Inter-varietal variation in elemental uptake by rice and its implications for public health: a case study of Dareta village, Zamfara State, Nigeria

THESIS

By

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This thesis is submitted to the School of Science, Engineering and Environment (SEE), the University of Salford in fulfilment of the requirements for the degree of Ph.D.

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ABSTRACT

Nigeria is ranked first among the countries affected by Pb poisoning since it was reported in 2010 in Zamfara State. The Pb poisoning epidemic in Zamfara received global attention from various stakeholders and dietary Pb intake through rice which was identified as a major exposure route. There is a need to understand the extent to which different rice varieties accumulate Pb and whether varietal selection could be used to reduce dietary Pb exposure.

Whilst the Pb poisoning is having a truly devastating consequence, the dietary transfer of other contaminants in Nigeria also needs to be understood. One group of contaminants that have received very little attention to date in Nigeria is anthropogenic radionuclides. Nigeria is developing nuclear power generation as part of its energy mix, there is a need to understand the potential food-chain transfer of radionuclides released into the environment. Two radionuclides of importance in both operational discharges and emergency (accident) situations are likely to be radio-caesium and radio-strontium. Therefore, in addition to Pb, this thesis provides an evaluation of inter-varietal variation in stable caesium (Cs) and stable strontium (Sr); stable isotopes are assumed to show the same environmental behaviour as their radioisotopes. The uptake of nine essential elements was also evaluated.

Site characterisation was conducted first in Dareta village Zamfara Nigeria to select a suitable site for the rice varieties' field trial; local rice samples were collected from four selected rice farms to examine Pb accumulation and partitioning in different parts of the rice plant (experiment 1). This was followed by the field trial for the 10 most commonly grown Nigerian rice varieties (experiment 2). The field trial was complemented by a pot trial for the same rice varieties at the University of Abuja, Nigeria (experiment 3). At maturity, the rice varieties were harvested together with their respective soil samples and analysed.

Experiment 1; Pb accumulation in the rice plant was in the order of root>shoot>seed. Pb accumulation in shoots and rice seeds exceeded the FAO/WHO permissible limits of 10 mg/kg and 0.3 mg/kg respectively. Bisalayi rice, ncro-49, ita-315 and art3-7l demonstrated low uptake and accumulation of Pb in both experiment 2 and 3, whereas nerica-l34, nerica-l19, wita-4 and sipi rice varieties were found to have high Pb uptake and accumulation. Statistically, there were no significant differences ($p>0.05$) in the uptake and accumulation of the stable Cs and Sr in both trials using the concentration ratio (CR). All the selected rice varieties were good source of the nine essential elements in terms of their percentage contribution to the recommended daily intake (RDI).

Dedication

To the victims of Pb poisoning across the globe

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Abbreviations and Acronyms

ATSDR – Agency for Toxic Substances and Disease Registry
BLL – Blood Lead Level
Bq/kg – Becquerel/kilogramme (Becquerel is SI derived unit of radioactivity. Specific activity per quantity of a radionuclide and it is a physical property of that radionuclide)
Ca – Calcium
Cmol^{+}/kg – Centimol per kilogramme
CDC – Centre for Disease Control
Cs - Caesium
Cu – Copper
FAO – Food and Agriculture Organisation of the United Nations
FDA - Food and Drug Administration of the United State Department of Health and Human Services
g - Gramme
GIS – Geographic Information System
ha - Hectare
HRW – Human Right Watch
ICP-MS – Inductively Coupled Plasma Mass Spectrometry
INCHEM – International Programme on Chemical Safety
IQ – Intelligence Quotient
kg - Kilogramme
LGA – Local Government Area
L – Litre
m – Metre
Mg – Magnesium
mg – Milligramme
mL – Millilitre
Mn - Manganese
mg/kg – Milligramme per Kilogramme (same measure as part per million)
MSF - Médecins Sans Frontières (MSF is an International, Independent, medical humanitarian organisation.
NAFDAC – National Agency for Food and Drugs Administration and Control
NERICA – New Rice for Africa
NIH – National Institute of Health (National Library of Medicine, United State)
OCHA – Office for the Coordination of Humanitarian Affairs (United Nations Agency)
P - Phosphorus
Pb – Lead
SON – Standard Organisation of Nigeria
Sr - Strontium
U5MR – Under 5 Mortality Rate
µg/g – Microgramme per gramme
µg/dL - Microgramme per decilitre
UNICEF – United Nations International Children's Fund
UNEP – United Nations Environmental Programme
USDL – United State Department of Labour
USEPA - United State Environmental Protection Agency
WHO – World Health Organisation
Zn - Zinc

CHAPTER ONE

Introduction

1.0 Background

Nigeria is currently ranked first in the world among the countries that are adversely affected by lead (Pb) poisoning (Cornelius, 2018). In March 2010, more than 400 people died in Zamfara State, northern Nigeria and as a result of Pb poisoning among which more than 100 were children under the age of 5 years (Dooyema et al. 2012). This is likely the largest Pb poisoning epidemic in the history (Grossman, 2012; MSF, 2012; Nigeria Centre for Disease Control and Prevention, 2013). The World Health Organization (WHO), the US Centres for Disease Control (CDC), the United Nations Children's Emergency Fund (UNICEF) and MSF in collaboration with the Federal and Zamfara State Ministries of Health investigated the matter and in their respective reports, all the bodies tested confirmed the Pb poisoning epidemic in Zamfara State (Getso et al., 2014; Mosadomi, 2016; Udiba et al., 2012; UNICEF, 2011).

Zamfara State has an estimated population of 3.7 million (NPC, 2016), of which 20 % are children under the age of 5 years (UNICEF, 2011). Dareta village, Anka Local government Area (LGA) of Nigeria (the study site for this research) is one of the highly Pb-polluted LGAs in Zamfara State with 142,280 people (NPC, 2016). In this village, about 10,000 people died, and more than 16,000 were affected by the Pb-poisoning epidemic between 2010 and 2013 (Nigeria Centre for Disease Control and Prevention, 2013). The major occupations of the people in this area are mining and farming (Clement & Patrick, 2017; O. Orisakwe, Oladipo, Ajaezi, & Udowelle, 2017) and rice farming is dominant (Dogo, 2014). Rice is a popular staple food in Zamfara State, and it is also used to make other foods such as fries and pastries (Lukman et al., 2017; Mani, Muhammad, & Haruna, 2018).

The soil in Dareta has extremely high Pb concentrations (>10,000 mg/kg) and concentrations of up to 145 mg/kg have been reported for plant foodstuffs (Plumlee et al., 2013; UNICEF, 2011). Rice has the potential to accumulate high levels of Pb (Ashraf et al., 2018; J. Fu et al., 2008; Lai et al., 2018; Liu, Li, Xu, Zhang, et al., 2003; Liu, Ma, Wang, & Sun, 2013). Whether the local rice variety and other improved rice varieties cultivated by the farmers in the study area have the same potential for Pb uptake and accumulation is one question to be answered. Existing literatures on how much Pb is accumulated by the varieties of rice grown in this affected area of Zamfara State prior to this study are not found (not available). Rice and other foods made from rice are children's favourite and they derive joy from eating rice as many times as possible (Lukman et al., 2017; Mani et al., 2018). This has probably exposed the affected children to high Pb (Simba et al., 2018). Reports show that more than 50% of the blood lead level (BLL) recorded in this area in children is ascribed to food contamination (MSF, 2012; Simba et al., 2018; UNICEF, 2011) which needs an urgent intervention.

According to Dooyema et al. (2012), an average of 4.2 children under the age of 5 years live in each household. About 25 % of these children in the affected communities died between 2009 to 2011 and most of the deceased children had incidents of convulsions before their deaths, a common symptom of Pb poisoning (Dooyema et al., 2012). Between 2009 and 2012, under five mortality rate (U5MR) for Pb poisoning was 12 per 10,000 children per day within this exposed population (Dooyema et al., 2012). Generally, between 2009 and 2012, U5MR in Nigeria was 100 per 1000 (10 %) (The World Bank, 2019). In 2012, 97 % of the children under the age of 5 years within the affected communities had BLL >45 µg/dL, which indicated an acute Pb poisoning situation (Dooyema et al., 2012). There is no safe limit or acceptable BLL (CDC, 2012). The BLL of 45 µg/dL is the threshold to keep the patient on admission in a hospital for treatment, 2 µg/dL BLL is the allowable limit and 5 µg/dL BLL is the action level threshold for investigation (CDC, 2012).

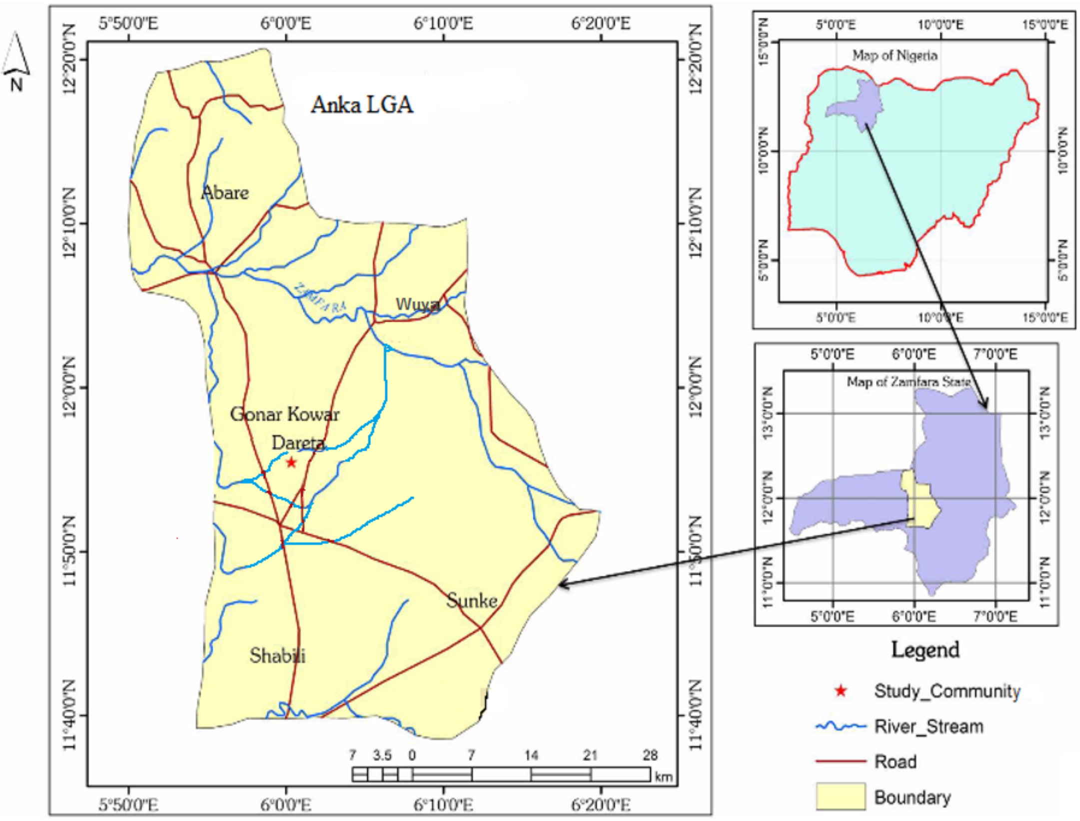
Investigation may involve determination of the source of contamination, route and period of exposure, while the treatment may involve administration of chelation therapy, which has been proven effective for elimination of Pb from the body (NIH, 2016). Some of the control measures initiated and implemented between 2010 and 2012 in Zamfara State were environmental remediation, chelation therapy, public health education and control of mining activities (Dooyema et al., 2012; Udiba et al., 2012; UNICEF, 2011). Sadly, the effect of the mining control policy, formulated by Zamfara state government to curb illegal mining, is not yet felt as indiscriminate mining is still ongoing (Jubril, Kabiru, Olopade, & Taiwo, 2017; Johnbull, Abbassi, & Zytner, 2019). UNICEF (2011). Tirima et al. (2016) strongly recommended further investigations on previous epidemics, remediation exercises and further research on interventions to reduce Pb exposure and poisoning in the affected population. The continuous unabated illegal mining activities is due to the abject poverty observed among the exposed population (Ingwe, Osonwa, &

Angiating, 2014) and strategies for reducing the resultant dietary Pb exposure of the local population are urgently required (Abubakar, Bagudo, Birnin Yauri, Sahabi, & Garba, 2015).

Whilst the Pb poisoning is having a truly devastating consequence, the dietary transfer of other contaminants in Nigeria also needs to be understood. One group of contaminants that have received very little attention to date in Nigeria is anthropogenic radionuclides, given that the country does not have any operational nuclear facilities. However, the country has plans to construct nuclear power stations and this will require prospective dose assessments and emergency planning. Current radionuclide transfer datasets have been derived based on a combination of data from radionuclides and their stable elements. Two radionuclides of importance in both operational discharges and emergency (accident) situations are likely to be radio-caesium and radio-strontium. Therefore, this PhD capitlises on the opportunities to undertake multi-elemental analysis of rice samples to determine the stable Caesium (Cs) and Strontium (Sr) transfer to rice grown in Nigeria and evaluates the extent to which varietal selection may help to reduce transfer should soils become contaminated with radio-caesium or radio-strontium.

1.1 Research Location

Zamfara state is one of Nigeria's 36 states and covers an area of 39,762 km^2 in the North Western Region of the country (Taft & Haken, 2015). The State has 14 Local Government Areas (LGA) including Anka LGA in which Dareta village (study site) is located (Figure 1.1); the Anka LGA is one of the two most Pb contaminated LGAs in Zamfara (Figure 1.2). The State has 62 districts and 147 political wards. Inter-state boundaries exist between Zamfara and Sokoto to the north-west, Katsina (north-east), Kaduna (south-east), Niger (south-south) and Kebbi State (south-west) (Aliyu, 2014). The State is mainly populated by Hausa and Fulani ethnic groups (Taft & Haken, 2015). Nigeria is a country of over 200 million people (World-Bank, 2016).



Geo-reference map of Dareta village, the studied community in Anka Local Government Area of Zamfara State (Salati, Mireku-Gyimah, & Eshun, 2014).

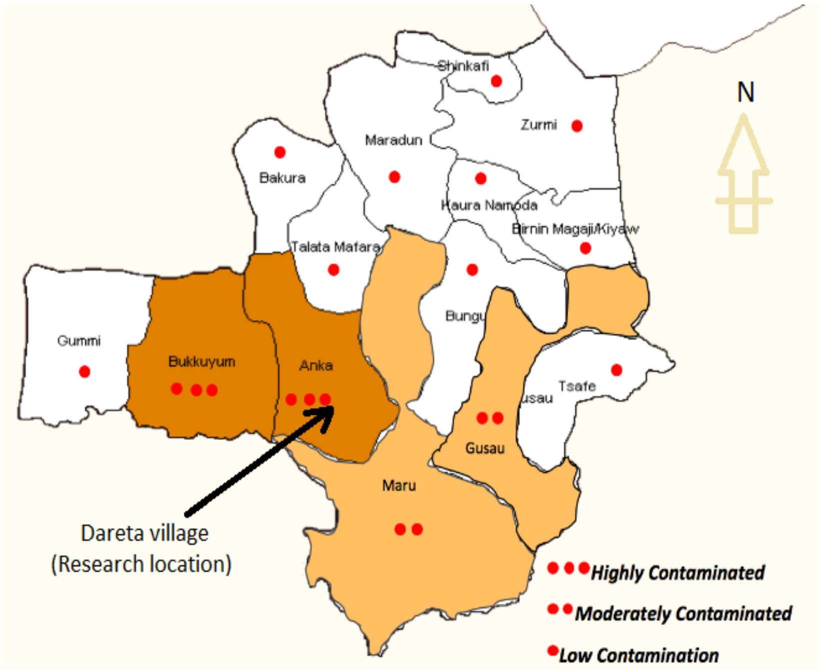


Figure 1. 1: Lead contamination ranking across Zamfara of Nigeria based on USEPA soil Pb permissible limit of 400 mg/kg (≤1000 mg/kg = low, ≤ 5000 mg/kg = moderate and ≥ 5000 = high) (Umar-Tsafe et al., 2013).

Zamfara State has a warm tropical climate with temperature rising to 38oC – 40oC between March and May every year (Dan-Ali, 2013). The annual raining season typically lasts from June to October while the dry season (windy harmattan) lasts from December to February (Aliyu, 2014). About 70 % of the population rely on farming for livelihood (Dan-Ali, 2013) and some on illegal mining of soil minerals (UNICEF, 2011). The major agricultural produce in the state are rice, guinea-corn, maize, cotton, tobacco, groundnut and beans (Adejumo & Raji, 2007). Industrial facilities in the State include rice mills, animal feed mills, rice flour factories and cotton factories which process farm produce into finished products (UNEP-OCHA, 2010). Zamfara State has many mineral resources comprising of gold (Au), copper (Cu), zinc (Zn) iron (Fe), tantalite (Ta_2O_6), manganese (Mn) among others (Salisu, 2010), which have been neglected by successive Federal and State Governments due to over-reliance on revenue from crude oil exploration from the southern part of the country (Odularu, 2008).

The high Pb in the soil in Dareta and other contaminated areas in Zamfara state (Figure 1.2) is traced to a long accumulation of Pb over the years and deposition of Pb dust emitted and waste from mining activities in the area which is one of the major occupation of the people of Zamfara (Simba et al., 2018; UNICEF, 2011). The local communities employ local methods to mine gold and other mineral resources domicile in the soil to sort themselves economically while unknowingly getting the enevironment contaminated (Udiba et al., 2013). Local mining sites is established almost in all the villages within the State (Plumlee et al., 2013). Dareta is one of the villages that is deeply engaged in the mining activities and about 70% of the adult males are involved (Udiba et al., 2013). Farming was the major occupation in Zamfara state prior to the discovery of mineral deposits in the late 1990s (Thisday, 2016). People used to engage in farming activities both in the rainy and dry seasons with irrigation (Orisakwe, Oladipo, Ajaezi, & Udowelle, 2017). However, active farming has been limited predominantly to raining season since the discovery of gold and only few farmers engage in irrigation during dry seasons (Okoh & Hilson, 2011). Artisanal mining is done all year round due to viability of the income generated from gold (Uriah, Kenneth, Rhoda, and Ayuba, 2013).

Income from mining is more than that from farming (Okoh & Hilson, 2011). The reason for the sudden increase in mining activities between 2009 and 2011, which led to the Pb poisoning crisis in the region has been traced to a sudden increase in the gold price due to high demand of gold globally (World Health Organization, 2016a). The gold price increased by 100 % from USD700/oz in 2009 to USD1400/oz in 2011 (Figure 1.3) and since then, it has remained above USD1000/oz (GoldPrice.com, 2018; US Bureau of Labour Statistics, 2017). It was also reported that the Nigerian gold production jumped from 1.3 to 4.0 metric tonnes per year between 2010 and 2011 (Philip, 2014).

Figure 1. 2: 10 years price of gold from 2009 (GoldPrice.com, 2018).

Extracting the gold from soil involves digging (which mixes the top and subsoils), grinding and crushing of rock materials which comprises of the gold ore, washing of the extracted gold ore in stagnant or running water and gold recovery (Amuda, Danbatta, & Najime, 2013). Through these procedures, the environment is contaminated and the contamination spreads with time (Udiba et al., 2012). The release of Pb from mining activities and its environmental mobility are discussed.

1.2 The mining process and the environmental contamination pathway

The process of exploring gold locally comprises of the following steps:

Extraction: This is the process of removing the mineral deposit from the underground and it is always in the form of sedimentary rocks or stones which are also called ‘ore’ (Mejia, 2015). The miners extract the best stones, based on their judgement, that are rich in gold by digging the soil in form of cave (Figure 1.4a), excavating a large areas of soil to expose the rock materials (Figure

1.4b) or tunnelling a man-hole down to the sediments underneath the soil (Figure 1.4c) to get the ore (Warra & Prasad, 2018). Figure 1.4 d shows how the deposit looks like under the soil before it is extracted and Figure 1.4 e & f reveals the image of the ore outside the soil. The ore contains more than 10 % Pb (United Nations Environment Programme, 2015).

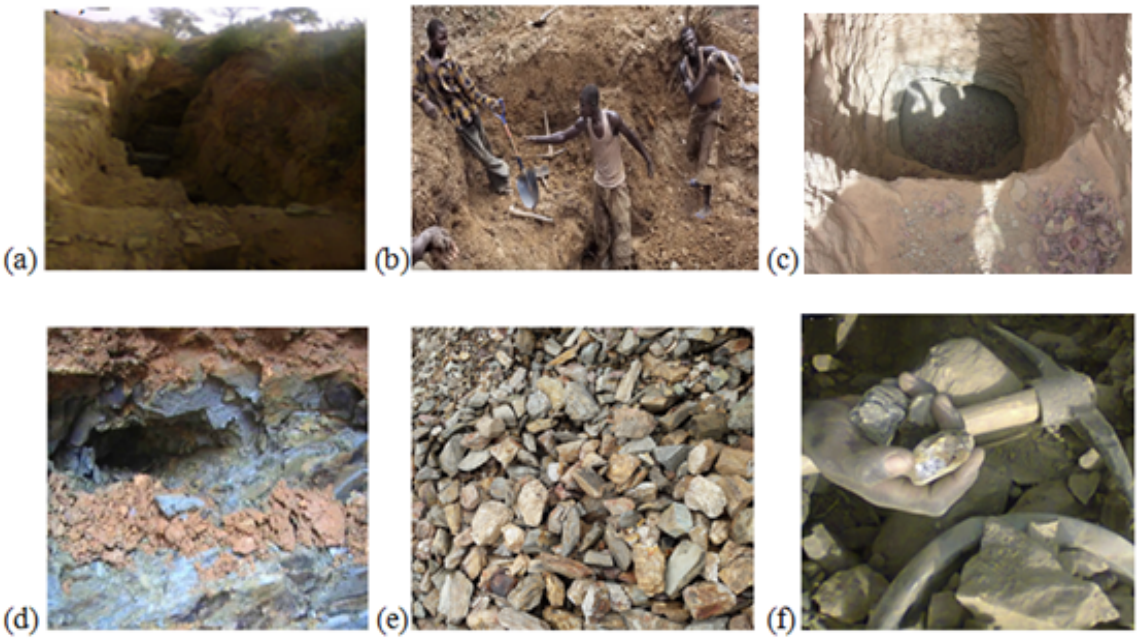


Figure 1.4: Extraction of the gold ore from soil (Gregg, 2009; Human Rights Watch, 2011; Warra & Prasad, 2018)

(a) digging the soil in form of cave, (b) excavating a large areas of soil to expose the rock materials, (c) tunnelling a man-hole down to the sediments underneath the soil (d) to get the ore, (e) shows how the deposit looks like under the soil before it is extracted, and (f) reveals the image of the ore outside the soil

By the time the aforementioned activity is completed, the topography is disrupted, and erosion is encouraged when it rains (Warra & Prasad, 2018). This is shown in Figure 1.5 captured during the fieldwork. Extraction mixes the top contaminated soil with subsoil and also reduces the grazing areas for animals (Udiba et al., 2013).

Figure 1.5: Look of the area where extraction takes place (photograph taken during the field work in 2017).

Crushing of the ore: To process the ore, it requires manual or machine crushing. Grinding and crushing of these materials involves manual application and the use of equipment such as chisels, hammers and the use of grinding machines to turn the ore to powder (MSF, 2012). During this process, there is heavy emission of dust which contains high Pb (United Nations Environment Programme, 2015). Crushing process is shown in Figure 1.6 (a) breaking of the rock materials into smaller forms making to make it easier to grind in the grinding machine, (b) emission of dust as the breaking of the rock materials takes place (c) grinding of the of the broken rock materials into fine particles and (d) emission of leaded dust as the grinding takes place (Human Rights Watch, 2011; Warra & Prasad, 2018). Most of the miners use the local fabricated grinding machines and there is no facility to protect the escape of the dust into the atmospheric space (Uriah et al., 2013). The Pb dust that is dispersed into the atmosphere travels miles away from the mining site due to the nature of heavy wind in Zamfara and the Northern part of Nigeria (MSF, 2012).

Figure 1.6: Crushing and grinding of the ore (Uriah et al., 2013; Udiba et al., 2012).

(a) breaking of the rock materials into smaller forms making to make it easier to grind in the grinding machine, (b) emission of dust as the breaking of the rock materials takes place (c) grinding of the of the broken rock materials into fine particles using grinding machine and (d) emission of leaded dust as the grinding takes place

Sluicing: This involves the washing of the grounded gold ore (the rock materials) with water in a sluice box to concentrate the liberated gold (Uriah et al., 2013). The locally made sluice box is made from car carpets, wooden planks joined with nails, two long wooden legs at one end of the wooden plank to create a sloppy medium and this is wrapped with rice bags at the upper part to allow free downward movement of the solution as shown in Figure 1.7a. Figure 1.7b reveals how some miners wash in groups into surface water. This solution that is been washed down into the water is a solution that contain high Pb sometimes between 15 % to 20 % (Udiba et al., 2012; Uriah et al., 2013).

Figure 1.7: Using a locally made sluice box to wash the gold ore (photograph taken during the field work in 2017).

(a) a small locally made sluice box for a single user (b) locally made sluice boxes operated by multiple users.

When this leaded solution is washed into the river or well or pond, it directly contaminates the water body (United Nations Environment Programme, 2015) and the water body serves as a transport medium to the Pb back onto the soil or directly into human body and to both land and aquatic animals (World Health Organization, 2011). If it is washed into a designated washing pots within the ore processing mills, there is no special way to treat the wastewater before it is disposed just as it was observed during the fieldwork for this research in Dareta, Zamfara. The wastewater is poured away on the surrounding soil at the gold processing mill and thus contaminates the soil directly. There is also a possibility that the unauthorised disposed wastewater from mining activities is washed into water bodies by erosion when it rains (UNICEF, 2011). For those established registered mining companies, gravity techniques with centrifuge or vibrating tables are used to discard wastewater and reduce environmental contamination (World Health Organization, 2016a) but no record of such is available around this area (Federal Ministry of Health, 2015).

Panning: This is the process by which the impurities is eliminated from the gold nugets recovered from sluicing (Uriah et al., 2013). This method is adopted by local miners because it is cheap and simple, although the productivity is low compared to the use of rocker box or electronic extractors

(Warra & Prasad, 2018). When substantial impurities have been removed, elemental mercury (Figure 1.8a) is added for the concentrate amalgamation (Uriah et al., 2013). This is done continuously as it revealed in Figure 1.8b captured during the fieldwork. bare hand is used to rub and wash the content against the pan while water is continuously added and decanted (Figure 1.8c) until gold nuggets shows (Figure 1.8d-f) (Gregg, 2009). At the end of this procedure, the added mercury together with the Pb and other metal components of the ore end-up as wastes in the environment while leaving the gold amalgam in the pan (Sousa et al., 2010). And the last stage is burning or flaming.

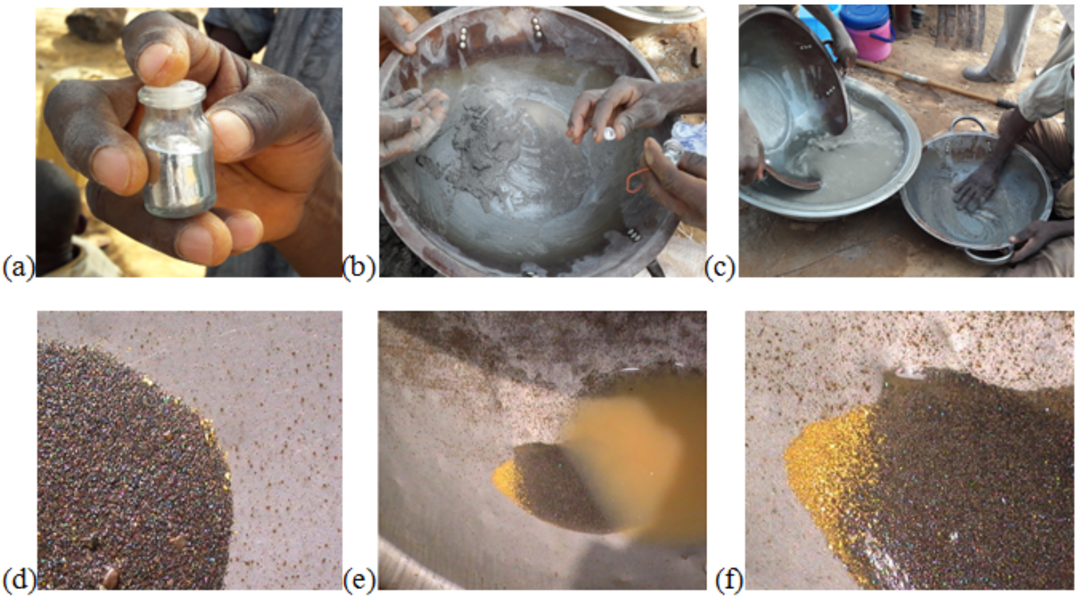


Figure 1.8: Gold panning techniques as observed in Dareta during the fieldwork (photograph taken during the field work in 2017).

(a) elemental mercury used for the concentrate amalgamation (b) using bare hand to rub and wash the amalgamated concentrate against the pan (c) addition of water to wash the concentrate in the pan (d) gold nuggets start to appear (e&f) gold nuggets appear more clearly

Burning/Flaming: This is the process of refining the gold by burning the amalgam with an open flame to recover the gold while the mercury is evaporated into the atmospheric air as shown in Figure 1.9a (Uriah et al., 2013). A gold nugets (amalgam) was captured during the fieldwork and it is shown in Figure 1.9b as it was placed on the palm of a miner. Figure 1.9c is the purified gold as captured by Warra and Prasad (2018).

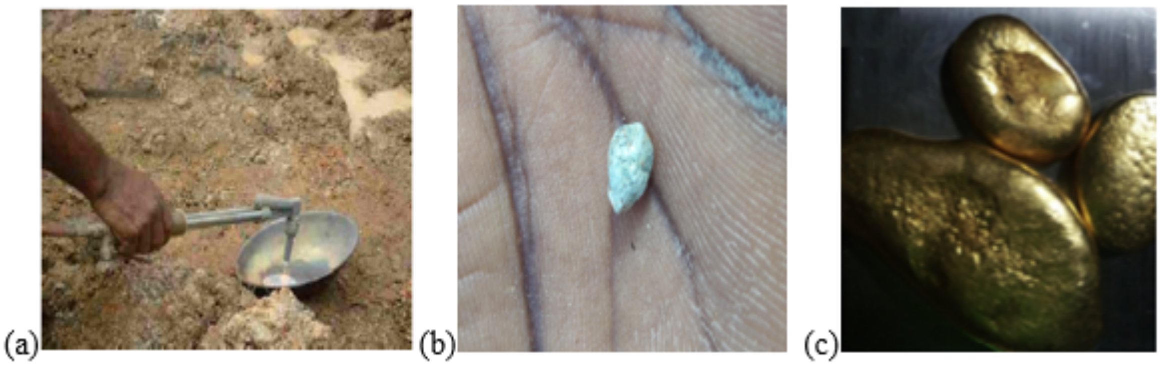


Figure 1.9: Burning of gold amalgam to a finished gold
(a) flaming of the gold nuggets to evaporate its mercury content into the atmosphere (b) the gold amalgam as it was captured during the field work (c) purified gold captured in Zamfara by Warra and Prasad (2018).

1.3 Reasons for Selecting Dareta Village

Dareta village has the highest number of Pb poisoning related deaths in Anka LGA and it has been one of the 8 villages recorded over 1000 deaths as a result of Pb poisoning (Federal Ministry of Health, 2015). These 8 villages are Dareta, Abare, Sumke, Duza, Tungar Daji, Bagega, and Yungar Euru and they were remediated between 2010 and 2012 based on the emergency remediation that was carried out (Nigeria Centre for Disease Control and Prevention, 2013) which is discussed in chapter 2, section 2.5. Logistics was one of the factors for selecting this village despite been one of the highly contaminated, Dareta was in a good location for easy accessibility. The village is located along Anka Bagega major road and this road was among the few access roads in the area at the time of this study compared to other villages. Dareta also close to Anka town, the local government headquarters.

Rice production was one of the major crops planted in this village every planting season. In terms of the security of life and properties as the government of the United Kingdom had marked Zamfara state as unsafe since early 2015 against foreign travel (GOV.UK, 2015). Dareta was fair and safe for researchers to work without a fear of ethnicity or religious crisis. At the time of this research, many parts of Zamfara were in a state of agony due to the activities of cattle rustlers, kidnappers who focus on Chinese nationals and other foreigners as their prey, armed bandits, armed robbery etc and many people have lost their lives (Jerrywright, 2016; ThePunch, 2018; ThisDay, 2016). All these were minimal in Anka LGA and Dareta village in particular before and during this study. Most importantly, farmers, leaders and the government officials were happy to see this research taking place on their land as this was stated in the approval letter issued in respect of this study by the government of Zamfara (Appendix C). The enthusiasm was right feasible from the first visit to the village head and to the farmers in the community for consultation.

1.4 Statement of the Problem

In Zamfara state, and specifically in the Dareta village, previous studies show that typical soil-Pb level ranges between 500 mg/kg and 300,000 mg/kg dry weight before 2012 (UNICEF, 2011; Udiba et al., 2012; Tirima et al., 2016). This had never happened in the global history of Pb pollution (UNEP-OCHA, 2010). As a result, emergency remediation of the affected villages was carried out between 2010 and 2012 in residential areas and mining sites where soil Pb was above 1000 mg/kg (UNICEF, 2011; Uriah et al., 2013). The remediation was simply the removal of the contaminated top soil (about 10cm) and replaced it with clean soil (the remediation is discussed in detail in chapter 2). Other areas remediated include worship centres, market places, drinking water points, playground and grinding mill sites (Tirima et al., 2016). No industrial site or arable land was included in the remediation plan due to limited resources (Getso et al., 2014; Udiba et al., 2012; UNICEF, 2011). Industrial site according to UNICEF (2011) report are the areas where the gold ores are been explored and processed while the arable land is the area that is being used for growing crops. Despite the high level of pollution, many of these contaminated soils are being used to grow crops in order to meet high demand for food due to global rapid population growth (Ran, Wang, Wang, Zhang, & Zhang, 2016). The need to meet up the family income with high cost of living in Zamfara state and Nigeria encourages the continuous cultivation of crops on the contaminated fields (Udiba et al., 2012; UNEP-OCHA, 2010; UNICEF, 2011).

In addition, the use of fertilizers to grow crops is very popular in Zamfara due to the fertilizer subsidy scheme introduced by the State Government (Liverpool-Tasie, Barrett, & Sheahan, 2014). In this subsidy scheme, the Government pays 40% of the fertilizer's cost while the farmers pay the remaining 60% (OEG-ZAMFARA, 2016). The subsidy scheme has encouraged the extensive use of fertilizers in Zamfara and this has been identified as one of the channels by which Pb in the soil can be mobilised as a result of lowering the soil pH (Liu, Li, Xu, Zhang, et al., 2003). Moreover, a recent study in Bagega, a border village to Dareta observes that the remediation techniques applied to clean the contaminated soil in Zamfara between 2010 and 2012 seemed not effective as most of the remediated areas still have elevated soil lead levels (SLLs) three years after the remediation exercise (Bartrem et al., 2014). Based on the soil Pb allowable limit of 420 mg/kg (United State Environmental Protection Agency, 2016), more than 50% of the remediated areas flags yellow and reds as shown in Figure 1.10. It is the Bagega village aerial map colour-coded with soil Pb concentrations measured by X-ray fluorescent spectrometer (XRF) (Bartrem et al., 2014). The orange colour indicates areas with 1000 – 4,900 mg/kg dry weight soil Pb concentrations while red indicates areas with 5,000 mg/kg dry weight soil Pb concentrations and above.

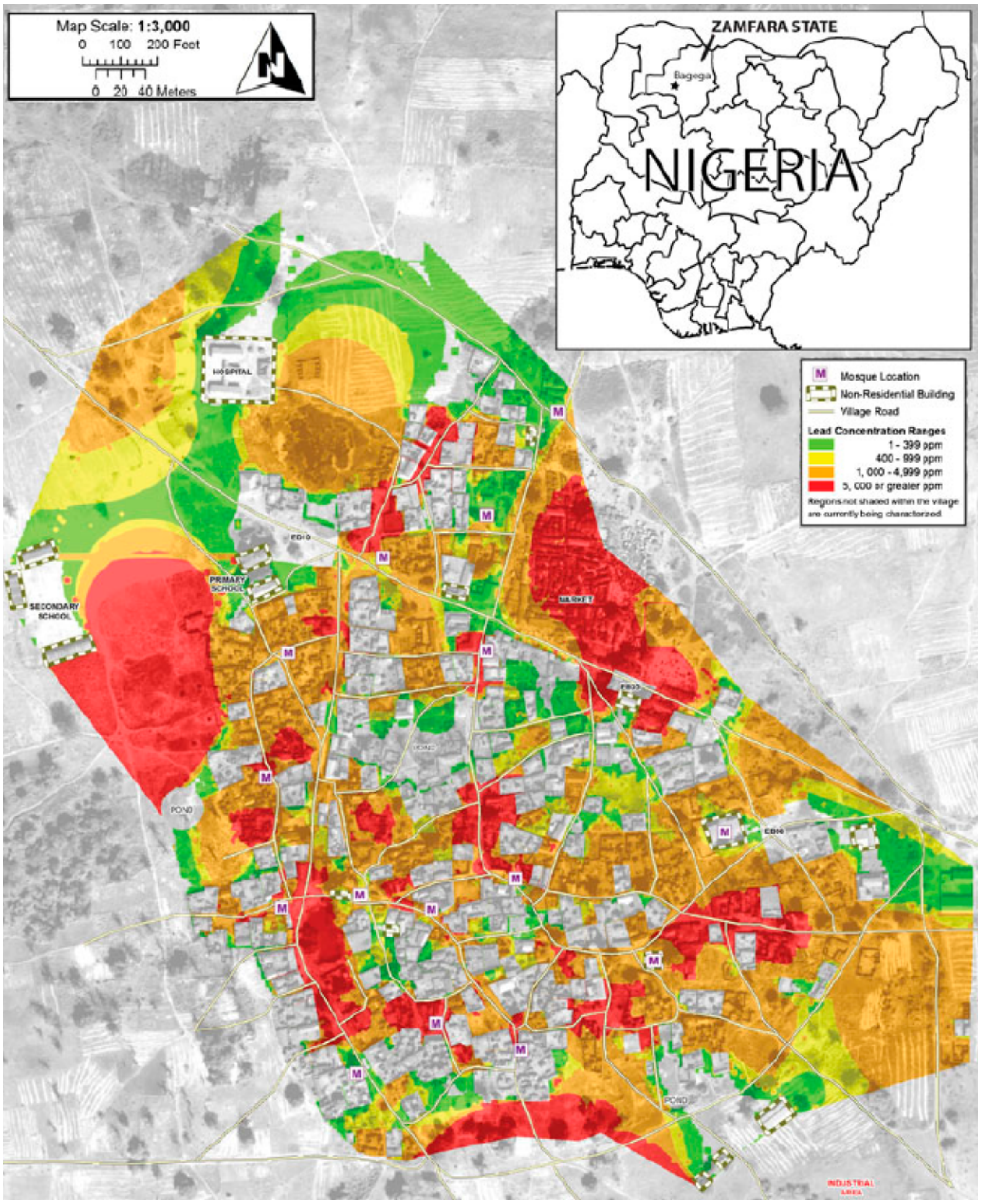


Figure 1.10: Aerial map of Bagega village showing the 2010 – 2012 remediated areas as it appears after three years of the emergency remediation (Bartrem et al., 2014).

Economically, Nigeria has been solely depending on crude oil since 1970s (Agbaeze, Udeh, & Onwuka, 2015) which accounts for 75 % of government revenue, 25% of Gross Domestic Product (GDP) and 95 % of the country's exports (TheEconomist, 2014). The African biggest economy (Nigeria) began to crash due to continuous fall of crude oil price globally from over USD100 to less than USD35 per barrel in 2015 (Timothy & Georgi, 2016). Technically, crude oil extraction cost was more than USD35 per barrel as at the time (Gail, 2016). Hence the negative effect on the Nigeria's economy. There was no other significant source of income for the country therefore, Government continues to produce and export oil, but a number of independent oil extraction companies could no longer continue due to high cost of production (Gail, 2016). As a result, the Nigerian government's revenue from oil was significantly reduced and government has chosen to diversify the economy into agriculture (Hussein & Vincent, 2016). Each state in Nigeria has priority areas to get involved in the Federal government's agricultural projects and the priority for Zamfara state was rice production (NigeriaMagazine, 2016; TheGuardian, 2016). Currently in Zamfara State, there was no land for agricultural purposes that was officially identified to be polluted (UNICEF, 2011). Hence, all the farmlands including the Pb contaminated ones are used to grow rice (Tirima et al., 2016).

Furthermore, according to Udiba et al., (2013) the State has placed a ban on mining activities, which is a more lucrative job than farming in the state, without providing an alternative source of income for those who were dependent on mining. This has increased the illegal mining activities in the affected communities. Mineral ores are still being processed within the affected communities and within the farmlands despite the Government's embargo on the mining activities (UNEP-OCHA, 2013).

Looking at the situation from the public health point of view, consumption of Pb contaminated food has posed a big threat to food safety in Nigeria (Aliyu, 2014). During the pilot study for this research, it was discovered that rice is the major food to the people of Zamfara and the children consumes rice more than four times a day (UNICEF, 2011). Eating rice more than once a day increases the risk of heavy metal poisoning for children (Meharg et al., 2008; Sun et al., 2009). Studies confirm Pb poisoning in farm animals that consume rice fodder (camels, cattle, goats and sheep) (Aliyu, 2014; Jubril et al., 2017). There is no research found that has been undertaken in this part of Nigeria to ascertain how much Pb is accumulated in rice fodder. On one of the 10 selected rice varieties, different parts (root, shoot, husk and seed) were analysed to provide the first data on Pd transfer to rice fodder in this region. This is presented in chapter 5.

Whilst the Pb poisoning is having a truly devastating consequence, the dietary transfer of other contaminants in Nigeria also needs to be understood. One group of contaminants that have received very little attention to date in Nigeria is anthropogenic radionuclides, given that the country does not have any operational nuclear facilities. However, the country has plans to construct nuclear power stations and this will require prospective dose assessments and emergency planning. Current radionuclide transfer datasets have been derived based on a combination of data from radionuclides and their stable elements. Two radionuclides of importance in both operational discharges and emergency (accident) situations are likely to be radio-caesium and radio-strontium. Therefore, this thesis also capitalises on the opportunities to undertake multi-elemental analysis of rice samples to determine the stable Caesium (Cs) and Strontium (Sr) transfer to rice grown in Nigeria and evaluates the extent to which varietal selection may help to reduce transfer should soils become contaminated with radio-caesium or radio-strontium.

1.5 Justifications

Currently, the world population is about 7.8 billion and Nigeria is 2.6% (Worldometer, 2019). It increases with 82 million people annually while Nigerian population increases with 5 million people on average every year for the past five years (Worldometer, 2019). It is important to note that the global population utilises rice as a major component of their diet, and this means it is important to understand how the dietary intake of contaminants via rice consumption can be minimised. Cultivar selection has become one of the best options to reduce the transfer of contaminants from soil to human via rice consumption (Norton et al., 2014).

Dareta village in Zamfara State and its neighbourhoods are predominantly rice growing communities with their soil contaminated with Pb and some other contaminants (UNEP-OCHA, 2010; UNICEF, 2011). It is important to obtain information on the concentrations regarding these contaminants in rice grown in Zamfara, some other parts of Nigeria and how this varies among several Nigeria rice varieties and its implication of consuming the rice varieties and their products. This is a critical time when government is diversifying the nation’s economy to agriculture from crude oil based. Nigeria is known globally as a nation that depends exclusively on oil (Ucha, 2010). Zamfara State Government makes a special budget to invest heavily in rice production locally every year (OEG-Zamfara, 2016) through a support from Presidential Initiative on Increased Rice Production (PIIRP) and the Nigerian National Rice Development Strategy for 2009 to 2020 (Cadoni & Angelucci, 2013). Through that, many Nigerians are getting engaged with rice production through government support (Shehu, 2016).

Although data is not available on the exact tonnes of rice that is produced from Dareta, it was confirmed in 2016 that Zamfara State produces more than 400,000 tonnes of rice per annum (NigeriaMagazine, 2016). With the current supports from the Federal government, research shows that the rice production in Nigeria including that of Zamfara has doubled the previous production for the past five years as at April 2019 (Russon, 2019). It is therefore necessary to determine how safe it is to consume rice that is produced in this mineral mining impacted area of Nigeria and how this varies among the various existing rice varieties in Nigeria.

Findings of this research contributes to the public health information associated with rice production in the mining region of Zamfara and, potentially, for other locations around the world where Pb concentrations and other contaminants in the soil are enhanced. Till date, data on the issue raised in this study are not available either to the government, public or agencies. It is observed that no research of this nature has been undertaken in Zamfara as well as affected regions in Nigeria.

This research is considering the effect of variety on the essential elements in rice. Toxic and essential elements in rice in Nigeria have recently been presented by Adedire et al. (2015), but the sampled rice was from market in the south-western part of Nigeria and the variety of rice investigated by Adedire (2015) is unknown. In addition, no study has looked into how soil properties influence the uptake of lead in rice in Zamfara State.

Finally, Pb is confirmed to be an issue of concern, but this study also involves other contaminants that need to be understood as the country plans to construct nuclear power stations across Nigeria which will eventually require dose assessment and emergency planning (Mjimba & Elum, 2016; Ohimain, 2015; Lowbeer-Lewis, 2010). Anthropogenic radionuclides have received little or no attention over the years in Nigeria due to the fact that there are no operational nuclear facilities in place (Olise, Akinnagbe, & Olasogba, 2016). Two radionuclides of importance in both operational dicharges and emergency (accident) situations are likely to be radio-caesium and radio-strontium (Mikami et al., 2015). Assessment of the stable isotopes of caesium (Cs) and strontium (Sr) can serve as analogue of the radioisotope counterparts of these elements as they have the same properties (Srinuttrakul and Yoshida, 2017).

1.6 Research Aim and Objectives

1.6.1 Aim

To explore inter-varietal variation in lead (Pb), caesium (Cs) and strontium (Sr) and nine essential elements uptake by rice grown in Nigeria and the potential implications for public health.

1.6.2 Objectives

- To evaluate the influence of soil physico-chemical properties on Pb uptake in rice
- To assess the localisation of Pb in different part of Nigerian rice plant (root, shoot and the seed).
- To establish the inter-varietal variation in the uptake of Pb, Cs, Sr and nine essential elements uptake among the varieties of rice grown in Nigeria.
- To examine rice varieties contribution to the recommended dietary intake of nine essential elements via rice consumption.

1.7 Thesis Structure

Data gathering for this study involved review of literature and recent articles on inter-varietal variation in lead uptake by rice across the world including the previous works done in Africa. No evidence of previous study on this topic in Nigeria based on the literature. In addition, there were tree major work conducted in line with the set-up objectives to actualise the aim of this study.

The first work was the site characterisation which involved the use of handheld X-ray Fluorescence (XRF) spectrometry to scan the soil of the rice farms to get an understanding of how the Pb concentration in the soil was distributed. Four rice farms were selected within Dareta village and characterised. Soil and Rice samples were collected from the four selected rice farms for laboratory analysis. Each selected farm was divided into four sections and samples were collected from five sampling points from each division (details in section 3.2.2). At every sampling point, after collection of the rice sample, the corresponding soil samples were collected at three different soil depths; 0-10 cm, 10-20 cm and 20-30 cm depth. The reason for sampling at different depth was to check the soil profile regarding Pb concentration at different soil dept within the reach of rice root as the study shows that rice root is domicile within 30cm soil depth (NCRI, 2017). The rice samples were also dissected into the root, shoot, husk and the seed. This was to evaluate the influence of soil physico-chemical properties on Pb uptake in rice and to access the localisation of lead in different part (root, shoot, husk and seed) of rice. The objective of this first work were majorly 3;

To select the appropriate site for the elemental transfer (varietal selection) experiment which would involve growing of the 10 selected Nigerian rice varieties.

To determine the influence of soil physico-chemical properties on the Pb uptake in rice. The goal was to focus on the popular Nigerian local rice (*Bisalayi*) which were already grown on the four rice farms that were selected for the site characterisation. As at the time of this study, no research of such was found in this area in Nigeria. More information about the method is presented in chapter 3 and the result is presented in chapter 4.

To assess the Pb concentration distribution and accumulation in rice pats (root, shoot, husk and seed) which is also called partitioning. The result about the method is presented in chapter 3 and the result is presented in chapter 5.

The second work was a field experiment (varietal trial) to establish the inter-varietal variation in the uptake of Pb, Cs, Sr and nine essential elements (Ca, Cu, Fe, Mg, K, Zn, Mn, Co and Se) uptake among the varieties of rice currently grown in Nigeria. Health risk assessment was conducted for Pb, Cs & Sr in rice and the rice varieties contribution to the recommended dietary intake of the nine essential elements via rice consumption was examined. Pb and the nine essential elements are presented in chapter 6 while Cs and Sr are presented in chapter 7.

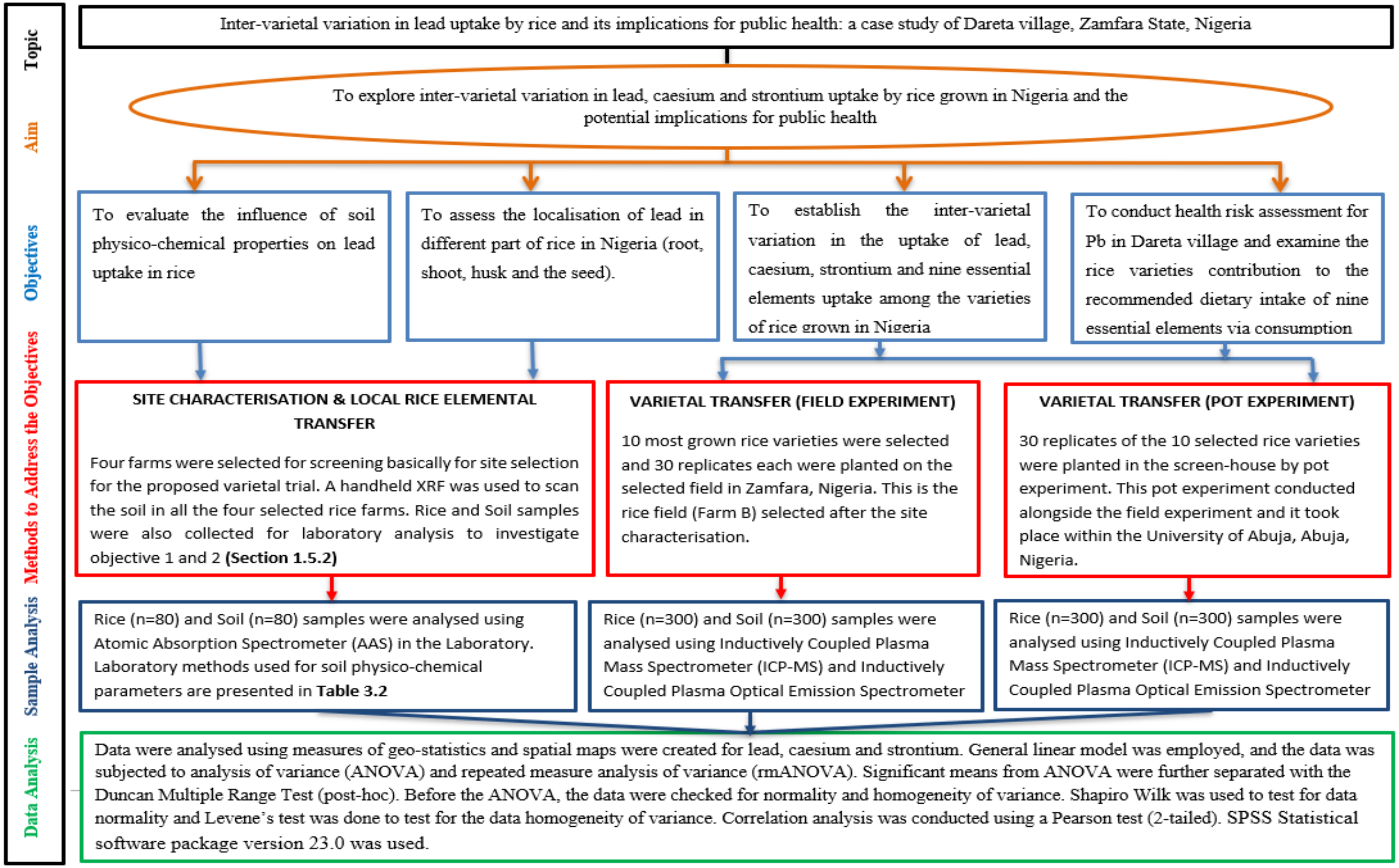
The third work was a pot experiment (varietal trial). Globally, millions of experiments are conducted under a controlled environmental condition which is popularly called greenhouse experiment (Poorter et al., 2012). Whether pots, boxes or bags are used to carry the growth medium, it is refers to as pot experiment and it has been one of the essential methods in pure, applied and life sciences especially in environmental management to assess environmental contaminants, soil and plants diseases (Mercier & Manker, 2005). The conventional practice for this method is to have all the abiotic environmental conditions for the plant growth such as the water supply, suitable temperature, relative humidity, minerals, CO2, and light under control (Postolache, Pereira, Girão, & Monteiro, 2012).

Notwithstanding, it is not in all cases that pot-experiments are suitable to be conducted under a controlled system of greenhouse (Friesl, Horak, & Wenzel, 2004). There are basic areas to consider for experimental set-up when it comes to pot experiment generally and these include but not limited to;

- Pot size required for the plant to be grown
- Type of rooting medium e.g. whether it is going to be water-based rooting (hydroponic), substrate based, or soil based.
- Type of the treatment that will be involved
- Where the rice will be grown
- How many replicates will be required

Among all the above listed basic areas, major consideration was given to the number of replicates because all the available greenhouses within the University would not be able to accommodate 300 plants appropriately with the adopted Randomised Complete Block Design (RCBD). At the same time, the aim was to compare with the same set of 10 selected rice varieties planted on the field where the soil-Pb concentration was not evenly distributed. In this out-door open air (screen-house) pot-experiment, the major aim of the study was to have a different trial with different soil but the same soil-Pb concentration (mg/kg) across all the pots (all replicates) for the trial. This was to see if the rice varieties would behave the same way as they did on the field. This method allowed everything to be naturally controlled under an open air as we had on the field to avoid multiple variations.

This work was to further establish the inter-varietal variation in the uptake of Pb, Cs, Sr and nine essential elements uptake among the varieties of rice currently grown in Nigeria. Little health risk assessment was conducted for Pb, Cs & Sr in rice and the rice varieties contribution to the recommended dietary intake of the nine essential elements via rice consumption was studied and compared with the result of the field experiment. This is presented also in Chapter 6. The Figure 1.11 summarises schematically the structure and the methods used in this study in brief while details are presented in chapter 3 (methodology chapter).



Inter-varietal variation in lead uptake by rice and its implications for public health: a case study of Dareta village, Zamfara State, Nigeria

To explore inter-varietal variation in lead, caesium and strontium uptake by rice grown in Nigeria and the potential implications for public health

To evaluate the influence of soil physico-chemical properties on lead uptake in rice

To assess the localisation of lead in different part of rice in Nigeria (root, shoot, husk and the seed).

To establish the inter-varietal variation in the uptake of lead, caesium, strontium and nine essential elements uptake among the varieties of rice grown in Nigeria

To conduct health risk assessment for Pb in Dareta village and examine the rice varieties contribution to the recommended dietary intake of nine essential elements via consumption

SITE CHARACTERISATION & LOCAL RICE ELEMENTAL TRANSFER

Four farms were selected for screening basically for site selection for the proposed varietal trial. A handheld XRF was used to scan the soil in all the four selected rice farms. Rice and Soil samples were also collected for laboratory analysis to investigate objective 1 and 2 **(Section 1.5.2)**

VARIETAL TRANSFER (FIELD EXPERIMENT)

10 most grown rice varieties were selected and 30 replicates each were planted on the selected field in Zamfara, Nigeria. This is the rice field (Farm B) selected after the site characterisation.

VARIETAL TRANSFER (POT EXPERIMENT)

30 replicates of the 10 selected rice varieties were planted in the screen-house by pot experiment. This pot experiment conducted alongside the field experiment and it took place within the University of Abuja, Abuja, Nigeria.

Rice (n=80) and Soil (n=80) samples were analysed using Atomic Absorption Spectrometer (AAS) in the Laboratory. Laboratory methods used for soil physico-chemical parameters are presented in **Table 3.2**

Rice (n=300) and Soil (n=300) samples were analysed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometer

Rice (n=300) and Soil (n=300) samples were analysed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometer

Data were analysed using measures of geo-statistics and spatial maps were created for lead, caesium and strontium. General linear model was employed, and the data was subjected to analysis of variance (ANOVA) and repeated measure analysis of variance (rmANOVA). Significant means from ANOVA were further separated with the Duncan Multiple Range Test (post-hoc). Before the ANOVA, the data were checked for normality and homogeneity of variance. Shapiro Wilk was used to test for data normality and Levene's test was done to test for the data homogeneity of variance. Correlation analysis was conducted using a Pearson test (2-tailed). SPSS Statistical software package version 23.0 was used.

Figure 1. 11: Schematic diagramme for this study

Chapter 1 contains a brief introduction and the background of the problem of the Pb poisoning in Zamfara State Nigeria (mortality, morbidity, and the prevalence), and how it is important in research. Research location, research justification and the problem statement. The pathway for the environmental contamination and the local mining process is discussed here. Study aim, and the objectives were presented in this chapter and the scope of the research work was highlighted too.

Chapter 2 presents the literature review on general physical and chemical properties of Pb, distribution and transportation in the environment, other likely sources of Pb contamination and exposure route, associated health challenges in human. Previous studies on the sources and the effects of Pb ingestion in children were presented, the emergency remediation that was conducted by the stakeholders previously in the area and post-remediation assessment conducted recently. The Pb in rice, the uptake including its permissible limit standards were discussed. Rice production in Nigeria, rice growing procedures, toxic and essential elements in rice, and elements that are of radio-ecological important were all presented in this chapter.

Chapter 3 is the section where the methods involved in this study was discussed. Soil characterisation, varietal trial (field and the pot experiment) including the experimental design, rice planting and transplantation, farm monitoring, harvesting. Statistical analysis and other essential calculations are discussed here. Sample collection, preparation and analysis are presented. The materials, tools, equipment/instrument used which include their calibration procedures were talked about.

Chapter 4 is where the investigation on the influence of soil physico-chemical properties on the accumulation of Pb in the Nigerian local bisalayi rice grown on Pb contaminated soil was reported. The general physical and chemical properties of soil in this area, Pb concentration in the bisalayi rice and their concentration ratio are discussed.

Chapter 5 presents Pb accumulation and distribution in the various part (root, shoot, husk and the seed) of Nigerian local bisalayi rice grown on Pb contaminated soil.

Chapter 6 focused on the inter-varietal variation in Pb uptake by the 10 selected rice varieties grown on the Pb contaminated field in Zamfara and those grown with the pot experiment, and a brief hazard assessment.

Chapter 7 presents the inter-varietal variation (IVV) in the essential element update among the 10 selected rice and examines the rice varieties contribution to the recommended dietary intake of nine essential elements analysed via the rice consumption. While

Chapter 8 was on the inter-varietal variation of the 10 selected rice variaeties on the uptake of stable Cs and Sr

Chapter 9 focuses on general discussion, summary of findings, conclusion and recommendations as well as the future research.

CHAPTER TWO

Literature Review

2.0 Brief Overview

Industrialisation has caused release of different pollutants into the environment including heavy metals and radionuclides. To support environmental regulation, exposure assessments and public health interventions, the environmental and human food chain mobility of these pollutants must be quantified (Khan et al., 2015), the level of exposures should be reduced and the exposure routes should be checked (World Health Organisation, 2016). As a result of industrial activities, soil in many places including those that are used for crop production are contaminated (Ran et al., 2016). Pb is considered by the World Health Organisation (WHO) as one of the major public health hazards currently (Mahar, Wang, Li, & Zhang, 2015). This thesis and the review of literature presented in this chapter primarily focuses more on Pb uptake in rice. However, the literature review also includes sections on Cs and Sr to provide additional context for the chapter that discusses the influence of rice varietal selection on the transfer of these two elements.

2.1 Physical and Chemical Properties of Pb

Pb is a ductile, soft, highly malleable heavy metal with bluish white shinning colour (Lenntech, 2015). It is a post-transition metal with atomic and mass number of 82 and 207.2 respectively (RSC, 2016). Pb exists naturally in solid form and in four observationally stable form (Isotope) of 204, 206, 207 and 208, but all have the same number of protons (82) (RSC, 2016). On the periodic table, it belongs to carbon group or group 14 elements and symbolically represented as Pb (Ducksters, 2016). The carbon group which in the modern IUPAC notation called Group 14, but in the field of semiconductor physics, it is called Group IV elements which comprises of the lead (Pb), silicon (Si), carbon (C), germanium (Ge), tin (Sn), flerovium (Fl), thulium (Tm) and mendelevium (Md) (RSC, 2016). Boiling point decreases as the mass of these metals increases (Cotton, Wilkinson, Murillo, Bochmann, & Grimes, 1999). Pb, being the heaviest (207 g/mol) has the least boiling point of 1755^{0}C compared to the lightest, Carbon (12 gmol-1) with the highest boiling point of 4827^{0}C (Housecroft & Constable, 2010). These two letters

(Pb) were derived from its Latin name Plumbum which is from the piping work “plumber” (Ducksters, 2016). In the history, Pb has been much involved as one of the contents in water steel-pipe manufacturing which prevents the pipes from rust and damage (Torrice, 2016). Due to the health impacts, using Pb in water pipes to prevent corrosion is now regulated globally (Brown & Margolis, 2012; Rosen, Pokhrel, & Weir, 2017).

A total of 118 elements are on the periodic table, out of which 77% are classified as metals (Lenntech, 2015). Generally, when metals are exposed to air, they react with oxygen to form oxides and these oxides are basic (alkaline) in nature compared to non-metals that react with oxygen to form acidic oxides (Passow, Rothstein, & Clarkson, 1961). Pb exists in the soil predominantly in +2 oxidation state and becomes less soluble with an increased pH in the soil solution due to complexation with organic matter, sorption on oxide and silicate clay minerals or precipitation as the carbonate, sulphate or phosphate (Alloway, 2013).

Pb is one of the human neurotoxic inorganic pollutant which can be mobilised within the soil and taken up by plants, together with other nutrients needed by the plants for its metabolic activities (Mahar et al., 2015; Torrice, 2016). Pb in the environment raises concern when it exceeds the standards of 0.05 mg/m^3 in air, 420 mg/kg in soil and 0.05 mg/l in water according to USEPA (1987) as cited in USDL (2004a). The ingestible limit in water provided by the World Health Organization (WHO) and the Standard Organisation of Nigeria (SON) is 0.01 mg/l (Standard Organisation of Nigeria, 2007; World Health Organization, 2015). Pb should not exceed 0.3 µg/g in vegetables (Abubakar et al., 2015), 0.20 mg/kg (fresh weight limit) and 0.24 mg/kg (dry weight limit) in cereals grains such as rice, wheat, husk, germ and maize (Adams et al., 2001). Pb is neither essential nor beneficial in the body of all living organisms (Alloway, 2013).

2.2 Pb and Gold Mining

Pb is the first metal to be extracted by man from its ores and extensively used for different purposes (Alloway, 2013). Silicate rocks or common sedimentary rocks shales have higher Pb of about 22 mg/kg than sandstones which has about 10 mg/kg (Alloway, 2013). Pb occurs in a variety of mineral phases, the most important of which are galena (PbS), cerrusite ($PbCO_3$), hydrocerussite ($Pb_3(CO_3)_2(OH)_2$), Pb hydroxide ($Pb(OH)_2$) and anglesite ($PbSO_4$) (INCHEM, 2016). Galena is the most important source of primary Pb (INCHEM, 2016). It occurs mostly in deposits associated with other minerals particularly those containing zinc and gold (INCHEM, 2016) which is the case in Zamfara gold mining environment (Mejia, 2015). Many of the above-mentioned compounds are present in Zamfara soil and they are very rich in Pb (Mejia, 2015). Apart from Pb in the gold ores, bismuth, antimony, arsenic, cadmium, tin, Nickel, Chromium, manganese, copper and zinc are among the other elements that may be present in large quantities (INCHEM, 2016).

2.3 Pb Transportation, Distribution in the Environment and the exposure routes

Pb is released into the environment by both natural and anthropogenic sources such as chemical conversions, leaching processes, mining, automobile exhaust and industrial activities (Figure 2.1) (Duruibe, Ogwuegbu, & Egwurugwu, 2007). Air transportation is one of the distribution routes (Plumlee et al., 2013) and Pb also gets into the air through the process involved in local mining process such as grinding and crushing of ores (section 1.3b, Figure 1.6) which emits Pb in form of dust into the air directly, emission from burning of leaded gasoline and emission from industries (Chambial, Shukla, Dwivedi, Bhardwaj, & Sharma, 2015). Pb particles, less than 2 µm in diameter can move over a long distance in air and result in contamination of the remote sites (INCHEM, 2016). This route also contributes to human exposure through contamination of food, water and polluted air by direct inhalation (Duruibe et al., 2007). The Pb particles spread by air can deposit and accumulate on soil and water with time and this may be at a significant rate (ATSDR, 2016). Particles containing Pb get into water through deposition from air, direct human contamination and run-off from soil (INCHEM, 2016). Pb composition and distribution in water depends on the organic content, the pH and the salt content of the soil which aids mobility of Pb (Park, Lee, & Kim, 2011). Naturally for surface water, hard water for instance may have as low Pb as 30 µg/l while soft water may have up to 500 µg/l because of its salt contents and pH level (INCHEM, 2016).

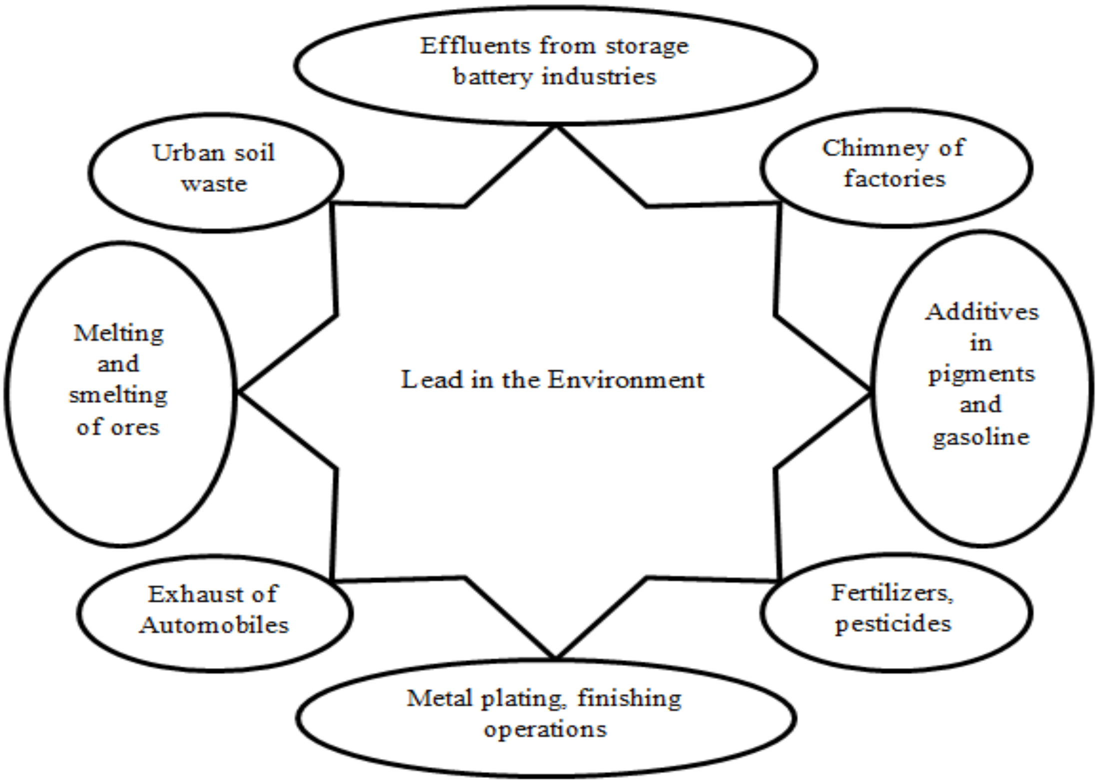


Figure 2. 1: Sources of Pb pollution in the environment adapted from Sharma and Dubey (2005).

Pb contaminates soil through air and water and human activities (Duruibe et al., 2007) which includes irrigation by contaminated water. The mean content of Pb in uncontaminated soils globally is estimated to be between 17 mg/kg and 50 mg/kg (Adams et al., 2001; Alloway, 2013). Poisoning and toxicity of Pb in man and animals occur frequently through exchange and coordination mechanism (Duruibe et al., 2007). It combines with biomolecules (proteins and enzymes) when ingested, to form stable bio-toxic compounds and thereby damaging their structures which obstructs enzymes and hormones from the bio-reactions of their normal functions (Duruibe et al., 2007). Figure 2.2 illustrates major exposure routes to Pb poisoning. Pollutants enter the food chain mainly through plants uptake (Penrose et al., 2015) and accumulation in the aquatic animals (Dang & Wang, 2009).

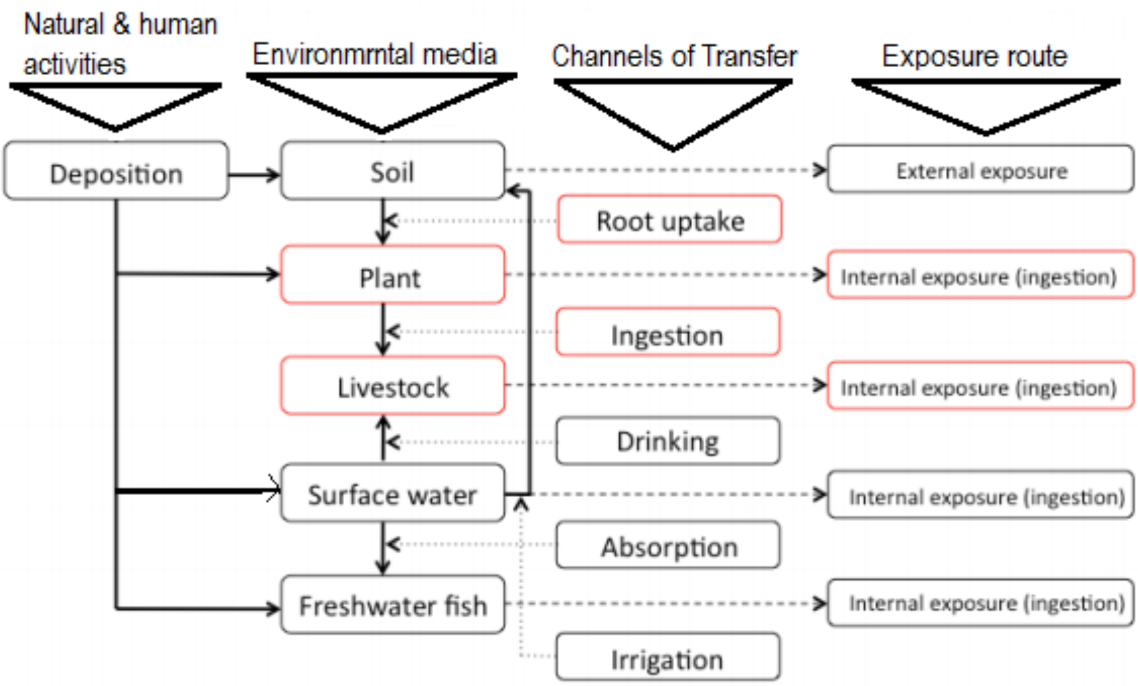


Figure 2. 2: Exposure routes for human and animal Pb poisoning adapted from Penrose (2015).

Globally, 60% of annual deaths were as a result of non-communicable diseases (NCDs) (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006) which accounts for 52.8 million in 2010 (Lozano et al., 2013). It rose to 68% in 2012 (WHO, 2015) and continues to rise due to people's lifestyles and environmental problems (WHO, 2015; WHO, 2017). Exposure to Pb accounts for 0.6% (about 500 thousand deaths on average) every year (World Health Organization, 2016b). Pathways of exposure and the resulting health effects are illustrated in Figure 2.3.

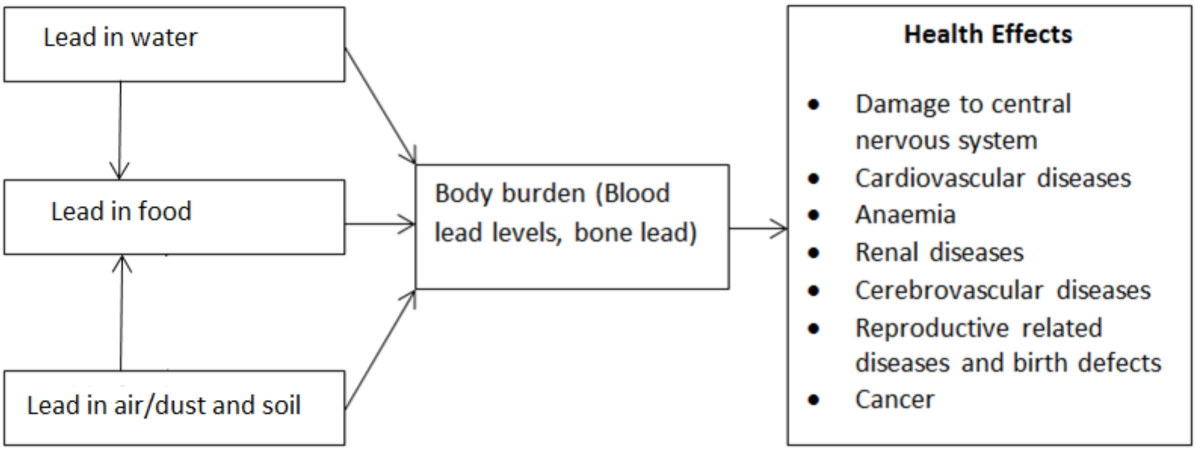


Figure 2. 3: Pathway to lead exposure and resulting health effects adapted from Fewtrell, Kaufmann, and Prüss-Üstün (2003).

Pb is toxic to human body systems (Hammond, 1977) in the sense that when it gains entrance, it inactivates many enzymes and this disturbs many metabolic processes (Otitoju, Otitoju, & Igwe, 2014). It is involved in the disruption of haem biosynthesis through alteration of activities of the two enzymes (delta-aminolevulinic acid dehydratase and ferrochelatase) that are directly involved in haem biosynthesis as revealed in Figure 2.4 (Adedire et al., 2015; Duru, Osinubi, Alebiosu, & Falana, 2015).

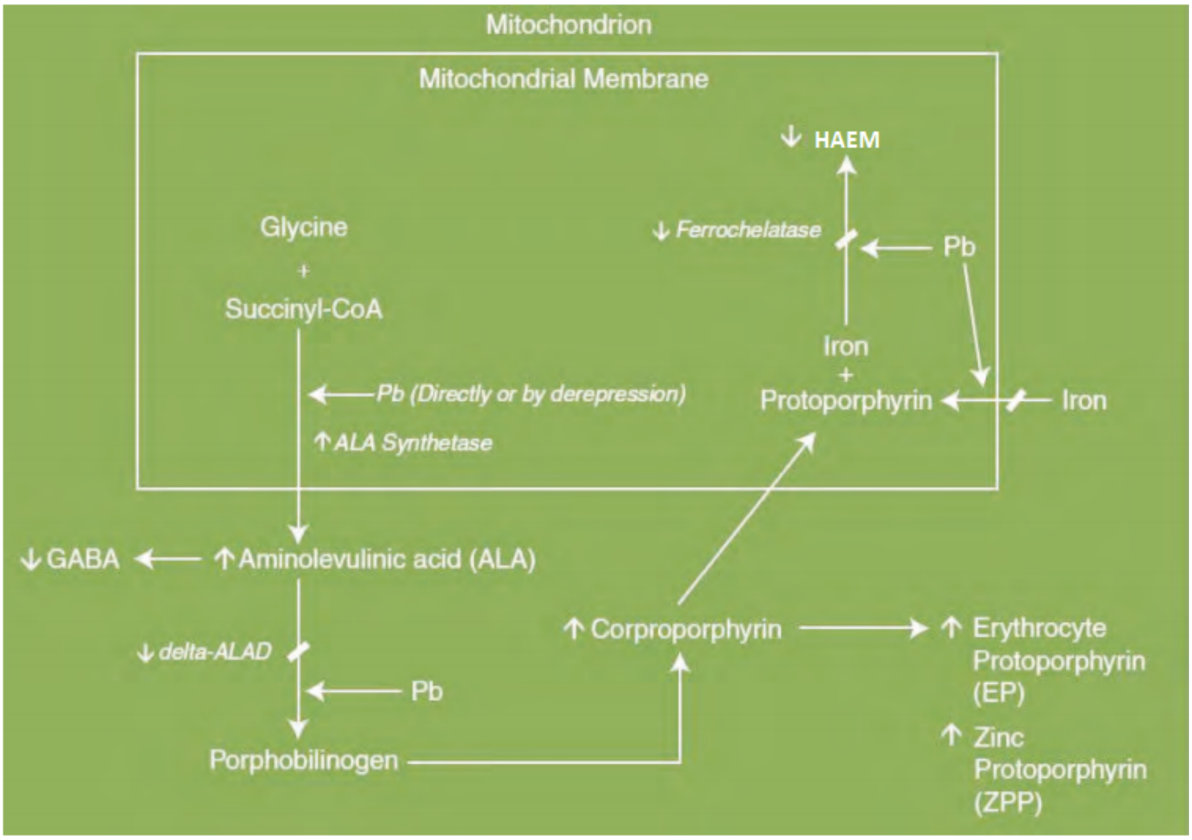


Figure 2. 4: Interruption of haeme biosynthesis by Pb (Duru et al., 2015).

No level of Pb is safe in human body especially in children whose vulnerability is high (McManus, Cummings, Visker, & Cox, 2015). The lowest detectable blood lead level (BLL) of <1 µg/dL may have effects on central nervous system which can produce significant negative effects on the normal mental and physical growth of a child and high exposure is capable of damaging the brain (McManus et al., 2015). Increase in blood Pb decreases Intelligent Quotient (IQ) in children (Parsons & Chisolm, 1997). An increase of approximately 1 µg/dL BLL is calculated to have an effect of 1.37 IQ-decrease in children under the age of 5 years (Gilbert & Weiss, 2006). Figure 2.5 illustrates the relationship between the BLL and the reaction of the

IQ in children under the age of 7 years while Table 2.1 reveals some previous studies on the effects of Pb poisoning on children. Anaemia, colic, muscle weakness, dementia, kidney damage, birth deformation and delayed sexual maturation are also associated with high exposure to Pb in children (ATSDR, 2016). Wang et al. (2017) explains that the Pb in the foetus cord has significant negative effects on the foetus as it affects the birth outcomes (Wang et al., 2017). Repeated low level of exposure causes abnormal behaviours in children (McManus et al., 2015) and blindness (MSF, 2012). This indicates that low level of Pb is also harmful (CDC, 2016). Repeated exposure to high Pb or long term repeated low level exposure can result in death of children and adults (CDC, 2007; World Health Organization, 2015).

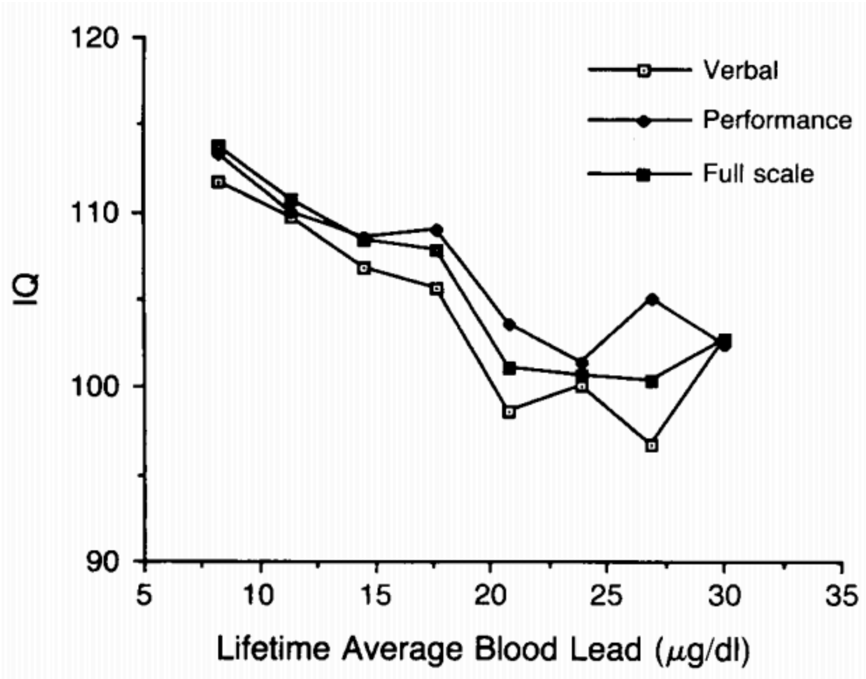


Figure 2. 5: Relationship between the blood lead levels and the reaction of IQ in under 7year old children (Baghurst et al., 1992).

Table 2. 1: Some previous epidemiological studies on the sources and effects of lead poisoning on children

Reference	**Region**	**Location**	**Methods, sample size, age and type of study**	**Result, comment and source of exposure**
Bose-O'Reilly et al. (2018)	Africa	Kabwe, Zambia	Health data from 3 health projects; Copperbelt, Zambia University and Pure Earth were analysed to provide a summary of BLLs. 12,378 Children (age<7), 7,919 Children (age<16) and 3,973 adults (age>16).	More than 95% of the children had BLLs above 2 µg/dL. About 50% had BLLs above 45 µg/dL. Lead poisoning is ongoing in Kabwe Zambia and multiple exposure routes were identified including food as major.
AbuShady et al. (2017)	Africa	Egypt	400 children (age 6 to 12) was studied using their blood samples from urban and industrial area for elevated BLLs	The children from the Industrial area have elevated blood lead levels (BLL) above 10 µg/dL and **it was traced to contaminated food source**.
Bello, Naidu, Rahman, Liu, and Dong (2016)	Africa	Nasarawa Nigeria	35 Children (age<7years) and 100 adults (age>18years) were examined from their blood samples for an elevated BLLs	Above 11% of the children and 14% of the adults possessed BLLs above 5 µg/dL. Also 68% of the adult population and 31% of the exposed children had their BLLs above 2 µg/dL. Ingestion of lead through the **food source was significant**.
Yabe et al. (2015)	Africa	Kabwe, Zambia	246 children (≤7years of age) were examined using their blood samples. The Study took place in the capital of the Zambia's Central Province called Kabwe	All were above the CDC'c action level of 5 µg/dL. The maximum recorded was 427.8 µg/dL. About 57 % had BLLs exceeding 65 µg/dL and 8 children had BLLs exceeding 150 µg/dL. The route of exposure was identified to be **contaminated food and environment.**
Greig, Thurtle, Cooney, Ariti, Ahmed, Ashagre, Ayela, Chukwumalu, Criado-Perez, Gomez-Restrepo, et al. (2014)	Africa	Zamfara state, Nigeria	972 children were studied. Children with BLLs ≥45 µg/dL tested between June 2010 and June 2011 were monitored as the treatment continues by MSF across the lead poisoning affected villages. Neurological assessment which included history of seizures, change in behaviour, delay or loss of development, peripheral neuropathies, gait, assessment of reflexes and level of consciousness was conducted.	35 % had BLLs ≥ 80 µg/dL. The maximum recorded was ≥ 708 µg/dL and before June 2011, 14 children among the study group have died. Those who died had BLLs between 104 and 460 µg/dL with consistent symptoms of encephalopathy. 83% of the children had mild neurological features while 24% had severe neurological feature. Mean BLL value for those who had severe neurological issues was >100 µg/dL. **Food contamination and poverty** were identified as part of the risk factors for elevated BLLs in the children.
Getso et al. (2014)	Africa	Zamfara state, Nigeria	A descriptive cross-sectional study of 307 children aged ≤6 years from Anka LGA of Zamfara state, Nigeria	92.5% of the children were lead poisoned (their BLLs > 5 µg/dL). Median value was 19.7 µg/dL and BLLs were from 3.3 – 372.3 µg/dL. It was concluded that these may be high exposure to lead ingestion. Risk factors identified were f**ood contamination, exposure to a contaminated environment, parent's low level of education and low income**.
Tuakuila, Lison, Mbuyi, Haufroid, and Hoet (2013)	Africa	Kinshasa, Democratic Republic of Congo (DRC)	275 individuals were examined via blood samples. 55 (20%) were children under 5 years from Kinshasa, the capital of DRC.	71% of the children under 5 years had BLL ≥ 10 µg/dL, 22% had BLL ≥ 20 µg/dL. The highest BLLs was found in children less than 3years old. The identified route of exposure was contaminated food and environment

Dooyema et al. (2012)	Africa	Zamfara State Nigeria	Survey of 119 family compounds, 463 children (<5years old) in gold-mining villages in Anka local government area of Zamfara was done due to massive death of children.	97% of the children have BLLs >45 µg/dL. It was concluded that the source of the elevated BLLs was lead poisoning from gold ore processing activities. **Ingestion through food and water** was identified as the exposure route.
Lo et al. (2012)	Africa	Zamfara State Nigeria	The research involved 74 villages in 3 local government area. 5 Children (ages 2months – 5years) were selected per village (5 × 74 = 370 Children)	81% of the villages had at least 1 child with BLLs >45 µg/dL and every village had more than 1 child with BLL ≥ 10 µg/dL. Every village had more than 1child who had died and had convulsions during the previous 12months. 71% of the villages process ore. Risk factors identified were **food and contaminated environment**.
Mathee, Röllin, Levin, and Naik (2007)	Africa	Johannesburg, South Africa	383 Johannesburg schoolchildren were screened for elevated BLLs. Home assessments and interviews with parents of the children were conducted.	BLLs ranged from 1.0 to 18.1 µg/dL. Mean value was 9.1 µg/dL. **Peeling paints in homes** was identified as a risk factor for elevated BLLs in the children.
Entzel, Fleming, Trepka, and Squicciarini (2003)	Latin America/ Caribean	Miami-Dade County, Florida	A retrospective cross-sectional study of medical records to conduct of blood test of 881 legally documented refugee children (<7years) who arrived newly in Miami-Dade refugee camp in the early year 2000.	22.9% of refugee children screened had elevated BLLs >10 µg/dL which is roughly 3 times higher than the US average of 7.6%. It was concluded that lead poisoning should be considered an important health problem among the refugee children arrived in Miami from Cuba. The risk factor could not be ascertained because the affected were just arriving the USA from another Country. The risk factor was suspected to be an **exposure to lead contaminated environment which could've further affected their food and drinking water source**.
Pfitzner et al. (2000)	Africa	Jos, Nigeria	A randomised cluster sampling of 218 children (urban children) of Jos, Nigeria (aged 6 – 35months) to evaluate elevated BLLs	70% of the children had BLLs >10 µg/dL. Mean BLLs was 15.2 µg/dL. Multiple factors associated with the increased BLLs were, **eating lead contaminated food, living near a battery smelter and living in contaminated prone geographic areas**.
Nriagu, Oleru, Cudjoe, and Chine (1997)	Africa	Kaduna, Nigeria.	Prevalence of elevated BLLs was determined in 154 children (age 1-6years). They were young urban children in Kaduna, North	2% have BLL >30 µg/dL. Mean BLLs was 10.6 µg/dL. Highest BLLs were found in age 5 children and was attributed to the tendency for this age group to stay longer in contaminated outdoor environments. Behavioural risk factor was **mainly ingestion of lead through food**.
Baghurst et al. (1992)	Australia	Port Pirie, Australia	IQ score of 494 seven-year-old children who live close to lead smelting site in Port Pirie were measured	Approximate deficit in IQ of 4 to 5% was recorded. Mean BLLs was 30 µg/dL. It was concluded that low level of exposure to lead during early childhood is associated with neuro-psychological development through the first seven years of life.

This table is adapted from World Health Organization (2007)

The blood lead level (BLL) is an indication of the quantity of accumulated Pb and it is useful as a guide for the purpose of medical intervention (ATSDR, 2016; Mitra, Haque, Islam, & Bashar, 2009). Unlike children, the associated effects of Pb poisoning in adults are chronic renal diseases, hypertension and nephritis, cerebrovascular diseases which includes stroke, transient ischaemic attack and vascular dementia (Gilbert & Weiss, 2006). Decreased fertility has been discovered in adult men with BLL of 46 µg/dL and above (ATSDR, 2016). Delay in conception and increased pre-term births are associated with BLL of 5 µg/dL and above in adult women (ATSDR, 2016; CDC, 2016; Mitra et al., 2009; NIH, 2016). There are strong relationships between arteriosclerotic heart disease, hearing loss, lungs and stomach cancer with high exposure to Pb among adults (ATSDR, 2016; NIH, 2016). About 50% of Pb inhaled may be absorbed in the lungs causing respiratory impairments (World Health Organization, 1995). Pb in blood may be bound to erythrocytes causing obstruction in blood flow (Kim et al., 2015). Pb that is accumulated in the body is slowly released from the body compartments with the aid of chelation therapy (Järup, 2003). Other ways suggested were cutting the route of exposure, which is aimed by this research, engaging in regular body exercise, fasting and taking good food with more water intake (ATSDR, 2016). Pb and other toxic substances in the body would be eliminated slowly from the body through excretes (sweat, urine and faeces) (Reid, 2016; Julie, 2015; Colbert, 2013). If the intake of Pb is left unchecked, the burden increases in the body throughout the lifetime (Hammond, 1977; CDC, 2016).

2.4 Sources of lead poisoning and exposure routes in Zamfara

Mining activities is a major source of Pb poisoning in Zamfara (Udiba et al., 2013; Udiba et al., 2012; UNICEF, 2011; Uriah et al., 2013). Globally, research has confirmed high BLL in individuals from mining areas (Clune, Falk, & Riederer, 2011; Fewtrell et al., 2003; Mitra et al., 2009). In Zamfara State, especially Anka LGA (research site), there are ongoing mining activities and hundreds of children under age 5 years including adults have been recorded with BLL higher than 350 µg/dL (Greig, Thurtle, Cooney, Ariti, Ahmed, Ashagre, Ayela, Chukwumalu, Criado-Perez, Gomez-Restrepo, et al., 2014; UNICEF, 2011). The CDC's BLL permissible limit is 2 µg/dL (Gilbert & Weiss, 2006). Almost everywhere in the minig region is affected by Pb contamination (UNEP-OCHA, 2010; UNICEF, 2011). In 2010, the soil samples collected from the residential compounds in the affected villages in Zamfara had soil lead levels (SLL) between 100,000 mg/kg and 379,000 mg/kg which called for emergency remediation (UNICEF, 2011). All the ponds in the area were tested with Pb levels between 5000 mg/m^3 to 50,000 mg/m^3 (UNICEF, 2011). Permissible limit of Pb in soil is 100 mg/kg, and 0.01 mg/L for water (EC, 2006; FAO/WHO, 2001), 420 mg/kg for soil and and 0.05 mg/L for water (USEPA, 2005), 100 mg/kg, and 0.01 mg/L for water (SON, 2007). The mining process and its contamination pathways in the environment has been previously discussed (chapter 1, section 1.3).

2.5 Emergency Remediation

As previously mentioned, the emergency remediation took place in Zamfara and the aim was to address the widespread unprecedented Pb poisoning across the affected regions and the LGAs between 2010 and 2013 (Tirima et al., 2016). In May 2010, an expert committee was set up by the federal government of Nigeria, the Zamfara state government and the international partners to set up an integrated health and environmental response system (Moszynski, 2010). The Federal and Zamfara State Ministries of Health and MSF set up clinics in strategic places across the affected villages (Tirima et al., 2016). The clinics focused on administration of chelation therapy which was limited to poisoned children under the age of 5 years and pregnant women (Thurtle et al., 2014). Chelating agents used in the detoxification treatment include intravenous (IV) calcium disodium versenate (CaNa2EDTA), intramuscular (IM) Dimercaprol and oral use 2,3-dimercaptosuccinic acid (DMSA, succimer) (Thurtle et al., 2014). Adult cases were reported to the general hospitals, the specialists and teaching hospitals (World Health Organization, 2011).

The United State Centre for Disease Control and Prevention (CDC Atlanta) recommended a U.S firm, TerraGraphics Environmental Engineering to champion the environmental remediation in collaboration with the Nigerian Federal and the Zamfara State Ministry of Environment to remediate both the soil and water bodies (UNEP/OCHA, 2010). Residential area in Dareta was remediated together with some other contaminated villages between 2010 and 2013 (Udiba et al., 2013). It was a simple process of taking off ten centimetres (10cm) layer of the contaminated top soil from the affected land area with soil Pb level above 1000 mg/kg and replacing it with clean soil having confirmed from previous investigations that the contamination was superficial (Udiba et al., 2012; von Lindern, von Braun, Tirima, & C., 2011). The evacuated contaminated top soil was buried in landfills as observed during our visit to the village in 2013. And during the raining season the landfill could be flooded (Figure 2.6), and the contamination could possibly spread to surrounding environment. The landfills were many (about 30 in the area visisted) and not of the same sizes. Some were 25ft (long) by 20ft (wide) and 20ft deep manually dug without lining. It is possible for the pollutants to leach through to the environment via the underneath water movement (Udiba et al., 2012). There are no boreholes around this area to allow monitoring of leaching.

Figure 2. 6: One of the Landfill sites where the removed contaminated top soil was disposed.

2.5.1 Effectiveness of the Emergency Remediation

Reports confirmed that no industrial site or arable land was remidiated due to limited resources (Simba et al., 2018; Uriah et al., 2013). In addition, the remediation exercise was found to be non-effective due to elevated Pb found in the study area after 3 years of the emergency remediation (Bartrem et al., 2014a). An assessment conducted by Udiba et al. (2012), a year after the remediation, reported high levels of heavy metals including Pb above the USEPA permissible limits. Pb concentrations in both soil and plants obtained in recent studies by

Johnbull, Abbassi, and Zytner (2018), Clement and Patrick (2017), Orisakwe et al. (2017), Abdulkareem, Abdulkadir, and Abdu (2015), Mohammed and Abdu (2014a), exceeded the EU acceptable limit of 300 mg/kg soil Pb and 0.03 mg/kg dry-weight Pb in food plant (Table 2.2).

According to MSF (2016) and Thurtle et al. (2014), oral chelation therapy is not 100% effective in the treatment of Pb poisoning (Kosnett, 2010). Re-exposure to Pb by the children under treatment has also been affecting the outcome of the chemotherapy effectiveness (Thurtle et al., 2014). Reducing the rate of exposure, total removal of the patient from a continuous exposure to Pb or total environmental remediation to eliminate any possible exposure remains the most important interventions to ensure a society free from Pb poisoning (Kosnett, 2012; Rogan et al., 2012).

2.5.2 Recent Studies on Post Remediation in Zamfara

There is a need for robust remediation procedures that will work globally (United Nations Environment Programme, 2015; World Health Organization, 2016a). Recently, a study shows that about 10% of the children in the affected villages in Zamfara State are still having severe neurological abnormalities due to Pb poisoning (Simba et al., 2018). MSF started medical treatment of both children and pregnant women since 2010 and more than 7000 have benefitted as at October 2016 (MSF, 2016), the early stage of this study.

In May 2015, about 30 children were reported dead in Nasarawa State (another state in Nigeria) as a result of Pb poisoning which was also discovered to be associated with mining (Thisday, 2016). Also, 28 Children died in Kawo and Magiro villages of Niger State having elevated blood Pb between April and May 2015 (Martin, 2016). There were similar incidences in many other states such as Kano and Benue States (Alkhatib et al., 2014; Ocheri & Ogwuche, 2012). All these confirmed that Pb poisoning is not peculiar to Zamfara State. Other States in Nigeria such as Kaduna, Bauchi, Kano, Kogi, Benue, Plateau, Edo, Osun, Oyo and Ebonyi, where mining activities are currently taking place, are also affected (Federal Ministry of Environment, 2015). The primary exposure routes for Pb poisoning have been identified to be: (i) incidental ingestion of contaminated soil (ii) consumption of food contaminated with Pb (iii) ingestion of Pb through drinking water (iv) inhalation of Pb contaminated air and (v) skin absorption

(Chambial et al., 2015; Qasim & Baloch, 2014; UNEP-OCHA, 2010). The most popular route through which Pb get into human food chain is through an uptake by plant via contaminated soil (Alloway, 1990). Globally, many agricultural soil are contaminated with high Pb (Alloway, 2013). Table 2.2 shows previous studies on plants relating to Pb uptake by food plants (crops) while Table 2.3 summarises recent studies on post remediation assessment of soil; all in Dareta village, Zamfara state (the study site).

Table 2. 2: Previous studies on plants, food stuff and lead uptake in Zamfara State

	Reference	**Study area/ community**	**Plant names/class**	**Importance of the plant**	**Average Pb concentration in the plant (mg/kg)**	**Concentration range (mg/kg)**	**Limit standard** (mg/kg)	**Standard name**
1.	Simba et al. (2018)	Bagega village (Neighbouring village to Dareta)	Rice grains	Food	0.73	-	0.2 *0.3	EU, (2006) *WHO/FAO, (2001)
			Millet grains	Food	0.41	-		
			Maize grains	Food	0.66	-		
			Guinea corn grains	Food	0.86	-		
			Cowpea	Food	0.39	-		
			Tapery beans	Food	0.08	-		
2.	Orisakwe et al. (2017)	Dareta, Bagega & Gusau	Vegetables (no name)	Food (Soup preparation and animal feed)	51.34	11.01 – 102.84		
3.	Abubakar et al. (2015)	Dareta village	Vegetable “*Adesonia ditata*”. Baobab (Kuka in Hausa)	Food (the leaves for soup)	2.53	1.43 – 3.63	0.3	EU, (2006) WHO/FAO, (2001)
			Vegetable “*Senna Occidentalis*”. Coffee senna (tafasa in Hausa)	Food (the leaves & stem for soup)	3.16	2.15 – 4.17	0.3	EU, (2006) WHO/FAO, (2001)
			Vegetable *“Amaranthus spinosus”*. Thorny pig weed (Alayyahu in Hausa)	Food (the leaves & stem for soup)	2.53	1.18 – 1.48	0.3	EU, (2006) WHO/FAO, (2001)
4.	Udiba et al. (2013)	Dareta village	Forage grasses	Farm animal feed	884.99	5.09 – 1312.73	0.5, 10	EC (2001), FAO (1983)

Table 2. 3: Recent studies on post remediation assessment of soil in Dareta village (study site), Zamfara state for Pb contamination

S/N	Place sampled (e.g residential, arable land)	Pb Concentration mean value (mg/kg)	Pb Conc. range (mg/kg) or (ppm)	Comment	Reference
1.	Farmland, Village square, Uncultivated land, Mining sites and Ore processing site		19.8 – 6,909	Soil was sampled at 30 cm depth across the sampling points	Johnbull et al. (2018)
2.	Residential Compound, Garden, Communal area, Industrial and Farm land,	-------	85.20 – 631.16	Soil sampled at 0-20 cm depth	Clement and Patrick (2017)
3.	Soil A (Mosque's Compound)	305.30	238.55 - 372.05	Samples from top soil were collected at a depth of 15 cm. Cone and quartering method was used at distance of 1 m × 1 m.	Abubakar et al. (2015)
	Soil B (Town centre)	246.40	239.29 – 253.51	‘’	‘’
	Soil C (Old mining processing site)	269.29	254.14 – 284.44	‘’	‘’
4.	Farmland - - - - - North South East West	- - - -	1627 – 2025* 1310 – 1586* 604 – 740* 625 – 876*	Soil samples were collected at depths of 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm from two selected farmlands on each cardinal point of the village (north, south, east and west). On each cardinal, this was done at distance of 50 metres (farm I) and 100 meters (farm II) respectively from the last building to the village. *Highest concentrations were recorded at the topsoil (0-20cm depth)	Abdulkareem et al. (2015)
5.	Farmland (Topsoil) - North South West East	- - - -	40 (1 km)* – 850 (30 m) 200 (150 m) – 730 (30 m) 30 (30 m) – 2,300 (50 m) 200 (30 m) – 1500 (50 m)	Soil samples were collected at depths of 20 cm from selected farmlands on each cardinal point of the village. On each cardinal, it was collected at (10, 30, 50, 150, 300, 500 and 1000) meters. Highest Pd conc. was obtained at 30m North (850 mg/kg), 50 m East (1,500 mg/kg) and 50 m West (2,300 mg/kg). *indicates distance of the sampling point to the village	Ibrahim Mohammed and Nafiu Abdu (2014)
	Farmlands (Subsoil) - North South West East	- - - -	40 (1.5 m)** – 350 (0.3 m) 40 (1.5 m) – 1000 (1 m) 200 (1 m) – 560 (2 m) 200 (2 m) – 600 (1 m)	The above-mentioned sampling directions were dug up to 3 metres (300 cm) depth and soil samples were collected. **indicates depth in meters at which the soil was sampled. **Note: permissible limits are 420 mg/kg (USEPA), 300 mg/kg (EU, SON, China NEPA & FAO standard)**	‘’
6.	Many locations were mapped out and examined using the handheld X-ray Fluorescence (XRF) spectrometer	89500	12 - 703000	90% of the sampling areas have lead above USEPA and EU standards for soil lead	C Bartrem et al. (2014)

7.	Residential, market, playground and ore-processing sites	54,400	5,420 – 58,900	Soil samples were collected at random within the community from Public places	Plumlee et al. (2013)
8.	Mineral ore processing sites	-	1,740 – 4,152	Soil samples were collected from various sampling points from 6 sites respectively at 20 cm depth.	Uriah et al. (2013)
	Mine site	-	1146 – 2,637	Soil samples were collected from various sampling points from 4 sites respectively at 20 cm depth.	‘’
	Village square (town centre)	-	19.57 – 3,326	Soil samples were collected from various sampling points from 5 sites respectively at 20 cm depth.	‘’
	Farmlands	-	19.8 – 2,892	Soil samples were collected from various sampling points from 5 farms respectively at 20 cm depth.	‘’
	Uncultivated lands	-	20.6 – 6,909	Soil samples were collected from various sampling points from 4 lands respectively at 20 cm depth.	‘’
9.	Residential Compound	370.62	85.20 – 631.16	The soil samples were collected from topsoil layer at depth of 0-10 cm from different location within the community (Dareta village). 5 locations were selected for Residential Compounds	Udiba et al. (2012)
	Market square/Play ground	479.46	81.65 – 684.27	6 locations were selected for Market square/Play ground	‘’
	Grinding Mills sites	553.42	429.29 – 662.61	7 locations were selected for Grinding Mills sites	‘’
	Around drinking water source (wells/ponds)	547.09	343.02 – 656.29	3 locations were selected for sampling around drinking water source (wells/ponds).	‘’
10.	Bagega industrial/farm area	21965.73	629 – 50775	15 locations were examined in Bagega, a village that bordered Dareta	UNICEF (2011)

2.6 Pb in rice

Rice is a monocotyledonous plant which comes from taxonomic genus Oryza and it is from the family of grass called Poaceae (Oko & Ugwu, 2011). More than 20 different wild species of rice exist but only two are generally cultivated globally (Cantrell & Reeves, 2002; Goff et al., 2002; Oko & Ugwu, 2011) which are bred into varieties in many countries (AfricaRice, 2011). The genetic information of Nigerian rice is explained in Appendix A, Table I. Historically, *Oryza sativa* was domesticated by the old people of China from the wild grass *Oryza rufipogon* in about 10,000 to 14,000 years ago (GRiSP, 2013). Genetic evidence shows that the two popular Asian rice (*japonica* and *indica*) are originated from a single event of domestication which occurred in about 14,000 year ago in the valley area of Pearl River, republic of China (GRiSP, 2013). Another popular specie of rice for many years is *Oryza glaberrima* which was domesticated in the West Africa around the same time (Li, Zheng and Ge, 2011)

Rice is the major food crop consumed by human race and it ranked third after wheat and maize in terms of worldwide production (Ejebe, 2013). The world most populated country China, according to the United Nation's estimates of over 1.4 billion people (18.72% of world population) consumes 148.5 million metric tonnes of rice per annum followed by India (1.3 billion people) which consumes 99.3 million metric tonnes (Statista, 2019; Worldometer, 2019). Nigeria (200 million people) is ranked 11th globally with consumption of 8.5 million metric tonnes of rice per annum (Statista, 2019; Worldometer, 2019). Apart from eating rice grains directly as food, it is useful in several other applications such as in the production of another food like gluten-free baked products (Sabanis & Tzia, 2009). Other various food products are made from rice such as rice syrup, rice milk and rice bran oil (Atli, 2016).

Research reveals presence of high concentration of Pb in some varieties of rice (Williams et al., 2009) and it can be accumulated in all rice organs or part (Figure 2.7). Rice has affinity to accumulate toxic metals more than other cereal crops (Mondal & Polya, 2008). Many communities depend on rice as their major food and more than 70% of the world population eats rice (Annapure, Singhal, & Kulkarni, 1998; Reilly, 2008; Statista, 2019). Rice has been identified as one of the major sources of lead intake especially to those that are highly dependent on it (Shimbo et al., 2001). The degree of toxicity depends on the frequency of consumption per body weight and time (Orisakwe, Nduka, Amadi, Dike, & Bede, 2012). Jianjie

Fu et al. (2008), Gorbunov et al. (2006), Orisakwe et al. (2012) and Otitoju et al. (2014) confirmed that Pb concentration could be very high in rice using the International standards. Norton et al. (2014) and Yang, Shu, Qiu, Wang, and Lan (2004) demonstrated that lead concentration in rice reduces with increasing distance from the source of pollution. However, soil contamination might not really depend on the known source alone, contamination in soil may exist for years without been noticed if there is no assessment of the farmland (Carsey Bartrem et al., 2014).

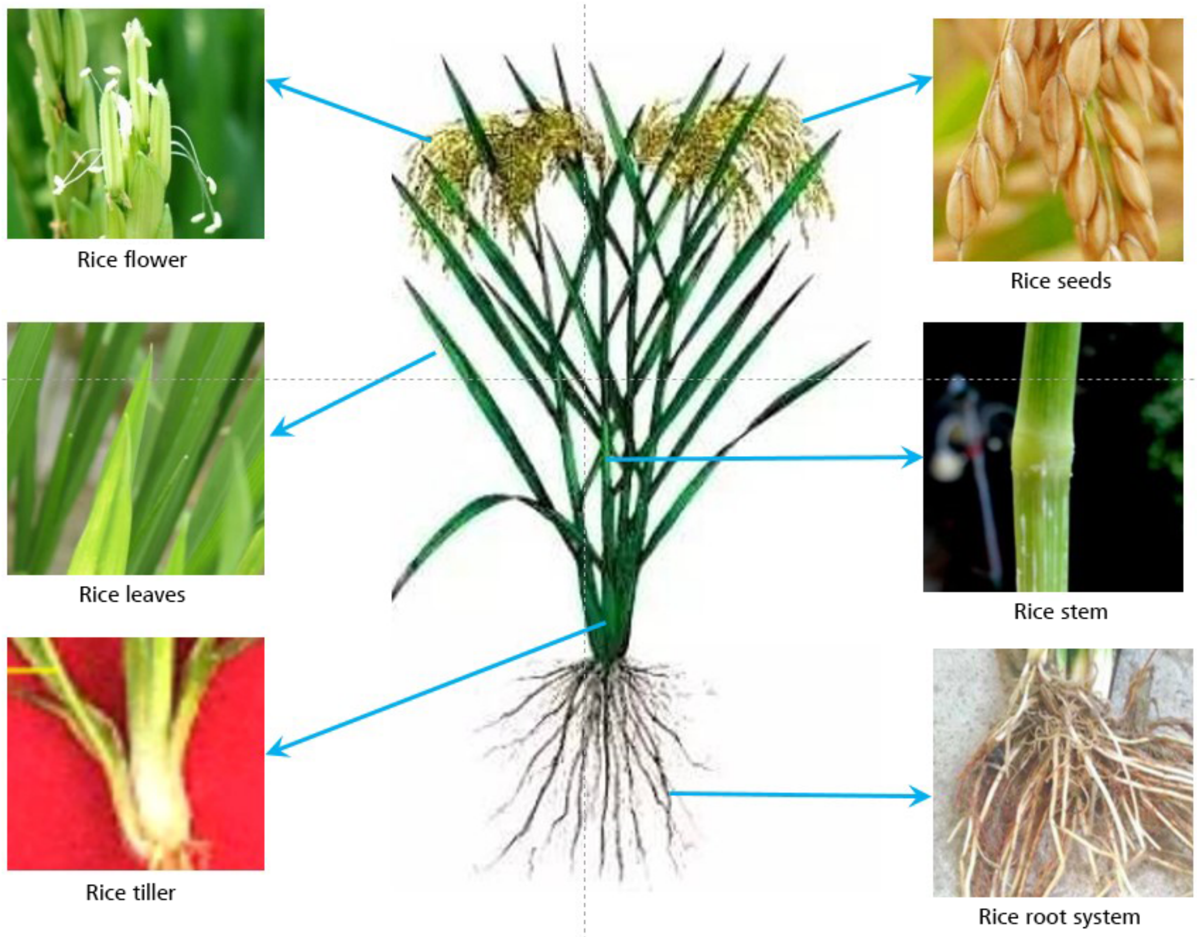


Figure 2. 7: Different parts of the rice plant adapted from ZEISS (2018)

However, for Nigeria, one may also think that the imported rice might be safer in terms of health risks but Otitoju et al. (2014) discovered high lead in some popular imported rice samples sampled across the northern Nigeria. Table 2.4 contains previous studies on rice, and lead uptake. Table 2.5 reveals lead and its permissible limits (standards) in the environmental media and some food while Table 2.6 illustrates other heavy metals and their limits by different regulatory agencies. Lead uptake in rice is significantly dependent on the rice variety (Liu et al., 2013; Liu et al., 2005).

Table 2. 4: Previous studies on rice and Pb uptake across the world

Reference	**Sampling and sample size**	**Study area**	**Country**	**Average Pb concentration in rice**	**Conc. Range (µg/g)**	**Limit standard**	**Regulatory body**
Alam et al (2002)	Already grown and harvested samples (rice) were collected	Jessore District	Bangladesh	7.7 µg/g	2.61 – 15.89		
Liu et al (2003)	20 cultivars (rice) from greenhouse experiment with soil-lead 800 µg/g	Greenhouse experiment	China	3.2 µg/g	2.7 – 4.8	0.2 µg/g	Chinese/EU standard
Cheng (2006),	12 cultivars (rice) grown in 3 different locations (269 rice samples collected). No greenhouse experiments.	Field experiment	China	1.135 µg/g	<DL	0.2 µg/g	Chinese/EU standard
Jianjie Fu et al. (2008)	Already grown rice sample were collected from E-waste recycling area	E-waste recycling site	China	0.69 µg/g	0.16 – 0.74	0.2 µg/g	Chinese/EU standard
Williams et al (2009)	Already grown rice samples were collected	11 mining districts	China	0.62 µg/g	0.051 – 0.784	0.2 µg/g	Chinese/EU standard
Williams et al (2012)	Harvested field rice samples were collected	Mine impacted sites in Guangdong province	China	0.246 µg/g	-		Chinese/EU standard
Liu et al (2013)	6 cultivars from greenhouse experiment with soil-lead 500 µg/g and 1000 µg/g	Greenhouse	China	3.5 µg/g and 5.1 µg/g		0.2 µg/g	Chinese/EU standard
Norton et al (2014)	Market samples (13 countries), Field samples (6 countries). Milled and un-milled was compared. 1,578 samples were analysed.	Both field and greenhouse but samples collected from the mine impacted region were excluded from the analysis	13 countries (Japan, Vietnam, Italy, Thailand, USA, Pakistan, France, Spain, SriLanka, India, Ghana, Nepal and china)	Not applicable	Some data were removed because they are very high and not reasonable for use to calculate PTTI	0.2 µg/g	FAO/WHO/EU standard
Otitoju et al. (2014)	Rice samples were collected from those imported from India, Vietnam, Brazil, Thailand, South Africa and USA	Kubwa (Abuja FCT), Jos (Plateau State), Jaba (Kano State), Wukari (Taraba State) and Kaduna (Kaduna State)	Nigeria	0.152 µg/g	0.014 (USA rice) – 0.383 (Thailand)	0.2 µg/g	SON Standard/State Environmental Protection Administration (2005)
Adedire et al. (2015)	23 Rice samples were collected from 4 major cities	Akure, Ondo, Ikare and Ore in Ondo State, South Western of Nigeria	Nigeria.	8.3 µg/g	23.14 – 52.0	0.2 µg/g	SON Standard
Simba et al. (2018)							

US-FDA: provisional total tolerable intake (PTTI) for Pb = 6 µg/day (children <7 yrs old), 15 µg/day (children >7 yrs old), 25 µg/day (pregnant women), 75 µg/day (other adults) www.fda.gov. **Note:** No previous research has been conducted on rice in Zamfara as regards environmental lead contamination. <DL= below detection limit.

Table 2. 5: Lead and its permissible limits (standards) in the environmental media and some food

Environmental media		US EPA	EU	WHO	SON/EU	Reference
Soil	Residential	400 mg/kg	100 mg/kg	100 mg/kg	-	United State Environmental Protection Agency (2016) FAO/WHO (2011) (Adams et al., 2001)
	Uncultivated and arable land	420 mg/kg	300 mg/kg	100 mg/kg	100 mg/kg	USDL (2004b) Anwarzeb Khan, Sardar Khan, Muhammad Amjad Khan, Zahir Qamar, and Muhammad Waqas (2015)
Water	Drinking/food process	0.05 mg/l	0.01 mg/l	0.01 mg/l	0.01 mg/l	United State Environmental Protection Agency (1987), World Health Organization (2015) and Standard Organisation of Nigeria (2007), Orisakwe et al (2012)
	Irrigation (surface water also supporting aquatic life)	0.0058 mg/l	-	0.01 mg/l	-	World Health Organization (2015) United State Environmental Protection Agency (1987)
Air	Rural	0.02 mg/m^3	-	0.05 mg/m^3	-	UNEP-OCHA, (2011)
	Urban	0.05 mg/m^3	-		-	UNEP-OCHA, (2011)
Plant	Cereals (grains: rice, maize, husk, germ and rice)	0.2 µg/g	0.2 µg/g	0.3 µg/g	0.2 µg/g	Adams et al. (2001) Norton et al (2014)
	Vegetables	-	0.3 µg/g	-	0.3 µg/g	Abubakar et al. (2015)
	Fruit	-	0.1 µg/g	0.3 µg/g	0.3 µg/g	EU, (2006) EC, (2001) FAO/WHO (2001; 2011)
	Tubers	-	0.3 µg/g	0.3 µg/g	0.3 µg/g	EU, (2006) WHO/FAO, (2001)
Animal product	Red meat and Poultry	-	0.1m g/kg	0.1 mg/kg	0.1 mg/kg	WHO/FAO (1978), Aliyu (2014)

*SON is Standard Organisation of Nigeria

Table 2. 6: International standard for lead and other heavy metals (mg/kg) in soil and plants

Heavy metals	**Commission Regulation (EC, 2006)**		**United State Environmental Protection Agency (2005)**		**FAO/WHO (1984, 2001a)**		**SEPA China (1995, 2005)**		**Indian standard (Awashthi, 2000)**	
	Soil	**Plant**	**Soil**	**Plant**	**Soil**	**Plant**	**Soil**	**Plant**	**Soil**	**Plant**
As	NA	NA	75	NA	20	0.1	30	0.5	NA	1.1
Cd	3	0.2	85	NA	0.3	0.1	0.6	0.1 – 0.2	3-6	1.5
Cu	100	20	NA	NA	100	73	100	20	135-270	30
Cr	100	1	3000	NA	100	2.3	200	0.5	NA	20
Ni	50	NA	NA	NA	50	66.9	50	10	75-150	1.5
Pb	100	0.30	420	NA	100	0.3	300	9	250-500	2.5

NA = Not Applicable

Source: Ewers, (1991), Khan, Khan, Khan, Qamar, & Waqas, (2015).

2.7 Rice production and varieties in Nigeria

Rice is generally known as rough rice, raw rice, paddy or paddy rice in its raw form (Ejebe, 2013). Paddy is the major raw material from which milled rice grains and rice products are obtained while this is produced by threshing after harvesting and pre-drying of the rice grain (Ejebe, 2013). From its exterior, a rough rice grain is mainly composed of the non-edible palea or husk, the husk layer, aleurone layer, the starchy endosperm and the embryo or germ as appears in Figure 2.8 (Ejebe, 2013; Ricepedia, 2017; Yoshida, 1981).

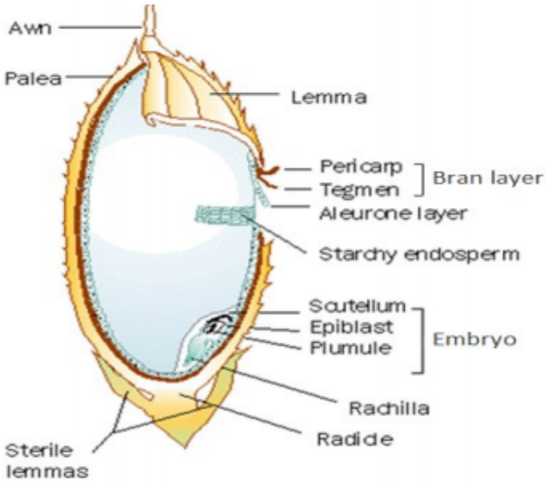


Figure 2. 8: A grain of paddy rice (Ricepedia, 2017)

In Nigeria, rice comes second after maize among the largest grains produced in the country and it is a staple food all over Nigeria (USAID-AfricaLead, 2016). Rice accounts for more than 20% of total food expenditure in Nigeria and it is grown on approximately 3 million ha of land (USAID-AfricaLead, 2016). Nigeria is the largest rice producer in Africa with 6.7 million metric tonnes (MT) and at an average of 2.2 MT/Ha (FAOSTAT (2016) cited in USAID-AfricaLead (2016)). All the regions; the north, south, west, east and the central grow rice (Ebuehi & Oyewole, 2007). Rice growing systems in Nigeria can be classified as rain-fed upland, irrigated lowland, rain-fed lowland, deepwater and mangrove swamp (Daramola, 2005). In all these systems, 47% of the country's rice production comes from rain-fed low-land; rain-fed upland accounts for 30%; and 16% comes from Irrigation. The remaining 7% is from mangrove swamp and deep-water systems (Daramola, 2005; USAID-AfricaLead, 2016). To meet-up with high demand for rice due to the country's rapid population and economic growth, Nigeria imports about a million metric tonnes annually from other rice producing countries such as United States, Thailand, India, China and some African countries to support the internally grown quantity (Makun, Dutton, Njobeh, Phoku, & Yah, 2011) making its total annual consumption to be about 8 million metric tonnes. The highest yield is from the northern part under which Zamfara is located (Makun et al., 2011). Zamfara State produces about 200,000 tonnes of rice annually (FAO, 2013) but in the year 2016 due to Federal Government support on agriculture, the state has increased its rice production to 450,000 tonnes (NigeriaMagazine, 2016). In Zamfara alone, about 150,000 farmers have engaged by the Federal Government in rice farming for 2016 through financial support (TheGuardian, 2016) and Government has trained additional 5000 Zamfara youths in rice farming to show their commitment to increased rice production (Shehu, 2016).

Asian rice, *Oryza sativa* and African rice, *Oryza glaberima* are the most popular cultivated rice in Nigeria. *O. sativa* is well recognised due to its massive yield (Makun et al., 2011; Oyetunji et al., 2012). This two rice species look alike morphologically but, on the field, *O. glaberima* is short and has tough ligules and few secondary panicle branches (Sano, Sano, & Morishima, 1984). *Oryza sativa* is always taller, up to 50cm in height and about 3m long in some floating swamp types (Burkill, 2016). Its ligules toughness is not as that of *O. glaberima* (Sano et al., 1984). It also bears an open nodding panicle of grains and it can adapt easily to numerous kinds of weather condition (Burkill, 2016). *Oryza sativa* was brought to the west Africa from Asia in about 500 years ago and it has technically displaced the native African rice, *O. glaberima* in many places (Burkill, 2016). In the South Western Nigeria, *O. sativa* is called "ofada" while *O. glaberima* is called "aroso" (Abulude, 2005; Ebuehi & Oyewole, 2007). Some varieties of *O. sativa* in the eastern Nigeria are been referred to as Sipi, Faro, Awilo and canada (Oko & Ugwu, 2011). But among the Northern states (mainly Hausa ethnic group which includes Zamfara), *O. sativa* is more grown, and it is called "shinkafa" or "koro shinkafa". This species is grown widely in the North because, apart from its high yield, it makes good fodder to feed their animals as many famers in this part of the country also engage in rearing of cattle, camel, donkeys, goats and sheep (Burkill, 2016).

Rice production started in Nigeria as far back as 14th century though the varieties available to farmers as at that time is not clear (Sharma, 2010). There is a local variety called Bisalayi rice which according to many farmers has been in existence for decades because of its taste and some other qualities such as resistant to rice common rice disease (Ejebe, 2013; NCRI, 2017). The rice is still among the popular varieties especially among the farmers in the Northern part of Nigeria (NCRI, 2017). Table I in Appendix A reveals the names, origin and some other morphological characteristics of all rice varieties in Nigeria from 1954 to the time the varietal trial of this study begins in June 2017.

2.8 Rice growing and harvesting

Rice planting generally involves about 13 steps, which are shown in Figure 2.9. Steps 1-10 demonstrate the activities from pre-planting to harvesting while 11 - 13 explain post-planting activities (IRRI, 2016) while different stages of growth in rice are illustrated in Figure 2.10. The stage 1 of the rice growth is the germination stage which occurs within 72 hours. Stage 2 (transplanting) can occur within 3 weeks after germination and stage 3 (maximum tiler formation) will complete within 30 to 50 days. The stage 4 (panicle formation) will happen between 50 and 80 days while stage 5 (flowering) will be experienced within 70 to 100 days. The last stage is when the rice is fully matured which is within 100 to 150 days (IRRI, 2017).

Figure 2. 9: Steps in rice production (IRRI, 2016).

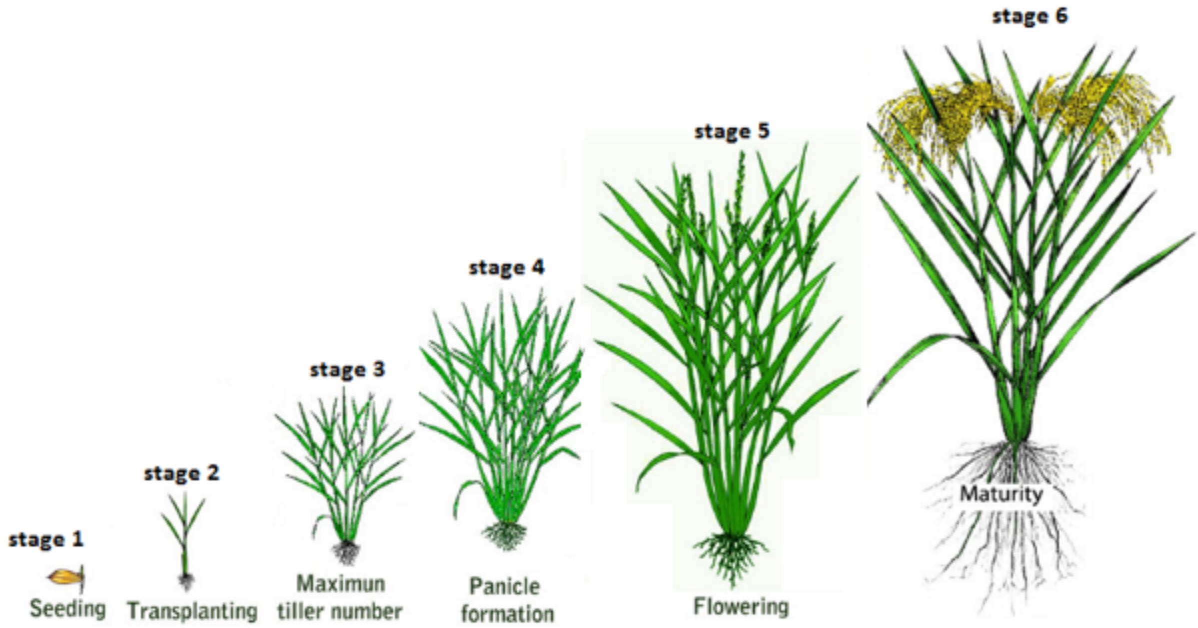


Figure 2. 10: Growth Stages of Rice (IRRI, 2017)

The rice seeds have to be soaked in water for about 48 hours at temperature between 20^0C and 25°C (Liu, Li, Xu, Zhang, et al., 2003). The seeds would then be removed from the water and covered with two layers of moist gauze at 32°C for another 30 hours to allow them to germinate (Liu, Li, Xu, Zhang, et al., 2003). The germinated seeds would then be transferred onto the soaked clean soil to grow for 30 days. Details about this is presented in chapter 3 (methodology), section 3.5.3.

Harvesting according to Ricepedia (2017a) is the process of collecting the mature rice crop from the field. Depending on the variety, a rice crop usually reaches maturity at around 105–150 days after crop establishment. Harvesting activities include cutting, stacking, handling, threshing, cleaning, and hauling (IITA/USAID, 2016). Good harvesting methods help maximize grain yield and minimize grain damage and deterioration (Ricepedia, 2017a). Harvesting is done either manually or mechanically (CGIAR, 2013). Manual one is the use of knives and sickles to cut the rice plant while the mechanical harvesting involves the use of reapers or combine harvesters (IRRI, 2016). Manual harvesting is labour intensive because it takes between 40 and 80 hours to harvest a hectare (Ricepedia, 2017a). Due to the nature of the experiments, manual harvesting was done for this study.

Drying is the next step in rice processing which is to reduce the moisture content from the grain to a safe level for storage and it is the most critical operation after harvesting a rice crop from farm (IRRI, 2016). Delays in drying, incomplete drying, or ineffective drying will reduce grain quality and result in losses (IRRI, 2016). Milling follows when the rice is dried. Milling is a crucial step in rice production and is basically the process of removing the husk and the husk layers to leave the edible and white rice kernel (Ricepedia, 2017a). It involves parboiling (optional), cleaning, hulling, polishing (commercial purpose), and grading as shown in Figure 2.11 (Ejebe, 2013).

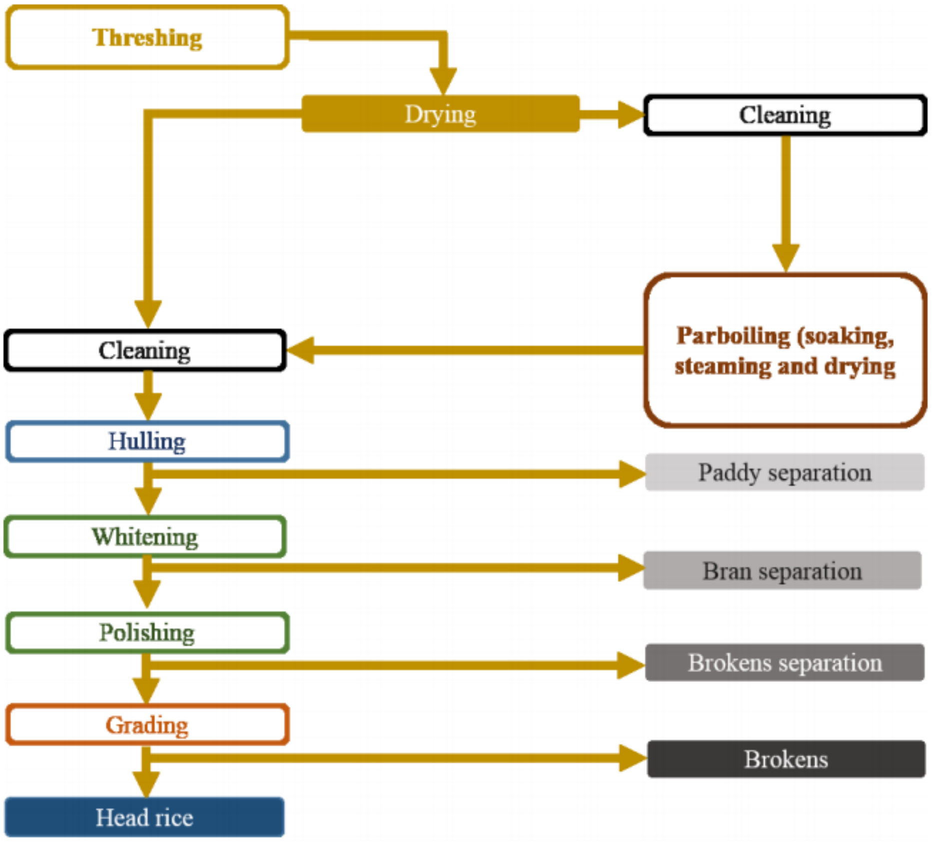


Figure 2. 11: Steps in commercial rice processing after harvesting (Ejebe, 2013)

2.9 Multiple uses and essentials of rice

Rice is the staple food among the communities in northern Nigeria, including Zamfara, and consume rice more than four times a day (Otitoju et al., 2014). Rice is used as key ingredient invarious foods from Zamfara, such as "Kunnu shinkafa", "tuwo shinkafa", "massa da miyya", "dambu", "fate", "garogaro" and Jolloff rice (ANR, 2016; Ronke, 2016). There is little information about how frequent the exposed population eat this group of food. However, the following information was gathered during field visits. Kunnu shinkafa is a porridge semi-liquid food made by over boiling rice with excess water. It is served hot as breakfast with sugar and this is very popular among all the families in Zamfara. Tuwo shinkafa is a solid food that is eaten with soup. It is prepared by mashing the rice with water after it has been overcooked until it becomes very soft. Hence, it becomes a solid food. It is then moulded into balls and eaten with soup (NF-TV, 2016). Tuwo is popularly taken as lunch and more than 90% of typical Hausa indigenes like the food (ANR, 2016). Massa is rice cake and it is taken anytime whether with stew (massa da miyya) or ordinarily (massa) (Ronke, 2016). Dambu is prepared from rice powder and it is also a solid food, but cooked by steaming (Ronke, 2016). Fate is prepared by grinding the rice half-way and then pouring into boiling water containing palm oil and some other ingredients. This is stirred continuously until it is solid and eaten like cake. Fate is sometimes eaten in combination with cooked beans; this combination is called “shinkafa da wanke” (rice and beans) (ANR, 2016).

Plants represents the primary source of food for all animals including man and cereals generally are indispensable among the categories of foods (Muhammad & Amusa, 2005). Rice is one of the cereal crops plays important roles in supplying some of the human body essential nutrients (Abulude, 2005). Essential nutrients in rice come from various classes of food such as proteins, carbohydrates, lipids, vitamins, minerals and water (diet.com, 2016). Food is considered to be nutritious when it is able to supply the key elements required for normal cellular metabolic activities in the body (Atli, 2016). These elements are required for maintenance of numerous physiological processes in humans and other organisms (Adedire et al., 2015; Ambrógi, Avegliano, & Maihara, 2016). Essential minerals (elements) in rice are mainly calcium (Ca), potassium (K), Iron (Fe), phosphorus (P), Zinc (Zn), Manganese (Mn), sodium (Na), Copper (Cu), Selenium (Se) and Molybdenum (Mo) (Adedire et al., 2015). Some members of this group are called microelements or trace elements due to the fact that, little quantity of them are required in the body (Adedire et al., 2015). For instance, chromium is known to enhance the action of insulin (the hormone that is responsible for regulating and storage of body sugar) while calcium is essential in bone and teeth formation, muscle contraction and cell signalling (Adedire et al., 2015). In this study, nine essential elements (Ca, Cu, Fe, Mg, K, Zn, Mn, Co and Se) in rice were studied, and the result is presented in chapter 6. Based on the literature, some of the elements found in rice that are essential in the human body are listed in Table 2.7 while Table 2.8 shows the essential elements in rice, amount in average per cup of rice, their nutritional importance and deficiency implication.

Table 2. 7: Essential macro and micro-nutrients found in rice

Micronutrients			Macronutrients		
SN	**Vitamins**	**Minerals**	**Amino acids**	**Energy sources**	**Electrolytes**
1	Ascorbic acid (Vitamin C)	Calcium	Histidine	Fatty acid	Sodium
2	Vitamin D, E, and K	Phosphorus	Isoleusine	Linolenic acid	Chlorides
3	Thiamine (Vitamin B1)	Magnesium	Leucine	α-linolenic acid	Ultra-trace elements such as sulphur, Hydrogen, Nitrogen etc also present
4	Riboflavin (Vitamin B2)	Iron	Lysine	Carbohydrate	
5	Niacin (Vitamin B3)	Zinc	Methionine		
6	Pantothenic acid (Vitamin B5)	Copper	Tryptophan		
7	Pyridoxine (Vitamin B6)	Manganese	Phenylalanine		
8	Folic acid (Vitamin B9 or Bc or M)	Iodine	Threonine		
9	Biotin (Vitamin H)	Selenium	Valine		
10	Cobalamin (Vitamin B12)	Molybdenum			
11	Retinol or retinoic acid (vitamin A)	Chromium*			

Table 2. 8: Essential elements in rice grains with their nutritional importance and deficiency implication

S/N	**Essential elements**	**Average amount in 1 cup of brown rice**	**Nutritional Importance**	**Deficiency implications**	References
1	Calcium (Ca)	15.8 mg	Building strong bones and teeth, blood clothing, improved sending and receiving nerve signals, squeezing and relaxing muscles, timely releasing of hormones and other chemicals and keeping a normal heart beat (USNLM, 2016)	Calcium deficiency symptoms are regarded as hypocalcaemia. Calcium deficiency may result to osteoporosis, brittle bones, eye damage, abnormal heart beat, sleepless night (insomnia), muscle cramps or muscle ache, weak nails, late sign of puberty and abnormal functions of the endocrine system (Healthline, 2016).	Healthline (2016; USNLM (2016)
2	Phosphorus (P)	68 mg	Calcium and phosphorus bound together to form the crystal that make-up the bones and teeth. Healthy bone formation, regulated excretion, improved digestion, protein synthesis, improved energy extraction, hormonal balance, cellular repair and optimized hormonal reactions are parts of the benefits of phosphorus	Weak bones and teeth, constipation, hormonal imbalance, abnormal protein synthesis, rickets in children, osteomalacia in adults. Others are energy generating systems disorder and decrease in red-blood cells function	VNC (2016).
3	Iron (Fe)	1.9 mg	Protein metabolism, production of red haemoglobin and blood cells,	Chronic anaemia, cough heart failure	VNC (2016)
4	Potassium (K)	55 mg	Normalises blood pressure, enhanced muscle strength, support electrolytic function, prevents heart and kidney disorders, guides against anxiety and stress and supports nervous systems.	Abnormal blood pressure (hypo/hypertension), muscle weakness, heart and kidney disorders, anxiety and stress and nervous system disorders	OrganicFacts, (2016)
5	Magnesium (Mg)	19 mg	It protects against heart attack, hypertension, kidney and gall stones, insomnia, constipation, osteoporosis, improves muscle functioning, protein synthesis, constricted airways in the lungs, improve the functions of parathyroid gland and also boost bioavailability of vitamin B6 and cholesterol. For women, it protects against premature labour, relief from symptoms of menopause and premenstrual syndrome.	Heart attack, hypertension, kidney and gall stones, insomnia, constipation, osteoporosis, constricted airways in the lungs and malfunctioning of parathyroid	HealthNewsmax (2016)
6	Zinc (Zn)	0.8 mg	Proper functioning of the immune and digestive systems, control of diabetes, reduction of stress levels, energy metabolism, and an increased rate of healing for acne and wounds. Zinc is also helpful in terms of pregnancy, hair care, eczema, weight loss, night	Low immunity, diabetes, stress, long healing of wounds, night blindness, and weight loss	OrganicFacts (2016)

			blindness, colds, eye care, appetite loss, protein synthesis and immunity boosting.		
7	Manganese (Mn)	0.7 mg	Healthy bone structure, bone metabolism, helps to create essential enzymes for building bones. Also acts as a co-enzyme to assist metabolic activity in the human body. Others are formation of connective tissues, absorption of calcium, proper functioning of the thyroid gland and sex hormones, regulation of blood sugar level, and metabolism of fats and carbohydrates.	high blood pressure, heart ailments, muscular contraction, bone malformation, high cholesterol, poor eyesight, hearing trouble, severe memory loss, shivers and tremors.	OrganicFacts (2016)
8	Sodium (Na)	1.6 mg	Improve nerve impulse, helps in contract and relax muscles, maintains the proper balance of minerals and water in the body system	It increases pressure in the blood vessels leading high blood pressure, heart attack, stroke, heart and kidney damage	HSPH (2016)
9	Copper (Cu)	0.1 mg	Improved cardiovascular systems, builds haemoglobin	Cardiovascular diseases	Klevay (2000)
10	Selenium (Se)	11.9 mg	The body needs selenium to produce enzymes called selenoproteins which are 25 in numbers. Glutathione peroxidase is one of them that work as antioxidants that prevents against cell damage. Those enzymes detoxicate hydrogen peroxides into harmless substance like water. Resistant to virus attack is another function of selenium.	High risk of prostate and lung cancer, cardiovascular diseases, prone to virus attacks such as HIV/AIDS. High amount of selenium is also toxic. Selenium deficiency may also result in cardiomyopathy.	Healthline (2014)
11	Chromium (Cr)	0.78 mg	It is known to enhance the action of insulin; the hormone that is responsible for regulating and storage of body sugar.	Diabetes	Shils & Shike (2006)

*More is available from World's Heathiest Food (2016) and SELFNutritionData (2016)

2.10 Elements that are of radio-ecological important (Caesium (Cs), Strontium (Sr)) in rice

Food has been identified as one of the main pathways through which radionuclides are taken into human body (Srinuttrakul & Yoshida, 2017). This is because the ionizing radiation; radionuclides (radioactive isotope that is unstable due to excess nuclear energy it possesses) could be accumulated by the food crops from their natural environment (Jibiri, Farai, & Alausa, 2007). Ingestion of radionuclides through food contributes significantly to the amount of radiation doses across several part of human body which has numerous long-term adverse health effects (Gupta & Walther, 2017). Examining the stable isotopes of Cs and Sr can serve as analogue of the radioisotope counterparts of these elements according to Srinuttrakul and Yoshida (2017). And exploiting the inter-varietal variation (variation within the species) in terms of contaminant's uptake is a potential remediation strategy to produce less contaminated food crops (Penrose et al., 2015a).

Rice which is currently dominant staple food in many countries globally is one of the critical foods for the intake of radionuclides by humans (Asaduzzaman, Khandaker, Amin, & Mahat, 2015; Uchida, Tagami, Shang, & Choi, 2009). Whether radioactive or stable, isotopes of the same element possess the same physical, chemical and reactivity properties (Hoefs, 2018). Measuring the concentration of the stable isotope of elements in food to precisely quantify the radionuclides accumulation potential in the food is now a popular area of research worldwide (Uchida et al., 2009). Examining how different varieties (cultivar) of rice vary in the uptake of stable Caesium (Cs) and Strontium (Sr) is an area that has not received good attention in sub-Sahara Africa (Ibikunle, Arogunjo, & Ajayi, 2019). Inter-varietal assessment for varietal selection has been proven as one of the best remediation strategies to minimise human exposure to environmental contaminants especially those that get into food chain via plant uptake (Penrose et al., 2017). There is little known globally in this study area on rice (Akinwale et al., 2011).

Radioactive caesium and strontium have been identified with long half-lives for both elements and they have the potential to harm the human body (Uchida et al., 2007). For instance, the Strontium ^{134}Cs has half-life of 2.06 years, ^{135}Cs has 2.3×106 years, ^{137}Cs has 30.17 years while ^{90}Sr has a half-life of 28.1years (Srinuttrakul & Yoshida, 2017). Radioactive half-life is the time required for a quantity of unstable atoms (such as radioactive atoms) to undergo radioactive decay (Martin, 2012). It is a measure of the tendency of the nucleus of an atom to dis-integrate or decay

(Knoll, 2010). When radioactive decay occurs, emission of radiation occurs and as the radioisotope disintegrate to a stable atom it emits radiation (Greenwood & Earnshaw, 2012). Toxicity of each depends on the dosage of the radiation and when it happens, the symptoms includes neurological disorder, body immune system been compromised, skin lesions, chromosomal malfunctions and the acute syndrome do come with diarrhoea, nausea and vomiting and death (Srinuttrakul & Yoshida, 2017).

There is a record of radioactive caesium causing fertility disorders, genotoxicity and cancer (Williams, 2004). Radio-strontium damages the bone when it is accumulated in the bone and it causes cancer because of likely damages on the cells of the DNA (Nadesan, 2014). Leukemia is another associated health risk of radiostrontium (Smith & Beresford, 2005), cancer risk is very high in exposure to radiostrontium (Raabe, 2004) and all these health risks can be long term (Kamiya et al., 2015).

Caesium (Cs) is an alkali metal, gold coloured, soft and it is found among the group I element on the periodic table (Zajacz et al., 2010). It has 40 Isotopes with mass numbers ranges between 112 and 151 but only ^{133}Cs is stable (Okuda et al., 2012) and it has the potential role as a predictor for radio-caesium behaviour (Salt, Kay, & Jarvis, 2004). This ^{133}Cs isotope is traced to be originated from a soil mineral called pollucite $Cs_4H_4Al_4Si_9O_{27}$ and this is always in abundance where gold ore is geologically domicile (Zajacz et al., 2010). Other 39 isotopes are not stable therefore, radioactive in nature (Kanter, Hauser, Michalke, Dräxl, & Schäffner, 2010). Breakdown of Uranium in fuel elements or nuclear explosions can produce ^{134}Cs and ^{137}Cs (ATSDR, 2019). Radio-caesium is one of the toxic elements of Public Health Importance (ATSDR, 2019).

Strontium (Sr) is an alkaline earth metal, silvery-white, soft and it is found among the group II element on the periodic table (Ropp, 2012). It has 33 isotopes with mass numbers ranges from 73 to 105 and among these are four stable isotopes which are 84Sr, 86Sr, 87Sr and 88Sr (Parsons, 2014). These are originated from soil mineral called Strontianite SrCO3 and celestite SrSO4 (Setoudeh, Welham, & Azami, 2010) and these two minerals are dominantly found to be rich in intermediate rock and where gold ore is in abundance (Bhuvana & Prakash, 2015) such as the research location for this study (Zamfara in Nigeria). According to the Agency for Toxic Substances and Disease Registry (ATSDR), radiostrontium belongs to the group of toxic elements of Public Health Importance (ATSDR, 2019).

Previous studies; Miyashita (2012), Saito et al., (2012), Fujimura et al., (2013), Endo, Kajimoto, and Shizuma (2013), Kondo et al., (2014), Fujimura et al., (2014) and Srinuttrakul and Yoshida (2017) examined the influence of soil 137Cs-137 and some other factors on 137Cs accumulation in rice (Oryza stiva) following the 2011 Fukushima's nuclear power station accident in Japan found that rice has potential to significantly accumulate radio caesium but this varies with the influence of different soil properties. There is no previous study that have checked rice varietal variation in Nigeria regarding the accumulation of radio-caesium or stable caesium. Tsukada et al. (2005), Uchida et al., (2009), Rinklebe, Shaheen and Yu (2016), Srinuttrakul and Yoshida (2017) also studied accumulation of radio Strontium and stable strontium in rice and all these studies looked into the influence of the soil parameters and not on varietal based.

2.11 Summary and Conclusion

Lead poisoning is a serious public health issue globally and Nigeria is currently ranked first among the countries of the world that are adversely affected. There have been many interventions but exposure through food consumption especially rice is still a big issue. Moreover, there is very limited data, if any, on the transfer of lead in different rice varieties grown in Nigeria. Findings from the literature shows that four major routes of exposure exist and these are; ingestion through water, ingestion through food, inhalation and skin absorption. For the case of Zamfara Pb poisoning, ingestion through food remains the biggest problem and rice was one of the suspected food.

Apart from farming, gold mining is the major activities in the Pb poisoning affected areas which has promoted the economic growth in the area but left the environment contaminated. The review of the literature shows that there was an emergency remediation previously to minimise the impact and the exposure of the affected population but the remediation only focused on the residential and the industrial area. Farmlands were untouched. It was also found that the remediation technique applied was not appropriate as all the areas previously remediated (cleaned) such as mentioned in chapter 1, section 1.4 paragraph 1 are still contaminated currently with Pb. Rice is one of the crops that is produced commercially in this Pb impacted area and previous studies (chapter 2 section 2.6, Table 2.4) confirmed that rice has the potentials to accumulate Pb significantly and varietal selection is suggested as one of the options to minimise human exposure to Pb via rice consumption. This research focuses on exploring the inter-varietal variation that may exist within 10 selected most grown Nigeria rice varieties for the purpose of varietal selection to minimise human exposure to Pb poisoning.

While minimising the Pb exposure via rice consumption is important, this research is exploring the dietary transfer of other contaminants such as the anthropogenic radionuclides. From the review of literature, Nigeria has a plan to construct nuclear power plants which may be operational sooner. This government plan requires prospective dose assessment and emergency planning which seems not in place as at the time this research commences. The most two important and associated contaminants (radionuclides) in both the operational discharges and emergency (accident) situations from nuclear power are likely to be radio-caesium and radio-strontium as identified in the literature review. Hence, the reason for their inclusion in this research. Therefore, the multi-elemental analysis of the 10 selected rice varieties explores also the intervaietal variation regarding the stable Caesium (Cs) and Strontium (Sr) transfer to rice and evaluates the extent to which varietal selection may help to reduce transfer should soils become contaminated with radio-caesium or radio-strontium in the future. No data was found in this area of research in Nigeria as at the time of this study.

Literature also shows that rice could be a good source of some of the required essential elements such as calcium (Ca), Iron (Fe), potassium (K), magnesium (Mg), Zinc (Zn), Selenium (Se), Manganese (Mn), Cobalt (Co) and Copper (Cu) in the human body through the rice consumption. The intervarietal variation analysis in this research considers these essential elements which will be included in the options for the varietal selection to establish the inter-varietal variation among the 10 selected varieties (cultivars) of rice currently grown in Nigeria. And in conclusion, this could be used as a public health intervention to reduce human exposure to Pb, radio-strontium and radio-caesium while some of the essentials are not compromised.

CHAPTER THREE

Methodology

3.0 Field Characterisation

The major aim of the field characterisation as mentioned in section 1.7 (thesis structure), was to select the best rice farm to set-up the experimental plot for the varietal trial (elemental transfer experiment) which involved growing of the 10 selected most grown Nigerian rice varieties. This was done between October 2016 and January 2017 on the contaminated farmlands in Dareta village, Zamfara State. Four rice farms were selected and the top soil across the four selected farms was scanned using the handheld X-ray Fluorescence (XRF) spectrometer at about 1m sampling distance and at an area of 100 square meters on every selected farm. This is described as a detail study (Esu, 1999). The XRF measured the total Pb concentration in the soil by scanning the soil directly. It provided the Pb distribution using the measured concentration obtained from the XRF scanning which helped to select the most suitable farm for the field experiment, design the test-plots. This was because the soil-Pb concentration and the distribution on farmlands in Zamfara were not previously studied. Result from previous studies (chapter 2 section 2.5.2, Table 2.3) show that the farmlands in Dareta village are contaminated.

3.1 XRF Field Scanning

As at the time of this study, though there were several studies which revealed that Dareta is contaminated across its residential, industrial and agricultural areas. Recent studies on post remediation assessment of soil in Dareta village (chapter 2 table 2.3) revealed some information regarding the Pb contamination but there was no study on the distribution of Pb across the farmlands which makes it difficult to map out the contamination. Residential, arable and industrial area have been explained previously in chapter 1, section 1.4. The use of the handheld X-ray Fluorescence (XRF) spectrometer was adopted as the best method to know the total Pb concentration across the four selected rice farms because the instrument is simple to carry, easy to use and allows the investigator a rapid and non-destructive measurement of metal concentration across a wide area within a short time (Rowe, Hughes, & Robinson, 2012; Shackley, 2010, 2011). Little or no sample preparation is required, and the cost of sample analysis is low (Nazaroff, Prufer, & Drake, 2010). It is also a multi-elemental analytical instrument for liquid and solid samples (Higueras et al., 2012; Hou, He, & Jones, 2004). The analyser measures the total concentration of metal (element) present in the sample by measuring the secondary fluorescent x-ray emitted from the sample when it is excited by a primary x-ray source (Paul, 2017). It involves the use of an x-ray source to irradiate the sample which in-turn fluoresces due to atomic excitation of the samples (Mejía-Piña, Huerta-Diaz, & González-Yajimovich, 2016).

This equipment works by producing a set of fingerprints for each element present in the sample, unique for each which can never be influenced by the fingerprint of other elements (Paul, 2017). Every element in the sample are measured uniquely at the same time (Mejía-Piña et al., 2016). Though this research is concerned about the plant available Pb but at this stage, the total Pb in the soil was enough to determine the Pb distribution across the four selected sites and for the purpose of site selection for the varietal trial experiment. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to analyse the soil samples after the varietal trial experiment to determine the plant available Pb and other elements in the soil.

Rice samples were collected alongside the XRF scanning (in-situ XRF measurement of soil) for the four selected farms shown in Figure 3.1. The rice that was sampled at this stage were those planted by the farmers on the four selected rice farms before this research work started in the area. Corresponding soil with the sampled rice were also collected together. This was done at different depth ie. 0 to 10 cm, 10 to 20 cm and 20 to 30 cm (more in section 3.3).

For the soil characterisation study, the first farm (A) was selected based on the closeness to the village. It was about 2 km away from Dareta village. The next two farms (B and C) were selected at about 5 km and it was based on their closeness to the ore processing sites. Farm B was close to a gold processing site and also close to a stream. Farm B and C were both about 50 meters to the major road that links Dareta and Bagega villages to Anka town (the LGA headquarters). The fourth farm (D) was about 8 km away from Dareta village and more than half km away from the road. Hence the farm (D) was neither close to the river nor to the ore processing sites or to the road. This is revealed in Figure 3.1.

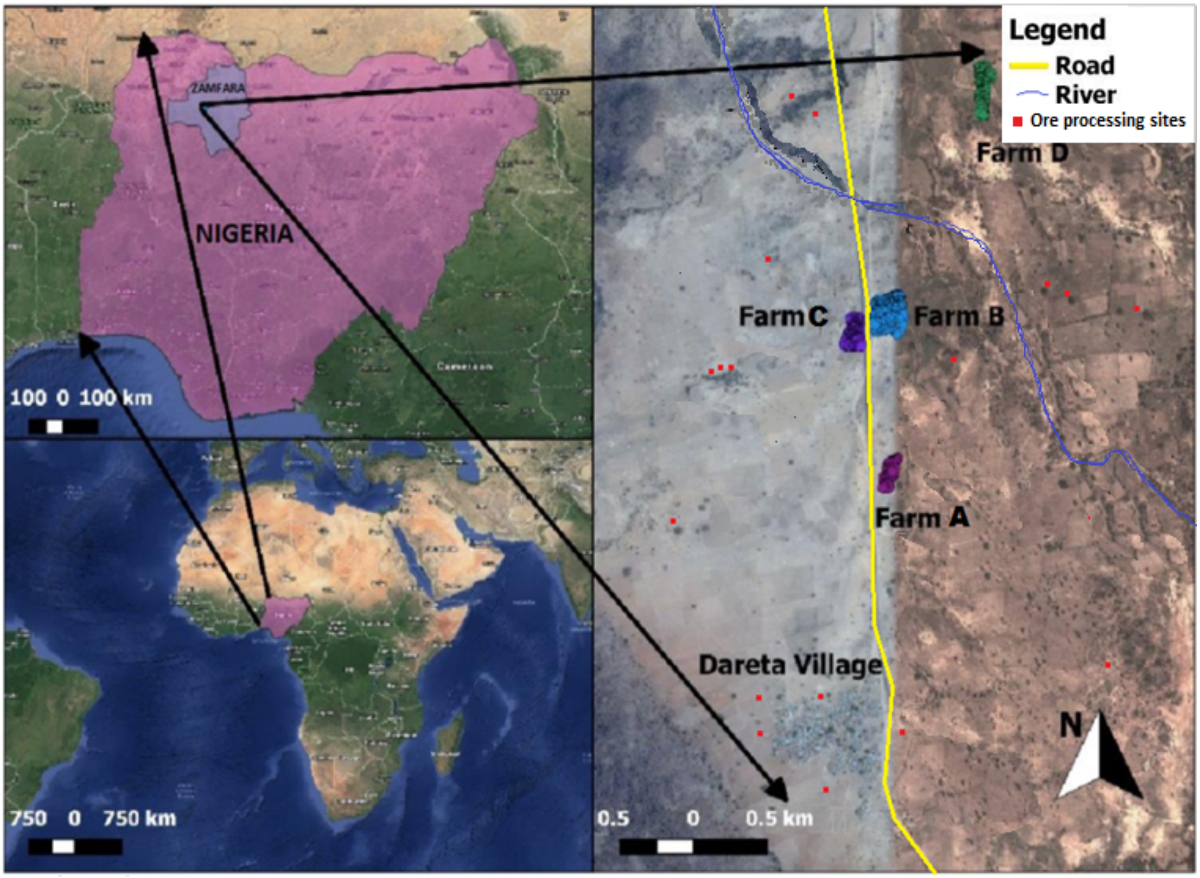


Figure 3. 1: The four selected Farms for the soil characterisation in Dareta village.

Olympus Innov-X DELTA Professional XRF (Olympus USA) (Figure 3.2) was used for this study. This equipment is very fast in its measurement with low limit of detection (LOD) and reliable (Shackley, 2011). It has a screen where the result is displayed (Figure 3.2 a), sample detecting area (Figure 3.2 b), 2 batteries (rechargeable, hot swap) and docking station with charger for both the equipment and the extra battery (OC, 2017).

Figure 3. 2: Olympus Innov-X DELTA Professional XRF used for this study

Other features are; large sample detecting area (Figure 3.2b) which could help in accommodating as many as possible elements within the scanning area or sampling-point, floating point processor which provides more calculations within a short time, powerful x-ray tube of 4 Watt and optimised beam settings, integrated wide area heat sinks which covered the body of the equipment for high power use in extreme hot weather, calibration modelling and advanced algorithms, advanced coloured LCD touchscreen for clarity and easy operation, rubber handle for easy grip, light and easy to carry (2 kg) and lastly, clear and very bright indicator light (Figure 3.3) for safety during analysis to help protect against radiation (International Business Connection, 2017). The green light shown in Figure 3.3a indicates no emission of radiation or not on analysis while the red light shown in Figure 3.3b indicates that radiation emission is active, scanning is ongoing.

Not Detected		
Sb	ND	< 69
Ag	ND	< 35
As	ND	< 7
Mo	ND	< 5.5
Rb	ND	< 3.3
Sr	ND	< 2.5
Zr	ND	< 5.2

Figure 3. 3: Indicator lights on XRF (photo during the field work)

3.2 Calibration of the Handheld XRF and the procedure of use.

The XRF calibration was done before the instrument was used according to the manufacturer's instruction. The calibration was quick and done with less energy. When the XRF case was opened, there were instruction manual, the XRF analyser, two batteries, the calibration check coupons, USB cable, flash drive that carried the XRF software for easy installation on computers, a foam-like cover which kept all the appliances in place and underneath were the docking station and the AC adapter.

The battery-lid was pulled off according to the operation manual and one of the two batteries was inserted and this was closed thereafter. The analyser was turned-on by pressing the power button on the top of the analyser which turns green (Figure 3.3a). Immediately after it was turned on, the analyser displayed the ionizing radiation notice for safety and the message reads; **"This instrument produces ionising radiation. It should only be used by trained technicians. Select START if you are a certified user".** Meanwhile, there were several trainings provided for the researcher of this study by the University of Salford on how to use this equipment, the associated safety precautions, and the general safety on the field about the instrument prior to the commencement of the fieldwork. The researcher was qualified to use the analyser at the time of this study.

The first step to using the analyser was to carry out the calibration. This was done by placing the analyser detector window against the calibration-check coupon that was already been positioned for this purpose in the analyser's docking station. After positioning the analyser, the calibration button (✓Cal) was pressed and it showed "**cal check……**" on the screen after which a calibration curve was produced as revealed in Figure 3.4c and the analyser was ready for use. The calibration was done within two minutes and it was repeated after every ten hours of use i.e every day before use. Each battery lasted for about 4 hours before replacement. The precautionary measures in the operation manual were adhered to strictly, to make sure that everyone on the field was safe from the effect of the ionizing radiation.

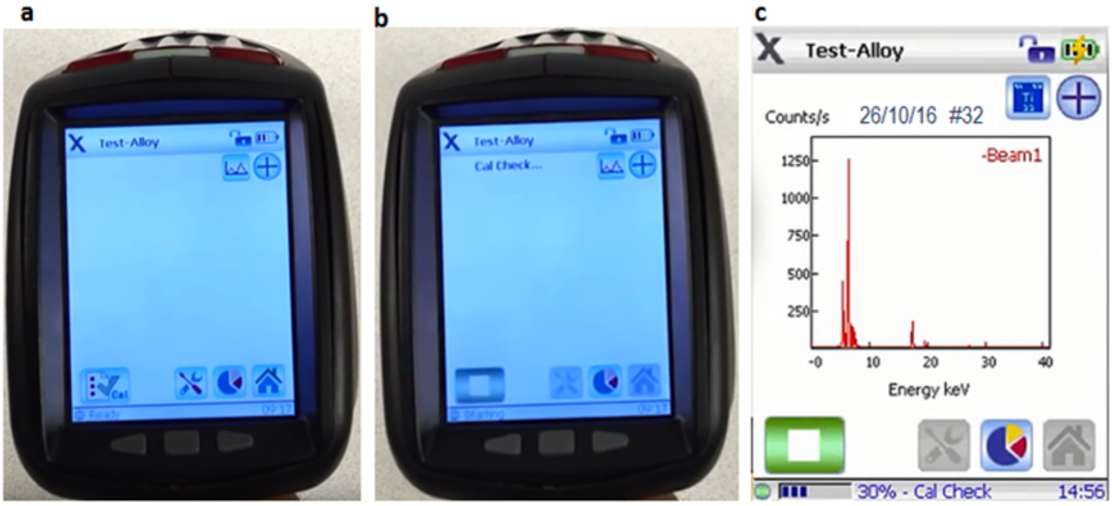


Figure 3. 4: Handheld XRF Calibration.

(a) the XRF screen showing the calibration button (b) calibration process taking place (c) the calibration curve produced at the end of the calibration.

The four selected rice farms were scanned with the XRF analyser at 1 meter intervals using grid sampling pattern as suggested by Alloway (2012). This was to determine the spread pattern of the soil Pb concentration to select appropriate location for the proposed field experiment (varietal trial). Figure 3.5 demonstrates the in-situ soil analysis using hand-held X-ray Fluorescent Spectrometer (XRF) while the reading for Pb was documented manually together with their corresponding GPS readings of every spot sampled (scanned). Image captured during the Calibration of the XRF on the field is Figure 3.5a while Figure 3.5b shows how the distance between the scanning points were measured and Figure 3.5c demonstrates how the records of the XRF readings were taken.

Figure 3. 5: Measuring the lead concentration on the four selected rice farms using the XRF Spectrometer (photo taken during the fieldwork).

3.3 Soil and Rice Sampling for the Field Characterisation

During the XRF soil scanning, rice and its corresponding soil were collected and this was done for the four selected rice farms that were characterised. To achieve detail coverage of the farms for soil sampling as recommended by Esu, (1999), each farm (about 100 m^2) was divided into four, and five samples were collected from each division (Figure 3.6). The rice collected from each point was dissected into root (n=80), shoot (leaves and stem together) (n=80) and the un-threshed paddy rice (n=80). The soil was collected at three different depths; 0 cm to 10 cm (n=80), 10 cm to 20 cm (n=80) and 20 cm to 30 cm (n=80) from the same sampling point which was by the root of the sampled rice plant. The aim was to determine the role of soil physico-chemical properties in Pb uptake by the Nigerian local rice (*bisalayi*) planted in this area as at the time of the soil characterisation. The result for this is presented in chapter four. No research was found on this in Nigeria as at the time of this research.

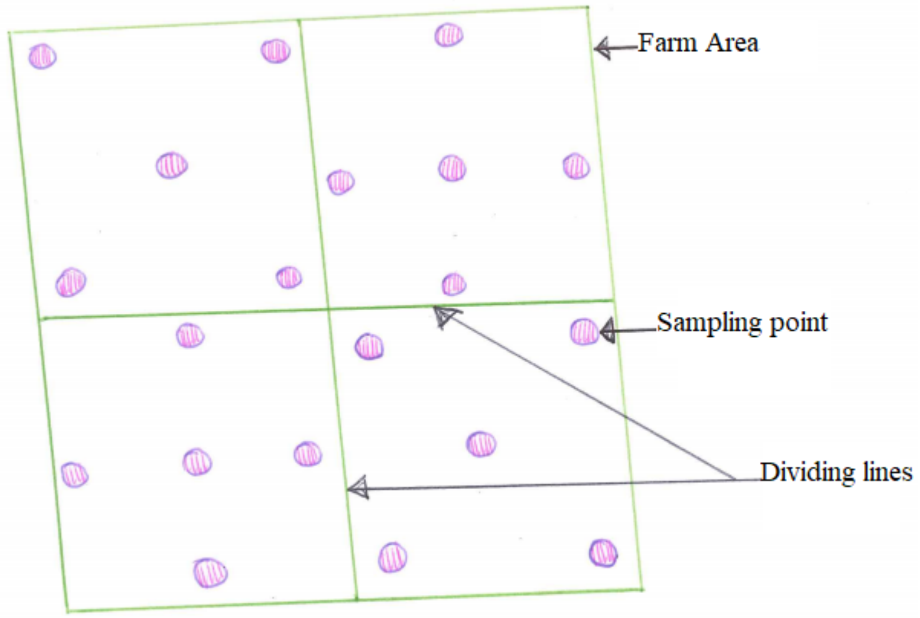


Figure 3. 6: Rice and soil sampling patern from each selected farm during field characterisation

The reason for sampling at different depth was to check the soil profile regarding Pb concentration at different soil dept within the reach of rice root as the study shows that rice root is domicile within 30 cm soil depth (NCRI, 2017). This was explained previously in chapter 1, section 1.7. Apart from the determination of the influence of the soil physico-chemical parameters of soil on the Pb uptake in rice (objective 1), this part of the analysis was also to assess the localisation of Pb in different parts of rice in line with the research objectives 2 as described previously in chapter 1, Figure 1.11. Procedure for sample collection and preparation was adopted from Alloway (2012). A maximum (plough) depth of 30 cm was recommended for an arable land regarding determination of heavy metal uptake in rice (Alloway, 2012; Esu, 1999; FAO, 2013). About 900 g of soil sample was collected from each point (300 g per depth and at the three depths). Figure 3.7 a reveals a sampling point as it was measured using a plastic ruler, Figure 3.7 b shows the rice root as it was collected from field and Figure 3.7 c shows how the un-threshed rice sample was collected together with the soil samples (in sealable sample bags) and the rice shoot from the same sampling point placed together in a big black sample bag, well labelled.

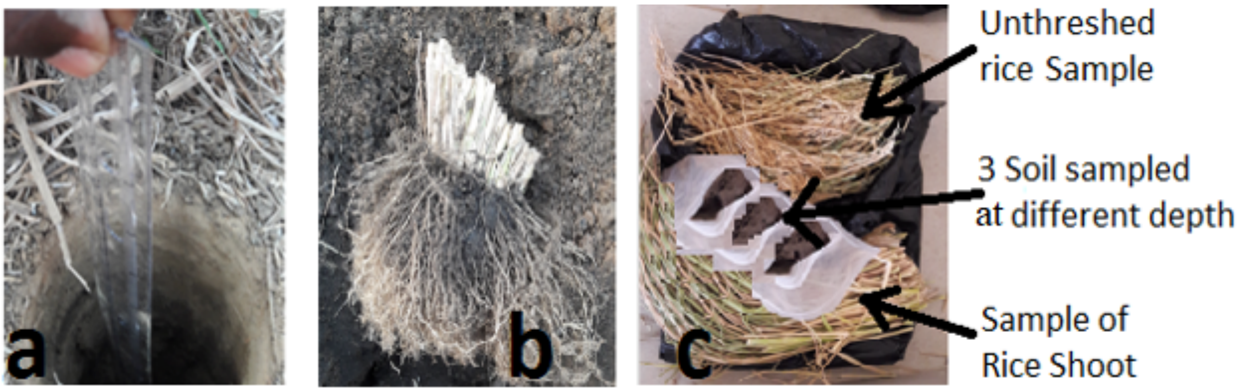


Figure 3. 7: Soil sampling at different soil depth (0-10 cm, 10-20 cm, 20-30 cm) using auger.

Aluminium auger, spade, shovel were the sample collection tools used and polyethylene bags were used to collect the samples from the field as this has been described as the best for the determination of heavy metals (Carter & Gregorich, 2008). Most of the sampling tools used were easy to clean, take equal volume of soil samples, adaptable to dry, sandy and wet sticky soil, rust resistant, bending resistant and easy to use as recommended (Alloway, 2012). These first group of samples were analysed in the laboratory using Atomic Absorption Spectrometer (AAS) and the digestion method was wet acid digestion which is detailed in section 3.4. Flame photometer was used to measure Na and K. Details about the elemental analysis including the caliberation and the instrument limit of detection (LOD) is presented in section 3.4.2. The physico-chemical analysis of the soil (soil test) is discussed in section 3.7.2, (Table 3.2 summarises it) while the soil and rice sample preparations are discussed in section 3.7.1 and 3.7.3 respectively. This part of the research work aimed to achieve the research object 1 and 2 and the result is presented in chapter 4 and 5.

3.4 Elemental Analysis by Atomic Absorption Spectrometer (AAS)

The elemental analysis for the rice and the soil samples collected for this research work (explained in section 3.3) was done by using Atomic Absorption Spectrometer (AAS). This was suitable for the type of investigation (chapter 4 and 5) this work aimed at, and due to limited resources.

3.4.1 Sample Digestion and the elemental analysis for AAS

As previously explained in the introduction (section 1.7) and in this chapter section 3.3 (above), rice and soil samples were collected from 20 sampling points from each farm of the four selected rice farms after the in-situ soil screening using the handheld X-ray Fluorescent Spectrometer (XRF) analyser. This amount to 80 soil samples (20 samples × 4 farms) and 80 rice samples (samples 20 samples × 4 farms). The soil was sampled at three different depths from each sampling points (0 cm to 10 cm, 10 cm to 20 cm and 20 cm to 30 cm depths) and each rice sample was also further segregated into root, shoot, husk and seed.

0.5 g of soil was weighed from each sample. A mixture of Nitric and per-chloric acid was prepared at ratio 2:1 and 10 ml each was added to the weighed samples in the test tubes under the fume cupboard. The test tubes were placed in the hot block and the hot block was electrically heated at 145^0C for 1 hour and further increased to 240^0C for another 1 hour. The temperature control knob on the hot block was turned to zero after 120 min and the mixture in the tubes were allowed to cool for 2 hours and filtered using whatman®42 filter paper. This was made up to 25 ml in the volumetric flasks with milli-Q water (deionised water). This was then analysed with Atomic Absorption Spectrophotometer (211VGP Buck Scientific USA). To get the concentration of lead in the digest, the formula (equation 1) below was used (IITA, 2016; Udo et al., 2009).

$$Concentration\ (Element)\left(\frac{mg}{kg}\right) = \frac{R\times V\times D}{\mathrm{SM}} \text{ ....equation 1}$$

R = AAS reading for the soil/plant samples – Blank
V = Digest volume used (25 ml)
D = Dilution factor
SM = Sample mass used (0.5 g).

0.5 g was also used for the rice samples and the same sample digestion procedure was followed according to IITA (2016). Per-chloric acid was added to the digestion to prevent the frequent excessive foaming that occurs when the nitric acid is used singly (Shaibur, Hasnat, Shamim, Huq, & Kawai, 2010). The main purpose of digestion in inorganic elemental determination is to destroy the organic matter in the medium and perchloric acid is more effective in digestion of organic complex (Shaibur et al., 2010).

3.4.2 Calibration of the Instrument, Atomic Absorption Spectrophotometer (AAS)

To prepare the working standard solution, 1000 mg/kg standard stock solutions of Pb, Cu, Fe, Zn, Mn, Cd and Cr provided by the manufacturer were used with appropriate dilutions. The manufacturers' instruction was followed to set up the AAS for each element that was analysed. These include acetylene (fuel) and oxygen gas (the oxidant) selection, best wavelength, type of burner and slit-with settings. A calibration curve was generated which was used to find the unknown concentration of those elements in the solution. The absorbance of each known solution was measured and then a calibration curve against absorbance was plotted according to the manufacturer's procedure. The instrument was calibrated, and a straight-line calibration curve was generated. Then, the sample solutions (digest) was injected into the instrument and the absorbance of the element in this solution was measured in line with Buck Scientific (2005). The limit of detection (LOD) for the instrument were 0.08 mg/kg for Pb, 0.01 mg/kg for Cd, 0.01 mg/kg for Ca, 0.04 mg/kg for Cr, 0.01 mg/kg for Cu, 0.05 mg/kg for Fe, 0.005 mg/kg for Mg, 0.03 mg/kg for Mn, and 0.005 mg/kg for Zn (Buck Scientific, 2005). While 0.005 mg/kg and 0.01 mg/kg were LOD used for Na and K respectively. (Scientific, 2016). Dry weight was used to calculate the concentration of metals in the digest of soil and plants.

3.4.3 Analytical Quality Assurance

In order to check the reliability of the analytical methods employed for heavy metals determination in both the rice and soil samples, Lichens coded IAEA-336 Certified Reference Materials (CRM) procured from International Atomic Energy Agency (IAEA) Vienna, Austria was used. It was digested and then analysed following the same procedure as we have done in the sample digestion according to the IAEA guides (Heller-Zeisler et al., 1999). 0.5 g of the CRM was weighed, digested in 3 replicates and the following elements were analysed from the digest: Pb, Zn, Cd, Mn and Cu. The result is presented in chapter 4 and 5.

3.5 Field Experiment (Varietal Trial)

3.5.1 Field Selection

The field experiment was set up on the selected rice farm. Based on the soil characterization previously conducted which is discussed from section 3.1 to 3.3, farm B was selected among the four farms examined. This farm possessed the required quality that was needed for the field experiment (varietal trial). The topography was flat, there was a stream flow across the base of the farm and based on the result obtained from the field characterisation, farm B was the best for the experiment in terms of the different grades in the spread of the elemental concentrations of Pb. Farm B was considered also for logistic reasons, easy access for maintenance of the experimental farm including planting and harvesting. The experimental set-up was done during rice growing season in Dareta village Zamfara in 2017 (between June and November). This is popularly called the wet season in the northern Nigeria.

3.5.2 Experimental Design for the rice growing (Varietal Trial)

This experiment was designed to have two different set of rice trials. The first set was planted on the Pb contaminated soil which was selected from the initial field characterisation (section 3.5.1 above) in Dareta village while the other set was planted in a pot experiment on soil contaminated artificially and this was carried out within the University of Abuja, airport road, Abuja Nigeria.

The rice on maturation were harvested together with their corresponding soil asmples from both the field and the pot experiment. The samples; both the rice (n=600) and the soil (n=600) i.e 300 rice and 300 soil each from both the field and the pot experiment were analysed at the toxicology laboratory, global centre for environmental remediation (GCER), the University of Newcastle, Callaghan, Australia. We have analysed few elements by the ICP-OES. This was not to validate or replicate the result from the ICP-MS but to avoid complex spectral interferences which may occur while using the ICP-MS for these selected elements (Nageswara & Kumar, 2007; Nardi et al., 2009). Research shows that major elements with high limits of detection could be analysed by the ICP-OES (Chaves et al., 2010; Lyra et al., 2010).

The elements analysed by ICP-OES in this study were four out of the nine essential elements analysed for this study. The four elements include calcium (Ca), iron (Fe), potassium (K), magnesium (Mg). All other elements were analysed by the ICP-MS including those elements that were predicted to have much lower elemental concentrations which may result to low limit of detection such as Pb and some other elements in the rice samples. Many samples could have shown a result of BDL (below detection limit) for Pb concentrations provided they were analysed by the ICP-OES. The detection limit for Pb by the ICP-OES is 0.5 µg/L while that of ICP-MS is 0.0005 µg/L (Thermo Fisher Scientific, 2017). Differences and similarities between the field and the pot experiments are presented in Table 3.1.

Table 3. 1: Differences and Similarities between the Field and the Pot Experiments.

Differences:

S/N	**Field Experiment**	**Pot Experiment**
1	Farmers normal/cultural method of rice growing in the region was followed	Researchers adopted scientific method of rice growing based on literature from the previous studies
2	Lead concentration in the soil was not the same across the field	Lead concentration in the soil was the same in all the pots
3	Rice plants were transplanted onto a contaminated soil (selected farm B) and before the transplanting, rice plants were already been planted by the farmers on the farm. Our selected rice varieties for this study were transplanted inside already established rice farm.	Rice plants were transplanted into pots (bags) filled with soil contaminated by the researcher
4	Took place in Dareta village, Zamfara State, Nigeria	Took place within the University of Abuja, Federal Capital Territory (FCT), Abuja, Nigeria
5	Handheld XRF and AAS was used for soil screening for an appropriate farm selection prior to setting-up of the field experiment on the selected farmland.	Only the laboratory based AAS screening was done on the pot soil before it was used for the pot experiment

Similarities:

Both the field plots and the pot experiment were fed with rain water normally in an open-air system of farming. The sunlight, the relative humidity and other natural environmental factors that are responsible for crop production were both naturally controlled.
The same varieties were used for both experiments.
Both experiments adopted Randomized Complete Block Design/System (RCBD) with the same number of replicates (30 replicates).

3.5.3 Rice Growing

Steps in rice planting and different stages of growth of rice plant have been explained in chapter 2 section 2.8, Figure 2.9, the same steps were followed in this study.

3.5.3.1 Germination

The planting technique and methods were adopted from the International Rice Research Institute (2017), Liu et al. (2003) and Liu et al. (2005). The rice seeds were soaked in water for 48 hours at room temperature of 30°C and removed from the water and covered with two layers of moist gauze at the same temperature for another 30 hours to germinate the seeds (CGIAR, 2013). Figure 3.8 (a-f) shows the pictorial illustration of how germination was done.

Figure 3. 8: Rice Germination

(a) The rice varieties in bags as it was supplied from the National Cereal Research Institute (NCRI) **(b)** Soaking of the 10 rice varieties for 48 hours at the room temperature **(c)** Spreading of the soaked rice seed on moist tissue **(d)** Covering the soaked rice seeds on the moist tissue with another moist tissue for 30hrs at the same room temperature **(e)** Removal of the top moist tissue after germination for proper aeration of the germinated seeds **(f)** Rice germination completed on the moist tissue.

3.5.3.2 Pre-planting

The germinated seeds were transferred onto moistened uncontaminated soils for 30 days for the rice plant to clearly form. This is revealed in Figure 3.9 (a – f). Before the selected rice varieties were pre-planted, there was a need to check the proposed clean soil to be sure of its cleanliness. 60 soil samples were collected randomly across the area of land marked to get the clean soil for rice pre-planting and pot experiment. The clean soil was collected from the open space behind the University Press, the University of Abuja campus and this was analysed using Atomic Absorption Spectrometer (211VGP Buck Scientific USA). The mean Pb for the soil samples (n=60) was 26 mg/kg dry weight. The international acceptable limit standard for EU is 100 mg/kg (EC, 2001). The instrument's (Atomic Absorption Spectrometer) limit of detection (LOD) for Pb was 0.08

mg/kg (Buck Scientific, 2005), and more than 50% of the soil samples showed below detection level (BDL) in the result. This was considered as a clean soil (Alloway, 2012).

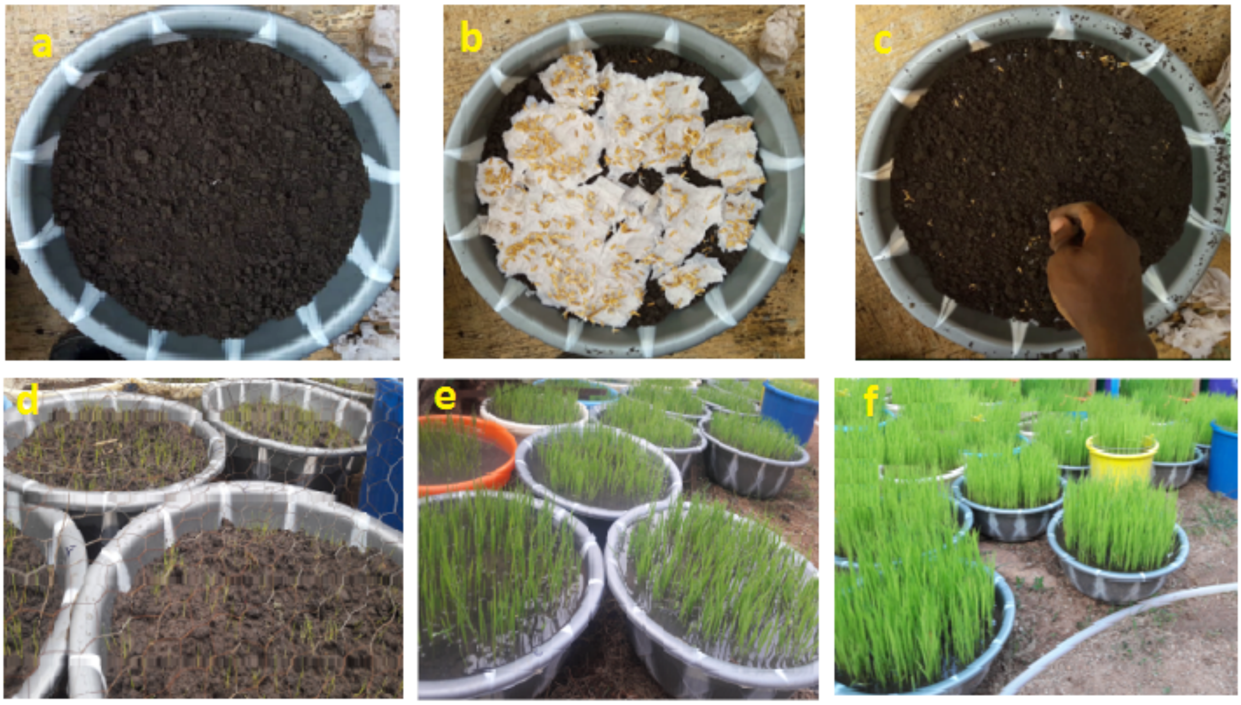


Figure 3. 9: Pre-planting of rice

(a) Pre-planting bowl with clean soil moistened with water and ready to receive the germinated seed **(b)** the clean soil in the pre-planting bowl was covered with the germinated rice seed **(c)** the germinated rice seed on the clean soil was covered with another layer of clean soil **(d)** the rice growing on the clean soil (a week old after germination) **(e)** the rice growing on the clean soil (2weeks old after germination) **(f)** the rice growing on the clean soil (a month old after germination).

3.5.3.3 Rice Transplanting (Field Experiment)

This was the process whereby the germinated rice seedlings (about a month old) were transferred from the non-contaminated soil in the nursery onto the contaminated soil on the field. The seedlings grown out of the 10 selected rice varieties were transplanted from the nursery where they were pre-planted on a clean soil onto the contaminated field (rice farm) in Dareta village. This was done based on previously used procedures (Liu et al., 2003; Liu et al., 2005) and the popular method adopted by the farmers in this area.

The rice was transplanted based on the original planting distance met on the field (15 - 20cm) which have already been set by the farmers who have planted their rice ordinarily. The pegs were positioned across the farm with the appropriate varietal code (allocated ID) for each variety that were planted around the peg. About 2 to 3 seedlings were planted on a spot around the labelled pegs according to Ricepedia (2017). The ten varieties were planted in 30 replicates each in line with the research design. The more the sample size, observation and the experiment, the more reliable the data is (Hopkins, 2010). This provided 300 rice plant on the field (10 varieties × 30 replicates = 300 rice plants). More replicates allow to estimate the variability of the result and it increases the accuracy of the estimate (Queen, Quinn, and Keough, 2002). Another reason why the replicate was large (30) was because it was necessary to establish the experimental error regarding the standard deviation and this would be difficult if the number of replicate was small (Manly, 2006).

The selected varieties were planted within an already established rice farm to avoid theft, attack by intruders and also to prevent against the edge effects (Fox et al., 2000). Every planting spot was identified using wooden pegs and the pegs were labelled with permanent marker to avoid cleaning off when it rains. The points where each variety was planted was measured using a measuring tape based on the adopted Randomised Complete Block Design (RCBD). This was done to make sure the varieties were well spread across the farm on the various soil lead concentrations which has been previously examined on the selected farmland.

After the positioning of the pegs which was based on tape-rule measurements, the farmer’s rice around the pegs were removed and replaced with the rice number that was coded on each peg. There were support personel on the farm who worked with us to transplant the rice. Each support personnel carry different variety of rice and the variety they carried were tagged on each person’s neck as it is revealed in Figure 3.10 to avoid confusion or mix-up during the rice transplanting. When the transplanting was completed, GPS coordinate for the location of the peg was recorded for record and easy monitoring. The planting points were in 16-feet (about 5meters) to each other and the whole layout covered about 150 meters by 80 meters when the transplanting was completed, and the layout was established.

Figure 3. 10: Rice transplanting on the field (pictures taken during the field work)

(a) Measuring the points where each variety would be planted based on the adopted Randomised Complete Block Design (RCBD) using meter rule **(b)** Positioning of the pegs on the field based on the points measured in “a” **(c)** Pegs were positioned **(d)** removing the farmer’s rice and replaced it with the under-studied variety based on the information on the pegs. Each support personnel carry different variety and the variety they carried were tagged on their necks as indicated in the pictures. This was to avoid confusion or miss-up during the rice transplanting **(e)** replacement of farmer’s rice with the research variety **(f)** recording the GPS coordinate of the peg for record and monitoring purposes.

3.5.3.4 Farm monitoring (Field Experiment)

Farmers in the area were involved in safeguarding the experimental plots throughout the whole period (about 5 months) as the selected varieties under investigation were planted within their farm. Randomized Complete Block Design/System (RCBD) was used in transplanting the seedlings with 30 replicates making it 300 experimental rice plants scattered within the farmer's farm. The recorded GPS coordinates documented during the transplanting of the rice was used to trace regularly the position of each variety by looking for the coded pegs as shown in Figure 3.11. The position of the labelled pegs signifies where the 30 replicates of every variety were located. During the weeding, the farmers were supported financially to get the work done carefully and to make sure that none of the experimental rice plants under the varietal trial were affected. Weeding is the removal of the unwanted plants grown within the rice plants which may affect the rice growth, yield, deprives the rice plants the required nutrients from the soil and can as well kill the rice (Hasanuzzaman, Islam, & Bapari, 2008).

Figure 3.11: One of the pegs was hiding during the farm monitoring conducted in September 2017 but later found with the help of GPS coordinate recorded during the rice transplanting.

3.5.3.5 Reasons for chosen RCBD

In this type of experiment, expected variations seems to be many, therefore there was a need to look for a method that would limit the variations to the focused varietal uptake of Pb, Cs and Sr. Based on the environmental factors, variation was expected from the selected variety, the soil type, soil properties, layout based on the topography, rainfall pattern, nutrient and metal distribution in the soil etc. Therefore, in Randomized Complete Block Design (RCBD), in all the blocks, all the replicates of the selected varieties are represented. The precision is higher with Randomized Complete Block Design than Complete Randomized Design (CRD) (Kravchenko, Robertson, Thelen, & Harwood, 2005). It means that the design has blocked multiple variations that may arise from the aforementioned factors. All the rice plants were having equal share of everything on the field (Anderson & McLean, 2018). It made the result more precise at the end of the experiment because the multiple sources of error have been taken care of (blocked). In this study, the blocks/replicates of the rice varieties were treated the same and no block or replicate missed during the experiment in both the field and in the pot experiment. Therefore, no block effect is expected at the end of the experiment according to Clovis (2007), Grant (2010) and Liu and Berger (2014)

The design for this study was 30 replicates of 10 varieties of rice in 3 blocks (Appendix B, table II) as there is no restriction on the number of replicates to have in varietal trial and when replicates are more, the missing plots (if any) are easily estimated and covered (Clewer and Scarisbrick, 2013). Two-way ANOVA was used to check the block effect on the experiment.

3.5.3.6 Harvesting of Rice and Soil Sampling (Field Experiment)

Harvesting was done by collecting not all the rice produced by our varieties but just the little that was required for the research purpose. The soil samples were collected alongside the rice samples at a depth of 30 cm from the sampling point (by the coded pegs) which was by the root of the sampled rice plant. A maximum plough depth of 30 cm was recommended for an arable land as regards heavy metal plant uptake analysis (Alloway, 2012; Esu, 1999; FAO, 2013).

Bulk of soil sample of about 600 g was collected at the base of all the rice varieties alongside their rice. The plant samples were dissected into root, shoot (leaves and stem together) and the un-threshed paddy rice. Paper envelope was used to collect the un-threshed rice seed to prevent been affected by excessive heat. A plastic pipe and a harmer were used to get the bulk of soil from the base of the rice. After removing the rice with its root, the plastic pipe of 30 cm in length was hit by rubber harmer down the soil until it enters the soil completely. This pipe was removed, and its soil content was emptied into the sealable polyethylene sample bags, sealed and labelled appropriately. This was placed together with the rice samples (root, shoot and un-threshed rice seed) that have been collected in the same big black polyethylene bag. Figure 3.12 (a-f) and Figure 3.13 (a-f) shows the pictorial illustration for the harvesting/sampling of rice and soil respectively from the field. Soil sample was colleceted from all the 300 rice replicates together with the rice samples. Therefore, the number of rice samples collected was 300 (n=300) and the number of soil samples collected was 300 (n=300) too from the field experiment.

Figure 3.12: Pictorial illustration for harvesting/sampling of rice from the field

(a) Using scissors to cut the rice **(b)** putting the rice inside a labelled paper bag to prevent been affected by heat **(c)** Using cutlass to cut the rice shoot **(d)** the rice shoot is kept in a big black sample polythene bag **(e)** the rice root was collected and kept in the same bag with the shoot **(f)** the bag was labelled appropriately with the variety number/ID, date and time.

Figure 3.13: Pictorial illustration for harvesting/sampling of soil from the field

(a) The plastic pipe of 30 cm long was hit with rubber hammer down the soil **(b)** the pipe in the soil **(c)** withdrawing the pipe from the soil with the soil sample **(d)** labelling of the sample bag **(e)** pouring of the soil in the sample bag **(f)** all the samples were kept in the big black bag and the bag was properly labelled.

3.6 Pot Experiment

This was to further work on the research objectives as to achieve the aim of this study. The soil-Pb concentration prepared was uniform for all the pots and across all replicates, since the average soil-lead in Dareta village was 1000 mg/kg (Bartrem et al., 2014) and based on the pilot study conducted prior to the field experiment. The soil was pre-treated manually with Pb by adding Pb-nitrate to the soil (Liu et al., 2003) to prepare the required concentration of 1000 mg/kg of soil Pb dry weight.

Lead (II) nitrate was used in this work due to its relevance in the mining environment (Rubo, Kellens, Reddy, Steier, & Hasenpusch, 2006). It is directly involved in gold cyanidation in mining (Rubo et al., 2006). Gold cyanidation is a metallurgical technique involved in extracting gold from low-grade gold ore (Rubo et al., 2006). $Pb(NO_3)_2$ is used as raw material in production of reagents for mineral processing to recover gold, silver, zinc and copper and it is involved in the industrial production of pesticides and that is why it is mostly present in the mining environment apart from the Pb component of the gold ore (Bosman, 2009). It commonly occurs as white powder but is colourless and soluble in water (Patnaik, 2003)., Therefore, lead II nitrate was recommended for experiments that involve examining of plant uptake of lead because of its ability to dissolve in water, its mobility in the soil and its bioavailability to plants (Liu, Qian, Cai, Yang, & Zhu, 2007).

This experiment was carried out at the University of Abuja, Airport road, Abuja, Nigeria. The screen-house (pot experiment) location was on $8^0$58’52”N, 7010’37.3E, an open space beside the Veterinary Teaching Hospital, Faculty of Agriculture, University of Abuja. . Differences in the two experiments were previously discussed in Table 3.1. The pot experiment had the soil Pb concentration of 1000 mg/kg for all the varieties, evenly distributed and polythene bags were used to plant the rice.

3.6.1 Soil Preparation and Contamination for the Pot Experiment.

The soil was contaminated with lead (II) nitrate powder (ACROS, Fisher Chemicals UK) and this was measured based on the calculations below:

One bag (planting bag) required 10 kg of dry soil
The design was for 30 replicates of 10 varieties of rice
10 kg of soil x 10 varieties x 30 replicates = 3000 kg. Therefore, 3000 kg of clean dry soil was obtained and transported to the allocated space for the pot experiment.

The space which was initially bushy was cleared and the holes for planting bags (Figure 3.14b) were created at one meter to each other along the tracks that were created for the replicates using twine and measuring tape to make sure the lines were straight and to avoid any error that might be caused by eye gauge. The reasons for burying the bags a little inside the soil were;

to prevent the bags from fallen-off the line
to prevent losing the content of the bags (spills) when they fall and
to localize any accidental spills.

The experimental design was to place 10 kg of soil in each planting bag. To calculate the percentage (%) of Lead II Nitrate required to contaminate 10 kg of soil, the formula below was used;

$$\frac{\%\ of\ Pb\ in\ the\ compound}{Molecular\ mass\ of\ Lead\ II\ Nitrate} X \frac{100}{1}$$

(Palipoch et al., 2011)

% of Pb in Lead (II) Nitrate = 62.6%, molecular mass of lead II Nitrate = 331.2098 (ConvertUnits.com, 2017)

$$\frac{62.5585}{331.2098} X \frac{100}{1} = 18.888\, g = 18.9\, g$$

18.9 g (18900mg) of Lead II Nitrate ($Pb[NO_3]_2$) was required for 1 kg of soil. Therefore, 18.888 X 10 = 188.9 g was used for 10 kg of soil (1 bag).

For the 3000 kg in total (10 kg (1 bag) X 10 varieties X 30 replicates (300 bags)), 18.888 g X 3000 = 56,664 g = $\left(\frac{56{,}664}{1000}\right) kg$ = 56.664 ≈ 56.7 kg of Lead (II) Nitrate was used to contaminate the 3000 kg of soil at 1000 mg/kg lead concentration level.

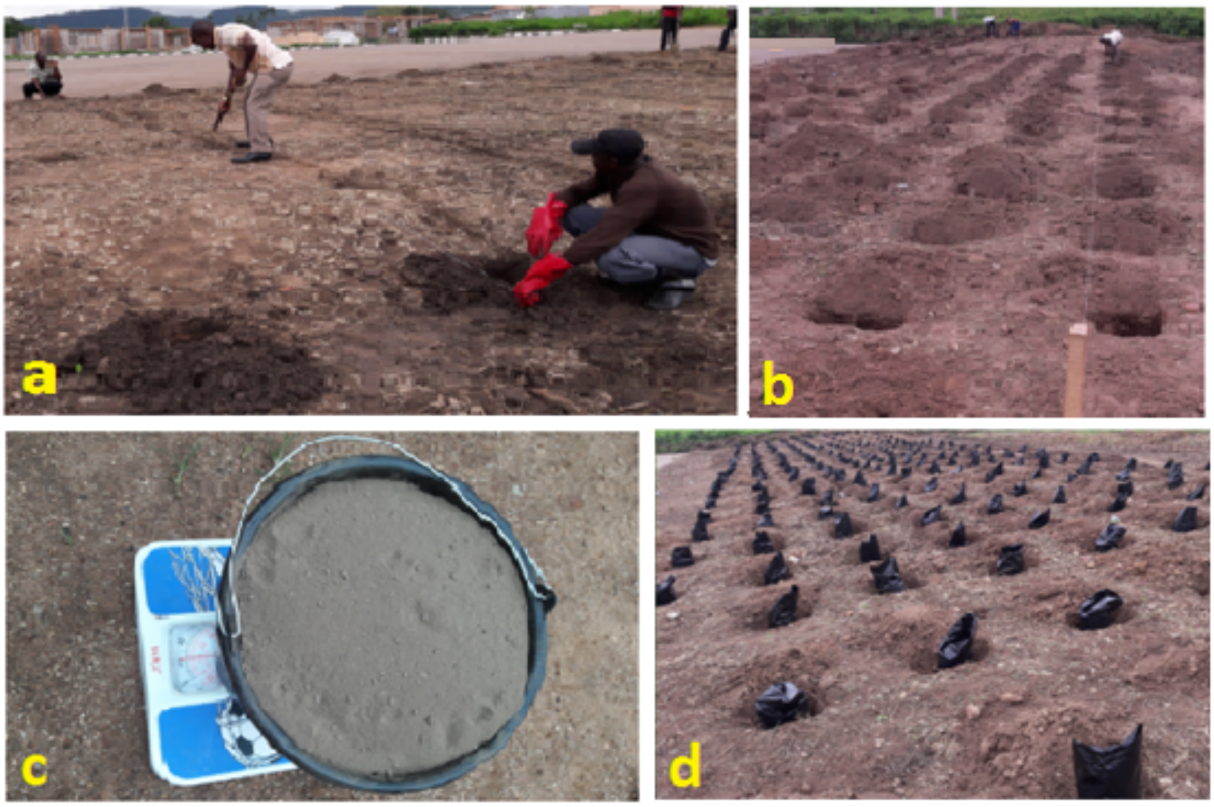


Figure 3.14: Land preparation for the screenhouse (pot experiment)

(a) creating lines and holes for planting bags using twine and tape rule **(b)** on the planting lines, holes were created for each bag **(c)** weighing of the clean soil for each bag using Hana weighing scale model J1509097852 China **(d)** the bags were filled with 5 kg clean soil and arranged appropriately.

3.6.2 Limitation during the soil contamination

Originally, 10 kg was planned but due to low supply of lead nitrate, 5 kg soil was used. Based on the calculation in section 3.6.1, 56.7 kg Lead nitrate was required for the contamination but this could not be supplied from the market with lots of efforts through other Universities and Research Institutes in Nigeria. Only 29.5 kg was supplied. Instead, half of the proposed soil for contamination was contaminated at 1000 mg/kg. 5 kg of clean soil was first placed in every bag then; each bag was top up with 5 kg of contaminated soil.

3.6.3 Steps in contaminating the Soil

- 10 kg soil was weighed into the bag while half of this was removed for contamination i.e half bag was poured on the floor for Pb contamination while the remaining half was left in the bag uncontaminated.
- The soil poured on the floor for lead contamination was divided into smaller units and Lead II Nitrate was spread on top across each portion (Figure 3.15b);
- The soil was mixed manually with shovel severally by two people (Figure 3.15c);
- The manually mixed soil was placed in a mechanical mixer (Figure 3.15e). A yard capacity manual cement mixer thoroughly cleaned was used. The mixer was washed thoroughly with tap water and then rinsed with deionised water before use.
- The contaminated soil that was initially mixed manually was filled in the mixer. The soil was mixed severally and consecutively with the mixer.
- Broom was used to sweep the remnant of the contaminated soil (Figure 3.15d) on the floor together to get the measured soil complete into the bags.
- The content of the bags was soaked for 3 days with rain water stored in water storage tank to get the bags ready for transplanting of the rice varieties
- Numbering of the bags was done after 3 days of soaking the bags with water in line with the adopted Randomised Complete Block Design (RCBD).

3.6.4 Rice Transplanting for the Pot Experiment.

The rice was transplanted on Saturday August 12, 2017 and all the soil bags in the screen-house that were already soaked with water were labelled with the variety number that was to be transplanted into them according to the adopted RCBD.

Steps in rice transplanting in the screen-house;

- The bag in the screen house were already well placed, soaked with rain water for 3 days;
- These bags were numbered based on the randomized complete block design (RCBD) used for rice planting in this study;
- After ensuring that the soil in the bag was completely soaked, the rice was transplanted onto it in line with the numbering;
- The varieties to be transplanted were placed in the car park beside the screen house and were numbered appropriately to avoid mix-up
- The roots of the rice seedlings were washed (Figure 3.16b) to get rid of the soil coming with it from the nursery before planting them in the bags. The roots were washed with rain water which was collected and stored in a plastic water tank (Round Geepee 5000 L tank, made in Nigeria).
- 30 Agricultural Science Students were trained of which 10 among them were selected to transplant the rice as in one person per variety each to avoid any error that may come from transplanting.

Pictorial illustration of how the soil was contaminated and the bags were prepared is shown in Figure 3.15 (a- i) and how the transplanting was done is shown in Figure 3.16 (a-f) and Figure 3.17 shows the rice varieties at 100 days after transplanting. At this stage, some were fully matured while some were flowering of which they needed more time to reach maturity.

Figure 3. 2: Soil contamination

(a) Dividing the clean soil into small units **(b)** spreading the Lead II nitrate on the soil **(c)** mixing the soil manually **(d)** using broom to sweep the remnants to get the contaminated soil together **(e)** loading the soil into the mechanical mixer **(f)** well mixed soil from the mixer **(g)** topping-up the bags with the 5 kg contaminated soil **(h)** soaking the bags with rain water from water storage tank **(i)** Numbering of the bags after soaking for 3 days.

Figure 3. 3: Rice transplanting for the pot experiment

(a) Varieties from the nursery (b) washing the rice roots with rain water from rain-water storage tank (c) rice roots washed (d) agricultural science students planting the rice (e) 10 students planted the 10 varieties (1person per variety) to avoid mix-up (f) the rice, a week after transplanting.

Figure 3. 4: Pot Experiment; rice varieties in the screenhouse at 100-days after transplanting.

The planting bags were 60 cm in height, 30 cm wide and non-perforated procured from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Figure 3.18 shows the dimension of the planting bag).

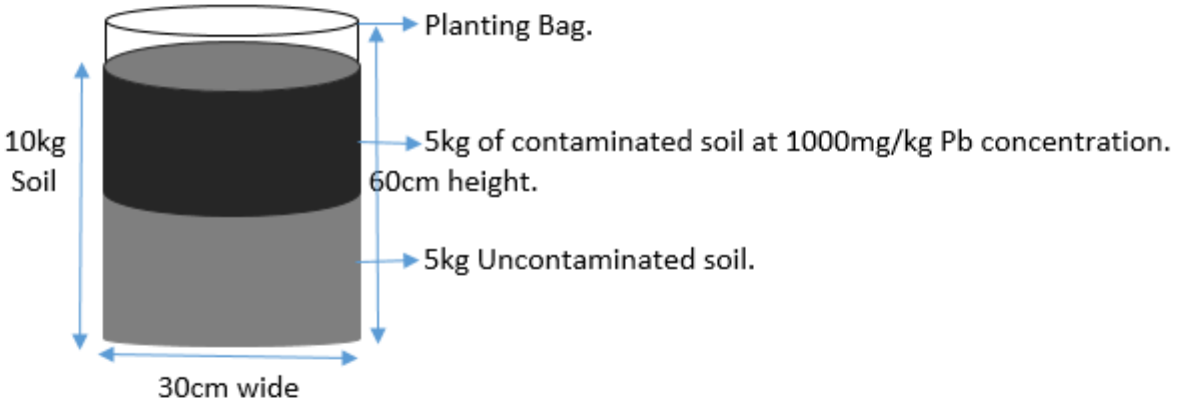


Figure 3.18: Dimension of the planting bag and the illustration on how it was filled with soil.

3.6.5 Farm monitoring (Pot Experiment)

The farm was fenced with wire gauze (Figure 3.19) to prevent intruders and the farm was monitored daily by the assigned students from the faculty of Agriculture University of Abuja.

Figure 3. 19: Screen-house secured with wire gauze to prevent animals and other intruders.

3.6.6 Harvesting of rice and Soil sampling (Pot Experiment)

Seeing the rice plants getting dried was a sign of maturity (Ricepedia, 2017) and prior to harvesting, the pots (bags) were renumbered again clearly according to the planting design (RCBD) using a white sticky tape. Plastic pipes of 60 cm was used to collect the soil sample from the bags. This enabled soil sampling done to the bottom of the bags. After hitting the pipe down into the bags, the pipe was taken out with the bulk of soil, and the soil in the pipe was poured into the sample bag, labelled and keep with other rice samples in the same big black sample bag labelled for all the samples from the same rice plant (Figure 3.20). Soil sample was colleceted from all the 300 rice replicates together with the rice. Therefore, the number of rice samples collected was 300 (n=300) and the number of soil samples collected was 300 (n=300) from the pot experiment just as we did for the field experiment.

Figure 3.20: Harvesting and sampling

(a) all the samples collected from each planting pot as it was labelled (b) all the samples collected as they were still lying in the screen house (c) aerating the samples in the laboratory.

Apart from that, the harvesting and sample collection technique followed the same procedure as described in the field experiment. The samples from the field and those from the pot experiment were taken to the soil science laboratory of the University of Abuja. The total number of the samples collected were 1,200 (field experiment: 300 rice and 300 soil, pot experiment: 300 rice and 300 soil). All the samples were air dried in the laboratory for about

40 days until constant weights were achieved for both vegetative and non-vegetative samples. The dried soil samples were missed thoroughly using cone and quartering method to get representative sample for the laboratory analysis.

3.7 Samples preparations and analysis

3.7.1 Soil samples preparation and analysis (all groups).

This section addresses the sample preparation for all the three groups of soil samples including the soil samples (n=80) from the field characteriasaion of which the result is presented in chapter 4 and 5, soil samples from the field experiment (n=300) of which the result is presented in chapters 6, 7 and 8 and the soil samples from the pot experiment (n=300) of which the result is presented together in chapters 6, 7 and 8.

The samples were air dried in the laboratory until constant weights were achieved according to Fakayode & Onianwa, 2002. The soil samples were crushed with mortar and pestle, sieved with 2 mm mesh sieve and stored at a room temperature. Figure 3.21 reveals some of the image captured during the soil sample preparation in the laboratory to get the sample ready for the analysis.

Figure 3.21: Laboratory sample preparation

(a) sample crushing using ceramic mortar and pestle (b) 2 mm sieve used for soil sieving (c) process of sieving the soil sample (d) discarding the unwanted materials from the soil samples.

3.7.2 Soil Analysis

Soil texture, colour, particle size, pH, organic matter content, cation-exchange capacity, available phosphorus, exchangeable acidity, organic carbon, nitrogen content, and electrical conductivity were done at the University of Ibadan, Ibadan Nigeria. Cation exchange capacity was determined by the summation of exchangeable bases which includes Ca, K, Mg, Na, and the exchangeable acidity (H). Table 3.2 shows the methods used in the laboratory for the soil test while the procedure for each method are explained in their respective sections.

Table 3. 2: Laboratory soil tests conducted, and methods used.

Laboratory soil test	**Methods used**	**Reference**
Particle size analysis	Bouyoucos hydrometer, Olsen's method	IITA (2016b) and Udo et al. (2009)
Soil colour	Munsell soil colour charts	Munsell (2000)
Soil texture	Soil textural triangle	US NIFA (2017), Berg and Gardner (1978) and Reuter et al. (1999)
pH	Electrometric method (glass electrode pH meter)	Udo, Ibia, Ogunwale, Ano, and Esu (2009)
Elemental analysis for site characterisation (Mn, Fe, Cu, and Zn, Mn (mg/kg))	Extraction method and then analysed by Atomic Absorption Spectrophotometer (AAS)	Udo et al. (2009)
Exchangeable Cations (Ca, Mg, Na and K (cmol/kg))	Extraction method and then analysed with AAS for Mg and Ca. Flame photometer (Clinical PFP7 Model M23400 by Richmond scientific) was used for Na & K.	IITA (2016b) and Udo et al. (2009)
Exchangeable acidity (cmol/kg)	KCl extraction method	Udo et al. (2009)
Available Phosphorus (mg/kg)	Murphey & Riley method	Murphy and Riley (1962) and IITA (2016b)
Cation Exchange capacity (CEC (meg/100g))	Summation method	Esu (1999) and Udo et al. (2009)

Organic Carbon (g/kg)	Titration method with Potassium dichromate ($K_2Cr_2O_7$) and Ferrous ammonium sulphate (Fe $(NH)_2(SO_4)_3$.6H2O).	IITA (2016b) and Udo et al. (2009)
Nitrogen (g/kg)	Macro-kjeldahl method	IITA (2016b) and Udo et al. (2009)
Electrical Conductivity (EC) (mS/m).	Saturation extraction method and the measurement was done by using conductivity meter	IITA (2016b) and Udo et al. (2009)
Heavy metals and the essential elements including the stable radio-nuclides	Wet Acid digestion method, filtered, diluted and analysed with ICP-OES and ICP-MS for metal concentration	Alloway (2013) and Udo et al. (2009)

3.7.2.1 Particle size

(a) Procedures for the Bouyoucos hydrometer method

The apparatus used were multimix machine (mechanical shaker) with baffled milkshake cup, 1 litre Bouyoucos glass cylinder, hydrometer and thermometer. Reagents used were sodium hexametaphosphate and 7 g of sodium carbonate (anhydrous) weighed into 500 ml of distilled water. This was stirred and made up to 1 litre. The solution was then filtered before use according to IITA (2016a).

50 g of 0.6 mm sieved oven dried soil sample was weighed and placed in the baffled cup. The cup was filled with distilled water half full and 50 ml of Sodium hexametaphosphate (reagent) solution was added. The baffled cup was placed on the mechanical stirrer and stirred for 10min until the soil aggregates were broken down. The suspension was transferred into the Bouyoucos cylinder (1 litre) and filled to the lower mark with distilled water while hydrometer was inserted and left on suspension. 50 g was used and therefore lower mark was reliable (Gee & Or, 2002). Upper mark of the cylinder would be used if 100 g of the soil was used (Glinski, 2018).

Determination of the % sand: The hydrometer was removed from the cylinder and a stopper was used to block it. The cylinder was mixed thoroughly by inverting it severally. The cylinder was later placed on desk and the time was recorded. It was left for 20 seconds and the hydrometer was inserted carefully to read the gravity. This was repeated at 40seconds and the reading was taken. The hydrometer was removed from the cylinder and the temperature of the suspension was taken. According to the manual, the temperature reading was above 20^0C, therefore 0.3 (constant) was added to the reading. If the temperature was below 20^0C, deduction of 0.3 was recommended from the temperature reading (Udo et al., 2009).

After 40 seconds, the sand was seen to be settled at the bottom of the cylinder. The hydrometer reading was taken, and this represented the amount of silt and clay in the suspension. The weight of sand in the sample was obtained by subtracting the corrected hydrometer reading from the total weight of the sample (50 g). The percentage sand was calculated by dividing the weight of sand by 50 g (the weight of the sample) and multiplied by 100. The weight of sand obtained after 20 seconds was used to calculate the percentage of coarse sand while that of 40seconds was used to calculate the percentage of the fine sand.

Determination of the % clay: The suspension was re-shaken, left for 2 hours, the hydrometer was inserted, and the reading was taken. The temperature of the suspension was taken and was corrected by adding 0.3 to it. At the end of the 2 hours, the silt in addition to the sand had settled out of the suspension in the cylinder. The corrected hydrometer reading at this time represented the grammes (weight) of clay in the sample. The percentage clay was calculated by dividing this weight by 50 g (the weight of the sample) and multiplied by 100.

Determination of the % silt: The sum of the percentages of both the sand and the clay was subtracted from 100 to get the percentage of silt.

Particle size was to determine the soil pore size classes and the sizes which provide the hydrological function of the soil (Jones, Jacobsen, & Olson-Rutz, 2001). This is classified by Hamblin (1986) and Glinski (2018) as bio-pores (very large; 500 – 5000 μm), macro-pores (large; 75-500 μm), meso-pores (medium; 30-75 μm), micro-pores (small; 0.5-30 μm) and residual (very small; <0.5 μm). Bio-pores gives rapid infiltration of water and inflow of air, macro-pores support infiltration of water, inflow of air, outflow of CO2, softening of soil to allow growth of roots to access water and nutrient while meso-pores supports drainage of water, flow of water and nutrient towards roots. Micro-pores provide storage of plant-available water and soil particles in residual are held together making the soil to be hard (Passioura, 1991; Reuter et al., 1999). This study adopted the Bouyoucos (1962) hydrometer method because it is more accurate and simpler (Gee & Or, 2002; Ryan, Estefan, & Rashid, 2007).

3.7.2.2 Soil Colour and Soil texture

(a) Soil Colour

Munsell soil colour chart was used to determine the colour of the soil samples. A paste of the soil to be tested was made by mixing about 10 g of the soil sample with water. This was colour-matched (Figure 22c) on the colour chat of the Munsell book to get the colour code. This colour codes were interpreted in the reading according to the Munsell colour chart. This is revealed in Figure 3.22.

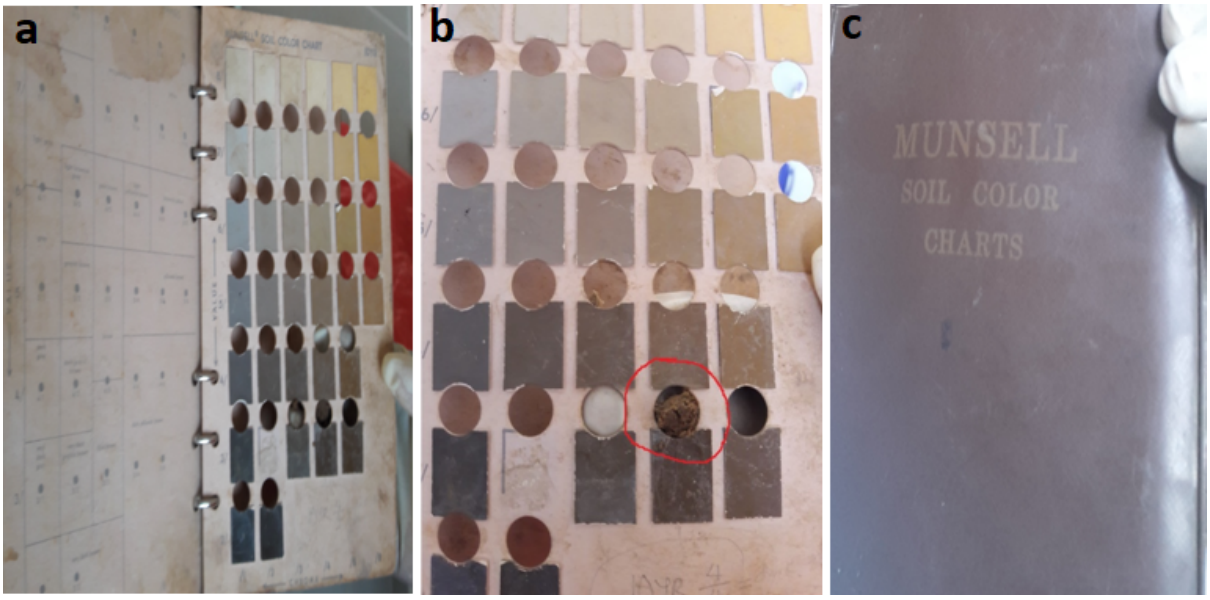


Figure 3.22: Soil Colour check

(a) one of the pages of the Munsell soil colour chart (2000) which shows both the colour- page (right) and the page with the colour code (left) **(b)** a sample was colour-matched (red circle) **(c)** back cover of the munsell colour chat.

(b) Soil texture: Texture triangle and Feel methods

(i) By Texture triangle

Texture was examined in the soil samples using the soil textural triangle (Figure 3.23) which is the best method recommended by NIFA (2017), IITA (2016a), and Reuter et al. (1999).

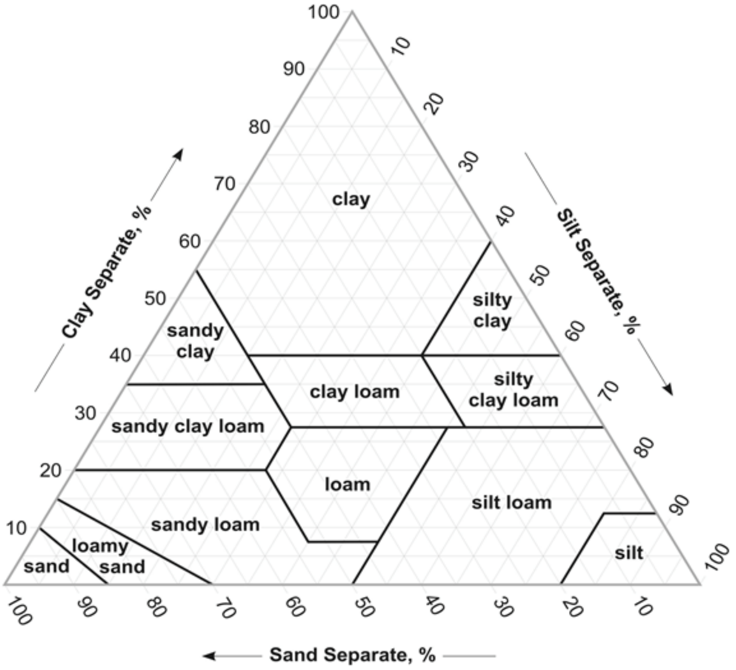


Figure 3.23: Soil textural triangle employed to examine the soil texture of the sampled soils.

Procedure

Percentage clay, silt and sand was determined from the previous particle size analysis. These values were used to determine the texture of the soil samples. For instance, the soil sample 1 out of the 80 samples collected for the site characterisation had 35.8% sand, 51.4% silt and 12.8% clay. A line was drawn from **point A** where 35.8 lies along the %sand line on the triangle to link **point B** where 12.8 lies on the %Clay line on the triangle. The **point A** was found between 35 and 40 along the %sand line and **point B** was found between 10 and 15. These two points were joined and then linked to the point C where 51.4% silt lies on the %silt line on the texture triangle. The intersection of the 3points (point A, B and C) gave the texture of the soil (Figure 3.24) (NIFA, 2017).

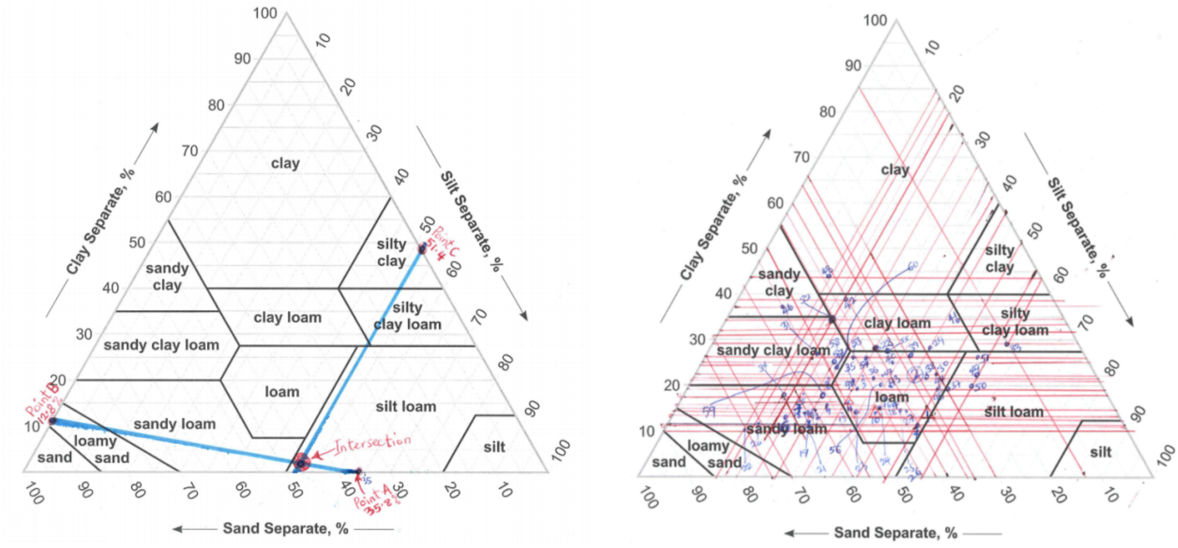


Figure 3.24: Using texture triangle

(a) soil texture determination of sample 1, silt loamy **(b)** One of the triangles used for this study

(ii) Soil texture determination by feel (hand feel)

Little soil was picked from the collected soil samples, placed onto palm (hand), gravels and stones were removed before the water was added. The soil on the palm was moistened with water and rubbed within the palm for about 2 minutes until it sticks to fingers. The soil on the palm continued to form a ball until there was no further change in the texture. According to Reuter et al. (1999) and NSW-OEH (2017), those that were handled like platicine and were moulded into rods without breaking, were classified as clay and this indicated that the plants roots growth may be restricted in it and it means water could be drained from it very slowly (NSW-OEH (2017). Those that were moderate, they were classified as medium clay and the stronger ones were classified as heavy clay.

The ones that were coarsed whereby the sand grains were seen with naked eye were classified as coarse sandy. Those that the sand grains were felt and heard when the bolus was manipulated, sand grains were seen clearly only under ×10 magnification hand-lens; they were classified as fine sandy. Those that the sand was low or fine, they were classified as gritty and the ones bigger and stony, were classified as gravel according to Reuter et al. (1999). The ones that looked loosed and moderately smoothy which its bolus comes together and by touching, scattered again, they were regarded as loamy soil. This is regarded as the best to grow plant with rain water because it allows air to reach the root (Jones et al., 2001). Some of the soil samples were seen to be mixtures of different textures such as sandy clay, sandy loam and clay loam in accordance with Franzen, Cihacek, Hofman, and Swenson (1998) submission.

Soil texture by feel was a method used also to confirm the texture of the soil (USDA, 2017). It determines the water holding capacity, water and nutrient detention or leaching capacity, compaction that may affect root growth and erodibility by feel (USDA, 2017). Fine sandy are easily carried by wind and coarse soil are heavier and require more force to be moved. This can be used to determine how fast the lead on the soil can turn to dust and move within the air from one place to another (Reuter et al., 1999). This was done according to procedures from Reuter et al. (1999) and all the samples agreed with the result gotten from the soil textural triangle.

3.7.2.3 Soil pH and Exchangeable acidity

Soil acidity is one of the factors that influence both the plant growth and pollutant uptake from the soil (UNH, 2015). Acid soil is the one that has its pH below 7.0 and it is determined by the hydrogen iron (H+) concentration (UNH, 2015). Soil acidity is of two components which are (i) active acidity and (ii) exchangeable or reserve acidity (Yuqing, Jianrong, & Keming, 2005). Active acidity is the concentration of H+ in the solution phase of the soil and this can be measured by pH but it cannot be used to determine the total soil acidity. The exchangeable acidity is the amount of the H+ on cation exchange sites of negatively charged soil components such as the organic matter and the clay (Yuqing et al., 2005). pH simply means “potential of hydrogen” is a numeric scale used to specify the acidity or basicity of an aqueous solution (Quan, Sanchez, Wasylkiw, & Smith, 2007). It is approximately the negative of the base 10 logarithm of the molar concentration, measured in units of moles per litre of hydrogen ions in a given medium or sample (Yuqing et al., 2005). pH is the measurement of the active acidity (hydrogen ion “H+”) or alkalinity of soil (Himmel, Goll, Leito, & Krossing, 2010). The pH and the exchangeable acidity are both very important when metal uptake is discussed in plants (Street, Sabey, & Lindsay, 1978).

(a) Determination of pH

There is no specific unit for pH. It is the negative logarithm of the hydrogen ion concentration in solution (Yuqing et al., 2005).

$pH = -\log_{10}(H^+)$
where H^+ = Activity of Hydrogen ion in moles/litre

Colorimetric and electrometric methods have been the most two popular methods to determine the pH values of a given soil (Thomas, 1996). Colorimetric method involves the use of suitable dyes or acid-based indicators of which the colour changes with the hydrogen ion activity (Thomas, 1996). These indicators or dyes only estimate pH ranges and indicates end points of acid-base titrations in clear solutions (Himmel et al., 2010; Thomas, 1996). Measurement of pH by colorimetric method is therefore less accurate and is used only for rapid testing of the soil in the field or in the laboratory (Haines, Akielaszek, Norton, & Davis, 1983). The electrometric method involves the use of the pH meters which measures the electrical potential between a reference solution and the soil solution by means of electrodes (Yuqing et al., 2005). The electrodes in pH meters could be a glass type or non-glass type. Two types of glass electrode pH meters exist. These are the potentiometric type which operates a null-balance and the direct reading type (Yuqing et al., 2005).

In the modern soil science systems, the direct reading types are popularly used (Yuqing et al., 2005). The glass electrode can be used in soil pastes and soil water mixture of various ratios. Many soil testing modern laboratories determine pH values in 0.01M CaCl2 or 1M KCl solution (Ryan et al., 2007). This is a way to provide constant soluble salts concentration and reduce the differences in pH values brought about by variation in soluble salt in soil water mixture of various ratios (Thomas, 1996). The non-glass type of pH meter comes as a handheld pH meter which is also digital (Carter, 2016). The most common non-glass pH meter is Soilstik (Marshall, 2014).

This study adopted the use of OHAUS glass electrode pH meter, model ST2100-F Starter 2100, Ohaus corporation, USA. The pH of the samples was checked in both distilled water (IITA, 2016a; Marx, Hart, & Stevens, 1999). Materials and tools used were glass-electrode pH meter, distilled water, weighing balance (Ohaus Model CS200).

Procedure

The soil to water ratio was made in ratio 1:1 (Udo et al., 2009). 20 g of the soil sample was weighed into 50 ml beaker and 20 ml of distilled water was added. This was allowed to stand for 30 minutes, and it was stirred with glass rod. The electrode of the pH meter was inserted into the partly settled suspension and the pH was measured. This was repeated for all the samples in line with (IITA, 2016b). Figure 3.25 shows the electrometric methods and the type of equipment used in the pH check.

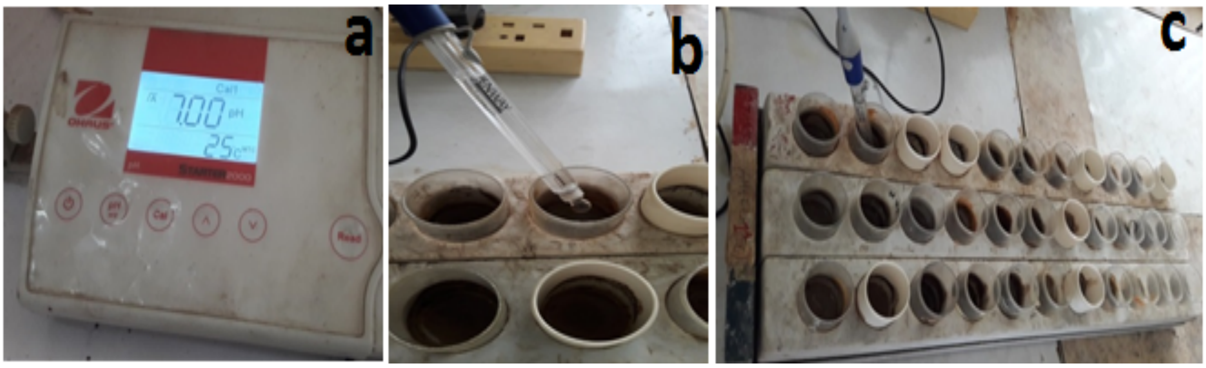


Figure 3.25: pH meter used during the study (image captured from the laboratory)

(a) equipment calibration **(b)** glass electrode in use **(c)** samples in extraction cups arranged in the analytical tray for pH measurement.

(b) Exchangeable acidity

The 2 mm sieved soil samples were weighed (2 g each) into centrifuge tubes and 20 ml of KCl (potassium chloride) solution was added to each centrifuge tube (1:10). The tubes were shaken on a reciprocating shaker for 15 min and the solution was filtered to drain into volumetric flask (KCl extract). 3 drops of phenolphthalein indicator was added. It was titrated against 0.01N NaOH solution to a permanent pink end-point. The titre value was the exchangeable acidity value. There was a need to check for the presence of Aluminium ion in the solution because the total exchangeable acidity in the medium is the total number of ions of both the H^+ and the Al^{2+} found in the medium (Udo et al., 2009). Therefore, 1 drop of 0.01N HCl and 5 ml of NaF (sodium fluoride) were added to de-colourised the solution while the solution was stirring continuously for 10min and waited to see whether the solution will further turn pink. There was no further colour change during this process, which indicates Al was not detected in the solution. The exchangeable acidity was necessary to calculate the Cation Exchange Capacity (CEC) as explained in the next section (Coscione, de Andrade, & van Raij, 1998). Exchangeable acidity was to determine the amount of Al present in the soil samples and this affects the Cation Exchange capacity of the soil (NSW-DPI, 2019).

3.7.2.4 Cation Exchange Capacity (CEC)

CEC = Total exchangeable bases (TEB) + Exchangeable Acidity (EA)

The exchangeable bases; Mg, Ca, were extracted from the soil samples then analysed with Atomic Absorption Spectrometer (AAS) while Na & K were analysed with Flame photometer, Clinical PFP7 Model M23400 by Richmond scientific. According to IITA (2016), it is more accurate to measure Na and K with flame photometer than AAS.

To get the total exchangeable bases, this was done by extraction method then it was analysed as stated above. The procedure goes thus:
1 g of each of the soil samples was weighed and 20 ml of 0.1M NH4OAc (ammonium acetate) was added, shaken for 10min and then filtered. The extract was in the filtrate which was analysed with Atomic Absorption Spectrometer (AAS). This instrument (AAS) was used for Mg^{2+} and Ca^{2+} while Flame photometer was used for Na^{2+} and K^{2+} as stated by IITA (2016). This procedure was used for determination of other metals (micro-elements) such as Mn^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} and Mn^{2+} (IITA, 2016).

In the second work, field experiment (varietal trial), the values obtained from ICP-MS analysis were in ug/kg (part per trillion or ppt) which was converted to mg/kg (part per million or ppm) by dividing the concentrations by 1000. To get the Cation Exchange Capacity (CEC), the formula below was used.

CEC = Exchangeable Acidity (EA) + Ca (cmol/kg) + Na (cmol/kg) + K (cmol/kg) + Mg (cmol/kg)

Meaning that to get CEC, there was a need to convert all the values of the exchangeable bases that were in mg/kg to cmol/kg. To convert the elemental concentration in mg/kg to cmol/kg,

$$\text{cmol/kg} = \frac{\text{Elemental value in mg/kg}}{\text{Equivalent weight (EW) of the element}} \qquad \text{(NSW-DPI, 2019)}$$

EW of an element = gramme atomic weight of the element divided by the valence of the element then, multiply by 10 (NSW-DPI, 2019)
EW (Na) Valence =1, atomic weight = 22.99.
EW (Na) = 22.99/1 × 10 = 229.9.
EW (Ca) Valence = +2, atomic weight = 40.08
EW (Ca) = 40.08/2 × 10 = 200.4
EW (Mg) Valence = +2, atomic weight = 24.31
EW (Mg) = 24.31/2 × 10 = 121.55
EW (K) Valence = +1, atomic weight = 39.09
EW (K) = 39.09/1 × 10 = 390.9

Na (cmol/kg) = measured Na value/229.9 = xNa value in cmol/kg.

Calculation for other metals from the instrument values:
The concentration of each micro elements Mn, Fe, Cu, Zn and Mn (in the extract), was calculated using the equation 1 as previously used in section 3.41.

3.7.2.5 Electrical Conductivity (EC)

(i) Calibration of the Electricity Conductivity Meter

DiST Electricity Conductivity Meter Model HI96301, ANNAH Instrument Italy was used and the Instrument was calibrated before use according to the manufacturer's instruction (HI, 2017). The protective cap was removed, and the instrument was turned on. The sensing probe was immersed in calibration solution up to the maximum immersion level without touching the bottom of the beaker. The instrument was used to stir the sample in the container gently until the instrument displayed "stabilised". After it reads stabilised, a small screwdriver was used to turn the calibration trimmer to match the value of the solution. Calibration completed at this point.

(ii) Procedure

10 g of the soil sample was weighed into analytical cups and 10 ml of distilled water was added to each sample (1:1). This was continuously shaken by the mechanical shaker and stirred for 10 min. The conductivity meter was then used to measure the conductivity of the soil samples after 10 min. The old battery of the conductivity meter was changed to new ones to avoid error in the reading. The reading was taken by inserting the sensor at the tip of the meter inside the sample solution (Figure 3.26). The sensor was continuously rinsed with distilled water in between samples to avoid cross contamination.

Figure 3.26: Electrical Conductivity meter

(a) battery replacement **(b)** Measurement of the electrical conductivity in the soil samples.

3.7.2.6 Determination of the Available Phosphorus in the Soil

2 g of 2 mm sieved soil was weighed into centrifuge tube and 20 ml of extracting solution was added to each centrifuge tube (1:10). The tubes were shaken vigorously by mechanical shaker for 15 min and it was filtered to drain. 5 ml was measured out of the filtrate into an extraction

cup and 5 ml of Murphy and Riley solution was added. 40 ml of distilled water was added and it was allowed to stand for 5 min to get the correct colour change. Before 5 min, the colour has turned blue and this was poured into sample cuvette (IITA, 2016; Murphy & Riley, 1962). Spectrophotometer (NV201 model, Ontario Canada) was used to read the phosphorus at wavelength of 882 nm according to IITA (2016) (Figure 3.27).

Figure 3.27: Spectrophotometer used to measure the available phosphorus in the soil samples

Calculations:

Available phosphorus = (Extraction factor × Dilution factor) × x ---------------- equation A

While;

Extraction factor = Extraction volume ÷ sample weight = 20 ÷ 2 = 10

Dilution factor = 50 ml ÷ 5 ml = 10

An equation was generated by the machine (Figure 3.27) from the slope of the standard stock solution prepared (0.2 mg/kg, 0.4 mg/kg, 0.6 mg/k, 0.8 mg/kg and 1 mg/kg) which was used to calibrate the machine (spectrophotometer).

The graph of the concentration from the standard solution was a straight-line graph. The equation generated was;

$Y = 0.784x + 0.012$ --------------- equation B

Y = sample reading

x = constant

To get x from the equation (B) above;

$x = \frac{Y - 0.012}{0.784}$ ---------------- equation C

Y was the reading gotten from the machine as the samples were placed and this value was substituted in the equation (C) and the value of x was calculated. The known value of x was then substituted for; in the equation (A) to get the available phosphorus in each soil sample (IITA, 2016).

3.7.2.7 Determination of the Nitrogen content by Macro-kjeldahl method

0.5 g of the soil samples was weighed into digestion tube and 5 ml of concentrated H_2SO_4 was added. 1 tablet of selenium was added as catalyst to each sample. The selenium tablet (catalyst) though it was toxic, but it makes digestion process faster. The digestion tubes were placed inside the digestion block (hot block) and this was done for 3 hours at 360^0C until the brown fumes disappeared and clear light amber colour solution was achieved. It was allowed to cool for 1 hour and was transferred into 250 ml standard volumetric flask. This was made to mark with distilled water and further transferred into extraction cups as stated by IITA (2016). This was the digest used.

Macro-kjeldahl Distillation

5 ml of boric acid indicator was measured into conical flask (100 ml capacity). The flame was ignited under the distillation flask and the water was allowed to boil to generate pressure. The conical flask containing the boric acid indicator was placed at the delivery end (Figure 3.28). 5 ml of the digest was measured and poured into the distillation chamber and 5 ml of 40% (v/v) NaOH was added. It was covered, and both were allowed to distil into the delivery chamber until 50ml distilled solution was achieved. This distilled solution was pitch-green in colour.

Figure 3.28: Macro-kjeldahl distiller during the lab work.

The distilled solution was removed from the delivery end of the distillation chamber and it was titrated against 0.01N HCl until the colour change from pitch-green to pink (Figure 3.29) and the titre value was recorded. Blank was also done; following the same steps but no sample digest was added.

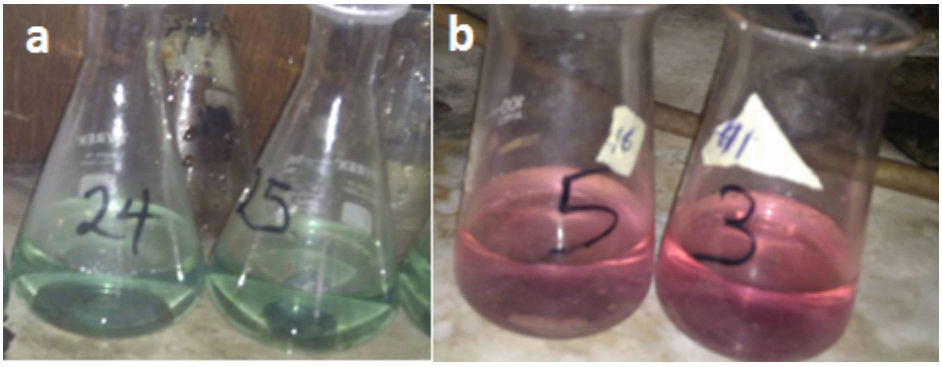


Figure 3.29: The colour-change during titration from pitch-green (a) to pink (b).

$$\% \text{ Total Nitrogen (TN)} = \frac{(T-B)\times\mathrm{N}\times\mathrm{R}\times 14.01\times 100}{Sample\ mass\times 1000}$$

T = Sample titre value

B = Blank

N = Normality of the acid used = 0.01

R = Distillation ratio = 50ml (distilled solution volume used) ÷ 5 ml (volume of the digest used

14.01 = Mass of Nitrogen

1000 = constant

3.7.2.8 Organic Carbon

A typical agricultural soil is made up of about 45 percent minerals, 25 percent water, 25 percent air, and 5 percent organic matter (UNH, 2015). The value for the percentage (%) organic matter content in the soil was calculated from the percentage organic carbon. Below is the procedure used to determine the organic carbon content of the soil samples.

Apparatus used were Burettes (50 ml capacity), Erlemeyer flask (250 ml), Pipette. The soil sample was passed through 0.5 mm-sieve and 1 g was weighed out of this into 250 ml conical (Erlemeyer) flask.

Solution A: 49.04 g of potassium dichromate ($K_2Cr_2O_7$) was dissolved in distilled water and diluted to 1 litre. 10 ml was measured out of this solution and was added to 0.5 g of soil samples in the conical flasks that were arranged under fume cupboard and 20 ml of concentrated sulphuric acid (H_2SO_4) was added to each of the samples in the conical flasks. They were allowed to cool for 30 min and distilled water was added to each solution in the flasks and they were made up to 150 ml. 3 drops of Orthophenanthroline-ferrous complex (0.025 M) were added to the solution.

Orthophenanthroline indicator: This indicator can also be called Ferroin indicator. Ferroin indicator was prepared by dissolving 14.85 g of Orthophenanthroline monohydrate and 6.95 g of $FeSO_4.7H_2O$ and diluted to 1litre.

Solution B (Ammonium ferrous sulphate): This was titrated against the solution A in the conical flask with the help of Orthophenanthroline indicator. 196.1 g of ferrous ammonium sulphate $Fe(NH)_2(SO_4)_3.6H_2O$ was dissolved in 800ml of distilled water containing 20 ml of concentrated H_2SO_4 and this was diluted to 1 litre.

Solution C (Blank): Blank was prepared by adding 10 ml of potassium dichromate ($K_2Cr_2O_7$) solution used in solution A and 20 ml of concentrated sulphuric acid and made up 150 ml with distilled water then indicator was added. It was called a blank because there was no sample in this. B was first titrated against blank and the titre value was recorded. Then B was titrated against A and C, the titre value was recorded.

$$\% \text{ Organic Carbon} = (\text{Titre value of Blank} - \text{Titre value of Sample}) \times x$$

While $x = \frac{volume\ of\ K2Cr2O7}{Titre\ value\ of\ blank} \times \frac{0.003 \times 1.33}{Sample\ mass} \times \frac{100}{1}$

Volume of $K_2Cr_2O_7$ was 10 ml
Titre value of blank was determined from the titration
Soil sample mass = 0.5 g
0.003 = Correction factor
1.33 = Relative Atomic Mass of carbon.
100 in the last fraction in the formula will take it to the percentage.
After calculating the % Organic carbon, the value was used to calculate the organic matter content of the soil. % Organic matter content of the soil sample = %Organic carbon × 1.729 (IITA, 2016; Udo et al., 2009).

3.7.3 Rice samples preparation and analysis (all groups)

This section addresses the sample preparation for all the three groups of rice samples including the rice samples (n=80) from the field characteriasaion of which the result is presented in chapter 4 and 5, soil samples from the field experiment (n=300) of which the result is presented in chapters 6, 7 and 8 and the soil samples from the pot experiment (n=300) of which the result is presented together in chapters 6, 7 and 8.

The well labelled segregated rice samples (root, shoot, and seed) were taken to the laboratory and the seed (paddy rice) were de-husked and further divided into the husk and the seed making it four samples all together from a rice plant. The root samples were washed with tap water and then rinsed with deionized water (Figure 3.21) before they were sundried for a day and later air dried in the laboratory until constant weight was achieved on all the samples.

Figure 3. 5: Root washing

(a) washing under running tap water and rinsed with deionized water **(b)** the roots were placed under the sun with their labelled bags **(c)** the rice roots were arranged outdoor within the University of Abuja for a day sun-drying before taken to the laboratory to oven dry (photo taken in 2017 after the harvest).

The above was repeated for the rice shoot (combination of leaves and stems) samples. It was washed with tap and deionised water consecutively to remove dust and soil particles in the samples to avoid secondary contamination. All the samples were dried until a constant weight was achieved. The samples were grinded into powder using electronic Binatone blender model BLG-450, China. The samples were double bagged, sealed, labelled more than once and the powdered sample bags were stored in sealed plastic containers to avoid moisture before the samples are analysed as recommended by Lstiburek and Carmody (1994).

The rice seed was threshed (rice seeds were removed from the rice plant) and de-husked using mortar and pestle, the husk was separated from the rice seeds manually by simple method called winnowing. Winnowing is a simple process of separating a de-husked rice seed from its husk using rice-winnowing tray (Carney & Carney, 2009; Das & Gangopadhyay, 2011). Winnowing was not only separating the husk from the rice seed, it also helps to remove dead insects, dirt and some other impurities that were present in the samples. After winnowing, the rice seed was washed in tap water and then deionised water to remove all the soil particles that may be present in the rice which may serve as source of secondary lead contamination. The rice samples were dried after washing and grinded to powder using electronic Binatone blender model BLG-450 China. Figure 3.27 (a-j) shows the pictorial illustration of the rice sample preparation in the Laboratory.

Figure 3. 6: Pictorial illustration of the rice sample preparation in the Laboratory

(a) Threshing of the rice sample with mortar and pestle **(b)** de-husking with mortar and pestle **(c)** Winnowing **(d)** the rice was separated from the husk **(e)** rice Washing (f) rice drying **(g)** prepared rice samples under air drying in the laboratory **(h)** grinding of the samples into powder **(i)** sample bagging after grinding **(j)** samples is ready for acid digestion.

3.8 Elemental Analysis by Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Digestion of the trace (Pb, Ba, Cr, V, Mn, Co, Cu, Zn, As, Se, and Sb) and the major metals (Al, Ca, Fe, K, Mg, and Na) in the rice and the soil samples after a successful varietal trial of the 10 selected rice varieties using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was carried out. The soil samples (n=600) was done following the USEPA 3051 microwave assisted extraction method (Rahman, Chen, & Naidu, 2009) while the digestion of the rice samples (n=600) was done in line with the procedure by Rahman, Owens, and Naidu (2009). Four elements (Ca, Fe, K, and Mg) were analysed using ICP-OES and this has been previously explained in chapter 3, section 3.5.2.

(a) Rice

0.5 g rice samples each were weighed into 75 ml digestion tube and a cold digestion was done by adding 5 ml of 65% (v/v) concentrated nitric acid (Mallinckrodt Chemicals, USA) to the samples in the digestion tubes under the fume cupboard and left overnight. The tubes were placed in the digestion hot block (Digestion System, AI Scientific AIM500). The temperature of the hot block was programmed to rise slowly with its heating system up to 140^0C and stay steadily for 8 hours. The digestion was monitored until small liquid content of about 1ml remained in each digestion tube. The digestion ended at this stage by removing the tubes from the hot block and they were allowed to cool and settled at the room temperature (30^0C) under the fume cupboard. 0.1% nitric acid, 20ml was added and this was mixed with the content of the tube and then filtered through Whatman filter paper number 42 into volumetric flasks and made up to 50 ml with Milli-Q deionised water (Millipore, USA), then analysed using Agilent 7900 (Agilent Technologies Tokyo, Japan) Inductively Couple Plasma Mass Spectrometer (ICP-MS) coupled with an auto-sampler (Agilent Technology). Few elements such as calcium (Ca), potassium (K), Iron (Fe), and magnesium (Mg) were analysed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) PerkinElmer Avio-200 with axial and radial dual view. The Standard Reference Materials (SRM) used for quality control in this study were NIST 1568b (rice flour) and NIST 2711a (Montana II soil) from the US National Institute of Standards and Technology (NIST). In each batch of analysis, blanks, certified reference materials (CRM), continuing calibration verification (CCV) and duplicates were included throughout the analysis and the standards were prepared daily. High purity argon gas (99.999%) was used to operate the instrument. The ICP-MS was operated with Nickel sampling and Skimmer cone. Standard solutions were made from Agilent multi-element stock solution which was used to generate the calibration curves. The carrier gas and dilution gas flow rate were 1.21 L/min and the 0.8 L/min respectively. The spray chamber temperature was 2^0C and the Nebulizer pump was set at 0.4 rps. The RF (radio frequency) power was 1500 W. For the sample introduction, the Nebulizer was Mira-Mist while the spray chamber was Scott type.

(b) Soil

The US Environmental Protection Agency (EPA)'s method 3051A (USEPA, 1997) was used to digest the soil. 1 g each was weighed out of the 2 mm sieved soil samples (n=600) batch by batch into the 14 Teflon microwave digestion vessel (HP500). Aqua regia 5ml was added and the vessels were closed. These closed vessels were placed into the microwave rotor, tightened securely. The rotor (loaded) was placed inside the microwave digester (MARS 5, CEM Corp, Mathews NC) and the microwave ramp for 3 stages as reveals in Table 3.3.

Table 3. 3: The stages of Microwave ramping

Stage	Power	Pressure	Time
Stage 1	1200W	300PSI	2min
Stage 2	1200W	300PSI	3min
Stage 3	1200W	300PSI	5min + hold

After the 5 min hold, the microwave was turned off and allowed to cool for about 30 min and then carefully, the microwave vessels were opened. The digests were transferred into 50 ml volumetric flask and make up to mark with Milli-Q water and then filtered through Millipore 0.45 membrane. The filtered digest was then analysed by the Agilent 7900 ICP-MS. When the analysis by the ICP-MS instrument was completed, the results were saved in a csv format and viewed through excel on the attached computer system. The formular below (equation 2) was used to calculate the elemental concentrations present in the samples:

$$\text{Element concentration } (\mu g/kg) = \frac{\text{Result – Blank X Dilution Factor (50)}}{\text{Sample Mass}} \text{ ....equation 2}$$

The values were divided by 1000 to convert parts per billion (ppb) elemental concentrations to parts per million (ppm).

3.8.1 Digestion and Elemental Analysis by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for Stable Caesium and Strontium.

Digestion and elemental analysis for stable caesium and strontium in both the rice and and the soil samples took place in private laboratory in Nigeria. This method used to digest the samples was adopted from Srinuttrakul & Yoshida (2017). 0.5 g each sample was used for the digestion and sample digestion was done in triplicates. The samples were placed in the tube and the two acids (10 ml of 68% (v/v) Nitric acid (HNO3) and 4 ml 38% (v/v) Hydrofluoric acid (HF) were added appropriately under the fume cupboard. These mixtures were placed in the hot plate at 800C for 3 hours for Organic carbon decomposition and it was allowed to cool. 0.5 ml of Hydrogen peroxide, 35% (v/v) was added and it were arranged in the microwave digester, digested for 10min at 1800C. After cooling for 30 min, these were arranged on the hot plate again and gradually heated to 1400C until the solution evaporated near dryness. HNO3 (1ml) was added with H2O2 (0.5 ml) and heated near dryness as well. 2% HNO3 (2 ml) was added to each tube to dissolve the residue and these were filtered into 50 ml standard flasks and made up to mark with Milli-Q water. CEM MARS5 Microwave digester was used.

The samples were diluted to 50ml after the acid digestion and the elemental determination was carried out using ICP-MS Agilent 7900 (USA). The operating conditions were set as follows; Nebulizer was Mira-mist, RF power was 1550 W, Carrier gas flow was 0.75 L/min, Dilution gas flow was 0.25 L/min, the spray chamber temperature was at 2^{0}C, the spray chamber was scott type, the Nebulizer pump was at 0.1 rps, Sampling cone and Skimmer cone were both Nickel. In (Indium) was employed during the operation as the internal standard to compensate for any change in the analytical signal. The rinse time was 2 min. The Agilent Multi-elemental standard solutions were used to get the calibration curve and 5-point external calibration was used to quantify the analysis. NIST 1568b Rice Flour Standard Reference Material from the National Institute of Standards and Technology, USA was used to validate (quality control) the method of analysis. The detection limit for both Cs and Sr were 0.016 µg/L and 0.214 µg/L respectively. The calculated limits of quantification were 0.706 µg/L and 0.052 µg/L for Sr and Cs respectively. Milli-Q water (Millipore Milli-Q plus USA) was used throughout the analysis. All the acid used were from Tama Chemicals Japan (TAMAPURE AA100), analytical ultrapure grade.

To guarantee quality and since there was no data for both elements (Sr & Cs) in the SRM (NIST 1568) used, the analysis was spiked with Sr and Cs and their recoveries agreed with the techniques as Sr and Cs were within ±15% acceptable limits. The spiked concentration for Cs was 0.25 mg/kg and the recovery was 96.7 ± 2.5% while the spiked concentration for Sr was 2.1 mg/kg and the recovery was 96.5 ± 2.2%. The spiking was carried out by adding 1 ml of 0.5 mg/L Strontium and 1 ml of 0.5 mg/L Caesium standard solution to 2 g of rice samples in the tubes which was subjected to aforementioned digestion procedure. The percentage recovery was calculated using the equation 3 (s = spiked sample concentration, u = unspiked sample concentration and c = concentration of the standard added.

$$\% \text{ Recovery (Element)} = \frac{s-u}{c} \times 100 \qquad \text{………. equation 3}$$

The procedures involved in this study regarding the elemental analysis is summarised in Table 3.4.

Table 3. 4: Summary of the elemental analysis in this study

Analysis	**Field Experiment**	**Pot Experiment**
Before the rice planting	XRF -> only soil (n= 774) of the 4 selected rice farms (pilot study). Conducted in-situ on the field.	AAS analysis (n=60), was done to check the Abuja soil if it was not contaminated before it was used for the pot experiment.
	AAS -> soil (n = 80) and rice (n = 80) that were collected from farmers of the 4 selected rice farms during the site characterisation. Root, shoot, husk and the seed of the rice samples and the soil samples were analysed	
After harvesting	ICP-MS & ICP-OES -> soil (n=300) ICP-MS & ICP-OES -> rice (n=300) **<u>Limitation</u>** Root, shoot and the husk were collected (separated) from the rice samples but not analysed due to limited resources.	ICP-MS& ICP-OES -> soil (n=300) ICP-MS & ICP-OES -> rice (n=300) **<u>Limitation</u>** Root, shoot and the husk were collected (separated) from the rice samples but not analysed due to limited resources.

3.8.2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has become valuable instrument to determine heavy metals concentrations in trace and ultra-trace levels of concentrations in different sample matrices because of its wide dynamic range and capability for multi-elemental analysis (Voica, Dehelean, Kovacs, & Lazar, 2012). According to Voica et al. (2012), ICP-MS can detect larger number of elements compare to Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and it can detect most of the elements including their isotopes including radioactive ones in less than 60seconds. It allows the determination of both major and trace elements at the same sample injection (Nardi et al., 2009).

ICP-MS provides simpler spectral interpretation and isotopic information better than other ICPs. In the analysis of heavy metals today, there are numbers of atomic spectrometry techniques among which are Atomic Absorption spectrometry (AAS), Flame Atomic Absorption spectrometry (FAAS), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), ICP-AES, ICP-OES and ICP-MS (Nageswara & Kumar, 2007; Nardi et al., 2009). GFAAS and FAAS are most extensively used (Jackson & Lu, 1998). Flame is used when the concentration of the analyte is assumed high and graphite is used when it is at trace level (Jackson & Lu, 1998). GFAAS has high detection limits and highly specific hollow cathode lamps are used for determination of each metal (Jackson & Lu, 1998). But all these cannot be used for multi-elements at the same time for multiple samples except the ICP-OES and ICP-AES (Nageswara & Kumar, 2007).

ICP-AES and the ICP-OES are also useful to do multi-elemental analysis but they are not as powerful as ICP-MS in terms of accuracy in measurement at ultra-trace levels due to problems of complex spectral interferences (Nageswara & Kumar, 2007). In summary, low limit of detection, high speed of analysis, multi-element capability, ability to perform isotopic analysis in a simple way with simple spectra and wide linear dynamic range are the areas where ICP-MS is superior than other ICPs (Nardi et al., 2009; Sutton & Caruso, 1999). Therefore, this study adopted the use of ICP-MS and the ICP-OES in examining Pb, the stable isotopes of Cs and Sr. The limits detection in the multi elemental analysis were calculated by analysing a spiked blank with six replicates and multiplying the standard deviation by 3.14, this was repeated three times and an averagewas taken. The result is shown in Tables 6.2, and 8.2.

3.9 Statistical Analysis and Other calculations

Different methods of data analysis were used as detailed in the respective chapters. During the data cleaning and formatting, below detection limit (BDL) in the data was replaced by limit of detection (LOD)/2 values (Norton et al., 2014; Płotka-Wasylka, Frankowski, Simeonov, Polkowska, & Namieśnik, 2018). This was recommended because below detection level (BDL) values are not zeros and if it is left blank, the mean values and some other calculations will be affected (Shrivastava & Gupta, 2011). Correlation analysis was conducted using a Pearson test (2-tailed) to check for both possible positive or negative relationships among variables.

3.9.1 Concentration Ratio (CR) and Inter-Varietal Variation (IVV)

The result for the concentration (mg/kg) of lead and other essential elements in rice were presented in arithmetic mean together with the standard deviation (SD). All data analysis was done with SPSS version 23.0 (SPSS Inc, Chicago, IL, USA) and Excel for windows 2016. The significance level was set at $p<0.05$ and 0.01 to present the result. The concentration ratio (CR) was calculated using equation (4) (Salem et al., 2014) to get the proportion of lead, caesium, strontium and the essential elements in the rice varieties and the soil. Equation (5) (Penrose, Beresford, Broadley, & Crout, 2015) was adapted and used to calculate the inter-varietal variation (IVV) of lead and the essential elements in the 10 rice varieties.

$$CR = \frac{Concentration\ of\ Lead\ in\ the\ rice\ sample\ \left(\frac{mg}{kg}\right)dm}{Exractable\ concentration\ of\ Lead\ in\ the\ soil\ sample\ \left(\frac{mg}{kg}\right)dm} \qquad \text{…………equation 4}$$

$$IVV = \frac{Mean\ concentarion\ ratio\ in\ the\ highest\ accumulation\ variety}{Mean\ concentarion\ ratio\ in\ the\ lowest\ accumulation\ variety} \qquad \text{....................equation 5}$$

3.9.2 Estimated Daily Intake (EDI)

EDI was calculated from the rice sample's average elemental concentration and the amount (weight) of rice that is consumed according to the individual body weight (kg). Equation 6 was used.

$$\text{EDI} = \frac{(FIR \times C)}{1000} \qquad \text{…………… equation 6}$$

3.9.3 Percentage contribution to the Recommended Dietary Intake (RDI)

The EDI was used to calculate the percentage contribution of an essential element to the recommended dietary intake (RDI) of the element. Equation 7 was used.

$$\%\ \text{contribution to RDI} = \frac{EDI}{RDI} \times 100 \qquad \text{……….. equation 7}$$

The values of RDI were from the United States Institute of Medicine, Department of Agriculture (USDA) Food and Nutrition Board, National Academies (USDA, 2000). In the calculation, the percentage contribution to the RDI was done for all the 10 varieties of rice and the age selected was the highest value for the applied age group as RDI varies among every age groups.

3.9.4 Average Daily Intake (ADI)

Quantifying the dosage of oral exposure to Pb per day, ADI (mg/kg/day) was used which was calculated from equation 8 (Dai, Song, Huang, & Xin, 2016; Song, Zhuang, Jiang, Fu, & Wang, 2015).

$$ADI = \frac{FAR\times\ EFr \times ED \times Ci}{BM \times AT \times 365} \qquad \text{……………… equation 8}$$

Where;

Ci is the mean concentration (mg/kg) of lead in rice

FAR is the average food assimilation rate (g/person/day) which is 0.389 for rice (Dai et al., 2016; Lin, Li, Liu, Jing, & Liu, 2004).

ED is the exposure duration for the ingestion measured in years. 30years is recommended for adult while the actual age is used for children (USEPA, 1997). Non-carcinogenic risk assessment was based on the adult.

EFr is the exposure frequency (meals/365days) which is 350 according USEPA (2000).

AT is the average time (years) for emerging of non-carcinogenic effects. Life expectancy for the country is used. Africa generally is 59 for male and 62 for female (Statista, 2018). This study used the average age of both female and male (60.5). For the children, their age was used which is 18.

BM is the body mass. Average body mass (kg) for rural adult according to Dai et al. (2016) is 58.1kg and 365 was used to convert the result from years to days.

3.9.5 Non-Carcinogenic Risk

Determination of carcinogenic health risk of Pb from rice consumption was done using the hazard quotient (HQ) which is which was calculated from equation 4 (USEPA, 1989).

$$HQ = \frac{ADI}{RfD} \quad \text{.................. equation 9}$$

RfD is the oral reference dose of Pb (mg/kg/day) which has been established by the United State Environmental Protection Agency (USEPA) and the World Health Organisation. RfD for Pb is 0.0035 (JECFA, 2011; USEPA, 2015).

This assessment is the associated risk potential of Pb in rice to compromise good health in human. Hazard quotient (HQ) total posed by food is referred to as Hazard Index (HI) which was derived from the Health Risk Assessment guidelines by the USEPA Chemical mixture (USEPA, 1989). The formula is shown in the equation 10.

$$\mathrm{HI} = \sum \ (HQ1 + HQ2 + HQ3 \ldots\ldots HQn) \quad \ldots\ldots\ldots\ldots \text{ equation 10}$$

The equation 10 provided a means to calculate the potential health risk through HI when the source of exposure from food is more than 1 (FAO/WHO, 2011). If HI is less than 1, it is unlikely that the exposed population is at risk adverse health effects. If HI is greater than or equals to one, there must be a proactive measure to mitigate the hazard because people are at risks of health effects (Dai et al., 2016; Shaheen et al., 2016).

3.9.6 Carcinogenic Risk

Pb is regarded as a carcinogen i.e. a cancer-causing agent (Zhang, Wei, Zhang, Liu, & Chen, 2014). It is listed by the International Agency for Research on Cancer (IARC) among the "Group B2" of possible human carcinogens (IARC, 2011b). Researches have revealed with experimental and epidemiological proof from their investigations that shows strong relationships between Pb exposure and different types of cancer (Rousseau, Parent, Nadon, Latreille, & Siemiatycki, 2007; Silbergeld, 2003; Silbergeld, Waalkes, & Rice, 2000).

The cancer risk in the exposed population (CR) was evaluated from the product of the Pb average allowable daily intake (ADI) mg/kg/day over a lifetime and the oral carcinogenic slope factor (Csfo) as revealed in equation 11. Total cancer risk (CR_{kt}) was calculated from equation 12. The probability of an individual to develop cancer over a lifetime period was CR_k (Dai et al., 2016). For instance, if the value of CR_k is 0.001 (10^{-3}), it means there is a probability that 1 person out 1000 population will develop cancer in their lifetime (Dai et al., 2016). ADI in this study was calculated based on adult parameters as the cancer cases are more in adults than the children (Gatta et al., 2009; Kaplan et al., 2013).

$$CR_k = ADI \times Csfo \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ equation 11}$$

$$CR_{kt} = \sum CR \qquad \text{………………… equation 12}$$

Where;

CR_k is the cancer risk (the risk of cancer for lifetime) from consumption of lead through rice.

ADI is the average daily intake of lead.

Csfo stands for the oral carcinogenic slope factors provided by the USEPA (2015) IRIS (Integrated Risk Information System) database. It is 8.5×10^{-3} mg/kg/day (0.0085) for lead (Pb).

CR_{kt} is the total cancer risk when the route of exposure is multiple e.g. when the carcinogens are more than one in the food. Only Pb is evaluated in this study.

3.10 Advocacy and Authorization

Royal Head of Dareta village was visited to seek permission on for conducting the field experiment in his Kingdom. Culturally, before anything could be done on people's land in Northern Nigeria especially in those affected villages such as Dareta, there must be an authorisation from the head of the community. The researcher's previous experience working with the communities in this part of Nigeria as a staff of the Federal Ministry of Health (FMoH) helped in managing this task. Previous studies from this area such as Udiba et al. (2012) also suggested this.

This was done in December 2015 during the first research visit to the area. To further create a mutual relationship with the dwellers of the village, the visit was repeated anytime our research team is in Dareta. Furthermore, the Zamfara Ministry of health, Ministry of environment and solid mineral, and the Ministry of Agriculture were also visited for possible collaboration and information. Rice farmers in the village were visited and discussed with. This study received supports from all. Summary of the research proposal was also submitted to both the Federal and Zamfara State Ministry of Health, Ministry of Environment and Solid Mineral including the Ministry of Agriculture for ethical approval. Clearance was issued by the Zamfara Ministry of Environment on behalf of others. The University of Abuja also granted permission (Appendix C). Having gotten the permission from the authority, there was a need to build a relationship with the individual farmers in Dareta village to have a cordial relationship with them. This was done through the Head of the department and the Director of Pollution Control, Zamfara Ministry of Environment who was also from the same Local Government Area (Anka).

3.10.1 Permission from the University of Salford and Risk Assessment

A thorough assessment of the research proposal was done by the University of Salford's research ethics committee and it was cleared for ethical issues. Risk assessment was conducted on then proposed field work prior to the commencement of the all the research works including the field experiment, the pot experiment and the laboratory work and an approval was granted.

CHAPTER FOUR

The Role of Soil Physico-chemical Properties in Pb Uptake by Rice

4.0 Methods and Statistical Analysis

The method of sample preparation for the soil samples was explained previously in section 3.7.1 and, the rice preparation and analysis in section 3.7.3 while the elemental analysis for this study was presented in section 3.4 all in chapter 3 (methodology). The data was subjected to analysis of variance (ANOVA) and the significant means from ANOVA were separated with the Duncan Multiple Range Test. Before the ANOVA, the data were checked for normality and homogeneity of variance. Shapiro Wilk was used to test for data normality and Levene's test was done to check for the data homogeneity of variance. Statistical confidence was set at $\alpha = 0.05$. Principal Component Analysis (PCA) was done using a statistical package called Facto-Minner to determine the general relationship observed among the four selected farms regarding the soil physico-chemical properties. Relationship between the soil properties on the Pb concentration in the rice seed was done by linear regression analysis using Excel Analyse-it. All other statistical analysis was done using SPSS Statistical software package version 23.0.

4.1 Results and Discussions

The analysis of the certified reference materials (CRM) was in agreement with the certified values (Table 4.1).

Table 4. 1: Result from the analysis of Lichen IAEA-336 CRM compared with the reference value

Element (mg/kg)	Pb	Zn	Cd	Mn	Cu
Experimental Values	5.14	28.86	1.05	62.06	5.21
	5.25	29.18	0.46	55.78	4.02
	5.03	30.42	0.28	58	3.52
Mean	5.14	29.49	0.60	58.61	4.25
Certified Values	4.2 - 5.5	27 - 33.8	0.1 – 2.34	56 - 70	3.1 – 4.1

Shapiro-Wilk normality test conducted reveals that the data was statistically normal ($p<0.05$). For the Levene's homogeneity of variance test, the p-value was larger than the alpha level ($\alpha > p$) which revealed that the null hypothesis that the data was homogenised stands. It means the variances are equal (homogenous data). Table 4.2 (A-C) states the summary of the soil properties with their mean values, ranges and standard deviation while Table 4.3 shows the mean concentration of Pb in both the soil and the rice samples in the four selected rice farms. The Table 4.4 presents the Pb concentration ratio (CR) and its ranges in the four selected rice farms.

Table 4. 2: Soil Physico-chemical Properties

A. General physical and chemical properties

	0-10 cm depth			10-20 cm depth			20-30 cm depth		
Properties	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**
Sand (mg/kg)	45.88	23.80 – 74.60	10.6	45.22	21.80 – 68.80	11.5	45.21	14.60 – 65.80	12.4
Silt (mg/kg)	36.17	6.80 – 54.80	11.5	35.04	17.40 – 54.80	9.7	33.34	14.8 – 57.4	10.9
Clay (mg/kg)	17.94	10.00 – 37.4	6.46	19.73	12.80 – 34.00	5.6	21.44	10.00 – 43.40	8.1
Soil Texture	NA	Sandy loam	NA	NA	Sandy loam	NA	NA	Sandy loam	NA
pH	6.74	5.11 – 7.92	0.6	6.69	5.12 – 7.89	0.6	6.66	5.12 – 7.72	0.6
CEC (mEq/100g)	2.83	1.88 – 4.22	0.5	2.62	1.82 – 3.67	0.4	2.48	1.57 – 3.60	0.5
Electrical Conductivity (mS/m).	1.22	0.4 – 2.5	0.3	1.11	0.6 – 1.6	0.2	1.01	0.5 – 1.5	0.2
Organic Carbon (mg/kg)	1.57	0.71 – 2.82	0.6	1.57	0.70 – 2.81	0.6	1.54	0.68 – 2.8	0.6
BS	81.3	67.39 – 89.92	5.1	81.3	53.06 – 91.03	7.7	82.56	72.7 – 91.02	4.9
Available Phosphorus (mg/kg)	14.40	12.03 – 17.72	1.7	13.37	10.99 – 16.71	1.7	16.70	9.90 – 16.70	1.7
N (mg/kg)	3.26	1.32 – 7.25	1.6	3.25	1.31 – 7.24	1.6	3.25	1.31 – 7.23	1.6
Exchangeable Acidity (cmol/kg)	0.56	0.31 – 0.81	0.1	0.52	0.20 – 0.73	0.1	0.51	0.11 – 0.81	0.1

NA = Not Applicable, SD = Standard Deviation

B. Cations

	0-10 cm depth			10-20 cm depth			20-30 cm depth		
Properties	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**
Ca (cmol/kg)	1.33	0.78 – 1.86	0.3	1.29	0.67 – 1.76	0.3	1.23	0.77 – 1.74	0.3
Mg (cmol/kg)	0.60	0.29 – 0.98	0.2	0.56	0.27 – 0.87	0.1	0.48	0.18 – 0.78	0.1
Na (cmol/kg)	0.23	0.12 – 0.53	0.09	0.18	0.11 – 0.42	0.06	0.23	0.14 – 0.53	0.08
K (cmol/kg)	0.12	0.02 – 0.28	0.08	0.08	0.02 – 0.17	0.04	0.08	0.02 – 0.17	0.04

SD = Standard Deviation

C. Metals

	0-10 cm depth			10-20 cm depth			20-30 cm depth		
Properties	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**	**Mean**	**Ranges**	**SD**
Pb (mg/kg)	598.15	136.11 – 6147.55	782.6	553.01	89.50 – 6120.7	752.6	423.06	39.66 – 2075.10	369.3
Cr (mg/kg)	338.69	26.38 – 1138.53	164.5	196.71	15.98 – 581.20	101.3	186.21	84.38 – 443.88	82.7
Cd (mg/kg)	0.541	0.02 – 2.01	0.3	0.41	0.05 – 0.75	0.2	0.38	0.03 – 0.78	0.2
Mn (mg/kg)	257.04	78 – 550	138.7	185.30	53.20 – 551	113.9	176.79	34.7 – 650	128.8
Fe (mg/kg)	576.04	185 – 1329	268.2	586.75	161 – 2455	557.7	446.60	153 – 2030	438.7
Cu (mg/kg)	19.26	8.31 – 165.5	33.7	12.36	8.04 – 29.40	5.2	23.13	8.06 – 260	54.5
Zn (mg/kg)	208.96	178.7 – 325.8	22.2	208.87	178.6 – 325.7	22.2	207.95	177.6 – 324.7	22.2

SD = Standard Deviation

Table 4. 3: Mean lead concentration (mg/kg) in soil and rice samples in the four farms

	Rice Pb			Soil Pb at 0-10 cm depth			Soil Pb at 10-20 cm depth			Soil Pb at 20-30 cm depth		
Sampled Farms	**Mean (mg/kg)**	**Ranges (mg/kg)**	**SD**	**Mean (mg/kg)**	**Ranges (mg/kg)**	**SD**	**Mean (mg/kg)**	**Ranges**	**SD**	**Mean (mg/kg)**	**Ranges**	**SD**
A (n=20)	1.05	0.26 – 2.04	0.5	629.78	141.41 – 1922.7	484	600.31	132.11–1866.5	435	485.62	39.66–1836.1	466
B (n=20)	1.07	0.32 – 2.81	0.8	908.89	146.85 – 6147.55	1346	846.24	140.75–6120.7	1332	767.50	115.94-6140.9	1311
C (n=20)	0.74	0.25 – 2.45	0.5	468.97	146.75 – 2586.35	539	407.26	89.50–2153.85	456	393.01	81.05-2075.10	433
D (n=20)	0.72	0.24 – 1.27	0.4	384.95	136.12 – 922.63	201	358.23	141.43-707.40	156	336.08	136.02-715.34	155
All (n=80)	0.90	0.24 – 2.81	0.6	598.14	136.12 – 6147.55	782	553.01	89.50 – 6120.7	752	495.56	39.66 – 6140.2	738

SD = Standard Deviation

Table 4. 4: Concentration Ratio (CR) (mg/kg) of Pb across the four selected farms.

	0-10 cm depth ± SD	0-20 cm depth ± SD	0-30 cm depth ± SD
Farm A	1.67E-3±2.00E-3	1.71E-3 ± 1.00E-3	1.83E-3 ± 2.00E-3
Farm B	1.18E-3 ± 7.00E-4	1.21E-3 ± 4.00E-4	1.27E-3 ± 8.00E-4
Farm C	1.58E-3 ± 1.00E-3	1.17E-3 ± 7.00 E-3	1.76 E-3 ± 2.00E-3
Farm D	1.87E-3 ± 2.00E-3	1.95E-3 ± 1.00 E-3	2.01 E-3 ± 2.00E-3

The concentration ratio (CR) was calculated using the equation 4 (chapter 3, section 3.9.1) by dividing the concentration of Pb (mg/kg) dry mass in the rice samples by the concentration of Pb (mg/kg) dry mass in the soil samples. In this case, the concentration of Pb (mg/kg) in the soil samples were calculated based on the three different soil depths applied i.e 0-10 cm, 0-20 cm and 0-30 cm. The first column (Table 4.4) represents the mean CR across all the farms for the sampling depth of 0 cm to 10 cm soil sampling depth. The second column represents the mean CR across all the farms for the sampling depth of 0 cm to 20 cm soil sampling depth while the third column represents the mean CR across all the farms for the sampling depth of 0 cm to 30 cm soil sampling depth.

In the soil, the mean Pb concentration based on the farms was in the order of Farm B > Farm A > Farm C > Farm D at the depth of 0 – 10 cm and 10- 20 cm which was slightly different at the depth of 20-30 cm where the order was Farm A > Farm B > Farm C > Farm D. Generally, the Pb concentration ranges between 39.66 mg/kg and 6147.55 mg/kg with the heighest soil Pb recorded on Farm B. The soil Pb concentration decreases as the depth increases in all the selected farms as shown in Figure 4.1. A very high soil Pb concentration ranging between 40,000 mg/kg and 100,000 mg/kg was previously found in the soil in Dareta village before the commencement of the 2011-2012 emergency remediation exercise ((UNICEF, 2011). Previous studies in the area have reported Pb concentration in the range of 604 mg/kg – 2025 mg/kg (Abubakar, Bagudo, Birnin Yauri, Sahabi, & Garba, 2015) which also falls within the result of our study. 40 mg/kg – 2,300 mg/kg range was reported by Mohammed & Abdu, (2014), 19.80 mg/kg – 2,892 mg/kg range was reported by Uriah, Kenneth, Gusikit, & Ayuba, (2013) for some farmland area in this same Dareta village, years after the emergency remediation took place. Our result confirms that the soil remediation did not take place on this lands as argued by Udiba et al., (2013).

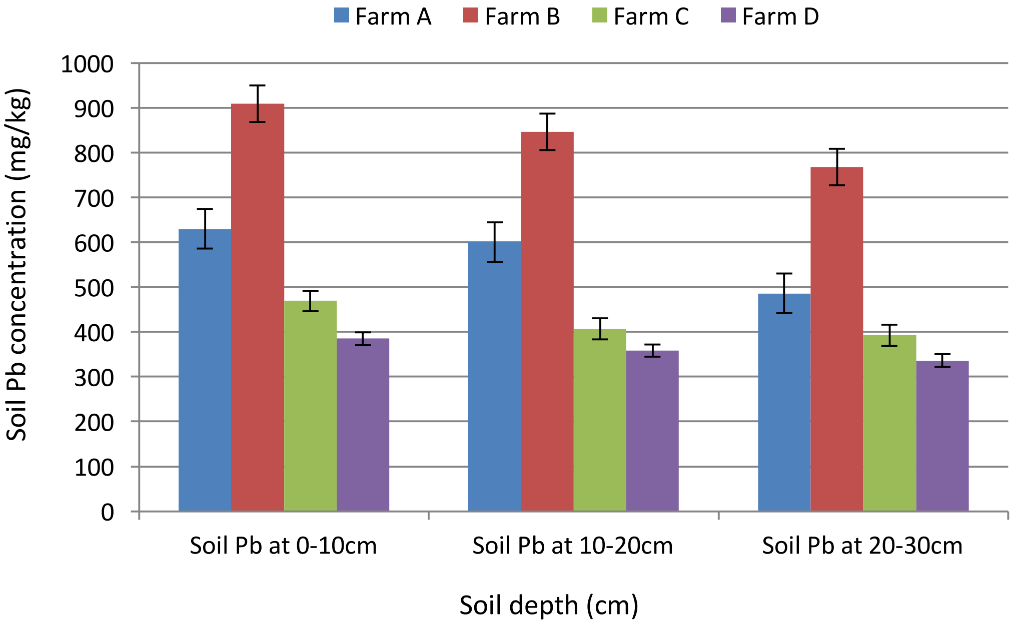


Figure 4. 1: Distribution of Pb based on the soil sampling depth for the four selected rice farms.

The concentration ratio (CR) for the Pb in rice and the soil Pb at the depth of 0-10 cm was the lowest across all the selected rice farms while CR at 0-30 cm depth was the highest (Figure 4.2). Our result confirms the fact that the rice root is domicile within 0 – 30 cm soil depth which is previously been presented by He et al. (2015) and Alloway (2013) as against Khan et al. (2010) that says the rice root is predominantly domicile within 0 to 15 cm soil depth. The 30 cm soil depth would not have shown a great effect on the CR if the root system of rice is domiciled within 15 cm soil depth. Bisalayi rice had its root system domiciled within 0 – 30 cm soil depth.

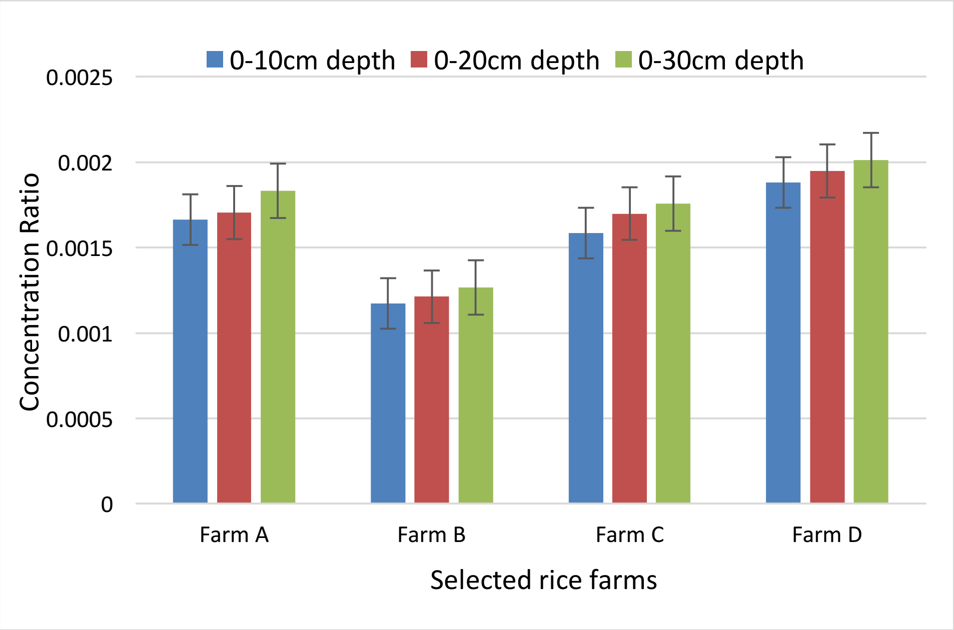
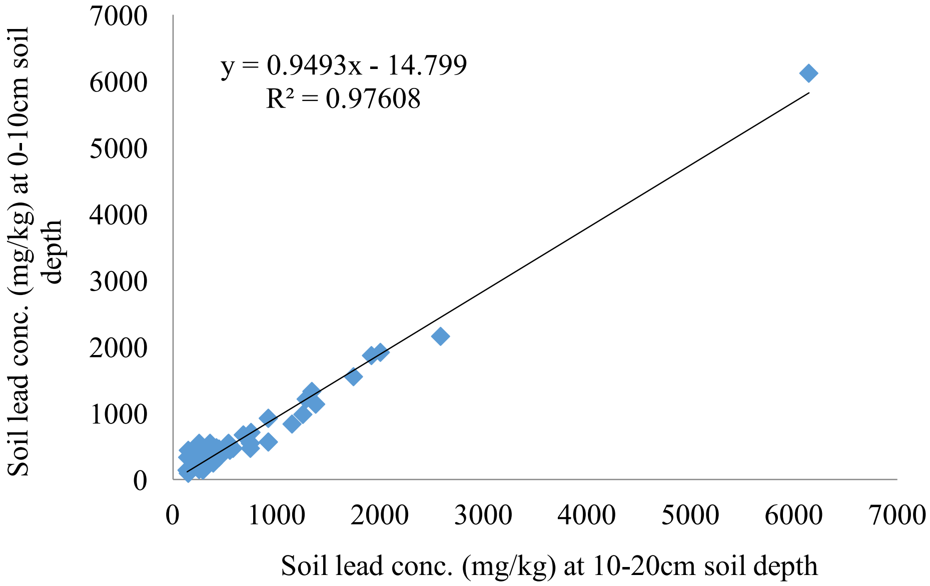
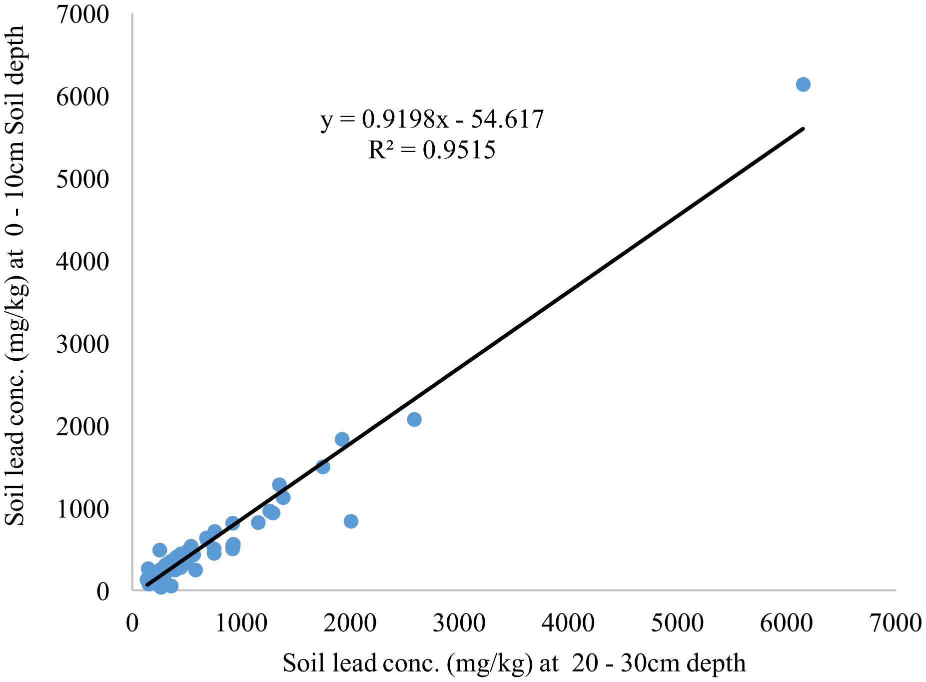


Figure 4. 2: Concertation Ratio (CR) across the four selected rice farms and depth

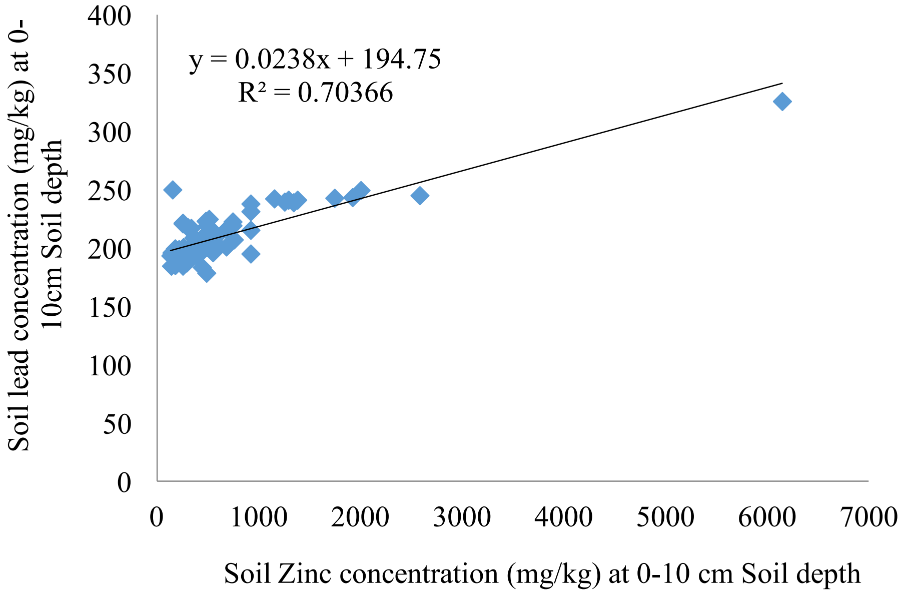
From the findings (Figure 4.3, 4.4, and Table 4.6), there were relationships among some soil parameters across the four selected rice farms. A positive relationship was found between soil Pb at between 0-10 cm depth against soil Pb at between 0-20 cm depth and soil Pb at between 0-20 cm depth against soil Pb at between 0-30 cm depth and the positive relationship was also recorded between the soil Pb at 10 cm depth and soil lead at 30 cm depth. Likewise, the soil Pb and the soil Zn concentration revealed a positive relationship, but a negative relationship was found between soil Pb concentration and the soil pH (Figure 4.3 (a – d)). These positive relationships show that the Pb concentration in the lower soil and down the soil profile depend on the Pb concentration at the top soil. This is similar to the findings by Mohammed and Abdul (2014).



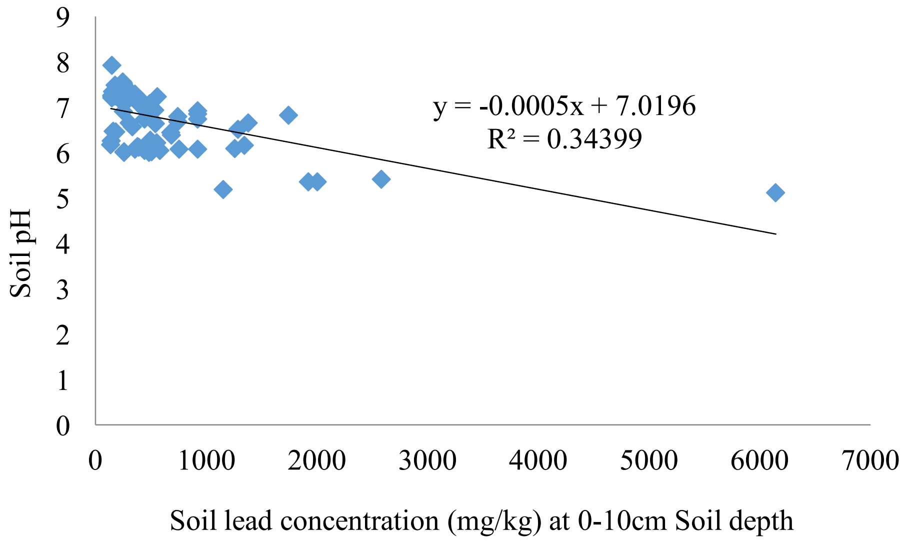
y = 0.9493x - 14.799
R² = 0.97608
Soil lead conc. (mg/kg) at 0-10cm soil depth
Soil lead conc. (mg/kg) at 10-20cm soil depth
0
1000
2000
3000
4000
5000
6000
7000



y = 0.9198x - 54.617
R² = 0.9515
Soil lead conc. (mg/kg) at 0 - 10cm Soil depth
Soil lead conc. (mg/kg) at 20 - 30cm depth
0
1000
2000
3000
4000
5000
6000
7000



c.



d.

Figure 4. 3: Regression analysis of a few soil properties

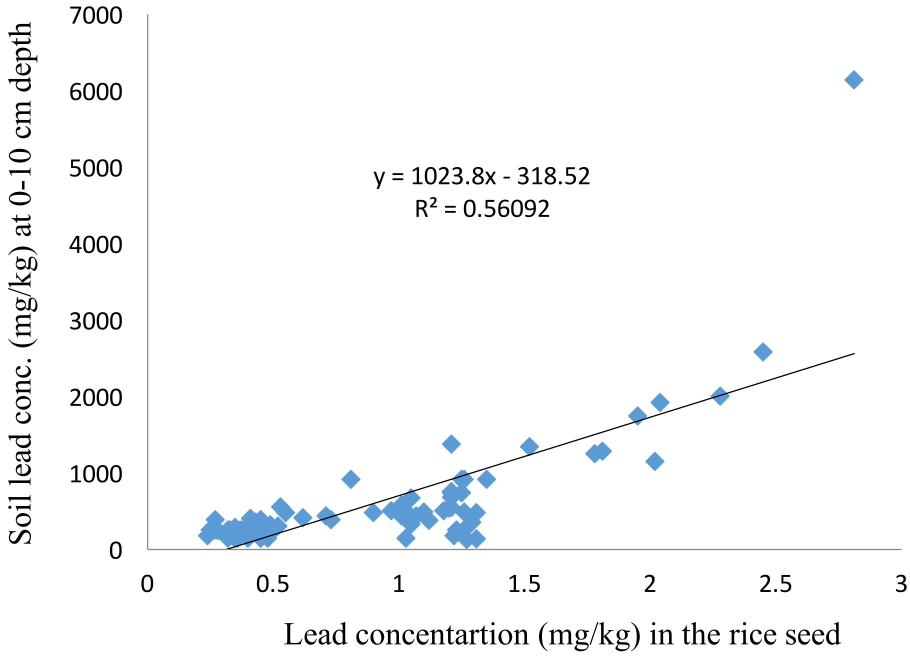
(a) Soil lead concentration (mg/kg) at 10cm depth with the Soil lead concentration (mg/kg) at 20cm depth (mg/kg) **(b)** Soil lead concentration (mg/kg) at 10cm depth with Soil lead concentration (mg/kg) at 30cm depth **(c)** Soil lead concentration (mg/kg) with Soil Zinc concentration (mg/kg) and 10cm dept **(d)** Soil lead concentration (mg/kg) with Soil pH.

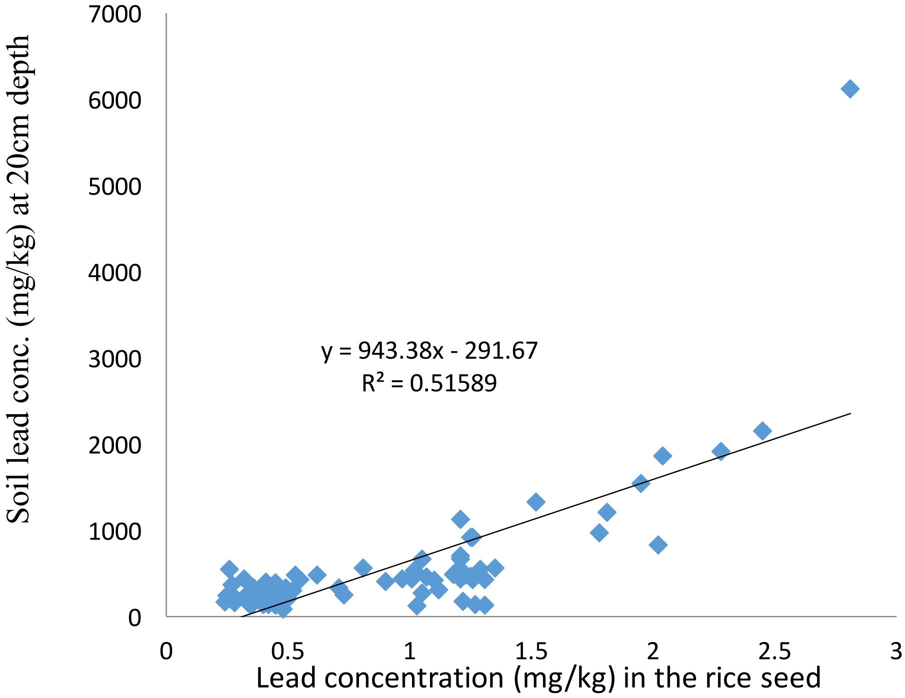
In the rice samples, the mean Pb concentration was in the order of Farm B > Farm A > Farm C > Farm D and there was no significant difference statistically in the Pb distribution in the soil across the sampled rice farms ($p > 0.05$). There was no significant difference in the Pb accumulation in rice across all the four selected rice farms in terms of the uptake. The mean rice Pb concentration of 1.05±0.5 mg/kg, 1.07±0.8 mg/kg, 0.74±0.5 mg/kg, and 0.72±0.4 mg/kg for the farm A, farm B, farm C and farm D obtained were above the permissible limit of 0.2 mg/kg (EC, 2001) and 0.3 mg/kg (USEPA, 2016; FAO/WHO, 2001) which could pose a health risk if the rice is consumed. The international standard for lead in soil and plant is presented in Table 4.5. Farm B has the highest lead uptake in both in terms of the concentration ratio (CR). A range of 23.14 mg/kg to 52.0 (mean 8.3 mg/kg) and 0.02 to 0.04 mg/kg (mean 0.2 mg/kg) have been previously reported for imported rice samples in South Western part of Nigeria by Adedire et al. (2015) and North Central Nigeria by Otitoju et al. (2014) respectively. The result obtained in this study was slightly higher than that obtained by Williams et al. (2009) that reported 0.62 (range 0.051 – 0.74 mg/kg) for 11 mining districts in China and much lower than that of Alam et al., (2002) that reported 7.7 mg/kg (ranges 2.61 – 15.89 mg/kg) for Jessore districts in Bangladesh.

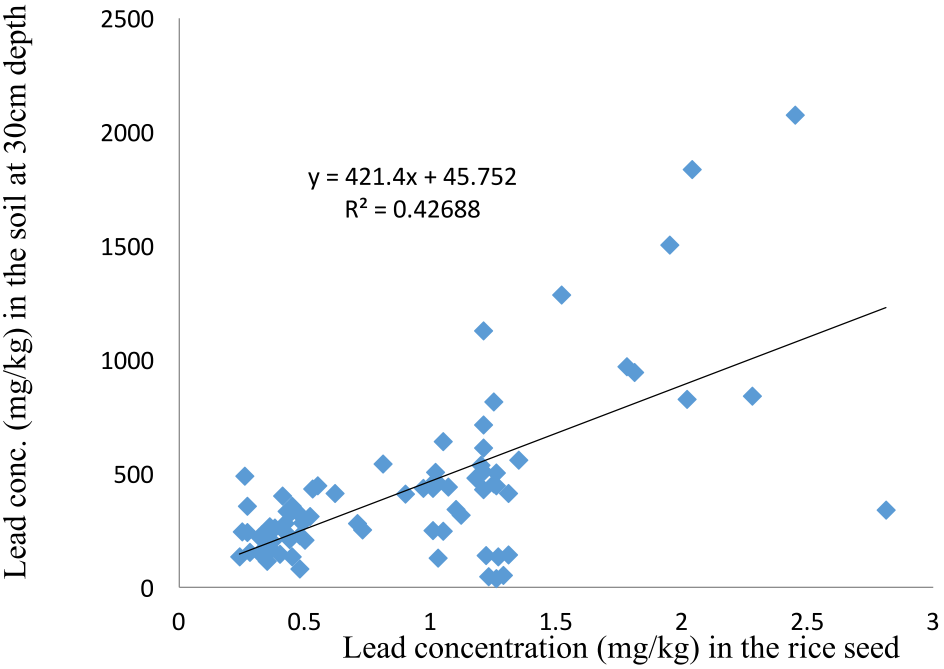
Table 4. 5: International standard for Pb concentration (mg/kg) in soil and plants

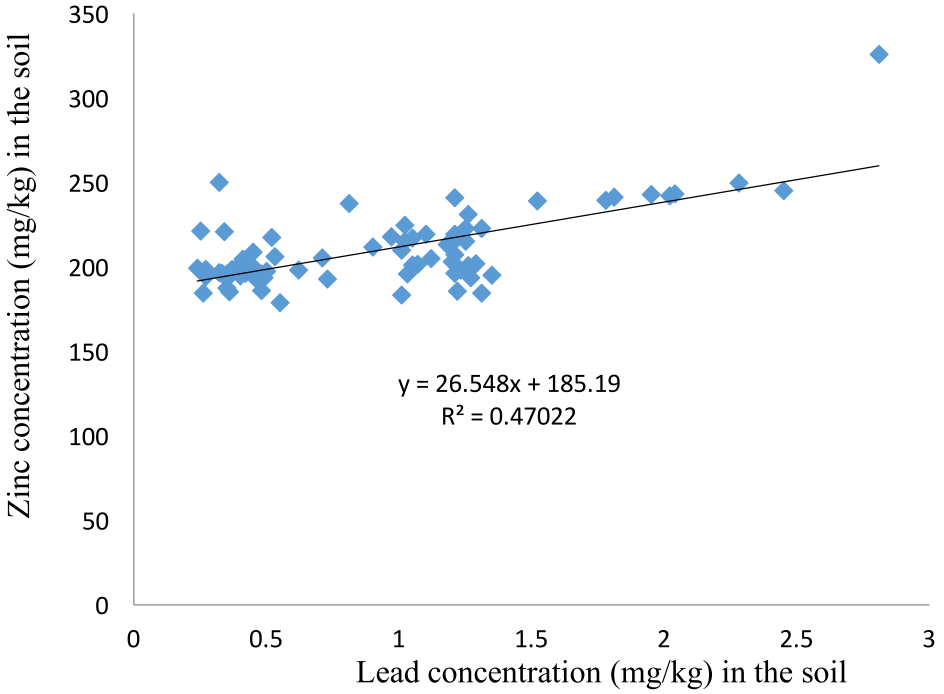
	EC (2006) Commission Regulation		FAO/WHO (2001)		USEPA (2016)		Indian Standard cited by Awashthi (2000)		**SEPA China (1995, 2005)**	
	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
Pb (mg/kg)	100	0.3	100	0.3	420	10	250-500	2.5	300	9

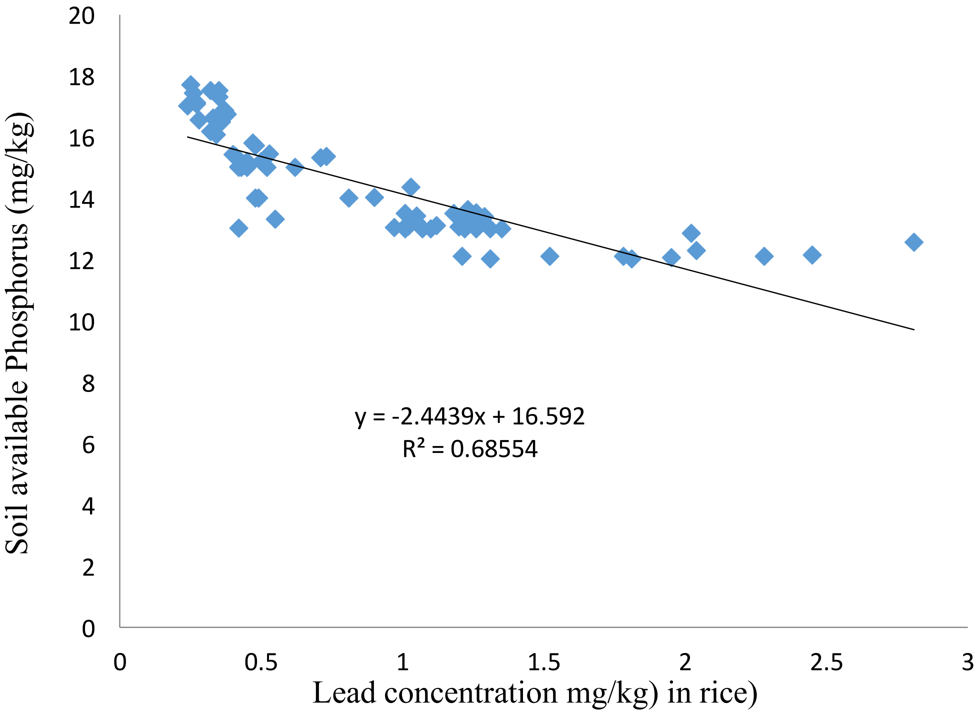
The study on the effect of soil properties on the Pb accumulation in rice grains, showed that there was a positive correlation between the Pb concentration accumulated in the rice with the soil lead at 0- 10 cm depth, the soil lead at 10 – 20cm depth, the soil lead at 20 – 30 cm depth and the Zinc, (Figure 4.4). However, Pb in the rice is negatively correlated with the soil available phosphorus, organic carbon and the pH, and the soil Nitrogen. Some other correlations are shown in Table 4.6.

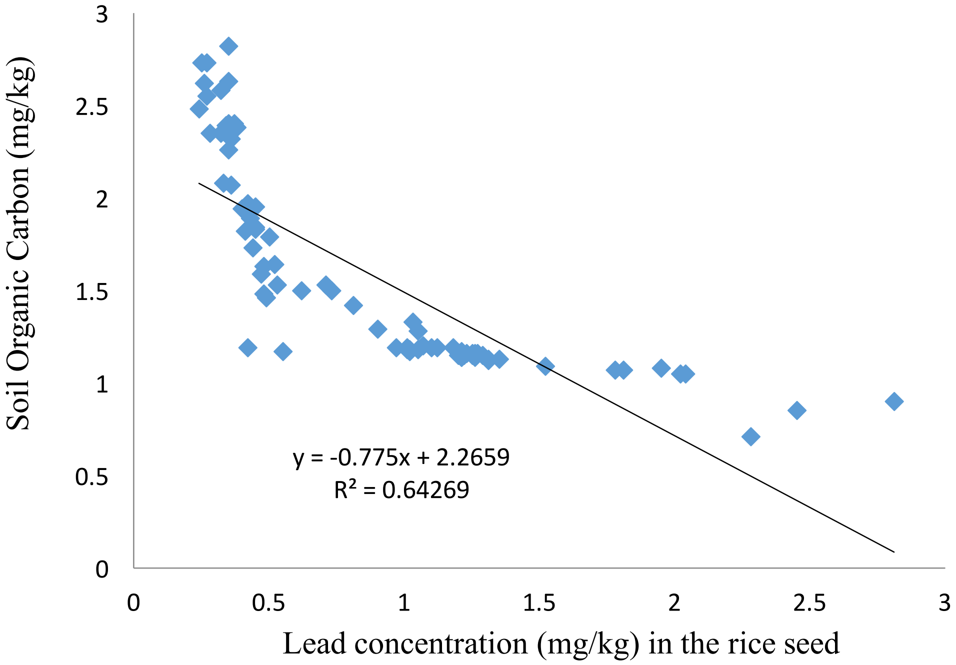

Soil lead conc. (mg/kg) at 0-10 cm depth
7000
6000
5000
4000
3000
2000
1000
0
y = 1023.8x - 318.52
R² = 0.56092
0
0.5
1
1.5
2
2.5
3
Lead concentartion (mg/kg) in the rice seed

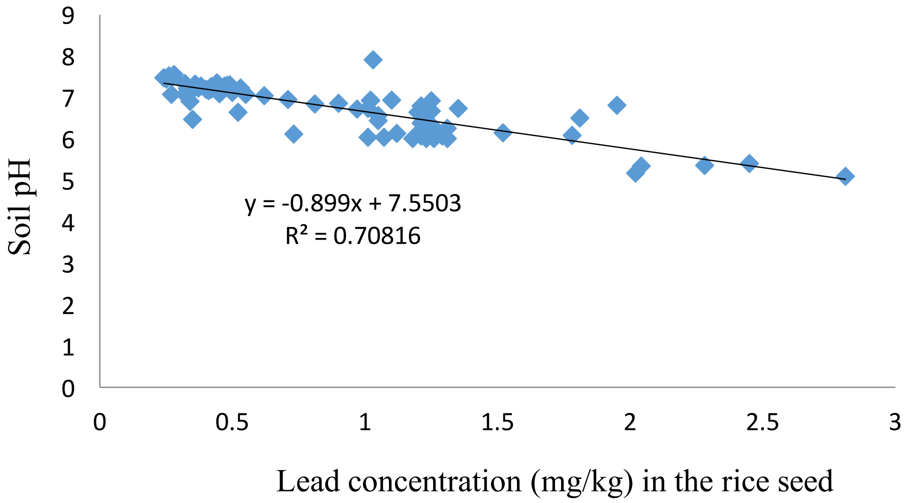

Soil lead conc. (mg/kg) at 20cm depth
7000
6000
5000
4000
3000
2000
1000
0
y = 943.38x - 291.67
R² = 0.51589
0
0.5
1
1.5
2
2.5
3
Lead concentration (mg/kg) in the rice seed


y = 421.4x + 45.752
R² = 0.42688
Lead conc. (mg/kg) in the soil at 30cm depth
Lead concentration (mg/kg) in the rice seed
0
500
1000
1500
2000
2500
0
0.5
1
1.5
2
2.5
3


y = 26.548x + 185.19
R² = 0.47022
Zinc concentration (mg/kg) in the soil
Lead concentration (mg/kg) in the soil
0
50
100
150
200
250
300
350
0
0.5
1
1.5
2
2.5
3


20
18
16
14
12
10
8
6
4
2
0
0
0.5
1
1.5
2
2.5
3
Soil available Phosphorus (mg/kg)
Lead concentration mg/kg) in rice)
y = -2.4439x + 16.592
R² = 0.68554


3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
Soil Organic Carbon (mg/kg)
Lead concentration (mg/kg) in the rice seed
y = -0.775x + 2.2659
R² = 0.64269



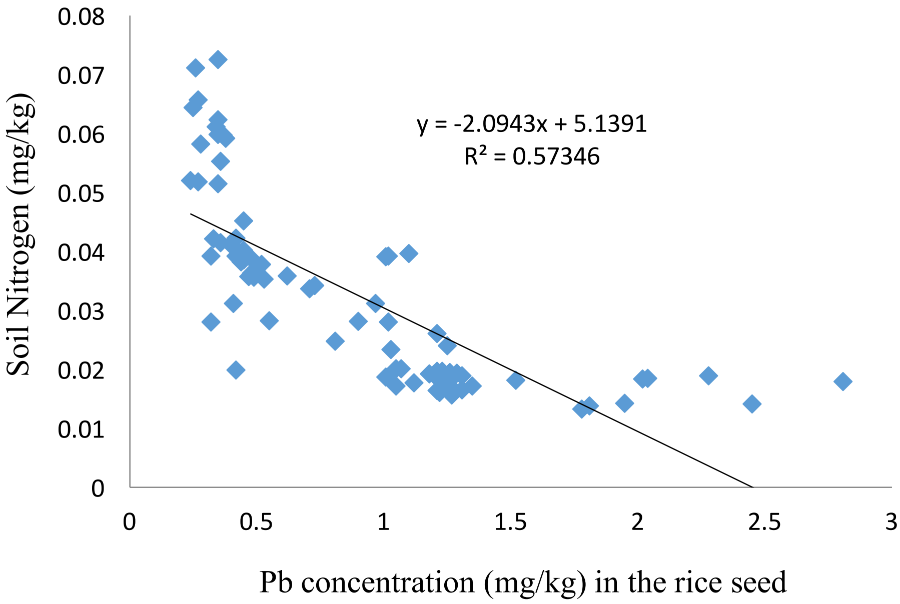


Figure 4. 4: Regression analysis of the soil properties and the lead in rice

(a) Soil lead concentration (mg/kg) at 0-10 cm depth with Pb concentration (mg/kg) in the rice seed (b) Soil Pb concentration (mg/kg) at 10-20 cm depth with Pb concentration (mg/kg) in the rice seed (c) Soil Pb concentration (mg/kg) at 20-30 cm depth with Pb concentration (mg/kg) in the rice seed (d) (e) Soil available phosphorus(mg/kg) with the Pb concentration (mg/kg) in the rice seed (f) Pb concentration (mg/kg) in rice with Soil Organic Carbon (mg/kg) (g) Pb concentration (mg/kg) in rice seed with the soil pH (h) Pb concentration (mg/kg) in the rice seed with the Soil Nitrogen (mg/kg).

Series of factors such as pH, organic carbon, soil Nitrogen, available phosphorus etc. are responsible for Pb's availability in the soil (Batista, De Oliveira Souza, Da Silva, & Barbosa, 2010). and the uptake and accumulation in plant is influenced by these parameters (Ferré-Huguet, Martí-Cid, Schuhmacher, & Domingo, 2008). The data shows that there were influence from soil Pb, the soil organic carbon, the soil Zn, pH and the soil Nitrogen on the seed Pb. The soil Pb also had a positive correlation with the soil-zinc, the soil phosphorus and pH as shown in Table 4.6. The result shows that higher Pb concentration in rice is associated with lower soil Nitrogen.

Table 4. 6: Result of the regression analysis to show the influence of few parameters of soil in the accumulation of Pb in rice

Correlation (Rice-lead vs Soil Properties)	At 0-10 cm depth		At 10-20 cm depth		At 20-30 cm depth	
	R^2	Regression Equation	R^2	Regression Equation	R^2	Regression Equation
Soil Lead	0.5609	y = 1023.8x - 318.52	0.5159	y = 943.38x - 291.67	0.4349	y = 850.09x - 265.6
Soil Phosphorus	0.6855	y = -2.4439x + 16.592	0.6855	y = -2.4439x + 16.592	0.6486	y = -2.457x + 15.273
Organic Carbon	0.6427	y = -0.775x + 2.2659	0.6427	y = -0.775x + 2.2559	0.6425	y = -0.7754x + 2.2384
Soil pH	0.7082	y = -0.899x + 7.5503	0.6778	y = -0.8551x + 7.461	0.6135	y = -0.8392x + 7.414
Soil Nitrogen	0.5735	y = -2.0943x + 5.1391	0.5734	y = -2.0944x + 5.129	0.5733	y = -2.0939x + 5.1247
Soil Zinc	0.4702	y = 26.548x + 185.19	0.4702	y = 26.548x + 185.19	0.4702	y = 26.548x + 184.18

Our result shows that the soil pH had significantly influenced the Pb in the rice seed in this study. The pH is negatively correlated with the Pb concentration in the rice seed, the organic carbon and the soil nitrogen (N). The mean pH across all the four selected farms was 6.7±0.6 which ranged from 5.11 to 7.92. As the acidity increases (decreasing pH scale), the Pb in the rice seed was seen to be increasing likewise the organic carbon and the soil nitrogen. This is similar to some previous studies outside Nigeria; Prasad (1999), Nigam, Srivastava, Prakash, and Srivastava (2001), Basta, Ryan, and Chaney (2005), Amini, Khademi, Afyuni, and Abbaspour (2005) and Khan, Khan, Khan, Qamar, and Waqas (2015). It is confirmed by Tsadilas, Karaivazoglou, Tsotsolis, Stamatiadis, and Samaras (2005) and Alloway (2012) that at low pH, the bioavailability and mobility of some metals including Pb increase. Soil pH is one of the factors that influence not only the mobility and availability of metals but also the availability of soil nutrient to plant (Peng, Song, Yuan, Cui, & Qiu, 2009). At low pH, the plants access more nutrients (Smith & Smith, 2011). Lofts, Spurgeon, Svendsen, and Tipping (2004) added that soil pH has a direct influence on toxicological effects of metal ions on plants.

The soil organic carbon ranged from 0.71 mg/kg to 2.82 mg/kg, 0.70 mg/kg to 2.81 mg/kg and 0.68 mg/kg to 2.80 mg/kg with mean value of 1.57±0.6 mg/kg, 1.57±0.6 mg/kg and 1.54±0.6 mg/kg across the three depths (10, 20 and 30 cm) respectively. Soil Organic carbon showed a negative correlation with the lead in rice. This means as the organic carbon in the soil decreases, the Pb concentration in the rice seeds increases. Research shows that the organic carbon component of the soil can keep the soil Pb immobile (Alloway, 2012; Khan et al., 2015). The Pb bioavailability decreases when there is an increased Organic carbon (Shaheen et al., 2016). It determines the amount of the organic matter in the soil. According to (Udo et al., 2009), about 60% of the organic carbon comes from the organic matter. The higher the organic matter, the higher the organic carbon. The major thing is that the organic matter is a sorbent to Pb and other metals (Park et al., 2011). Meanwhile statistically, the correlation is not that strong between the organic carbon and the soil-Pb which may be due to the season (dry season) at which the sample was collected (Alloway, 2013).

Availability of metals including Pb is induced also by the soil texture (Kashem & Singh, 2001). High clay content (clay soil) can make the metal ions unavailable in the soil (Vega et al., 2010) but with mild organic matter content, it forms humous together the clay content. Retention of

Pb may occur when Pb^{2+} (lead ions) are adsorbed onto clay particles, making it unavailable (Sammut et al., 2010; Uzu, Sobanska, Aliouane, Pradere, & Dumat, 2009). The soil test result shows that 45% of the soil samples was loamy soil while 55% was sandy soil on the Farm A. On the farm B which was later the selected farm for the field trial, 45% of the soil samples was loamy soil, 20% was sandy clay, and clay loam, 15% was silt loam and 20% was sandy loam and sandy clay loam. On the other hand, loamy soil was 55%, sandy loam was 20%, clay was 15% and silt was 10% on the farm C. Farm D had 55% loamy soil, 25% sandy loam, 10% each of the clay loam and silt clay. The result of the particle size and the soil colour are shown on Table II Appendix D. The four farms demonstrated wide distribution of same soil colour.

A significant difference was found in the accumulation of Pb across different segment of the rice plant. A significant increase in Pb concentration was observed from the root region up to the seed region. This is revealed in Figure 4.5 and more details about this are presented in chapter 5.

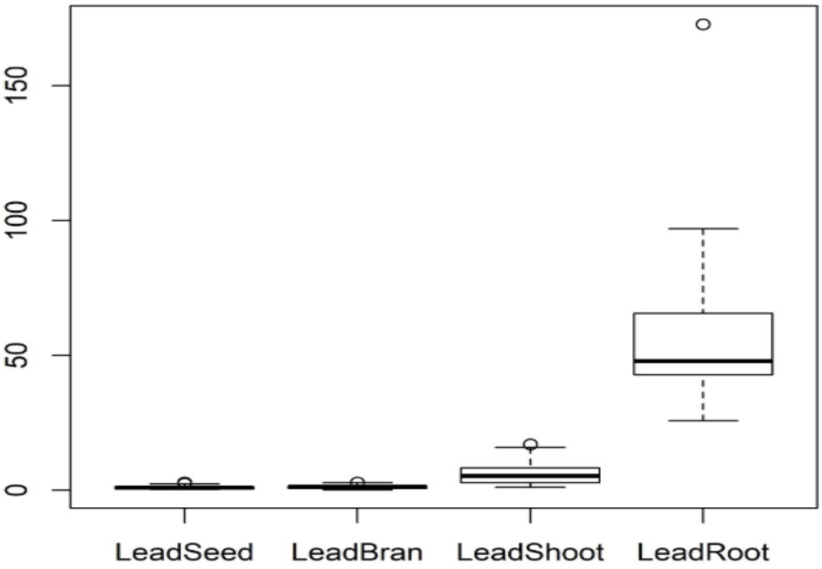


Figure 4. 5: Accumulation of lead in different part of the rice plant.

Each box represents 25th and 75th percentile (interquartile range), the band represent 50 percentiles while whisker represent the 5th and 95th percentile.

4.6 Principal Component Analysis

The result from the two-dimensional multivariate data analysis, principle component analysis (PCA) showed the variations among the four selected farms as regards the soil properties. PCA was to capture as much as possible variables to determine the inter-relationships within the soil characteristics (Pan, Bosch, & Ma, 2017). The result shows that the farms were closely related by general characteristics. Farm A, B and C were closely related in the general characteristics while D was seen to be slightly different (Figure 4.6). In terms of sampling depth, there was no significant difference statistically observed between the four selected rice farms. The soil physico-chemical properties were similar across the depths based on the observed sample clusters. The selected rice farms were not statistically different from each other in terms of location within the village (Dareta). All these are revealed in Figure 4.6.

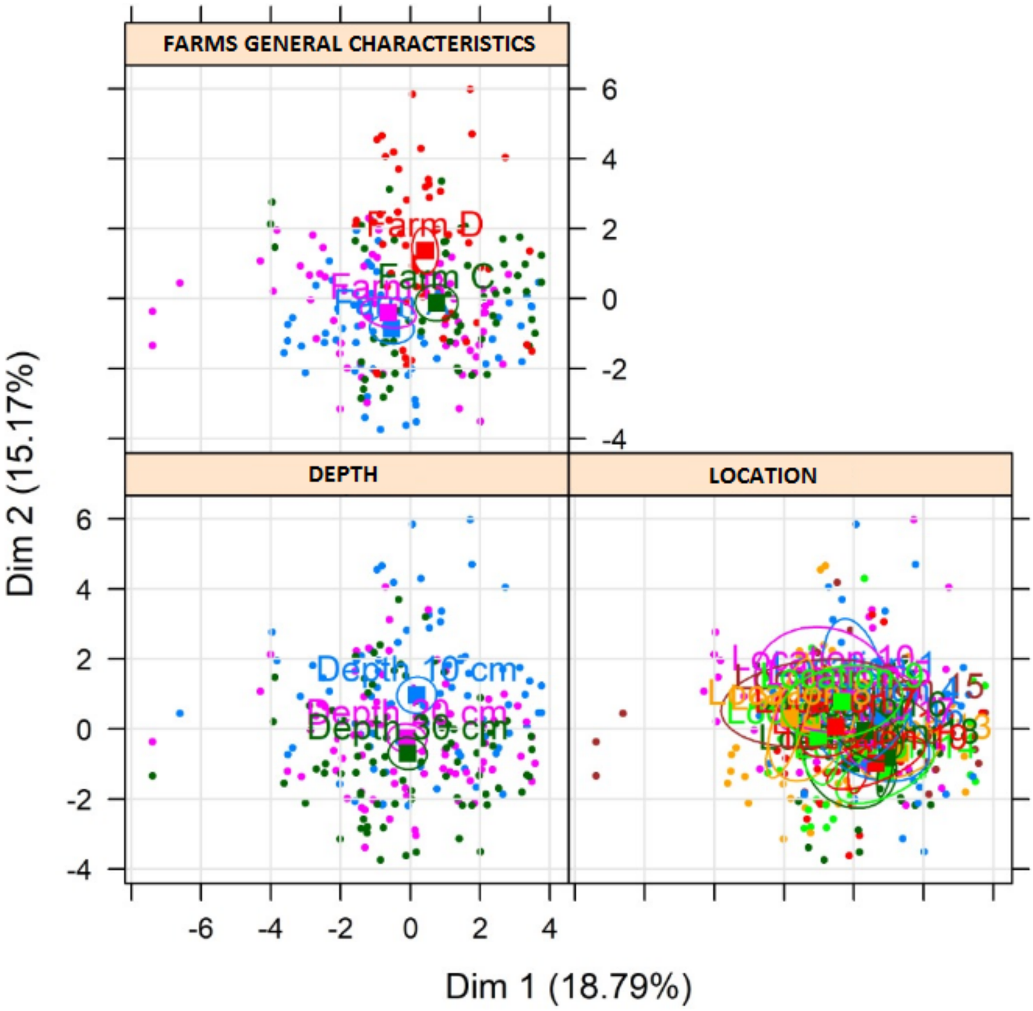


Figure 4. 6: General relationship observed among the four selected rice farms for soil characterisation.

In the variable factor map of the PCA, there were clusters observed which explained further the variations among the physico-chemical properties (parameters) of the soil. The soil Pb was observed along the same dimension with other three metals such as cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn). This simply means that whatever parameter of the soil that has affected the soil Pb may also affect those aforementioned toxic metals. The exchangeable bases (Na, Mg, K and Ca) were also observed along the same dimension together with the Cation Exchange Capacity (CEC). This confirm the fact that the more the exchangeable bases, the bigger the CEC and CEC has been one of the factors stated by the previous study (Fontes & Alleoni, 2006) that determined the bioavailability of Pb in the soil. The pH, available phosphorus, soil nitrogen and the Organic carbon were found on the same dimension. This has been explained previously by the regression analysis. Iron (Fe) and manganese (Mn) behaved the same way which was expected and in line with Zeng et al. (2011). These are shown in Figure 4.7.

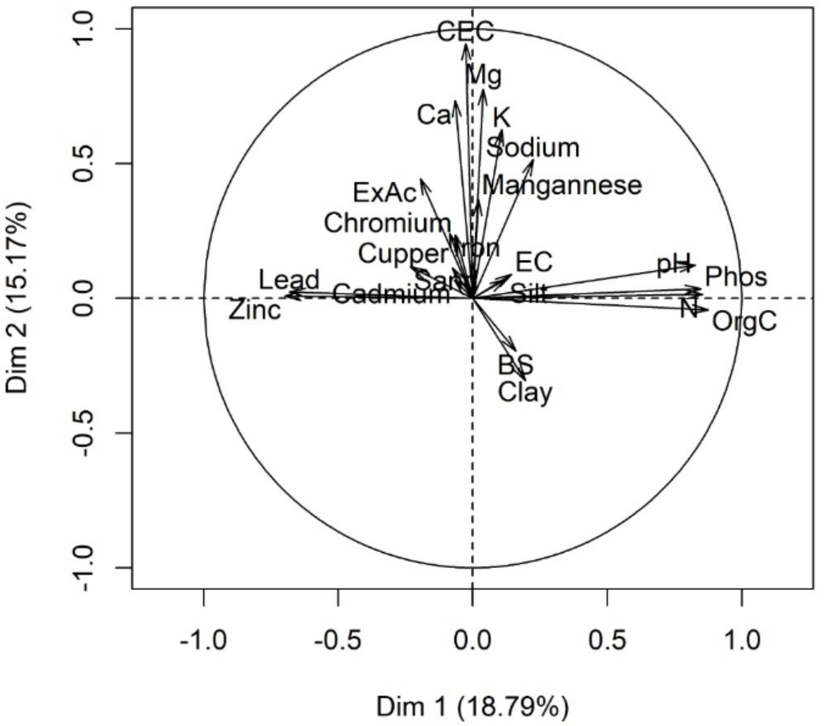


Figure 4. 7: Variable Factor Map showing similar soil parameters across the four selected rice farms.

4.7. Conclusion and Recommendations

This study is the first attempt in Nigeria to check the influence of soil physico-chemical properties (parameters) regarding the Pb uptake in rice. It was not clear how much Pb is lost down the soil profile but based on the three sampling depths in this study (0-10 cm, 20-30 cm and 20-30 cm), less than 4% drop was found between the three selected sampling depths. This may be because loss of several activities such as ploughing, biotic factors (plant and animal activities), and erosion during the raining season influence mixing of the top soil up to the plough depth. It will be interesting to know how much Pb is retained in the top-soil (0-30 cm plough depth) against leaching, ploughing activities and how much is lost further to other soil components down the soil profile. This study suggests a study of soil Pb concentration up to 1 meter depths down the soil across the arable land in Dareta village. It was found that there are other crops such as yam, cassava and some other tuber crops that root up to 1 meter and more in the soil. It will be beneficial to include the study of Pb uptake by these group of plants in the future study.

The findings of this study established a strong relationship between low pH of the soil (soil acidity) and an increased Pb uptake. We are suggesting that a known contaminated soil with Pb should be assessed for its pH and if the pH is low, the soil should not be used to grow rice unless a thorough risk assessment is conducted. One option presented by Rengel (2003) is to treat the soil for acidity before using it and this may reduce the excessive mobility and bio-availability of Pb to rice within the soil. There was no statistically significant difference ($p>0.05$) for pH in all the four selected rice farms. The mean Pb concentration in rice for the local bisalayi was above both the EU (0.2 mg/kg) and the FAO/WHO (0.3 mg/kg) limit. This study concludes that the four selected rice farms characterised are producing rice that are not appropriate for Pb concentration content. The contamination levels in all the farms are high enough to cause concern from human exposure risk assessment perspective.

CHAPTER FIVE

Lead Accumulation and Distribution in Rice

5.0 Brief

Exploring the Pb accumulation and distribution in the parts of popular Nigerian local rice (bisalayi) grown on Pb contaminated soil was to further understand the Pb uptake from root and its distribution across to different parts of rice. To quantify the concentration of Pb in rice root, shoot, husk and the seed and to ascertain the level of significance of the variations that exist in the Pb concentration within different part of rice plant. Also, to determine the extent of Pb mobility within the parts of the rice plant.

5.1 Materials and methods

This study shares the same methodology with the previous chapter (chapter 4). It was part of the work conducted during the field characterisation which was done preliminary to select the appropriate site for the experiment on varietal selection. Four selected rice farms were selected, studied, details have been discussed in chapter 3 (section 3.3).

5.2 Statistical analysis

The data were checked for normality and homogeneity of variance. Shapiro Wilk was used to test for the data normality and Levene's test was done to test for the data homogeneity of variance before any analysis. A one-way repeated measure analysis of variance (ANOVA) was conducted to test the null hypothesis that there was no difference in Pb translocation/partitioning across the various part of rice plant. To evaluate statistically significant differences among the mean values, one-way ANOVA was done with Duncan Multiple range test (post hoc test) to verify significance of the variation between the dependent variables at a probability level of 0.05. Correlation analysis was conducted using a Pearson test (2-tailed). The rice ability to uptake Pb from the soil and translocate it within its body parts was evaluated according to Bonanno (2011) by the Index of Bioaccumulation or Translocation Factor (TF), expressed in the following ratios: $[\text{Lead}]_{\text{root}}/[\text{Lead}]_{\text{soil}}$ and $[\text{Lead}]_{\text{rice-parts}}/[\text{Lead}]_{\text{root}}$. The level of Pb uptake was measured by concentration ratio (CR) using the equation 4 (Chapter

3, section 3.9.1). All other statistical analysis was done on the IBM SPSS Statistical software package version 23.0 of SPSS Inc. Chicago, USA.

5.3 Results and Discussions

The result for the certified reference materials (CRM) was presented in the previous chapter (Table 4.1). Table 5.1 presents the summary of Pb concentration (mg/kg) in the root (Pb-Root), the Shoot (Pb-Shoot), the husk (Pb-Husk), and the seed (Pb-Seed) at 10 cm soil sampling depth (soil10Pb), 20 cm soil sampling depth (soil20Pb), and 30 cm soil sampling depth (soil30Pb) and their concentration ratio (CR) at 0-10 cm soil sampling depth (PbCR10 cm), 0-20 cm soil sampling depth (PbCR20 cm) and 0-30 cm soil sampling depth (PbCR30 cm).

The Pb distribution and accumulation varied among the four parts of the rice plant and this was in the order of root > shoot > husk > seed. Based on the sampling depth in all samples, the soil-Pb concentration (mg/kg) was highest (598.15±782.69) at the 0-10 cm sampling depth and lowest (495.55±738.04) at 21-30 cm sampling depth. The highest mean soil-Pb concentration found was from the farm B which was 908.89±1346.59 mg/kg at 0-10 cm depths, 846.24±1332.30 mg/kg at 10-20 cm depth and 767.50±1311.54 at 20-30 cm sampling depth. Farm D was 384.95±201.08 mg/kg at 0-10 cm, 358.23±155.80 mg/kg at 10-20 cm and 336.08±154.97 mg/kg at 20-30 cm sampling depth and this was the lowest. As expected, the result shows lowest concentration ratio (0.0021±0.0016) at the top soil (10 cm sampling depth).

Statistical Summary of lead concentration (mg/kg) in the plant parts; root (Pb-Root), shoot (Pb-Shoot), husk (Pb-Husk), the seed (Pb-Seed), 10 cm depth soil samples, 20 cm depth soil samples and 30 cm depth soil samples. Their Concentration Ratio (CR) at 0-10 cm (PbCR10cm), 0-20 cm (PbCR20cm) and 0-30 cm (PbCR30cm) soil sampling depth and the translocation factors [Pb concentration mg/kg in the rice root ratio Pb concentration in the soil, Pb concentration mg/kg in the rice parts (root, shoot, husk and the seed) ratio Pb concentration mg/kg in the rice root]

Table 5. 1: Statistical Summary of Pb concentration (mg/kg) in the plant parts.

									Concentration Ratio			Translocation Factors			
		Pb-Root	**Pb-Shoot**	**Pb-Husk**	**Pb-Seed**	**Soil10Pb**	**Soil20Pb**	**Soil30Pb**	**PbCR10cm**	**PbCR20cm**	**PbCR30cm**	**Root/Soil**	**Shoot/Root**	**Husk/Root**	**Seed/Root**
Farm A	Mean	56.67	4.982	1.282	1.0485	629.783	600.305	485.6185	0.0023	0.001105	0.002505	0.13072	0.10485	0.10485	0.01857
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Std. Deviation	19.88092	4.297079	0.603408	0.528157	484.4049	435.3259	465.7025	0.002014	0.000959	0.002033	0.05283	0.113316	0.113316	0.008725
	Minimum	31.4	1.06	0.08	0.26	141.41	132.11	39.66	0.0009	0.0003	0.0006	0.048	0.022	0.022	0.006
	Maximum	90.25	16.95	2.75	2.04	1922.7	1866.45	1836.1	0.0093	0.0048	0.0094	0.231	0.456	0.456	0.042
	Variance	395.251	18.465	0.364	0.279	234648.1	189508.6	216878.8	0	0	0	0.003	0.013	0.013	0
Farm B	Mean	63.291	5.1605	1.359	1.065	908.886	846.243	767.499	0.001735	0.000895	0.001865	0.14086	0.08952	0.08952	0.01589
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Std. Deviation	31.78425	3.480532	0.824927	0.749031	1346.587	1332.301	1311.541	0.000701	0.000365	0.000775	0.078229	0.067194	0.067194	0.007544
	Minimum	41.55	1.85	0.4	0.32	146.85	140.75	115.94	0.0005	0.0002	0.0005	0.028	0.021	0.021	0.007
	Maximum	172.75	13.9	2.86	2.81	6147.55	6120.7	6140.15	0.0031	0.0017	0.0037	0.317	0.322	0.322	0.032
	Variance	1010.238	12.114	0.681	0.561	1813295	1775025	1720139	0	0	0	0.006	0.005	0.005	0
Farm C	Mean	53.2555	7.54	1.038	0.744	468.973	407.2625	393.007	0.002015	0.001085	0.00225	0.19266	0.14941	0.14941	0.01348
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Std. Deviation	14.5504	3.940164	0.501142	0.533463	539.1126	455.6201	433.613	0.001332	0.000712	0.001491	0.100671	0.08749	0.08749	0.006791
	Minimum	34.55	1.75	0.53	0.25	146.75	89.5	81.05	0.0009	0.0005	0.001	0.041	0.028	0.028	0.004
	Maximum	92.15	15.8	2.6	2.45	2586.35	2153.85	2075.1	0.007	0.0037	0.0076	0.401	0.394	0.394	0.027
	Variance	211.714	15.525	0.251	0.285	290642.4	207589.7	188020.2	0	0	0	0.01	0.008	0.008	0
Farm D	Mean	50.643	7.69	1.1005	0.724	384.9465	358.233	336.079	0.002305	0.00117	0.0024	0.16429	0.1704	0.1704	0.01582
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	Std. Deviation	18.20482	4.904949	0.405378	0.358541	201.0759	155.7973	154.969	0.002032	0.001026	0.002087	0.08243	0.125195	0.125195	0.009513
	Minimum	25.65	1.77	0.43	0.24	136.12	141.43	136.02	0.0007	0.0004	0.0007	0.056	0.024	0.024	0.003
	Maximum	97	15.82	1.64	1.27	922.63	707.4	715.34	0.0093	0.0046	0.0092	0.393	0.439	0.439	0.037
	Variance	331.415	24.059	0.164	0.129	40431.5	24272.8	24015.39	0	0	0	0.007	0.016	0.016	0
Total Average	Mean	55.96488	6.34313	1.19487	0.89538	598.1471	553.0109	495.5509	0.002089	0.001064	0.002255	0.15713	0.12854	0.12854	0.01594
	N	80	80	80	80	80	80	80	80	80	80	80	80	80	80
	Std. Deviation	22.16749	4.303432	0.606947	0.572576	782.6871	752.0401	738.0443	0.001603	0.000799	0.001668	0.082406	0.104241	0.104241	0.008255
	Minimum	25.65	1.06	0.08	0.24	136.12	89.5	39.66	0.0005	0.0002	0.0005	0.028	0.021	0.021	0.003
	Maximum	172.75	16.95	2.86	2.81	6147.55	6120.7	6140.15	0.0093	0.0048	0.0094	0.401	0.456	0.456	0.042
	Variance	491.398	18.52	0.368	0.328	612599.1	565564.4	544709.4	0	0	0	0.007	0.011	0.011	0

The result shows that the Pb mobility from soil to the root was higher than the Pb mobility within the rice parts. The mean Pb concentration accumulated in the shoot, the husk and the seeds in this study was more than the WHO/FAO international permissible limits of 10 mg/kg. Generally, in all the four selected rice farms (characterised farms), the Pb transfer rate from the soil to the root was higher, follows by the transfer betwwen the root to the shoot and the root to the husk while that of the root to the seed was the least (Table 5.1). There was no significant difference in the translocation rate shoot across the rice parts in all the selected four rice farms. The concentration ratio across the sampling depth did not show any significant difference ($p>0.05$) across all the four farms.

5.4 Source of Pb in rice parts

No correlation was found between the concentration ratios and the Pb concentration in the various parts of rice except the root Pb concentration (mg/kg) which was negatively correlated with the concentration ratio at 0-30 cm sampling depth. But the result shows a positive relationship between the Pb concentration (mg/kg) in the whole rice plants (root + shoot + husk + seed) and the Pb concentration (mg/kg) in the soil from the regression analysis (Figure 5.1). This shows that the major Pb up-taken by rice in this study was from the soil. There may be other sources of Pb in plant as speculated by Choi et al., (1998) cited in IAEA-TRS 472, (2010) which states that the Pb in plant could also be through the foliar uptake.

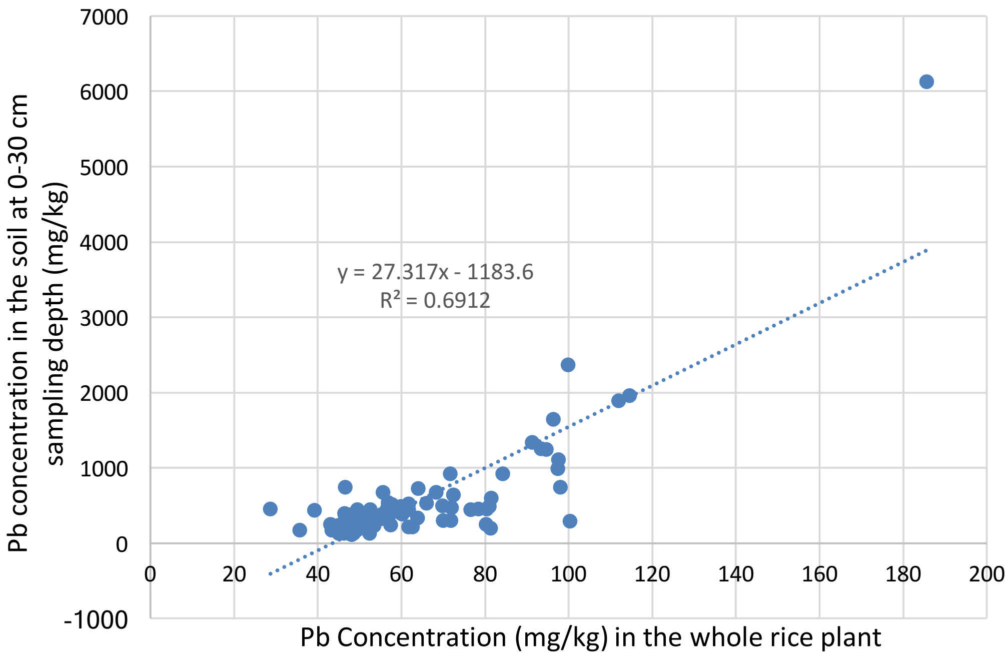


Figure 5. 1: Regression analysis between the Pb concentration in the whole rice plant and the Pb concentration in the soil.

Pb uptake through the rice leaves cannot be ignored as the atmosphere is confirmed to have particles of Pb (Udiba et al., 2013). The Pb aerosols and dry air (dust) that contains Pb could deposit on the rice leaves and these could get absorbed through the leaves (Feng et al., 2011; Schreck et al., 2014). If at all in this study there were folia uptake and leaves absorption of Pb through the rice leaves, using the soil samples has accounted for all the uptake because the concentration ratio was used to calculate the rate of uptake. This is the ratio of the Pb concentration (mg/kg) in the whole rice plant and the Pb concentration (mg/kg) in the soil samples. The concentration ratio represents what is in the rice (whole rice) and what is in the soil. The soil may not be the only source but the amount of Pb concentration in the soil is a good representative of the amount of Pb that has been deposited from all sources (Kovacheva et al., 2000; Alloway, 1990).

The seed Pb concentration (mg/kg) showed positive correlation with the root Pb concentration (mg/kg), the husk Pb concentration (mg/kg) and the soil Pb concentration (mg/kg) at all the sampling depths. The result of the Pearson correlation (2tailed) is presented in Table 5.2. There were other relationships found between the CRs and the TFs with the soil properties. This is presented in Table 5.3

Table 5. 2: Result of the correlation analysis using Pearson test (2tailed)

	Pb-Root	Pb-Shoot	Pb-Husk	Pb-Seed	Soil10Pb	Soil20Pb	Soil30Pb	PbCR10cm	PbCR20cm	PbCR30cm	Root/ Soil	Shoot/ Root	Husk/ Root	Seed/ Root
Pb-Root	1													
Pb-Shoot	0.656**	1												
Pb-Husk	0.635**		1											
Pb-Seed	0.686**		0.857**	1										
Soil10Pb	0.839**		0.664**	0.749**	1									
Soil20Pb	0.824**		0.633**	0.718**	0.988**	1								
Soil30Pb	0.790**		0.572**	0.660**	0.975**	0.978**	1							
PbCR10cm	-0.308**				-0.284*	-0.254*	-0.269*	1						
PbCR20cm	-0.320**				-0.281*	-0.273*	-0.269*	0.978**	1					
PbCR30cm	-0.305**				-0.274*	-0.261*	-0.276*	0.984**	0.986**	1				
Root/Soil	-0.298**		-0.414**	-0.555**	-0.515**	-0.487**	-0.471**	0.404**	0.408**	.410**	1			
Shoot/Root	-0.373**	0.873**	-0.230*	-0.221*	-0.227*							1		
Husk/Root	-0.373**	0.873**	-0.230*	-0.221*	-0.227*							1.000**	1	
Seed/Root			0.533**	0.695**				0.580**	0.572**	0.601**	-0.377**			1

Table 5. 3: Relationship between the soil parameters, the concentration ratio and the translocation factors

Soil	Concentration Ratio (CR)				Translocation Factors (TF)		
Parameters	**PbCR10cm**	**PbCR20cm**	**PbCR30cm**	**Root/Soil**	**Shoot/Root**	**Bran/Root**	**Seed/Root**
Soil10Pb	**-0.284***	**-0.281***	**-0.274***	**-0.515****	**-0.227***	**-0.227***	0.201
Soil20Pb	**-0.254***	**-0.273***	**-0.261***	**-0.487****	-0.213	-0.213	0.184
Soil30Pb	**-0.269***	**-0.269***	**-0.276***	**-0.471****	-0.182	-0.182	0.141
Soil10Zn	**-0.308****	**-0.320****	**-0.305****	**-0.298****	**-0.373****	**-0.373****	0.018
Soil20Zn	**-0.307****	**-0.320****	**-0.304****	**-0.298****	**-0.372****	**-0.372****	0.018
Soil30Zn	**-0.308****	**-0.320****	**-0.305****	**-0.298****	**-0.373****	**-0.373****	0.018
Soil10Phos	-0.215	**-0.228***	**-0.244***	**0.534****	0.096	0.096	**-0.757****
Soil20Phos	-0.213	**-0.226***	**-0.243***	**0.536****	0.095	0.095	**-0.758****
Soil30Phos	**-0.265***	**-0.286***	**-0.295****	**0.502****	0.112	0.112	**-0.747****
Soil10Mg	**0.241***	**0.248***	**0.239***	0.19	-0.057	-0.057	0.097
Soil20Mg	**0.236***	**0.243***	**0.241***	0.183	-0.124	-0.124	0.113
Soil30Mg	**0.220***	**0.232***	0.217	0.092	-0.047	-0.047	0.135
Soil10ExAc	-0.104	-0.118	-0.138	**-0.235***	-0.067	-0.067	0.12
Soil20ExAc	-0.131	-0.147	-0.158	**-0.266***	-0.159	-0.159	0.139
Soil30ExAc	-0.068	-0.091	-0.102	-0.181	-0.028	-0.028	0.093
Soil10CEC	0.198	0.193	0.172	0.119	0.083	0.083	0.109
Soil20CEC	0.204	0.196	0.184	0.111	-0.008	-0.008	0.146
Soil30CEC	0.199	0.193	0.174	0.085	0.073	0.073	0.136
OrgCSoil10	**-0.231***	**-0.240***	**-0.268***	**0.526****	0.121	0.121	**-0.753****
OrgCSoil20	**-0.231***	**-0.240***	**-0.268***	**0.526****	0.121	0.121	**-0.753****
OrgCSoil30	**-0.230***	**-0.238***	**-0.266***	**0.526****	0.121	0.121	**-0.752****
Soil10pH	**-0.233***	**-0.227***	-0.14	**0.468****	0.19	0.19	**-0.624****
Soil20pH	**-0.233***	**-0.227***	-0.194	**0.414****	0.167	0.167	**-0.614****
Soil30pH	**-0.233***	**-0.227***	**-0.257***	**0.381****	0.094	0.094	**-0.629****
Soil10N	**-0.272***	**-0.266***	**-0.295****	**0.420****	0.18	0.18	**-0.699****
Soil20N	**-0.272***	**-0.266***	**-0.295****	**0.420****	0.18	0.18	**-0.699****
Soil30N	**-0.272***	**-0.266***	**-0.295****	**0.420****	0.18	0.18	**-0.699****

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

The mean Pb concentration in all the rice seed samples (n=80) was 0.90±0.57 ranging from 0.24 to 2.81mg/kg. Among the four rice farms selected in terms of rice Pb concentration (mg/kg), farm B was also the highest with 1.07±0.75 (0.34 – 2.81) mg/kg followed by farm A with 1.05±0.53 (0.26 – 2.04) mg/kg, farm C with 0.744±0.53 (0.25 – 2.45) mg/kg and farm D with 0.72±0.36 (0.24 – 1.27) mg/kg for the rice seed. The Pb and its international permissible limits are presented in Table 5.4 which have been exceeded by the mean Pb concentration in both the rice and the soil samples

Table 5. 4: Pb and its permissible limits (standards) in soil and plant

Environmental media		US EPA mg/kg	EU mg/kg	WHO/FAO mg/kg	SON/EU mg/kg	Reference
Soil	Residential	400	100	100	-	United State Environmental Protection Agency (2016) FAO/WHO (2011) (Adams et al., 2001)
	Uncultivated and arable land	420	300	100	100	USDL (2004) Khan, Khan, Khan, Qamar, and Waqas (2015)
Plant	Cereals (grains: rice, maize, husk, germ and rice)	0.2	0.2	0.3	0.2	Adams et al. (2001)
	Vegetables	-	0.3	-	0.3	Norton et al (2014)
	Fruit	-	0.1	0.3	0.3	(Abubakar, Bagudo, Birnin Yauri, Sahabi, & Garba, 2015)
	Tubers	-	0.3	0.3	0.3	EC (2006)
	Animal feed	-	-	10.0	-	WHO/FAO, (2001)
Animal product	Red meat and Poultry	-	0.1	0.1	0.1	EU, (2006)

*SON is Standard Organisation of Nigeria

For the Pb concentration (mg/kg) in the shoot, the mean Pb concentration generally across all shoot samples (n=80) was 6.34±4.30 ranging between 1.06 and 16.95 mg/kg. Farm D was the highest in terms of the Pb concentration in the rice shoot with 7.69±4.91 (1.77 – 15.82) mg/kg followed by the farm C with 7.54±3.94 (1.75 – 15.80) mg/kg, farm B with 5.16±3.48 (1.85 – 13.90) mg/kg and farm A with 4.98±4.30 (1.06 – 16.95) mg/kg. Though the mean Pb-concentration values in the rice shoot from the four selected rice farms were within the WHO/FAO limits of 10 mg/kg in almost all the samples. Similar results were previously presented by Liu et al., (2003) and Lee et al., (2016).

The Pb partitioning within the soil to root region was the highest and the lowest was found within the root and the seed. In the overall result for all samples (n=80), the Pb partitioning rate which can also be referred to as translocation factor (TF) of 0.16±0.08 (16%) was observed at the root (root/soil) followed by the shoot (shoot/root) with 13% [0.13±0.10 (0.02 – 0.46)], then the husk (husk/root) with 13% [0.13±0.10 (0.02 – 0.46)], and the seed (seed/root) with 2% [0.02±0.01 (0.003 – 0.042)]. The TF for the shoot and the husk appeared similar generally. This may be because those plants parts are very close to each other on the rice plant and the rate at which the nutrients are translocated within their tissue may be similar. On the farm A, the TF for the root was 13% (0.13±0.05), was 10% (0.10±0.11) for both the shoot and the husk respectively while only 1% (0.01±0.01) was obtained for the seed. Farm B was slightly different as the TF for the root was 14% (0.14±0.08), was 9% (0.09±0.07) for both the shoot and the husk respectively while 2% (0.02±0.01) was obtained as well for the seed. The TF was higher on the farm D as 16% (0.16±0.08) was obtained for the root, 17% (0.17±0.12) each was recorded for both the shoot and the husk respectively while 2% (0.02±0.01) was obtained for the seed. The highest TF was from the farm C of which 19% (0.19±0.10) was obtained for the root, 15% (0.15±0.09) each was recorded for both the shoot and the husk respectively while 1% (0.01±0.01) was obtained for the seed. This sequence was similar to what Wang, Wang, Gao, and Wang (2016) reported for Chinese rice.

The multivariate "within the effect test" provided a significant Pb-partitioning within the subject effect (root, shoot husk and seed) for the Pb partitioning within bisalayi rice grown on four different Pb contaminated soil. All the multivariate within the effect test yielded the same result but the most commonly reported is Wilks' Lambda. The Wilks' Lambda was 0.94, F (3, 77) = 248.10, $p<0.01$, and $n^2 = 0.91$. Therefore, there is a significant evidence to reject the null hypothesis and it is concluded that a reliable statistically significant effects was provided. Based on this test using degree of freedom of 3 and with an observed power of 1.000, the Pb partition among the rice parts were significantly different ($p<0.01$). The result is presented in Table 5.6 with its Wilk Lamba values.

Table 5. 5: Result of multivariate within effect test confirming significant difference in the Pb partitioning.

	Value	**F**	**Hypothesis df**	**Error df**	**Sig. (*p*-value)**	**Partial Eta Squared**	**Noncent. Parameter**	**Observed Power[b]**
Pillai's trace	0.906	248.102[a]	3.000	77.000	0.000	0.906	744.306	1.000
Wilks' lambda	0.094	248.102[a]	3.000	77.000	0.000	0.906	744.306	1.000
Hotelling's trace	9.666	248.102[a]	3.000	77.000	0.000	0.906	744.306	1.000
Roy's largest root	9.666	248.102[a]	3.000	77.000	0.000	0.906	744.306	1.000

Each F tests the multivariate effect of Pb-partitions. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means. a = Exact statistic, b = Computed using alpha = 0.05

5.5 Differences in the Pb concentrations of the different rice parts

The Pb accumulation, and the partitioning in the rice parts (root, shoot, husk and rice) were significantly different ($p<0.05$) but no significant difference statistically found in Pb accumulation, and the partitioning among the different selected rice farms. No significant difference found in the concentration ratios (CR) among all the selected rice farms (Table 5.7). This indicated that the Pb uptake and the accumulation in the rice plants were similar in all the farms in Dareta village. The Pb concentration in the root was the highest and it agreed with the previous studies that confirms more than 50% of the Pb taken by rice plants remains in the rice roots due to the retention capacity of the root (Lee et al., 2016; Kibria et al., 2006).

Table 5. 6: Result of the Duncan multiple range post ANOVA test

Selected Farms	Pb in rice root (mg/kg)	Pb in rice shoot (mg/kg)	Pb in rice husk (mg/kg)	Pb in rice seed (mg/kg)	CR at 0-10cm depth	CR at 0-20cm depth	CR at 0-30cm depth	Soil to Root TF	Soil to shoot TF	Soil to husk TF	Soil to seed TF
A	a	a	a	a	a	a	a	b	b	b	a
B	a	a	a	a	a	a	a	ab	ab	ab	a
C	a	a	a	a	a	a	a	ab	ab	ab	a
D	a	a	a	a	a	a	a	a	a	c	a

The reason for the high Pb in the root is likely to be due to adsorption than mobility in the plant according to Bonnano (2011) that examined accumulation and distribution of trace elements including Pb in the common reed. Reed and rice are the same family of grasses. According to MacFarlane (2007), metal accumulation and partitioning patterns are similar across the same families in plants. Also, the variation and the differences in the Pb concentration accumulated in the various rice part may be as a result of the regulatory metabolic control in the plant, to prevent toxicity in the rice plant (MacFarlane 2007).

5.6 Conclusion

A decreased transfer rate was observed in bisalayi rice from the root region towards the seed. It was found that the Pb accumulation, and the partitioning in the rice parts (root, shoot, husk and rice) were significantly different. The Pb concentration in the root was very high while that of the seed was very low though the mean Pb concentration in the seed was higher than the EU (0.2 mg/kg) and FAO/WHO (0.3 mg/kg) standard permissible limit of Pb in the grains. The shoot had its mean Pb concentration (6.3 mg/kg) within the FAO/WHO standard permissible limit of 10 mg/kg dry mass. This shows that it is safe for the farmers to use as fodder to feed their farm animals such as goats, sheep and cattle with this vegetative rice parts.

The root was the highest accumulator among the different parts of the rice plants. The result shows that rice is a root bio-accumulator of Pb and soil was found to be the major source of Pb in the sampled rice. Mobility and translocation of Pb was higher from the root to the shoot and lowest between the husk and the seed as the seed accumulate at the lowest rate. This suggest a strong metabolic system that restricts toxic elements from translocating into the seed. The positive correlation of Pb concentration between the soil and the whole rice plant (Figure 5.1) is useful for pollution monitoring of Pb in rice especially in an area that is known to be polluted with Pb and also produce rice at commercial quantity. If the rice root could be removed after every rice harvesting and its Pb content could be recovered or disposed off appropriately, rice plant may be considered promising for bioremediation alternative for Pb contaminated soil.

CHAPTER SIX

Inter-varietal Variation in Pb Uptake (Field and Pot Experiment)

6.0 Materials and Methods.

The method involved in this study is presented in chapter 3, from section 3.5.

6.1 Data analysis and few other calculations

The result for the Pb concentration (mg/kg) in the rice samples were presented in arithmetic mean together with the standard deviation (SD). The significance level was set at $p<0.05$ and 0.01 to present the result. The concentration ratio (CR) was calculated by dividing the concentration of Pb (mg/kg) dry mass in the rice samples by the concentration of Pb (mg/kg) dry mass in the soil samples. The inter-varietal variation (IVV) of Pb in rice was calculated by dividing the mean Pb concentration ratio of the highest accumulated rice variety by the mean Pb concentration ratio of the lowest accumulated rice variety. Other calculations have been stated in chapter 3. A one-way analysis of variance (ANOVA) was carried out to detect significant statistical differences the inter-varietal variation that exist among the 10 varieties using the mean Pb concentration. For the RCBD used for rice planting (chapter 3, section 3.5.3.5), two-way ANOVA was used to check for the block effect on the experiment. The data were checked for normality and homogeneity of variance before ANOVA and correlation analysis was conducted using a Pearson correlation test (2-tailed).

6.2 Result and discussion

The result of the percentage recovery of Pb (n=6), the Limit of detection (LOD), and the limit of quantification (LOQ) are presented in Table 6.1.

Table 6. 1: Result of the LOD, LOQ and the percentage recovery of Pb

	LOD	**LOQ**	**% Recovery NIST 1568b**	**% Recovery NIST 2711a**
Pb (mg/L)	0.05	0.17	110	120

6.2.1 Inter-varietal variation of Pb in the field experiment

The mean Pb concentration in the rice was 0.74±0.33 mg/kg which varied across the varieties (1-10) ranging between 0.03 mg/kg and 2.51 mg/kg. In the soil, the mean concentration of Pb generally was 285.45±465.62 mg/kg, (ranges from 0.47 to 1468.37) mg/kg. The soil physico-chemical parameters are presented in Table 6.2. Statistical summary of the result is presented in Table 6.3. The soil was majorly dark yellowish brown and Sandy-loam based on the Munsel colour chart analysis and the textural test conducted using soil-texture triangle (Appendix D, table III and IV). Regarding the randomised complete block design (RCBD) used in the rice planting, it is recommended to check the block effect if the treatment is assigned randomly to the experimental units within a block and maybe a block missed one or more treatment (Clovis, 2007; Liu and Berger, 2014). In this study, the blocks/replicates of the rice varieties were treated the same and no block or replicate missed in both the field and in the pot experiment.

Table 6. 2: Result for the soil physico-chemical parameters for the field experiment.

Soil (n=300)	pH (H_2O) 1:1	^{a}EC (dS/m)	%Organic Carbon	Av-P_2O_2	Exchangeable cations (cmol /kg)				Exchangeable Acidity	CEC	Particle size			N	Pb (mg/kg)
					Ca	K	Mg	Na			%Clay	%Silt	%Sand		
Mean	6.58	1.19	4.00	7.77	1458.58	1400.28	1759.41	27.12	0.65	26.10	46.63	39.74	45.63	3.12	1759.41
SD	0.86	0.36	0.90	2.84	307.33	707.97	586.80	46.37	0.19	7.89	7.26	14.64	16.84	1.59	586.80
Min	4.51	0.3	1.47	0.620	971.64	318.55	684.68	0.05	0.34	12.85	2.00	2.00	11.50	1.32	684.68
Max	8.51	2.5	7.67	14.00	2505.62	3046.49	3015.34	296.82	2.37	43.32	37.4	69.1	94.00	7.25	3015.34

SD = Standard Deviation, Max = Maximum, Min = Minimum, n= Number of samples, EC= Electrical Conductivity, Av-P_2O_2 = Available Phosphorus, N = Nitrogen

Table 6. 3: Summary of the result of Pb concentrations (mg/kg) in the 10 rice varieties and their corresponding soil samples.

SD = Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples.

Rice Varieties		Parameters	Pb
IRAT_170	RICE	Mean± SD	1.12±0.010
Variety 1	(N=30)	Min, Max	0.97, 1.34
		CV	0.009
	SOIL	Mean± SD	299.91±500.63
	(N=30)	Min, Max	0.57, 1375.32
		CV	1.67
SIPI_692033	RICE	Mean ±SD	0.63±0.20
Variety 2	(N=30)	Min, Max	0.38, 1.28
		CV	0.31
	SOIL	Mean ±SD	309.04±471.66
	(N=30)	Min, Max	0.89, 1333.67
		CV	1.53
ITA_315	RICE	Mean ±SD	0.87±0.32
Variety 3	(N=30)	Min, Max	0.45, 1.48
		CV	0.37
	SOIL	Mean	247.41±456.31
	(N=30)	Min, Max	0.78, 1290.65
		CV	1.84
WITA_4	RICE	Mean± SD	0.61±0.26
Variety 4	(N=30)	Min, Max	0.36, 1.38
		CV	0.42
	SOIL	Mean± SD	290.05±469.44
	(N=30)	Min, Max	0.47, 1247.60
		CV	1.62

Rice Varieties		Parameters	Pb
NERICA_L19	RICE	Mean± SD	0.72±0.33
Variety 5	(N=30)	Min, Max	0.42, 1.70
		CV	0.45
	SOIL	Mean± SD	314.21±479.07
	(N=30)	Min, Max	0.80, 1311.20
		CV	1.52
NERICA_L34	RICE	Mean± SD	0.56±0.33
Variety 6	(N=30)	Min, Max	0.14, 1.24
		CV	0.59
	SOIL	Mean± SD	258.88±452.78
	(N=30)	Min, Max	0.97, 1319.30
		CV	1.75
NCRO_49	RICE	Mean± SD	0.92±0.35
Variety 7	(N=30)	Min, Max	0.23, 2.51
		CV	0.38
	SOIL	Mean± SD	286.39±509.54
	(N=30)	Min, Max	0.90, 1468.37
		CV	1.78
ART3_7L	RICE	Mean± SD	0.88±0.26
Variety 8	(N=30)	Min, Max	0.58, 1.40
		CV	0.30
	SOIL	Mean± SD	306.38±487.68
	(N=30)	Min, Max	0.88, 1422.74
		CV	1.59

Rice Varieties		Parameters	Pb
ART_15	RICE	Mean± SD	0.74± 0.14
Variety 9	(N=30)	Min, Max	0.53, 1.27
		CV	0.19
	SOIL	Mean± SD	274.50±461.14
	(N=30)	Min, Max	0.65, 1338.46
		CV	1.68
BISALAYI	RICE	Mean± SD	0.38±0.18
Variety 10	(N=30)	Min, Max	0.03, 1.13
		CV	0.46
	SOIL	Mean± SD	267.71±429.17
	(N=30)	Min, Max	0.71, 1308.75
		CV	1.60
Total	RICE	Mean± SD	0.74±0.33
Average	(N=300)	Min, Max	0.03, 2.51
		CV	0.44
	SOIL	Mean± SD	285.45±465.62
	(N=300)	Min, Max	0.47, 1468.37
		CV	1.63

Pb concentration (mg/kg) was significantly different ($p < 0.05$) among all the 10 rice varieties but similar ($p>0.05$) in the soil samples across all replicates on the field. This means that the varieties behaved differently in terms of Pb accumulation despite the similar soil conditions. A wide variation in Pb concentration (min 0.03 and max 1.13 mg/kg) was found in the local variety, bisalayi rice (variety 10) and the minimum mean Pb concentration was recorded for the same variety. The highest Pb concentration was found in Irat-170 (variety 1) and the mean Pb concentration recorded for the 10 selected rice varieties (0.74±0.33 mg/kg) in this study is similar to 0.73 mg/kg Pb concentration previously reported for the same rice in Zamfara by Simba et al. (2018). This previous study collected its rice samples from Bagega village, a neighbouring village to Dareta, used similar method of analysis (ICP-MS) and Bagega village shares similar contamination record with Dareta village, the study site for this research. The result of our study is also similar to that of Norton et al. (2014) who reported mean Pb concentration of 0.68±0.80 mg/kg for Chinese rice. Our result was lower than the mean Pb concentration reported for market rice samples (2.70 mg/kg) in Nigeria by Adedire et al. (2015) and it was higher than the one reported for Ghanaian rice (0.007±0.01 mg/kg) and rice from USA (0.021±0.31 mg/kg) by Norton et al. (2014). Also, Brazilian rice (0.185±0.05 mg/kg), Thailand rice (0.383±0.01 mg/kg), and Vietnamese rice (0.308±0.01 mg/kg) reported by Otitoju, Otitoju, and Igwe (2014).

All the rice varieties from the field accumulated Pb above the standard international permissible limit. The standard international permissible limit for the Pb in rice is 0.2 mg/kg (EC, 2001, 2006; USEPA, 2000) and 0.3 mg/kg (FAO/WHO, 2001, 2011). The result of the Duncan multiple range test for Pb concentration (mg/kg) in all the rice samples (n=300) at $p<0.05$ confidence level shows that 3 varieties (Ita-315 (variety 3), ncro-49 (variety 7) and art3-7L (variety 8)) were statistically not different from each other. Sipi-692033 (variety 2), wita-4 (variety 4), Nerica-L19, Nerica-L34 and art-15 appear to be similar statistically too. All these are revealed in Figure 6.1. Different letters in the data labels shows that the rice varieties were different statistically and same letters means they are not different statistically. Based on the result for the soil parameters (Table 6.2) also discussed in chapter 4, (table 4.2), the soil was slightly acidic and therefore, mobility and bio-availability of Pb in the soil was suspected to be enhanced according to Khan et al. (2015). Acidic soil supports bioavailability of Pb in the soil. The Pb accumulation among the rice varieties was in the order of bisalayi < Nerica-L34 < Wita-

4 < Sipi-692033 < Nerica-L19 < Art-15 < Ita-315 < Art3-7L < Ncro-49 < Irat-170. The inter-varietal variation (IVV) obtained for Pb among the 10 rice varieties was 2.71 folds.

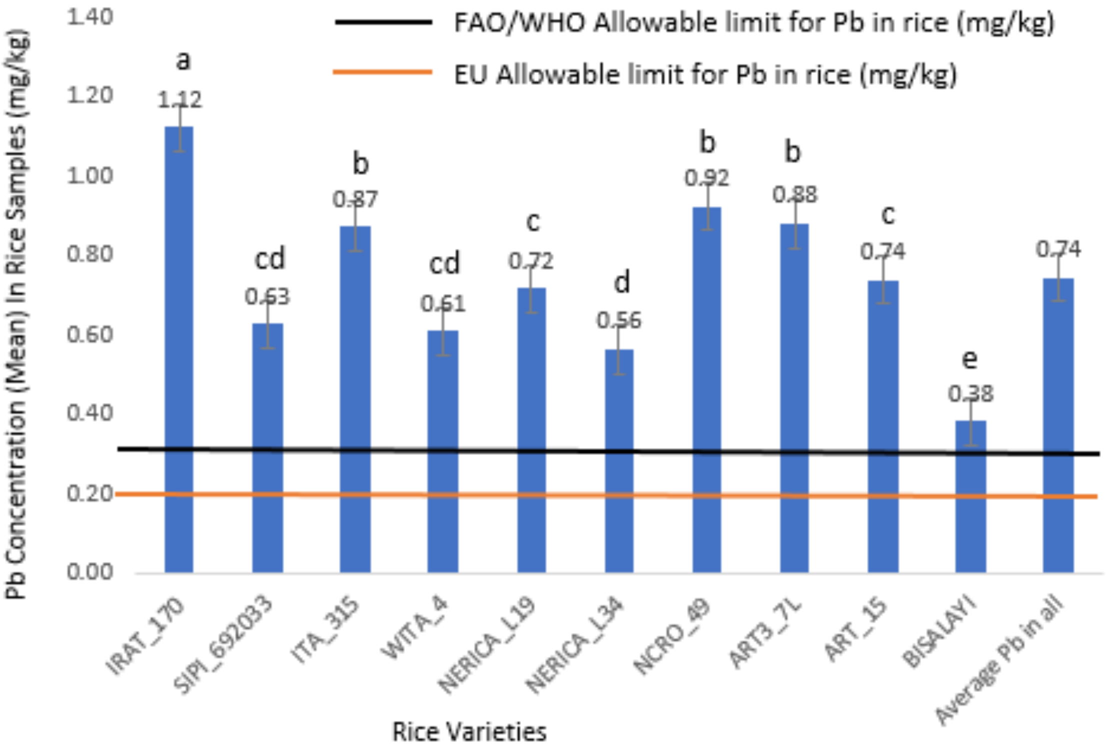


Figure 6. 1: Pb concentration in rice samples in comparison with the standard international permissible limit together with the result of the Duncan multiple range post-hoc test.

6.2.2 Inter-varietal variation of Pb in the pot experiment

The mean Pb concentration in the rice was higher (1.56 mg/kg) in pot experiment while a bit lower in the field experimental result (0.74±0.33 mg/kg) but the two were above the European Union (EU), United State Environmental Protection Agency (USEPA) standard permissible limit of 0.2 mg/kg for the Pb in rice (EC, 2001, 2006; USEPA, 2000) and the joint World Health Organization (WHO) and Food and Agriculture Organisation (FAO) of the United Nation standard permissible limit of 0.3 mg/kg (FAO/WHO, 2001, 2011). This is revealed in Figure 6.2.

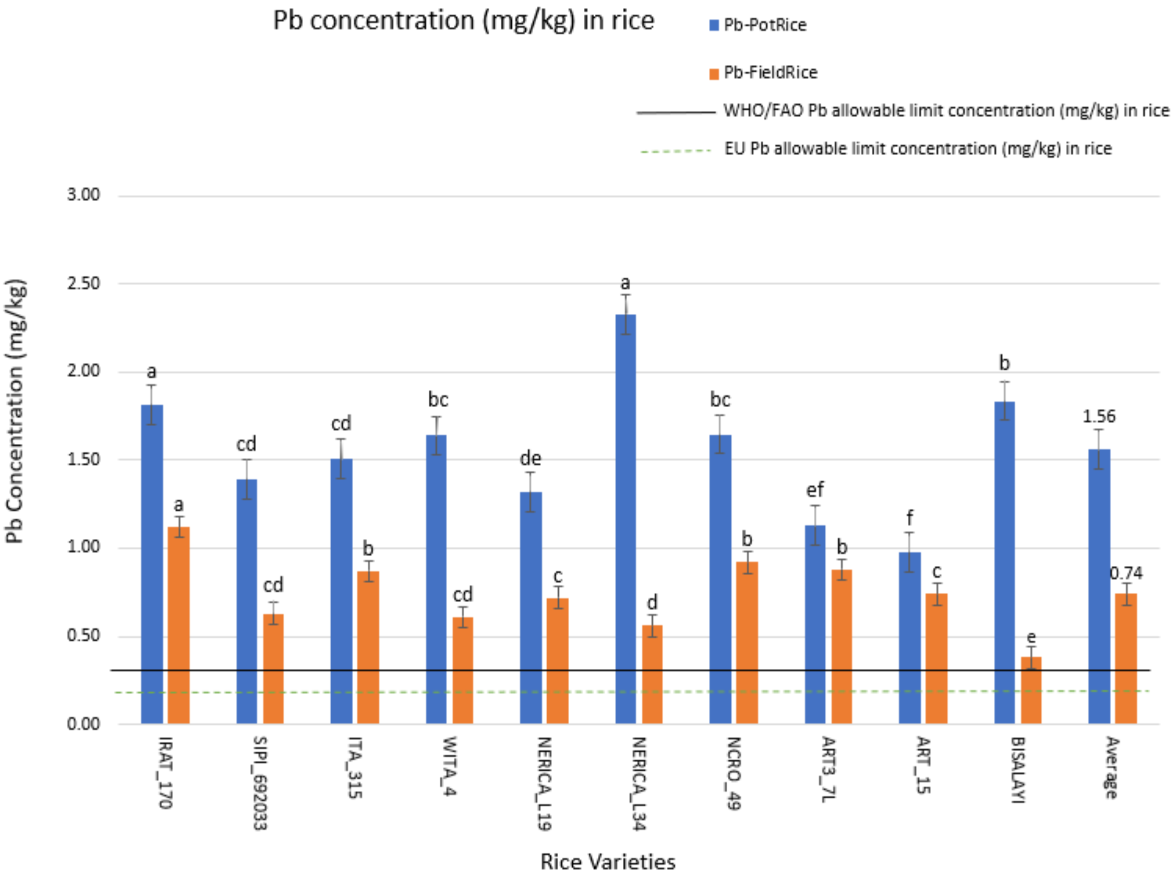


Figure 6.2: Pb concentration in rice samples (field and pot experiment) in comparison with the international permissible limit.

The Pb concentration in rice was from 0.01mg/kg to 7.93mg/kg in the pot trial among the 10 selected rice varieties. The highest Pb concentration (mg/kg) in rice in the pot experiment was found in Nerica-L34 with 2.33±1.25 mg/kg ranging from 0.00 mg/kg to 7.93 mg/kg while the highest Pb concentration (mg/kg) observed in rice in the field trial was Irat-170 with 1.12±0.010 mg/kg ranging from 0.97 mg/kg to 1.34 mg/kg. Based on the Duncan multiple range test, these two varieties (Nerica-L34 and Irat-170) were not significantly different. The lowest Pb accumulator in the field trial was bisalayi rice with 0.38±0.18 mg/kg ranging from 0.03 mg/kg to 1.13 mg/kg and the lowest Pb in rice in the pot experiment was Art-15 rice with 0.98±0.19 mg/kg ranging from 0.84 mg/kg to 1.90 mg/kg and there was a significant different statistically among these two varieties following the result of the Duncan post ANOVA test. Art-15 and Bisalayi rice were significantly different in both the field and the pot experiment respectively. In both experiments, the Pb concentration in rice across the 10 selected rice varieties was significantly different ($p < 0.05$) but Pb concentration is similar ($p > 0.05$) across all the soil samples in both experiments. The result shows that the soil used for the pot experiment was more acidic than the field soil (Table 6.2 and 6.4). Soil physico-chemical properties (parameters) of the soil were examined before planting (pre-planting) and after the rice growing (post-planting) and this is presented in Table 6.4. The statistical result summary of the pot experiment for Pb concentrations (mg/kg) in the 10 Nigerian rice varieties and their corresponding soil samples as measured by the ICP-MS is presented in Table 6.5. The soil samples were dark in colour and similar in texture with the soil samples from the field (Appendix D, Table III and IV). The pre-planting soil samples were 60 (n=60) while post planting soil samples were 300 (n=300) in the pot experiment. In the soil, the mean concentration of Pb in the pot experiment was 1859.25 ± 282.60 mg/kg, ranging from 0.01 mg/kg to 2546.98 mg/kg while the mean concentration of Pb in the field soil was 285.45±465.62 mg/kg, ranging from 0.47 mg/kg to 1468.37 mg/kg. The inter-varietal variation (IVV) obtained for Pb among the 10 rice varieties in the pot experiment was 2.4 folds.

Table 6. 4: Result of the soil physico-chemical analysis before and after the pot experiment.

	Soil (n=60) (n=300)	**pH (H_2O) 1:1**	**^{a}EC (dS/m)**	**%Organic Carbon**	**Av-P_2O_2**	**Exchangeable cations (cmol /kg)**				**Exchangeable Acidity**	**CEC**	**Particle size**			**N**	**Pb (mg/kg)**
						Ca	**K**	**Mg**	**Na**			**%Clay**	**%Silt**	**%Sand**		
Pre Planting Soil Analysis	Mean	5.75	0.17	2.65	8.85	7.69	0.08	2.37	0.04	1.38	11.58	5.96	12.77	81.23	3.50	35.17
	SD	0.49	0.19	0.72	7.08	1.61	0.03	0.50	0.01	0.32	2.14	2.30	3.56	4.50	0.52	5.40
	Min	4.17	0.05	1.38	1.33	5.00	0.10	1.50	0.02	1.06	7.81	3.36	3.94	68.68	0.08	24.04
	Max	6.29	1.12	6.01	26.32	12.00	0.12	4.00	0.06	2.72	18.83	15.46	19.94	90.72	8.50	44.96
Post Planting Soil Analysis	Mean	5.76	0.98	2.63	7.94	1789.81	2015.39	2129.36	26.50	0.66	32.55	7.35	20.29	72.36	2.49	1859.25
	SD	0.70	0.52	0.72	4.17	304.93	230.46	248.05	36.27	0.17	3.21	2.72	5.90	6.66	1.84	282.60
	Min	3.98	0.05	1.35	0.62	0.03	0.05	0.03	0.05	0.36	23.03	3.36	3.94	55.62	0.06	0.01
	Max	7.38	2.30	6.23	26.32	3191.93	2662.48	2889.50	278.75	2.37	44.88	16.49	37.98	90.72	7.25	2546.98

SD = Standard Deviation, Max = Maximum, Min = Minimum, n= Number of samples, EC= Electrical Conductivity, Av-P_2O_{2} = Available Phosphorus, N = Nitrogen

Table 6. 5: Result summary of Pb concentrations (mg/kg) in the 10 rice varieties and their corresponding soil samples as measured by ICP-MS.

Rice Varieties	Sample	Parameters	Pb
IRAT_170	RICE	Mean± SD	1.82±0.11
Variety 1	(N=30)	Min, Max	1.61,2.06
		CV	0.06
	SOIL	Mean± SD	1789.31±388.26
	(N=30)	Min, Max	383.11, 2423.08
		CV	0.22
SIPI_692033	RICE	Mean ±SD	1.39±0.19
Variety 2	(N=30)	Min, Max	1.16, 2.08
		CV	0.13
	SOIL	Mean ±SD	1902.74±252.71
	(N=30)	Min, Max	1516.11±2546.98
		CV	0.13
ITA_315	RICE	Mean ±SD	1.51±0.19
Variety 3	(N=30)	Min, Max	1.29, 2.29
		CV	0.12
	SOIL	Mean	1807.62±423.21
	(N=30)	Min, Max	0.01, 2413.09
		CV	0.23
WITA_4	RICE	Mean± SD	1.64±0.29
Variety 4	(N=30)	Min, Max	0.47, 2.45
		CV	0.18
	SOIL	Mean± SD	1862.56±245.97
	(N=30)	Min, Max	1306.10, 2278.15
		CV	0.13
NERICA_L19	RICE	Mean± SD	1.32±0.27
Variety 5	(N=30)	Min, Max	1.07, 2.29
		CV	0.20
	SOIL	Mean± SD	1937.72±233.85
	(N=30)	Min, Max	1498.62, 2445.57
		CV	0.12
NERICA_L34	RICE	Mean± SD	2.33±1.25
Variety 6	(N=30)	Min, Max	0.00, 7.93
		CV	0.54
	SOIL	Mean± SD	1901.65±193.99
	(N=30)	Min, Max	1446.32, 2311.64
		CV	0.10
NCRO_49	RICE	Mean± SD	1.65±0.24
Variety 7	(N=30)	Min, Max	1.44, 2.66
		CV	0.15
	SOIL	Mean± SD	1851.45±256.10
	(N=30)	Min, Max	1232.23, 2306.13
		CV	0.14
ART3_7L	RICE	Mean± SD	1.13±0.08
Variety 8	(N=30)	Min, Max	0.95, 1.32
		CV	0.07
	SOIL	Mean± SD	1797.74±283.44
	(N=30)	Min, Max	1191.73, 2274.28
		CV	0.16
ART_15	RICE	Mean± SD	0.98±0.19
Variety 9	(N=30)	Min, Max	0.84, 1.90
		CV	0.20
	SOIL	Mean± SD	1899. 11±168.44
	(N=30)	Min, Max	1507.22, 2188.65
		CV	0.09
BISALAYI	RICE	Mean± SD	1.84±0.62
Variety 10	(N=30)	Min, Max	1.06, 4.79
		CV	0.34
	SOIL	Mean± SD	1842.59±280.48
	(N=30)	Min, Max	1313.51, 2403.47
		CV	0.15
Total	RICE	Mean± SD	1.56±0.60
	(N=300)	Min, Max	0.00, 7.93
		CV	0.38
	SOIL	Mean± SD	1859.25±282.60
	(N=300)	Min, Max	0.01, 2546.98
		CV	0.15

SD = Standard Deviation, Max = Maximum, Min = Minimum, N= Number of samples, CV= Coefficient of Variance.

6.3 Effect of flooding on the field experiment

There was a time during the experimental period (rice cultivation time) when the rainfall was at its peak, a part of the field was submerged in water. This means that some of the replicates lied within the flooded area for a short period (about 3 weeks). The temporary flooded area was towards the lower part (south) of the field. Whereas, some of the replicates were in an unflooded area of the field. The result shows that this condition impacted the soil samples only as the flooded part shows high Pb. This appears clearly in the spatial distribution of the Pb concentration in the soil as revealed in Figure 6.3. The samples collected within the flooded area of the field were analysed separately and then compared with the result of the unflooded area of the field.

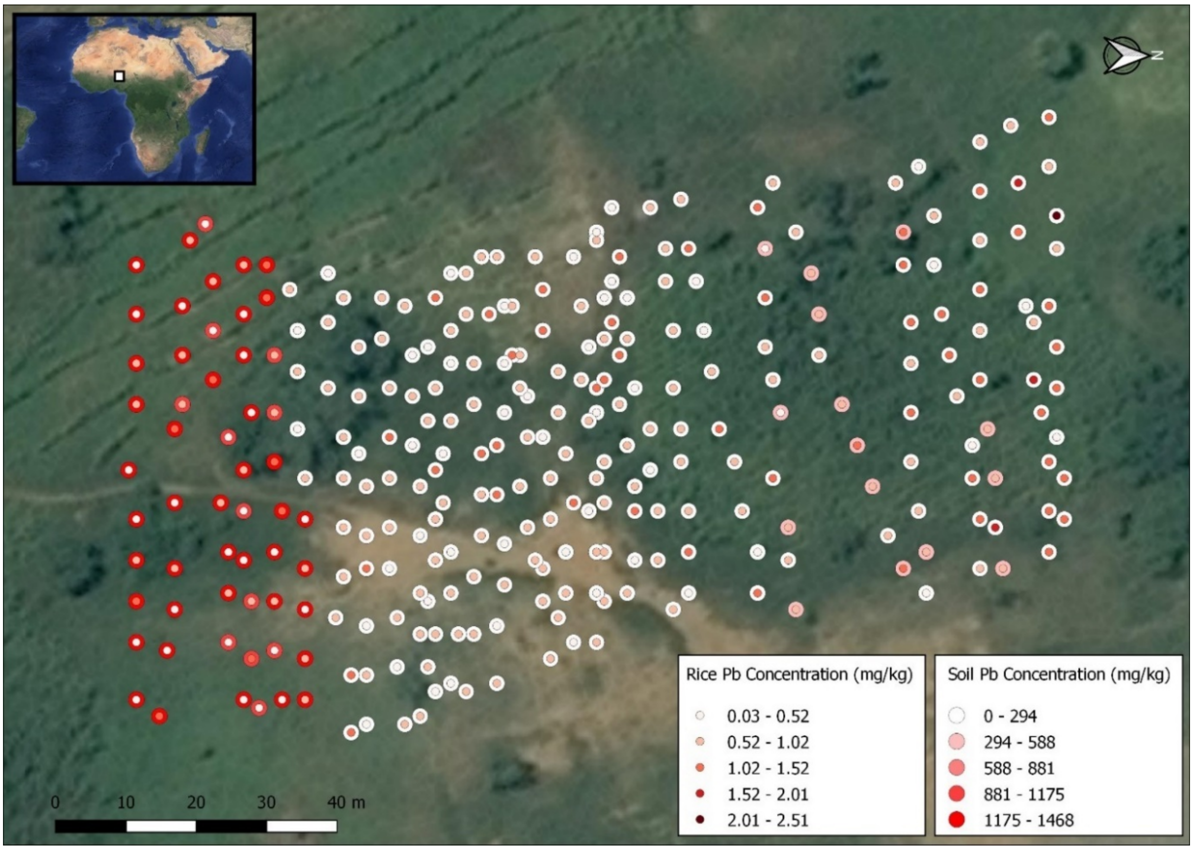


Figure 6. 3: Spatial distribution of Pb in rice and soil across the experimental farm in Dareta

The dots (coloured circles) in Figure 6.3 represent the planting/sampling points. The outer circle represents the Pb concentration in the soil (n=300) while the inner circle represents the Pb concentration in rice (n=300).

Statistically, there was no significant difference (p>0.05) between the two set of data (flooded and unflooded area) of the field experiment for Pb CR across the 10 varieties of rice, the Duncan multiple range post-hoc test confirms (Table 6.6). Also, the result shows no significant Pb uptake and accumulation in the rice samples collected from the flooded area of the field as expected. Generally, the inter-varietal variation (IVV) of Pb among the 10 selected rice varieties was 2.7 folds. The IVV recorded for the flooded area (2.2) was 18.5% lower while that of the unflooded area (7.5) was 177.7% higher compare to the general result. This shows that the 10 varieties of rice varied widely in the unflooded area of the field while this was not the case in the flooded area of the field.

The lowest mean CR of 0.07±0.14 was found in SIPI_692033 (variety 2) and Bisalayi (variety 10) respectively as the two varieties provided same CR while the highest mean CR of 0.22 ± 0.39 was observed in art-15 (variety 9). SIPI-692033 was originally from Taiwan and it was improved upon and adopted in Nigeria (Appendix A, Table I). Sipi is one of the improved rice varieties (Appendix A, Table I) and Bisalayi is the most popular local rice variety in the northern states and it has been with farmers for many years. The highest to lowest mean CR for the field experiment was found in the order of art-15 > irat-170 > ita-315 > art3-7L > wita-4 > nerica-L19 > nerica-L34 > ncro-49 > sipi-692033 > bisalayi. Summary of the result for the general mean concentration ratio (CR) for the field experiment, flooded and unflooded area of the field is presented in Table 6.7 while Figure 6.4 compares the general mean CR, mean CR for the flooded area and the mean CR for the unflooded area across the 10 selected rice varieties.

Table 6. 6: Result of the Duncan multiple range post-hoc test

Rice Varieties	N	Flooded area Pb-CR	Unflooded area Pb-CR
Irat_170 (1)	15	A	a
Sipi_692033 (2)	15	A	a
Ita_315 (3)	15	A	a
Wita_4 (4)	15	A	a
Nerica_L19 (5)	15	A	a
Nerica_L34 (6)	15	A	a
Ncro_49 (7)	15	A	a
Art3_7l (8)	15	A	a
Art_15 (9)	15	A	a
Bisalayi (10)	15	A	a
IVV	150	**2.2**	**7.5**

Result of the Duncan multiple range post-hoc test to check for disparity between the flooded and the unflooded field data and the inter-varietal variations among the 10 rice varieties. IVV = Inter-varietal variation, N=sample number, CR=Concentration Ratio. Different letters = significant difference, Same letters = no significant difference.

Table 6. 7: Mean Concentration Ratio (CR) of Pb across the 10 rice varieties for the field experiment.

General			Flooded Area		Unflooded Area	
Rice Varieties		**Pb-CR**	**Rice Varieties**	**Pb-CR**	**Rice Varieties**	**Pb-CR**
IRAT_170	Mean± SD	0.19±0.36	**IRAT_170**	0.13±0.16	**IRAT_170**	0.15±0.27
Variety 1	Min, Max	0.00, 1.73	Variety 1	0.00,0.58	Variety 1	0.00,0.79
(N=30)	CV	1.89	(N=15)	1.23	(N=15)	1.80
SIPI_692033	Mean± SD	0.07±0.14	**SIPI_692033**	0.24±0.44	**SIPI_692033**	0.03±0.06
Variety 2	Min, Max	0.00, 0.70	Variety 2	0.00,1.29	Variety 2	0.00,0.24
(N=30)	CV	2.01	(N=15)	1.83	(N=15)	2.00
ITA_315	Mean± SD	0.16±0.27	**ITA_315**	0.11±0.14	**ITA_315**	0.08±0.19
Variety 3	Min, Max	0.00, 0.84	Variety 3	0.00,0.46	Variety 3	0.01,0.74
(N=30)	CV	1.71	(N=15)	1.27	(N=15)	2.37
WITA_4	Mean± SD	0.13±0.32	**WITA_4**	0.19±0.45	**WITA_4**	0.04±0.06
Variety 4	Min, Max	0.00, 1.41	Variety 4	0.00,1.73	Variety 4	0.00,0.24
(N=30)	CV	2.54	(N=15)	2.37	(N=15)	1.50
NERICA_L19	Mean± SD	0.10±0.20	**NERICA_L19**	0.14±0.21	**NERICA_L19**	0.04±0.09
Variety 5	Min, Max	0.00, 0.84	Variety 5	0.00,0.70	Variety 5	0.00,0.36
(N=30)	CV	1.90	(N=15)	1.50	(N=15)	2.25
NERICA_L34	Mean± SD	0.10±0.16	**NERICA_L34**	0.17±0.26	**NERICA_L34**	0.08±0.12
Variety 6	Min, Max	0.00, 0.63	Variety 6	0.00,0.81	Variety 6	0.00,0.34
(N=30)	CV	1.57	(N=15)	1.53	(N=15)	1.50
NCRO_49	Mean± SD	0.09±0.18	**NCRO_49**	0.32±0.49	**NCRO_49**	0.04±0.04
Variety 7	Min, Max	0.00, 0.85	Variety 7	0.00,1.41	Variety 7	0.00,0.11
(N=30)	CV	1.86	(N=15)	1.53	(N=15)	1.00
ART3_7L	Mean± SD	0.13±0.23	**ART3_7L**	0.13±0.23	**ART3_7L**	0.14±0.27
Variety 8	Min, Max	0.00, 0.83	Variety 8	0.00,0.84	Variety 8	0.00,0.83
(N=30)	CV	1.71	(N=15)	1.77	(N=15)	1.92
ART_15	Mean± SD	0.22±0.39	**ART_15**	0.11±0.16	**ART_15**	0.15±0.35
Variety 9	Min, Max	0.00, 1.29	Variety 9	0.00,0.46	Variety 9	0.00,1.13
(N=30)	CV	1.83	(N=15)	1.45	(N=15)	2.33
BISALAYI	Mean± SD	0.07±0.13	**BISALAYI**	0.19±0.27	**BISALAYI**	0.02±0.04
Variety 10	Min, Max	0.00, 0.54	Variety 10	0.00,0.85	Variety 10	0.00,0.16
(N=30)	CV	1.93	(N=15)	1.42	(N=15)	2.00
Average	Mean± SD	0.13±0.26	**Average**	0.17±0.30	**Average**	0.08±0.19
(N=300)	Min, Max	0.00, 1.73	(N=150)	0.00,1.73	(N=150)	0.00,1.13
	CV	2.03		1.76		2.37

SD = Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples, CR = Concentration Ra

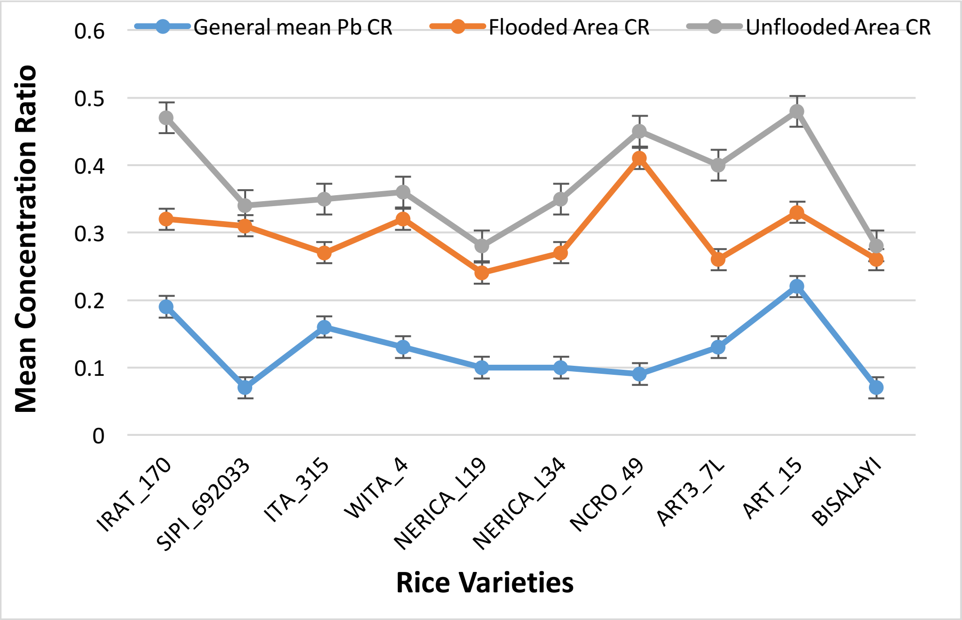


Figure 6.4: Comparing the mean CR of the flooded and unflooded area with the mean CR of the general field.

6.4 Behaviours of the rice varieties in both experiment (field and pot)

The behaviour recorded for the field experiment where the rice varieties behaved differently having similar soil conditions also repeated itself in the result of the pot experiment. The Pb concentration in rice was from 0.03 mg/kg to 2.51 mg/kg in the field trial while ranged from 0.01 mg/kg to 7.93 mg/kg in the pot trial among the 10 selected rice varieties. The highest Pb in rice in the field experiment was found in Irat-170 with 1.12±0.010 mg/kg ranging from 0.97 mg/kg to 1.34 mg/kg while the highest in the pot trial was found in Nerica-L34 rice with 2.33±1.25 mg/kg ranging from 0.00 mg/kg to 7.93 mg/kg. According to the Duncan multiple range test, the two rice varieties with the highest Pb content (Figure 6.5) in both experiments were significantly different from the lower accumulating counterparts (Table 6.8). The lowest in the field trial was bisalayi rice with 0.38±0.18 mg/kg ranging from 0.03 mg/kg to 1.13 mg/kg and the lowest Pb in rice in the pot trial was found in Art-15 rice variety with 0.98±0.19 mg/kg ranging from 0.84 mg/kg to 1.90 mg/kg. Art-15 and Bisalayi rice were significantly different in both the field and the pot trial.

Table 6. 8: Result of the Duncan multiple range post-hoc test for both the field and pot experiments.

Rice Varieties	N	Field	Pot
1. IRAT-170	30	a	b
2. SIPI-692033	30	cd	cd
3. ITA-315	30	b	cd
4. WITA-4	30	cd	bc
5. NERICA-L19	30	c	de
6. NERICA-L34	30	d	a
7. NCRO-49	30	b	bc
8. ART3-7L	30	b	ef
9. ART-15	30	c	f
10. BISALAYI	30	e	b

Different letters indicate significant differences in the Pb concentrations in rice among the rice varieties and same letters indicates no significant difference.

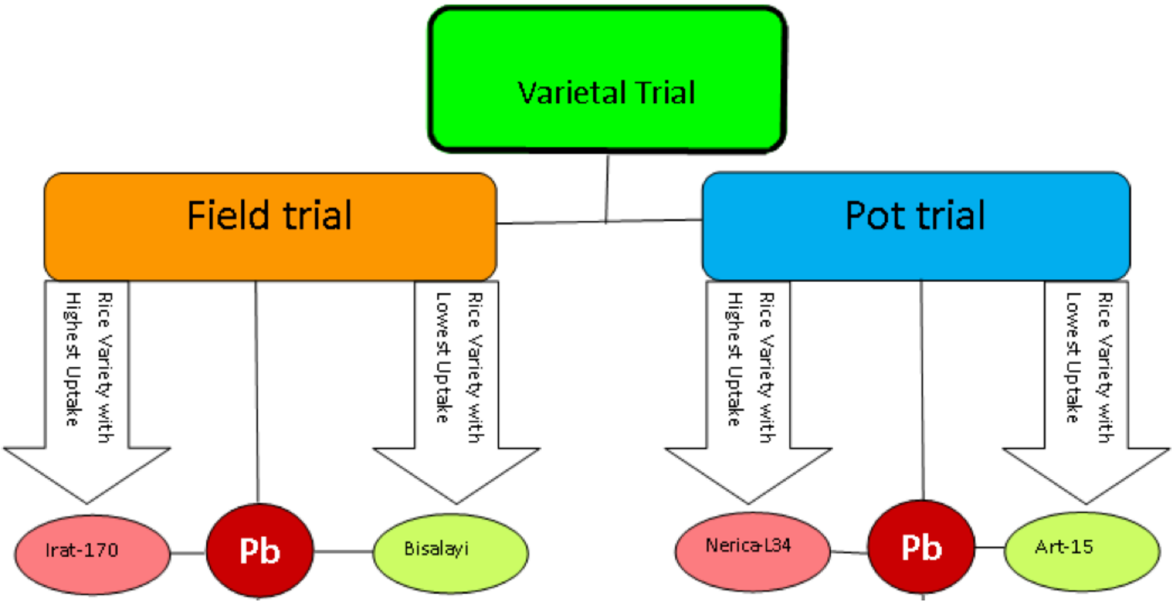


Figure 6.5: A diagrammatic representation of the rice varieties with the highest and the lowest Pb content. Red colour = not beneficial, green colour = beneficial to human.

6.4.1 Concentration ratio (CR)

In the field experiment, the mean Pb concentration ratio (CR) in all the 10 rice variety samples was observed to be 0.13±0.26 (min 0.07, max 1.73). The highest CR was found in art-15 (variety 9) and it was in the orders of art-15 > irat-170 > ita-315 > art3-7L > wita-4 > nerica-L19 > nerica-L34 > ncro-49 > sipi-692033 > bisalayi. The lowest of 0.07±0.14 was found in SIPI_692033 (variety 2) and Bisalayi (variety 10). These two varieties provided similar CR. SIPI-692033 was originally from Taiwan, it was improved upon genetically and has been adopted in Nigeria for more than 5 decades (Appendix A, Table I). Bisalayi is the local rice variety in Nigeria, Zamfara state and it has been with farmers for many years too (Ejebe, Danbaba, & Ngadi, 2015).

In the pot trial, the mean concentration ratio (CR) of 0.0009±0.0004 was observed across the 10 rice varieties which is very low compare to that of the field experiment (0.13). This was expected because the result shows that the soil mean Pb concentration (mg/kg) in the soil was higher (1859.25±282.60 mg/kg) in the pot experiment compare to that of the field experiment (285.45±465.62 mg/kg). These were presented previously in Table 6.3 and Table 6.5. From the pot experiment, the highest to the lowest CR was in the orders of Nerica-L34 > Irat-170 > Bisalayi > Ncro-49 > Wita-4 > Ita-315 > Sipi-692033 > Nerica L19, Art3-7L > Art-15 respectively. The highest mean Pb CR of 0.22±0.39 in the field samples was found in Art-15 rice and the highest of 0.0012±0.0006 was found in Nerica-L34 rice for the pot experiment (Figure 6.6) while the lowest in the field experiment was found in bisalayi rice and Art-15 rice respectively as revealed in the Pb CR result summary for the both experiment (Table 6.9). The lowest in the pot experiment was also Art-15 with 0.0005±0.0001 ranging from 0.0004 to 0.0009.

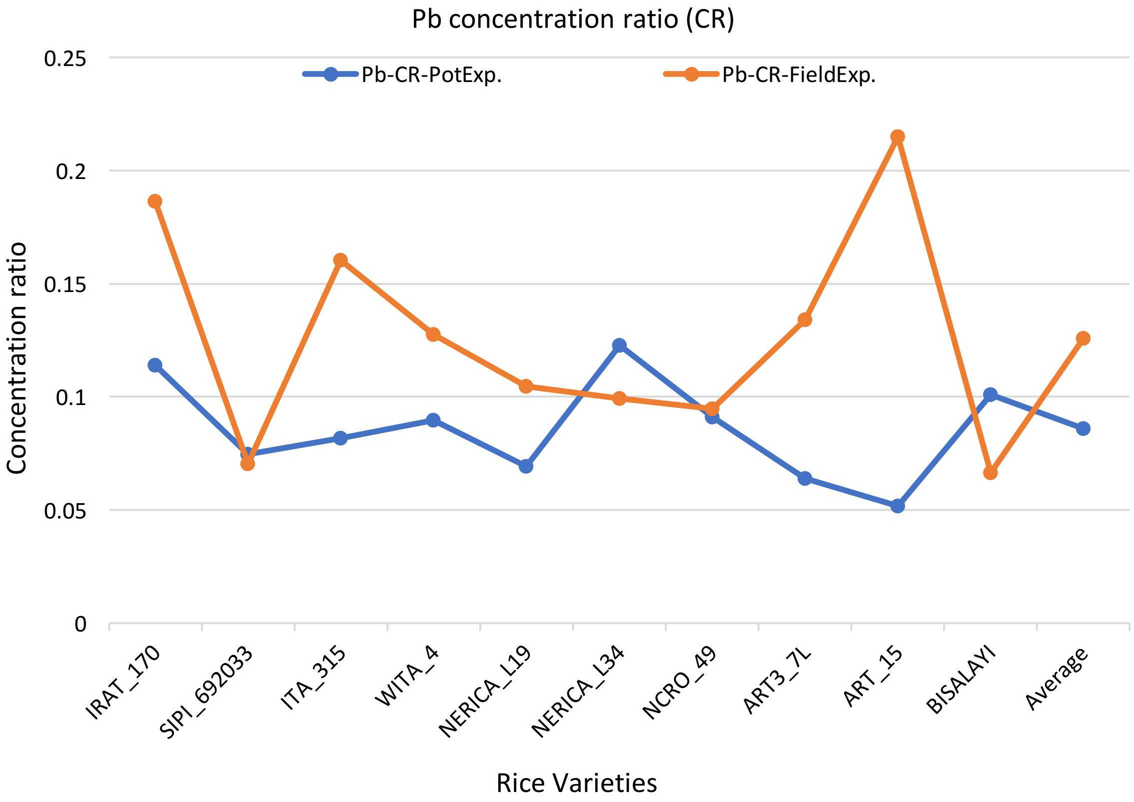


Figure 6.6: Pattern in the Pb CR for both the field and pot experiment in the 10 selected rice varieties.

Table 6. 9: Result of the concentration ratio (CR) for both experiments.

Rice Varieties		Field Experiment	Pot Experiment
IRAT-170	Mean± SD	0.19±0.36	0.0011±0.0007
Variety 1	Min, Max	0.00, 1.73	0.0008, 0.0048
	CV	1.89	0.6239
SIPI-692033	Mean± SD	0.07±0.14	0.0007±0.0002
Variety 2	Min, Max	0.00, 0.70	0.0005, 0.0012
(N=30)	CV	2.01	0.2041
ITA-315	Mean± SD	0.16±0.27	0.0008±0.0002
Variety 3	Min, Max	0.00, 0.84	0.0004, 0.0012
	CV	1.71	0.1984
WITA-4	Mean± SD	0.13±0.32	0.0009±0.0002
Variety 4	Min, Max	0.00, 1.41	0.0003, 0.0013
	CV	2.54	0.2276
NERICA-L19	Mean± SD	0.10±0.20	0.0007±0.0002
Variety 5	Min, Max	0.00, 0.84	0.0005, 0.0012
	CV	1.90	0.2303
NERICA-L34	Mean± SD	0.10±0.16	0.0012±0.0006
Variety 6	Min, Max	0.00, 0.63	0.0000, 0.0041
	CV	1.57	0.5281
NCRO-49	Mean± SD	0.09±0.18	0.0009±0.0002
Variety 7	Min, Max	0.00, 0.85	0.0007, 0.0018
	CV	1.86	0.2455
ART3-7L	Mean± SD	0.13±0.23	0.0006±0.0001
Variety 8	Min, Max	0.00, 0.83	0.0005, 0.0009
	CV	1.71	0.1863
ART-15	Mean± SD	0.22±0.39	0.0005±0.0001
Variety 9	Min, Max	0.00, 1.29	0.0004, 0.0009
	CV	1.83	0.1907
BISALAYI	Mean± SD	0.07±0.13	0.0010±0.0004
Variety 10	Min, Max	0.00, 0.54	0.0006, 0.0026
	CV	1.93	0.3634
Average	Mean± SD	0.13±0.26	0.0009±0.0004
(N=300)	Min, Max	0.00, 1.73	0.0000, 0.0048
	CV	2.03	0.4742

SD = Standard Deviation, Max = Maximum, Min = Minimum,
N= Number of replicates/samples, CV= Coefficient of Variance.

However, the rice varieties were grouped based on the similarities observed through the Duncan multiple range post hoc test using the mean concentration ratio. For the field experiment Ita-315 (variety 3), Art3-7L (variety 8), Ncro-49 (variety 7) and Bisalayi (variety 10) appear to be the varieties with lower CR (low uptake varieties). Irat-170 (variety 1) was slightly low in terms of the Pb uptake while Art-15 (variety 9), Wita-4 (variety 4), Nerica-L19 (variety 5), Sipi (variety 3) and Nerica-L34 (variety 6) appear to be the rice varieties with the high uptake in the field experiment. In the pot experiment, Art3-7L (variety 8), and Art-15 (variety 9) appear to be the varieties with lower CR (low uptake varieties) while Nerica-L34 (variety 6) appear to be the only rice variety that appear at the high side in terms of the uptake of Pb from soil. The remaining rice varieties are slightly low. This is revealed in Figure 6.7.

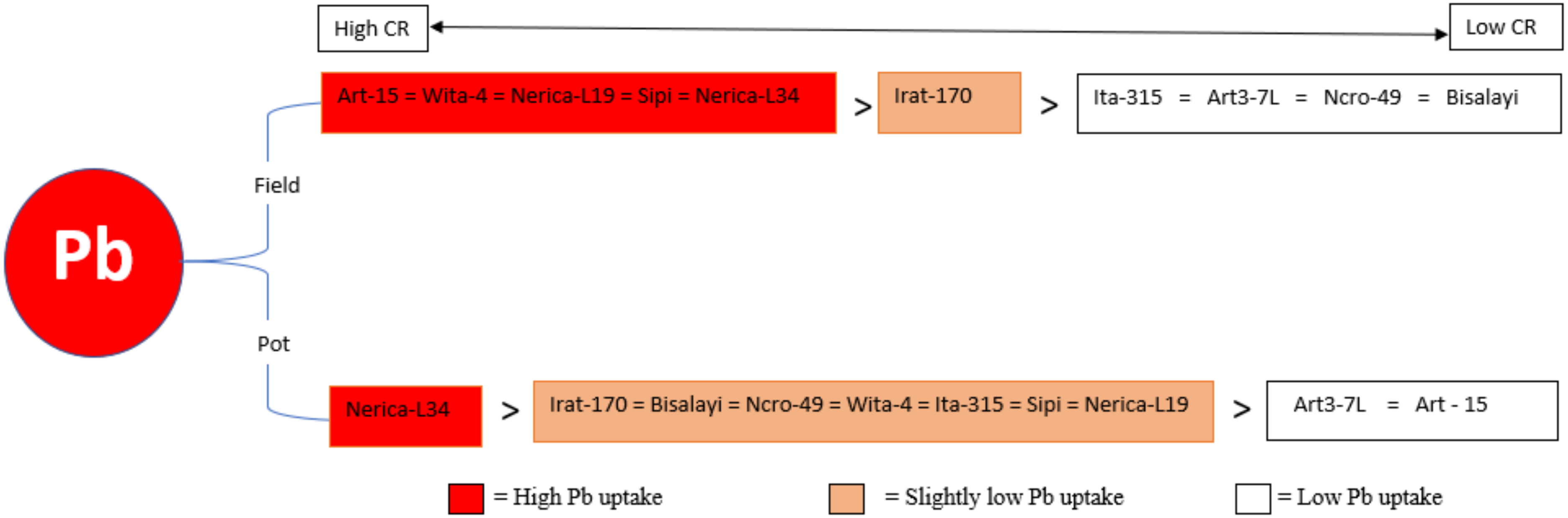


Figure 6.7: Rice varietal selection for Pb using the Concentration Ratio (CR).

6.5 Pb uptake and the yield

Measurement of yield was not part of the objectives of this study but interestingly, the rice varieties identified with low CRs (low uptake) also demonstrated good yield in both experiments. Bisalayi (variety 10) which was still the local variety in the area took the first position in terms of the yield in both experiment. The seeds were small in sizes compare to others but it produced rice seeds more than others. Following Bisalayi were Irat-170 (variety 1) and Art3-7L (Variety 8) which appears similar in terms of the yield and they both produced rice seeds very well. In terms of the Pb uptake, they were both within the group characterised with low Pb uptake in both experiment. Irat-170 seeds were a bit bigger than Bisalayi rice but not as bigger as Art3-7L. Ita-315 (variety 3) was the next in terms of the yield followed by Wita-4 (variety 4) and they all made it to the low uptake rice varieties from the result of both experiment. Art 315 (variety 9) was the least in terms of the yield on the field for the field experiment. And unexpectedly, this variety behaved differently in the pot experiments with the yield and in terms of Pb uptake. Art-315 was grouped as one of the low Pb uptake rice varieties in the pot experiment with good yield as well. Therefore, it was clear from the result that yield also influences the Pd uptake among the 10 selected rice varieties. This shows that, at high yield or high seed production, the Pb uptake in rice becomes diluted. This observation was in line with some previous studies on yield and Pb uptake by rice crops (Zhou et al., 2015; Li et al., 2007; Srivastava et al., 2014; and Lei et al., 2011).

This result could be that rice regulates the transfer of Pb based on the varietal capacity no matter the amount of the elemental quantity within the soil as presented in the previous studies (David et al., 2019; Khan et al., 2015; Ogbuagu et al., 2015; and Liu et al., 2013). May be the rice varieties have taken enough of what their capacity could consume from soil and they have reached a limit where they couldn’t take more.

6.5 Estimated Daily Intake (EDI) Carcinogenic and Non-carcinogenic Risk

The Estimated Daily Intake (EDI) of Pb for the 10 selected rice varieties was calculated (Table 6.4) using the FDA (Food and Drug Administration of the United State Department of Health and Human Services) Pb maximum ingestible daily allowable limit (Pb-MIDAL) which is 3 μg/day (0.003 mg/day) for children and 6 μg/day (0.003 mg/day) for adult (EFSA, 2010; FDA, 2018) and by the application of equation 6 (chapter 3 section 3.9.2). These were used to calculate the percentage daily Pb dose ingested (chapter 3, section 3.9.3, equation 7) from all the selected rice varieties from both experiments. The result shows that the consumers of the 10 selected rice varieties consume Pb everyday more than a thousand folds higher than the Pb maximum ingestible daily allowable limit (Pb-MIDAL). This is shown in Figure 6.8. In the pot experiment, the rice varieties were about 53% higher than the result of the field experiment, which means the situation is about half more in the pot experiment compare to the field experiment as shown in Table 6.5.

Table 6. 10: Mean Pb concentration in rice, and EDI for Children (<18years old) and adult (>18yeays)

	Rice Varieties	Field Experiment	Pot Experiment
Mean Pb Conc. (mg/kg)	**Variety 1 (**IRAT-170)	1.123	1.815
	Variety 2 (SIPI-692033)	0.627	1.392
	Variety 3 (ITA-315)	0.874	1.506
	Variety 4 (WITA-4)	0.610	1.639
	Variety 5 (NERICA-L19)	0.718	1.32
	Variety 6 (NERICA-L34)	0.564	2.328
	Variety 7 (NCRO-49**)**	0.924	1.646
	Variety 8 (ART3-7L)	0.881	1.129
	Variety 9 (ART-15)	0.738	0.98
	Variety 10 (BISALAYI)	0.381	1.835
		Pb-EDI	**Pb-EDI**
Children	**Variety 1 (**IRAT-170)	0.112	0.1815
	Variety 2 (SIPI-692033)	0.063	0.1392
	Variety 3 (ITA-315)	0.087	0.1506
	Variety 4 (WITA-4)	0.061	0.1639
	Variety 5 (NERICA-L19)	0.072	0.132
	Variety 6 (NERICA-L34)	0.056	0.2328
	Variety 7 (NCRO-49**)**	0.092	0.1646
	Variety 8 (ART3-7L)	0.088	0.1129
	Variety 9 (ART-15)	0.074	0.098
	Variety 10 (BISALAYI)	0.038	0.1835
		Pb- EDI	**Pb- EDI**
Adult	**Variety 1 (**IRAT-170)	0.225	0.363
	Variety 2 (SIPI-692033)	0.125	0.2784
	Variety 3 (ITA-315)	0.175	0.3012
	Variety 4 (WITA-4)	0.122	0.3278
	Variety 5 (NERICA-L19)	0.144	0.264
	Variety 6 (NERICA-L34)	0.113	0.4656
	Variety 7 (NCRO-49**)**	0.185	0.3292
	Variety 8 (ART3-7L)	0.176	0.2258
	Variety 9 (ART-15)	0.148	0.196
	Variety 10 (BISALAYI)	0.076	0.367

Table 6. 11: Percentage contribution to Pb maximum ingestible daily allowable limit (Pb-MIDAL) based on sex and age group.

	Sex/Age group	Pb-MIDAL (mg/day)	% Contribution to Recommended Daily Intake (RDI)									
			Variety 1	Variety 2	Variety 3	Variety 4	Variety 5	Variety 6	Variety 7	Variety 8	Variety 9	Variety 10
Pb **Field**	Infant (0-12)	0.003	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Children (1-9)	0.003	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Male (10-18)	0.003	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Female (10-18)	0.003	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Male (>19)	0.006	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Female (>19)	0.006	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Pregnant W	0.006	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
	Lactating W	0.006	3744.1	2090.4	2914.0	2033.4	2391.8	1879.6	3078.9	2936.8	2461.3	1271.4
Pb **Pot**	Infant (0-12)	0.003	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Children (1-9)	0.003	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Male (10-18)	0.003	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Female (10-18)	0.003	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Male (>19)	0.006	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Female (>19)	0.006	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Pregnant W	0.006	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7
	Lactating W	0.006	6050.0	4640.0	5020.0	5463.3	4400.0	7760.0	5486.7	3763.3	3266.7	6116.7

Adapted from EFSA (2010); and FDA (2018).

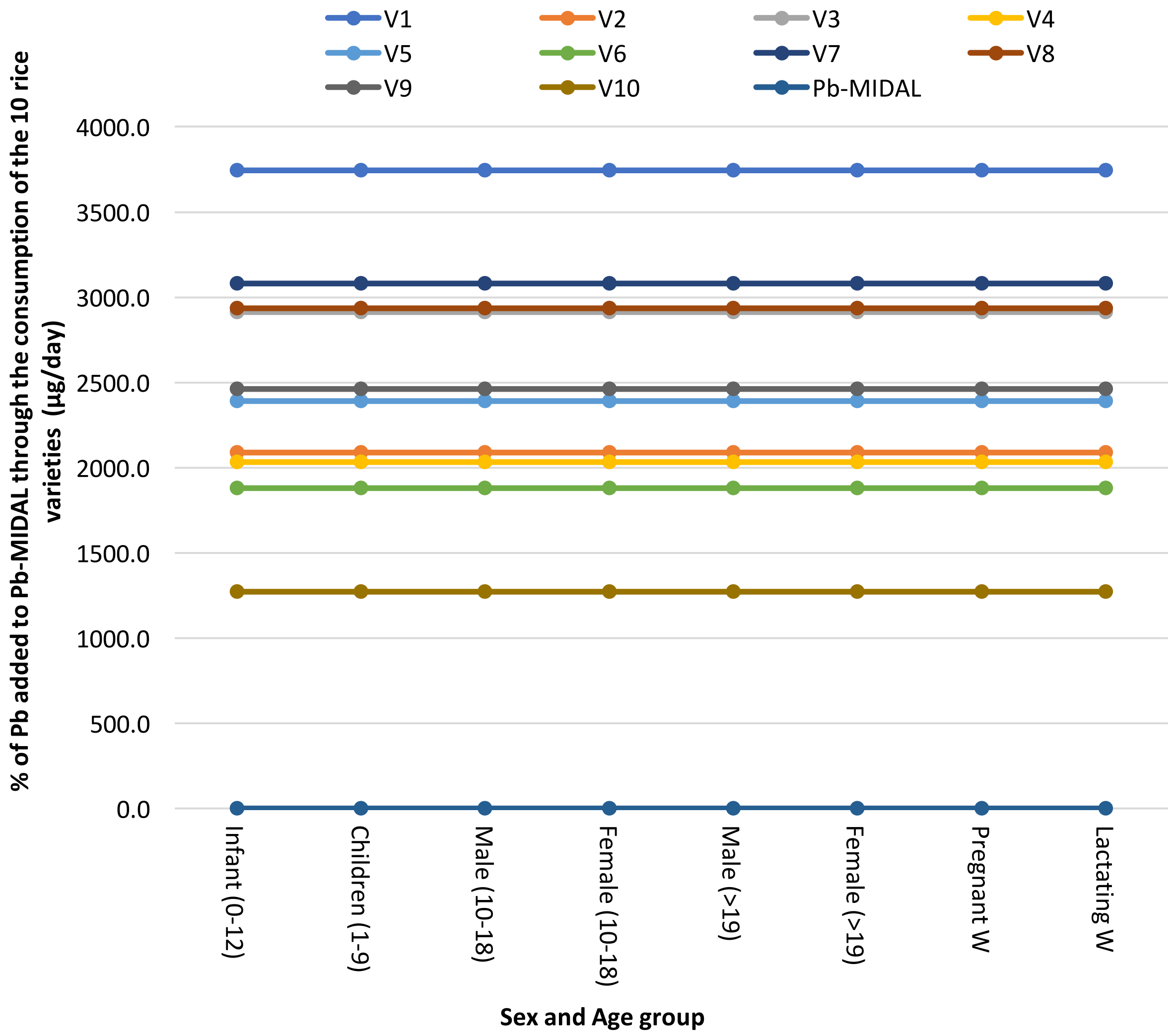


Figure 6.8: Percentage of Pb added to Pb maximum ingestible daily allowable limit (Pb-MIDAL) through the consumption of the 10 selected rice.

Quantifying the dosage of oral exposure to Pb per day, the average daily intake (ADI) mg/kg/day dose of Pb was calculated using the equation 8 (chapter 3 section 3.9.4) (Dai, Song, Huang, & Xin, 2016; Song, Zhuang, Jiang, Fu, & Wang, 2015). And determination of non-carcinogenic health risk of lead from rice consumption was then done using the hazard quotient (HQ) which was calculated from equation 9 (chapter 3 section 3.9.5) (USEPA, 1989) while the RfD for Pb was 0.0035 (JECFA, 2011; USEPA, 2015). Sum total of hazard quotient (HQ) of food is referred to as Hazard Index (HI) which was derived from the Health Risk Assessment guidelines by the USEPA Chemical mixture (USEPA, 1989). The formula is shown in the equation 10 (chapter 3 section 3.9.5). This assessment is the associated risk potential of lead in rice to compromise good health in human. The equation 10 provided a means to calculate the potential health risk through the hazard index (HI) when the source of exposure from food is more than 1 (FAO/WHO, 2011). If HI is less than 1, it is unlikely that the exposed population is at risk of adverse health effects. If HI is greater than or equals to one, there must be a proactive measure to mitigate the hazard because people are surely at risks of health effects (Dai et al., 2016; Shaheen et al., 2016).

Hazard Quotient and the Hazard Index for the consumption of Pb with the 10 selected rice varieties were calculated from the equation 11 and 12 (section 3.9.6, chapter 3) respectively. The risk of having cancer within the exposed population (CR_k) was determined from the hazard index and the result is presented in Table 6.6 for the field experiment and Table 6.7 for the pot experiment.

Table 6. 12: Result of the Average Daily Intake (ADI), Hazard Quotient (HQ), Hazard Index (HI) and Cancer Risk (CR_k) for the 10 selected rice varieties in the field experiment.

Rice Varieties	**Mean Pb in rice**	**ADI (mg/kg/day)**	**HQ**	**HI**	**CR_k**
Variety 1 (IRAT-170)	1.12	0.003576	1.021681	1.021681	3.04E-05
Variety 2 (SIPI-692033)	0.63	0.001997	0.570432	0.570432	1.7E-05
Variety 3 (ITA-315)	0.87	0.002783	0.795165	0.795165	2.37E-05
Variety 4 (WITA-4)	0.61	0.001942	0.554878	0.554878	1.65E-05
Variety 5 (NERICA-L19)	0.72	0.002284	0.652659	0.652659	1.94E-05
Variety 6 (NERICA-L34)	0.56	0.001795	0.512891	0.512891	1.53E-05
Variety 7 (NCRO-49**)**	0.92	0.002941	0.840163	0.840163	2.5E-05
Variety 8 (ART3-7L)	0.88	0.002805	0.801378	0.801378	2.38E-05
Variety 9 (ART-15)	0.74	0.002351	0.671643	0.671643	2E-05
Variety 10 (BISALAYI)	0.38	0.001214	0.346946	0.346946	1.03E-05

Table 6. 13: Result of the Average Daily Intake (ADI), Hazard Quotient (HQ), Hazard Index (HI) and Cancer Risk (CR_k) for the 10 selected rice varieties in the pot experiment.

Rice Varieties	Mean Pb in rice	ADI (mg/kg/day)	HQ	HI	CR_k
Variety 1 (IRAT-170)	1.815	0.005778	1.650909	1.650909	4.91145E-05
Variety 2 (SIPI-692033)	1.392	0.004432	1.266152	1.266152	3.7668E-05
Variety 3 (ITA-315)	1.506	0.004794	1.369845	1.369845	4.07529E-05
Variety 4 (WITA-4)	1.639	0.005218	1.490821	1.490821	4.43519E-05
Variety 5 (NERICA-L19)	1.32	0.004202	1.200661	1.200661	3.57197E-05
Variety 6 (NERICA-L34)	2.328	0.007411	2.117529	2.117529	6.29965E-05
Variety 7 (NCRO-49)	1.646	0.00524	1.497188	1.497188	4.45413E-05
Variety 8 (ART3-7L)	1.129	0.003594	1.026929	1.026929	3.05511E-05
Variety 9 (ART-15)	0.98	0.00312	0.8914	0.8914	2.65191E-05
Variety 10 (BISALAYI)	1.835	0.005842	1.669101	1.669101	4.96557E-05

Since the source of hazard in this study is one (Pb in rice), the HQ = HI. If HI<1, it is unlikely that the exposed population are at the risk of adverse health effect as a result of rice consumption (USEPA, 1989; Dai et al., 2016). The result (Table 6.6) shows that the hazard index (HI) in all the selected rice varieties were less than 1 except the variety 1 (Irat-170) which is also approximately 1 for the field experiment. This means that it is unlikely that the exposed population is at risk of adverse health effect from Pb hazard for consuming the 10 selected rice varieties grown on the field. Therefore, there was no risk of Pb poisoning from the consumption of the 10 selected Nigeria rice varieties grown on the field. But, for the pot experiment (Table 6.7), only the variety 9 (Art-15) is less than 1. This indicates that growing those 10 Nigerian rice varieties in pots is not a good idea because their consumption poses great health risk of Pb poisoning to human.

The cancer risk varied among the 10 rice varieties in the field experiment. The variety 1 (Irat-170) and 7 (NCRO-49) demonstrated the probability of having 3persons in 100,000 exposed population to develop cancer while variety 2 (Sipi-692033), variety 3 (Ita-315), variety 4 (Wita-4), variety 5 (Nerica-L19), variety 6 (Nerica-L34), variety 8 (Art3-7L), and variety 9 (Art-15) indicated that probably, 2 persons may develop cancer while variety 10 (Bisalayi) shown 1 in 100,000 population over a period of 60.5 years' life expectancy (Statista, 2018) was used.

For the pot experiment, the cancer risk also varied among the 10 rice varieties. The variety 1 (Irat-170), 7 (Ncro-49) and 10 (Bisalayi) demonstrated the probability of having 5 persons in a population of 100,000 to develop cancer while variety 6 (Nerica-L34) revealed 7 persons out of 100,000 population. Variety 8 (Art3-7L) and Variety 9 (Art-15) revealed probability of 3 persons to develop cancer in a population of 100,000 while others revealed 4 persons each that may develop cancer out of population of 100,000 in their lifetime.

6.6 Conclusion

After a comprehensive analysis, the inter-varietal variation of Pb uptake recorded among the 10 rice varieties in the pot experiment was 2.4 fold. For the field experiment, the inter-varietal variation of Pb uptake recorded among the 10 rice varieties was 2.7 fold. But when we compared the unflooded area of the field with the flooded area, the variation in the unflooded area was higher by 29% (7.5 fold) than the flooded area (2.2 fold). The wider variation in the unflooded area was suspected to be the different in the water supply within the short time as the varietal variation in the flooded area (2.2 fold) of the field experiment appeared to be similar to the varietal variation among the 10 rice varieties recorded for the pot experiment (2.4) and the pot experiment was not flooded at any time. Statistically, in terms of Pb uptake, there was no significant difference between the rice varieties harvested from the flooded area of the field experiment and the rice varieties harvested from the unflooded area of the field (Table 6.6). At the end of the experiment, Bisalayi rice (the local rice variety) appeared consistently with low Pb uptake in both experiment and this is the rice variety that is already grown by the farmers in this affected area of Zamfara state especially the Dareta village. Therefore, it is concluded that there is no action needed to be taken regarding the variety currently grown in Dareta village Zamfara State.

This study also demonstrated that people consume more Pb daily from the 10 selected rice varieties than the maximum ingestible daily allowable limit (Pb-MIDAL) based on sex and age group according to EFSA (2010); and FDA (2018) as presented in Table 6.5. For the non-carcinogenic health risk, only one variety (Irat-170 rice) had the highest probability to compromise the consumers' health as its hazard Index was greater than 1. Other varieties do not pose health risks to Nigerians and any other consumers. Two varieties (Irat-170 rice and Ncro-49 rice) posed a probability of having 3 in 100,000 population developing cancer in their lifetime for consuming those two varieties. One variety (bisalayi rice) shows 1 in 100,000 population to develop cancer in their lifetime while other varieties revealed 2 in 100,000 population to develop cancer in lifetime for consumption of those rice using 60.5 as the average lifetime age (life expectancy). The result for the pot experiment shows that the Pb concentration in the 10 selected rice varieties were likely to pose both carcinogenic and non-carcinogenic health risks on the exposed population (HI>1). This means that every adult has probability to suffer one health challenge or the other among those health challenges that are related to Pb accumulation (Pb poisoning) in the body (chapter 2 Figure 2.3). There is at least 3 in 100,000 population probably to develop cancer in their lifetime.

6.7 Limitation

The method employed in this study has provided a simple way to assess the risk of Pb in rice to human health. However, there are limitations in the applications of this method which has to do with accuracy of the exposure duration, rice ingestion rate for different age groups and the information on the impact of which cooking may have on the Pb concentration in rice during consumption. 100g of rice per day ingestion rate was used for children (0 to 18 years) and 200g was used for adults (18 and above) as previously used by AfricaRice (2005) and Norton et al. (2014) and this seems not realistic looking at the rate at which rice is been consumed in Africa generally especially Zamfara state.

CHAPTER SEVEN

Inter-varietal Variation of the Essential Elements in Rice (Field and Pot Experiment)

7.0 Materials and Methods.

The method involved in this study has been presented in chapter 3, from section 3.5. The nine essential elements in rice in this study were calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), zinc (Zn), selenium (Se), manganese (Mn), cobalt (Co), and copper (Cu).

7.1 Data analysis and few other calculations

The result for the essential elements concentration (mg/kg) in the rice samples were presented in arithmetic mean together with the standard deviation (SD). The significance level was set at $p<0.05$ and 0.01 to present the result. The concentration ratio (CR) was calculated by dividing the concentration of the essential element (mg/kg) dry mass in the rice samples by the concentration of the essential element (mg/kg) dry mass in the soil samples (chapter 3, section 3.91, equation 4). The inter-varietal variation (IVV) of the essential element in rice was calculated by dividing the essential element's mean concentration ratio of the highest accumulated rice variety by the essential element mean concentration ratio of the lowest accumulated rice variety (chapter 3, section 3.91, equation 5). Other calculations have been stated in chapter 3. A one-way analysis of variance (ANOVA) was carried out to detect significant statistical differences the inter-varietal variation that exist among the 10 varieties using the essential elements' mean concentration. For the RCBD used for rice planting (chapter 3, section 3.5.3.5), two-way ANOVA was used to check for the block effect on the experiment. The data were checked for normality and homogeneity of variance before ANOVA and correlation analysis was conducted using a Pearson correlation test (2-tailed). Percentage recovery according to Naseri, Vazirzadeh, Kazemi, & Zaheri (2015) was calculated from the analytical result of the Standard Reference Materials (SRMs; NIST 1568b (rice flour) and NIST 2711a (Montana II soil)) by dividing the observed concentration by the certified concentration then multiplied the result by 100 [(observed/certified × 100) %]. Each element was analysed in 6 replicates and the means data were recorded in the observed column.

7.2 Result and discussion

The result of the percentage recovery of the essential elements (n=6), the Limit of detection (LOD), and the limit of quantification (LOQ) are presented in Table 7.1.

Table 7. 1: Result of the LOD, LOQ and the percentage recovery of the essential elements

		Elemental concentration (mg/L)								
		Ca	**Fe**	**K**	**Mg**	**Zn**	**Se**	**Mn**	**Co**	**Cu**
	LOD	0.05	0.01	0.01	0.05	0.01	0.2	0.01	0.05	0.02
	LOQ	0.17	0.03	0.3	0.17	0.03	0.67	0.03	0.17	0.07
NIST 1568b	**% Recovery**	106	101	92	82	96	118	97	104	106
NIST 2711a	**% Recovery**	112	116	98	90	108	102	106	89	98

7.2.1 Inter-varietal variation of the essential elements in rice (field experiment)

The mean concentration of the essential elements in all the rice samples was 131.45±18.38 mg/kg for Ca, 25.43±17.33 mg/kg for Fe, 4.66±1.28 mg/kg for Cu, 901.99±104.31 mg/kg for Mg, 2025.95±242.99 mg/kg for K, 25.05±3.99 mg/kg for Zn, 23.24±2.99 mg/kg for Mn, 0.07±0.03 mg/kg for Co, and 0.11±0.02mg/kg for Se. The ranges (mg/kg) were from 56.32 to 204.5 for Ca, 1.55 to 164.32 for Fe, 1.49 to 19.80 for Cu, 341.94 to 1347.29 for Mg, 756.01 to 3238.54 for K, 9.09 to 39.55 for Zn, 9.82 to 34.54 for Mn, 0.02 to 0.14 for Co, and 0.07 to 0.21 for Se respectively.

In the soil, the mean concentration of Ca was 1458.58±307.33 mg/kg (ranges from 971.64 to 2505.62), Fe was 16678.60±3273.49 mg/kg (ranges from 10626.26 to 26641.42), Cu was 10.73±11.40 mg/kg (ranges from 0.33 to 79.99), Mg was 1759.41±586.80 mg/kg (ranges from 684.68 to 3015.34), K was 1400.28±707.97 mg/kg (ranges from 318.55, 3046.49), Zn was 11.43±9.85 mg/kg (ranges from 0.36 to 83.88), Mn was 184.96±177.81 mg/kg (ranges from 6.34 to 1800.53), Co was 4.33±3.90 mg/kg (ranges from 0.19, 34.24), and Se was 0.22±0.134 mg/kg (ranges from 0.06 to 0.91) respectively. The soil physico-chemical parameters are presented in Table 7.2. Statistical summary of the result is presented in Table 7.3. The soil was majorly dark yellowish brown and Sandy-loam based on the Munsel colour chart analysis and the textural test conducted (chapter 3, section 3.7.2) using soil-texture triangle (Appendix D, Table III and IV).

Table 7. 2: Result for the soil physico-chemical parameters for the field experiment.

Soil (n=300)	pH (H_2O) 1:1	^{a}EC (dS/m)	%Organic Carbon	Av-P_2O_2	Exchangeable cations (cmol /kg)				Exchangeable Acidity	CEC	Particle size			N
					Ca	**K**	**Mg**	**Na**			**%Clay**	**%Silt**	**%Sand**	
Mean	6.58	1.19	4.00	7.77	1458.58	1400.28	1759.41	27.12	0.65	26.10	46.63	39.74	45.63	3.12
SD	0.86	0.36	0.90	2.84	307.33	707.97	586.80	46.37	0.19	7.89	7.26	14.64	16.84	1.59
Min	4.51	0.3	1.47	0.620	971.64	318.55	684.68	0.05	0.34	12.85	2.00	2.00	11.50	1.32
Max	8.51	2.5	7.67	14.00	2505.62	3046.49	3015.34	296.82	2.37	43.32	37.4	69.1	94.00	7.25

SD = Standard Deviation, Max = Maximum, Min = Minimum, n= Number of samples, EC= Electrical Conductivity, Av-P_2O_2 = Available Phosphorus, N = Nitrogen

Table 7. 3: Summary of the result of the essential elements in the rice samples

Rice Varieties		Parameters	Mn	Co	Cu	Zn	Se	Ca	Fe	K	Mg
IRAT_170	RICE	Mean± SD	20.92±1.75	0.05±0.01	5.27±0.56	26.79±1.99	0.11±0.02	138.03±9.40	23.41±4.65	1938.47±76.07	809.92±44.06
Variety 1	(N=30)	Min, Max	17.70, 24.05	0.03, 0.06	4.58, 6.82	23.18, 30.70	0.08, 0.16	118.39, 159.44	16.53, 39.84	1753.25, 2084.36	727.21, 895.18
		CV	0.08	0.18	0.11	0.07	0.15	0.07	0.20	0.04	0.05
	SOIL	Mean± SD	179.78±146.99	4.07±3.20	10.49±11.22	11.04±8.80	0.23±0.18	1487.12±293.87	16599.38±3266.79	1470.76±735.76	1819.36±604.47
	(N=30)	Min, Max	8.63, 441.09	0.24, 8.68	0.40, 45.51	0.42, 21.46	0.07, 0.91	1077.40, 1994.07	11783.01, 23245.73	364.75, 3033.15	718.97, 3015.34
		CV	0.82	0.79	1.07	0.80	0.78	0.20	0.20	0.50	0.33
SIPI_692033	RICE	Mean ±SD	23.68±1.99	0.11±0.02	5.02±0.59	27.62±3.01	0.11±0.03	120.79±11.98	20.21±3.38	2189.19±117.99	971.76±51.21
Variety 2	(N=30)	Min, Max	21.10, 29.36	0.03, 0.14	4.28, 6.54	23.81, 35.58	0.08, 0.21	106.43, 164.15	13.06, 29.05	1969.49, 2485.97	880.12, 1062.16
		CV	0.08	0.21	0.12	0.11	0.25	0.10	0.17	0.05	0.05
	SOIL	Mean ±SD	238.25±324.50	5.55±6.34	11.65±10.63	12.23±8.81	0.23±0.13	1479.12±325.70	16517.51±3097.35	1486.94±707.56	1860.14±575.40
	(N=30)	Min, Max	9.19, 1800.53	0.26, 34.24	0.38, 35.44	0.48, 27.10	0.07, 0.46	971.64, 2020.55	11387.78, 21719.98	562.39, 2900.89	915.28, 2884.71
		CV	1.36	1.14	0.91	0.72	0.55	0.22	0.19	0.48	0.31
ITA_315	RICE	Mean ±SD	24.63±2.51	0.06±0.02	4.68±0.68	24.21±2.81	0.10±0.01	139.62±19.25	19.19±3.52	2021.44±195.32	900.91±75.17
Variety 3	(N=30)	Min, Max	18.47, 28.94	0.03, 0.09	3.60, 6.22	18.82, 31.23	0.07, 0.13	113.21, 177.45	11.65, 26.13	1697.41, 2435.01	755.18, 1047.32
		CV	0.10	0.34	0.14	0.12	0.13	0.14	0.18	0.10	0.08
	SOIL	Mean	193.11±210.72	4.81±5.31	8.63±8.22	10.87±9.51	0.21±0.13	1474.10±339.52	17093.06±3587.47	1337.58±711.58	1707.59±592.00
	(N=30)	Min, Max	9.71, 960.45	0.25, 20.44	0.35, 25.34	0.45, 24.02	0.07, 0.42	1022.88, 2505.62	11270.43, 24250.48	500.30, 3046.49	813.59, 3003.49
		CV	1.09	1.10	0.95	0.87	0.61	0.23	0.21	0.53	0.35
WITA_4	RICE	Mean± SD	21.60±1.57	0.07±0.02	4.35±3.02	22.30±2.94	0.11±0.02	129.61±17.77	17.95±5.06	1879.77±145.40	828.98±71.20
Variety 4	(N=30)	Min, Max	18.51, 24.91	0.04, 0.10	2.87, 19.80	17.61, 28.70	0.08, 0.15	110.43, 181.55	10.88, 34.43	1660.21, 2288.54	705.41, 1032.88
		CV	0.07	0.22	0.69	0.13	0.15	0.14	0.28	0.08	0.09
	SOIL	Mean± SD	177.05±144.40	4.01±3.14	10.04±10.24	10.89±8.78	0.22±0.14	1487.87±331.51	16989.50±3891.84	1428.92±746.08	1788.62±618.95
	(N=30)	Min, Max	7.92, 387.56	0.25, 8.25	0.36, 39.03	0.38, 22.05	0.07, 0.56	1015.94, 2111.52	11801.07, 26641.42	318.55, 2881.54	684.68, 2913.68
		CV	0.82	0.78	1.02	0.81	0.64	0.22	0.23	0.52	0.35
NERICA_L19	RICE	Mean± SD	23.26±2.05	0.07±0.01	4.77±0.84	24.49±4.07	0.12±0.02	139.81±18.93	25.29±26.55	2049.16±170.23	919.17±74.46

Variety 5	(N=30)	Min, Max	20.97, 29.72	0.03, 0.09	3.92, 6.94	20.05, 35.99	0.09, 0.15	120.55, 189.54	14.07, 164.32	1794.58, 2499.73	808.02, 1105.63
		CV	0.09	0.19	0.18	0.17	0.17	0.14	1.05	0.08	0.08
	SOIL	Mean± SD	181.35±137.31	4.28±3.11	12.11±11.71	11.92±9.01	0.23±0.13	1457.66±321.24	16483.37±3798.09	1488.45±758.19	1833.62±604.22
	(N=30)	Min, Max	9.43, 393.93	0.23, 8.59	0.35, 38.81	0.41, 23.16	0.06, 0.44	977.24, 2082.53	11398.27, 25626.50	456.07, 3044.71	808.15, 2967.52
		CV	0.76	0.73	0.97	0.76	0.55	0.22	0.23	0.51	0.33
NERICA_L34	RICE	Mean± SD	20.27±2.99	0.07±0.03	3.92±1.12	23.33±3.92	0.11±0.02	116.09±10.52	19.04±3.15	1842.32±122.96	821.26±88.17
Variety 6	(N=30)	Min, Max	14.10, 27.73	0.03, 0.10	2.75, 6.55	18.64, 35.95	0.08, 0.13	97.45, 148.60	12.30, 24.91	1654.92, 2257.51	625.95, 990.70
		CV	0.15	0.37	0.29	0.17	0.15	0.09	0.17	0.07	0.11
	SOIL	Mean± SD	181.18±170.90	3.98±3.36	9.91±10.03	10.62±9.11	0.20±0.12	1428.17±291.84	16456.89±2596.03	1365.85±695.67	1730.37±598.08
	(N=30)	Min, Max	6.34, 700.10	0.19, 9.38	0.36, 33.85	0.41, 24.65	0.07, 0.39	978.26, 2120.210	11373.27, 20359.09	498.85, 2910.89	832.40, 2971.59
		CV	0.94	0.84	1.01	0.86	0.59	0.20	0.16	0.51	0.35
NCRO_49	RICE	Mean± SD	24.03±4.25	0.07±0.02	4.37±1.36	25.68±6.66	0.11±0.01	143.40±22.19	23.35±12.62	2129.67±474.45	914.76±185.24
Variety 7	(N=30)	Min, Max	9.82, 34.54	0.02, 0.11	1.50, 7.24	9.09, 39.55	0.08, 0.13	56.32, 196.83	5.70, 73.07	756.01, 3238.54	341.94, 1347.29
		CV	0.18	0.35	0.31	0.26	0.13	0.15	0.54	0.22	0.20
	SOIL	Mean± SD	180.18±151.53	4.24±3.36	11.23±15.44	11.22±9.03	0.21±0.13	1455.25±277.84	16878.83±3265.74	1356.43±652.35	1723.25±544.41
	(N=30)	Min, Max	8.60, 532.12	0.22, 10.21	0.33, 79.99	0.42, 22.96	0.07, 0.42	1014.78, 1964.79	10626.26, 26127.43	482.97, 2841.16	838.01, 2865.92
		CV	0.84	0.79	1.38	0.80	0.60	0.19	0.19	0.48	0.32
ART3_7L	RICE	Mean± SD	23.84±2.59	0.08±0.03	4.50±0.87	26.66±3.75	0.11±0.01	134.45±16.91	23.24±10.17	2189.20±150.83	974.41±67.12
Variety 8	(N=30)	Min, Max	19.47, 28.67	0.03, 0.14	3.47, 6.30	19.46, 34.73	0.08, 0.12	112.60, 166.70	13.68, 65.22	1903.11, 2618.45	842.59, 1123.19
		CV	0.11	0.39	0.19	0.14	0.10	0.13	0.44	0.07	0.07
	SOIL	Mean± SD	177.04±138.93	4.31±3.59	10.96±12.05	11.23±9.01	0.23±0.13	1411.87±306.88	15868.63±2856.06	1353.23±732.10	1677.81±608.12
	(N=30)	Min, Max	9.27, 452.05	0.27, 13.61	0.36, 43.97	0.46, 32.48	0.06, 0.44	1006.15, 1876.66	10692.10, 21733.05	636.94, 2825.74	897.75, 2803.75
		CV	0.78	0.83	1.10	0.80	0.58	0.22	0.18	0.54	0.36
ART_15	RICE	Mean± SD	25.26±2.50	0.06±0.01	5.18±0.45	26.86±2.43	0.11±0.01	130.50±11.25	23.08±6.03	2206.37±126.42	976.54±63.27
Variety 9	(N=30)	Min, Max	19.54, 30.79	0.03, 0.07	4.39, 5.90	22.80, 33.16	0.08, 0.14	110.98, 162.48	1.55, 37.30	1913.02, 2594.33	867.27, 1152.58
		CV	0.10	0.14	0.09	0.09	0.13	0.09	0.26	0.06	0.06
	SOIL	Mean± SD	166.65±143.20	3.88±3.28	10.54±10.96	12.88±16.20	0.20±0.12	1431.16±312.10	16815.67±2864.65	1338.07±727.08	1704.20±614.95
	(N=30)	Min, Max	7.17, 387.37	0.20, 8.66	0.34, 38.31	0.36, 83.88	0.07, 0.42	998.66, 2139.55	12034.00, 22711.03	348.60, 2875.36	723.61, 2875.81
		CV	0.86	0.84	1.04	1.26	0.59	0.22	0.17	0.54	0.36
BISALAYI	RICE	Mean± SD	24.95±2.19	0.08±0.01	4.56±0.44	22.62±1.86	0.11±0.01	122.22±19.89	59.51±24.94	1813.93±99.47	902.16±55.30
Variety 10	(N=30)	Min, Max	19.65, 30.33	0.06, 0.10	3.96, 5.81	18.44, 27.52	0.09, 0.14	95.52, 204.51	25.85, 150.09	1490.83, 1943.07	749.51, 993.19
		CV	0.09	0.12	0.10	0.08	0.13	0.16	0.42	0.05	0.06
	SOIL	Mean± SD	175.03±139.02	4.12±3.23	11.79±13.28	11.40±9.21	0.22±0.14	1473.47±304.04	17083.12±3563.41	1376.62±691.33	1749.13±563.12
	(N=30)	Min, Max	9.25, 388.92	0.24, 8.91	0.34, 53.90	0.45, 23.57	0.07, 0.67	1059.80, 2202.58	11143.72, 24861.65	564.08, 2872.01	929.28, 2919.34
		CV	0.79	0.78	1.13	0.81	0.65	0.21	0.21	0.50	0.32
Total	RICE	Mean± SD	23.24±2.99	0.07±0.03	4.66±1.28	25.05±3.99	0.11±0.02	131.45±18.38	25.43±17.33	2025.95±242.99	901.99±104.31
Average	(N=300)	Min, Max	9.82, 34.54	0.02, 0.14	1.49, 19.80	9.09, 39.55	0.07, 0.21	56.32, 204.5	1.55, 164.32	756.01, 3238.54	341.94, 1347.29
		CV	0.13	0.35	0.27	0.16	0.16	0.14	0.68	0.12	0.12
	SOIL	Mean± SD	184.96±177.81	4.33±3.90	10.73±11.40	11.43±9.85	0.22±0.134	1458.58±307.33	16678.60±3273.49	1400.28±707.97	1759.41±586.80
	(N=300)	Min, Max	6.34, 1800.53	0.19, 34.24	0.33, 79.99	0.36, 83.88	0.06, 0.91	971.64, 2505.62	10626.26, 26641.42	318.55, 3046.49	684.68, 3015.34
		CV	0.96	0.90	1.06	0.86	0.61	0.21	0.20	0.51	0.33

SD = Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples

The mean concentration ratio (CR) which determines the proportion of the elemental concentration that is taken up by rice from the soil in all the 10 rice variety samples (soil n=300, rice n=300) was observed for Ca to be 0.10±0.02 (min 0.06, max 0.13). The lowest of 0.08±0.02 was found in nerica-l34 (variety 6) and the highest CR was recorded from nerica-L19 (variety 5) which is similar to irat-170 (variety 1), sipi-692033 (variety 2), ita-315 (variety 3), wita-4 (variety 4), ncro-49 (variety 7), art3-7l (variety 8), and art-15 (variety 9). The concentration ratio (CR) of the nine essential elements investigated in the rice samples for the 10 varieties across the field is presented in Table 7.4, CR for the unflooded area of the field is presented in Table 7.5 while that of the flooded area of the field is presented in Table 7.6. The result shows no significant difference among the two set of data for the flooded and the unflooded area of the field.

Table 7. 4: The concentration ratio (CR) of the essential elements in the 10 selected rice varieties for the whole field.

		Concentration Ratio								
Rice Varieties		**Mn-CR**	**Co-CR**	**Cu-CR**	**Zn-CR**	**Se-CR**	**Ca-CR**	**Fe-CR**	**K-CR**	**Mg-CR**
IRAT_170	Mean± SD	0.70±0.81	0.07±0.08	4.39±5.39	17.58±20.62	0.78±0.52	0.10±0.02	0.001±0.000	1.66±0.90	0.50±0.19
Variety 1	Min, Max	0.05, 2.58	0.01, 0.23	0.10, 14.73	1.08, 58.88	0.11, 1.74	0.06, 0.13	0.001, 0.003	0.58, 5.24	0.24, 1.19
(N=30)	CV	1.16	1.17	1.23	1.17	0.67	0.21	0.314	0.54	0.38
SIPI_692033	Mean± SD	0.63±0.80	0.13±0.17	3.45±4.80	14.21±18.84	0.75±0.58	0.09±0.02	0.001±0.000	1.78±0.75	0.58±0.19
Variety 2	Min, Max	0.01, 2.42	0.00, 0.44	0.15, 13.76	1.01, 55.59	0.24, 2.54	0.06, 0.13	0.001, 0.002	0.68, 3.83	0.31, 1.05
(N=30)	CV	1.27	1.31	1.39	1.33	0.77	0.26	0.252	0.42	0.33
ITA_315	Mean± SD	0.91±0.98	0.11±0.12	4.33±5.06	18.10±20.08	0.77±0.51	0.10±0.03	0.001±0.000	1.90±0.86	0.59±0.21
Variety 3	Min, Max	0.02, 2.53	0.00, 0.33	0.22, 15.02	0.91, 50.77	0.24, 1.83	0.05, 0.16	0.001, 0.002	0.60, 4.09	0.28, 1.08
(N=30)	CV	1.08	1.13	1.17	1.11	0.66	0.28	0.314	0.45	0.35
WITA_4	Mean± SD	0.71±0.83	0.11±0.12	3.82±6.96	13.64±16.54	0.79±0.55	0.09±0.03	0.001±0.000	1.76±1.19	0.54±0.25
Variety 4	Min, Max	0.05, 2.61	0.01, 0.30	0.12, 36.26	0.85, 56.92	0.15, 1.82	0.05, 0.16	0.000, 0.002	0.58, 6.04	0.25, 1.26
(N=30)	CV	1.16	1.13	1.82	1.21	0.70	0.31	0.333	0.68	0.46
NERICA_L19	Mean± SD	0.67±0.84	0.08±0.11	3.60±4.71	14.51±18.30	0.81±0.60	0.10±0.03	0.002±0.002	1.72±0.80	0.56±0.19
Variety 5	Min, Max	0.06, 2.57	0.00, 0.31	0.13, 13.40	0.96, 53.64	0.26, 2.09	0.06, 0.17	0.001, 0.014	0.62, 4.22	0.28, 1.06
(N=30)	CV	1.26	1.28	1.31	1.26	0.75	0.29	1.406	0.46	0.35
NERICA_L34	Mean± SD	0.82±0.93	0.13±0.15	3.43±3.88	15.34±16.88	0.81±0.54	0.08±0.02	0.001±0.000	1.67±0.73	0.54±0.20
Variety 6	Min, Max	0.03, 3.28	0.00, 0.44	0.13, 10.76	0.91, 46.47	0.26, 1.82	0.05, 0.14	0.001, 0.002	0.59, 3.54	0.27, 1.03
(N=30)	CV	1.13	1.12	1.13	1.10	0.67	0.23	0.222	0.44	0.38
NCRO_49	Mean± SD	0.74±0.85	0.10±0.12	2.93±3.52	15.74±18.16	0.80±0.56	0.10±0.03	0.001±0.001	1.91±0.86	0.59±0.23
Variety 7	Min, Max	0.03, 2.77	0.00, 0.35	0.08, 10.35	0.50, 54.74	0.25, 1.96	0.03, 0.16	0.000, 0.007	0.29, 4.51	0.13, 1.20
(N=30)	CV	1.14	1.17	1.20	1.15	0.70	0.28	0.783	0.45	0.39
ART3_7L	Mean± SD	0.70±0.85	0.12±0.15	3.49±4.50	16.46±20.22	0.73±0.51	0.10±0.02	0.002±0.001	2.03±0.85	0.66±0.24
Variety 8	Min, Max	0.05, 2.46	0.00, 0.39	0.11, 12.70	0.88, 52.16	0.21, 1.62	0.06, 0.14	0.001, 0.004	0.70, 3.49	0.30, 1.17
(N=30)	CV	1.22	1.28	1.29	1.23	0.69	0.23	0.429	0.42	0.36
ART_15	Mean± SD	0.97±1.09	0.09±0.10	5.10±5.87	20.69±23.87	0.79±0.50	0.10±0.02	0.001±0.001	2.15±1.21	0.65±0.25
Variety 9	Min, Max	0.07, 3.35	0.01, 0.28	0.15, 17.62	0.34, 70.02	0.25, 1.64	0.06, 0.14	0.000, 0.003	0.68, 6.25	0.30, 1.32
(N=30)	CV	1.12	1.10	1.15	1.15	0.63	0.21	0.369	0.56	0.39
BISALAYI	Mean± SD	0.80±0.92	0.11±0.12	3.92±4.72	15.43±18.05	0.77±0.49	0.09±0.02	0.004±0.001	1.61±0.67	0.57±0.19
Variety 10	Min, Max	0.07, 2.55	0.01, 0.33	0.09, 13.08	0.92, 43.71	0.16, 1.62	0.05, 0.17	0.002, 0.008	0.54, 3.27	0.27, 1.01
(N=30)	CV	1.16	1.16	1.20	1.17	0.64	0.28	0.405	0.42	0.33
Average	Mean± SD	0.77±0.89	0.10±0.13	3.85±4.99	16.17±19.08	0.78±0.53	0.09±0.03	0.002±0.001	1.82±0.90	0.58±0.22
(N=300)	Min, Max	0.01, 3.35	0.00, 0.44	0.08, 36.26	0.34, 70.02	0.112.54,	0.03, 0.17	0.000, 0.014	0.29, 6.25	0.13, 1.32
	CV	1.16	1.20	1.30	1.18	0.68	0.27	0.770	0.50	0.38

SD = Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples, CR = Concentration Ratio

Table 7. 5: The concentration ratio (CR) of the essential elements among the rice varieties in the unflooded area of the field.

			Concentration Ratio										
	Rice Varieties		**Mn-CR**	**Co-CR**	**Cu-CR**	**Zn-CR**	**Se-CR**	**Ca-CR**	**Fe-CR**	**K-CR**	**Mg-CR**	**Cs-CR**	**Sr-CR**
Unflooded Field Area	**IRAT_170**	Mean± SD	0.55±0,69	0.05±0.06	3.51±5.14	14.19±18.77	0.74±0.51	0.11±0.02	0.00±0.00	1.66±0.37	0.50±0.09	0.05±0.03	0.15±0.04
	Variety 1	Min, Max	0.05, 1.78	0.00, 0.19	0.16,13.82	1.28, 49.07	0.11,1.48	0.08,0.13	0.00,0.01	1.04,2.57	0.33,0.70	0.01,0.11	0.09,0.26
	(N=15)	CV	1.26	1.20	1.46	1.32	0.51	0.18	1.00	0.22	0.18	0.60	0.26
	SIPI_692033	Mean± SD	0.44±0.62	0.08±0.12	2.08±3.44	9.77±14.55	0.67±0.47	0.10±0.02	0.00±0.00	1.85±0.35	0.59±0.10	0.11±0.12	0.13±0.01
	Variety 2	Min, Max	0.01, 1.62	0.00, 0.32	0.15, 9.64	1.33, 39.45	0.27,1.63	0.07,0.13	0.00,0.01	1.16,2.35	0.38,0.76	0.01,0.53	0.12,0.17
	(N=15)	CV	1.40	1.50	1.65	1.49	0.70	0.20	1.00	0.19	0.17	1.09	0.08
	ITA_315	Mean± SD	0.61±0.87	0.07±0.11	2.43±3.54	11.10±16.35	0.72±0.56	0.12±0.03	0.00±0.00	1.92±0.61	0.60±0.15	0.08±0.04	0.13±0.01
	Variety 3	Min, Max	0.02, 2.33	0.00, 0.27	0.22,10.47	0.94, 47.85	0.24,1.83	0.07,0.16	0.00,0.01	1.29,3.75	0.42,1.04	0.01,0.11	0.11,0.14
	(N=15)	CV	1.42	1.57	1.46	1.47	0.78	0.25	1.00	0.32	0.25	0.50	0.08
	WITA_4	Mean± SD	0.55±0.69	0.09±0.11	4.01±9.23	10.38±13.32	0.72±0.53	0.11±0.03	0.00±0.00	1.68±0.29	0.54±0.09	0.05±0.04	0.13±0.02
	Variety 4	Min, Max	0.05, 1.86	0.01, 0.27	0.12, 36.26	1.06,33.89	0.15,1.55	0.06,0.16	0.00,0.01	1.37,2.31	0.43,0.77	0.01,0.11	0.12,0.18
	(N=15)	CV	1.25	1.22	2.30	1.28	0.74	0.27	1.00	0.17	0.17	0.80	0.15
	NERICA_L19	Mean± SD	0.50±0.74	0.06±0.09	1.91±3.03	9.85±15.52	0.70±0.58	0.12±0.03	0.00±0.00	1.96±0.41	0.62±0.12	0.05±0.03	0.14±0.02
	Variety 5	Min, Max	0.09, 2.15	0.00, 0.25	0.13, 9.58	1.15,43.98	0.31,2.08	0.08,0.17	0.00,0.01	1.35,2.91	0.45,0.89	0.01,0.10	0.13,0.18
	(N=15)	CV	1.48	1.50	1.59	1.58	0.83	0.18	1.00	0.21	0.19	0.60	0.14
	NERICA_L34	Mean± SD	0.62±0.82	0.10±0.13	2.57±3.51	11.55±15.01	0.77±0.59	0.10±0.02	0.00±0.00	1.78±0.51	0.55±0.17	0.07±0.04	0.13±0.02
	Variety 6	Min, Max	0.03, 2.06	0.00, 0.13	0.13, 9.15	1.17,37.36	0.26,1.82	0.08,0.13	0.00,0.01	1.03,2.82	0.29,0.85	0.01,0.11	0.10,0.19
	(N=15)	CV	1.32	1.30	1.37	1.30	0.77	0.18	1.00	0.28	0.31	0.57	0.15
	NCRO_49	Mean± SD	0.53±0.70	0.07±0.10	1.87±2.59	11.66±16.19	0.78±0.62	0.11±0.02	0.00±0.00	2.04±0.51	0.62±0.15	0.07±0.04	0.13±0.01
	Variety 7	Min, Max	0.05, 1.76	0.00, 0.27	0.08, 7.59	1.25,39.66	0.27,1.95	0.080.16	0.00,0.01	1.43,3.47	0.45,1.02	0.02.0.11	0.12,0.17
	(N=15)	CV	1.32	1.42	1.39	1.39	0.79	0.18	1.00	0.25	0.24	0.57	0.08
	ART3_7L	Mean± SD	0.70±0.93	0.11±0.15	3.08 ±4.40	14.52±20.05	0.74±0.55	0.11±0.02	0.00±0.00	2.22±0.61	0.70±0.17	0.06±0.03	0.13±0.01
	Variety 8	Min, Max	0.06, 2.46	0.00, 0.38	0.11, 12.04	0.88,52.16	0.21,1.62	0.07,0.14	0.00,0.01	1.46,3.49	0.48,1.04	0.01,0.10	0.11,0.14
	(N=15)	CV	1.32	1.36	1.43	1.38	0.74	0.18	1.00	0.27	0.24	0.50	0.08
	ART_15	Mean± SD	0.80±1.09	0.07±0.09	3.79±5.63	15.88±22.39	0.73±0.54	0.10±0.02	0.00±0.00	2.20±0.88	0.67±0.20	0.04±0.03	0.13±0.01
	Variety 9	Min, Max	0.09, 3.35	0.01, 0.28	0.14, 17.62	0.34,59.43	0.25,1.64	0.08,0.14	0.00,0.01	1.44,4.65	0.47,1.12	0.01,0.11	0.11,0.17
	(N=15)	CV	1.36	1.29	1.49	1.41	0.74	0.20	1.00	0.40	0.30	0.75	0.08
	BISALAYI	Mean± SD	0.42±0.64	0.05±0.08	1.98±3.59	8.49±14.52	0.63±0.47	0.10±0.03	0.00±0.00	1.56±0.23	0.56±0.07	0.07±0.04	0.14±0.03
	Variety 10	Min, Max	0.08, 1.78	0.01, 0.22	0.09, 11.40	1.02,42.14	0.16,1.49	0.07,0.17	0.00,0.01	1.24,2.00	0.43,0.67	0.01,0.11	0.10,0.18
	(N=15)	CV	1.52	1.60	1.81	1.71	0.75	0.25	1.00	0.15	0.13	0.57	0.21
	Average	Mean± SD	0.57±0.78	0.07±0.11	2.73±4.70	11.76±16.55	0.72±0.53	0.11±0.02	0.00±0.00	1.89±0.54	0.59±0.15	0.06±0.05	0.14±0.02
	(N=150)	Min, Max	0.01, 3.35	0.00, 0.38	0.08, 36.26	0.34,59.43	0.11,2.08	0.06,0.17	0.00,0.01	1.03,4.65	0.29,1.12	0.01,0.53	0.09,0.26

SD=Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples, CR = Concentration Ratio

Table 7. 6: The concentration ratio (CR) of the essential elements among the rice varieties in the unflooded area of the field.

			Concentration Ratio										
	Rice Varieties		**Mn-CR**	**Co-CR**	**Cu-CR**	**Zn-CR**	**Se-CR**	**Ca-CR**	**Fe-CR**	**K-CR**	**Mg-CR**	**Cs-CR**	**Sr-CR**
Flooded Field Area	**IRAT_170**	Mean± SD	0.88±0.82	0.14±0.13	4.25±4.21	20.45±19.08	0.81±0.47	0.09±0.03	0.00±0.00	1.80±1.04	0.59±0.31	0.06±0.04	0.13±0.02
	Variety 1	Min, Max	0.03,2.20	0.01,0.39	0.13,11.40	0.50,49.73	0.25,1.43	0.03,0.14	0.00,0.01	0.29,3.44	0.13,1.17	0.01,0.11	0.11,0.18
	(N=15)	CV	0.93	0.93	0.99	0.93	0.58	0.33	1.00	0.58	0.53	0.66	0.15
	SIPI_692033	Mean± SD	0.92±1.09	0.12±0.14	4.66±5.68	22.10±25.62	0.77±0.49	0.09±0.02	0.00±0.00	1.98±1.52	0.61±0.32	0.08±0.12	0.14±0.02
	Variety 2	Min, Max	0.05,3.09	0.01,0.34	0.25,15.12	0.92,70.02	0.26,1.47	0.06,0.13	0.00,0.01	0.63,6.25	0.25,1.32	0.01,0.52	0.10,0.19
	(N=30)	CV	1.19	1.17	1.22	1.16	0.63	0.22	1.00	0.77	0.52	1.50	0.14
	ITA_315	Mean± SD	0.99±0.98	0.11±0.11	5.60±5.48	20.42±20.20	0.83±0.52	0.08±0.01	0.00±0.00	1.55±0.78	0.54±0.22	0.07±0.03	0.14±0.02
	Variety 3	Min, Max	0.05,2.43	0.01,0.29	0.19,12.10	0.92,45.05	0.2,1.62	0.05,0.10	0.00,0.01	0.63,2.79	0.30,0.92	0.01,0.11	0.12,0.19
	(N=15)	CV	0.98	1.00	0.98	0.99	0.62	0.13	1.00	0.50	0.40	0.42	0.14
	WITA_4	Mean± SD	1.05±1.01	0.14±0.14	5.38±5.08	21.13±20.36	0.85±0.51	0.08±0.02	0.00±0.00	1.82±1.26	0.59±0.27	0.07±0.04	0.14±0.02
	Variety 4	Min, Max	0.07,2.58	0.01,0.33	0.31,13.08	1.06,58.88	0.32,1.74	0.05,0.11	0.00,0.01	0.54,5.24	0.27,1.19	0.01,0.11	0.12,0.18
	(N=15)	CV	0.96	1.00	0.94	0.96	0.60	0.25	1.00	0.69	0.45	0.57	0.14
	NERICA_L19	Mean± SD	0.80±0.89	0.10±0.12	5.07±5.77	19.54±21.86	0.77±0.51	0.08±0.02	0.00±0.00	1.53±1.02	0.49±0.26	0.07±0.06	0.13±0.03
	Variety 5	Min, Max	0.05,2.42	0.00,0.44	0.10,14.73	1.01,55.59	0.24,1.46	0.06,0.12	0.00,0.01	0.58,3.83	0.24,1.05	0.01,0.26	0.05,0.19
	(N=15)	CV	1.11	1.20	1.14	1.12	0.66	0.25	1.00	0.67	0.53	0.86	0.15
	NERICA_L34	Mean± SD	1.11±1.05	0.20±0.19	5.91±5.54	23.20±21.91	0.90±0.66	0.08±0.02	0.00±0.00	1.77±1.08	0.57±0.24	0.06±0.03	0.14±0.02
	Variety 6	Min, Max	0.06,2.53	0.01,0.44	0.27,13.20	0.91,51.67	0.24,2.54	0.06,0.11	0.00,0.01	0.60,4.09	0.28,1.08	0.01,0.11	0.11,0.18
	(N=15)	CV	0.95	0.95	0.94	0.94	0.73	0.25	1.00	0.61	0.42	0.50	0.14
	NCRO_49	Mean± SD	1.17±1.00	0.13±0.12	5.76±5.47	25.34±22.36	0.91±0.55	0.09±0.02	0.00±0.00	2.33±1.64	0.67±0.33	0.08±0.11	0.13±0.01
	Variety 7	Min, Max	0.05,2.61	0.00,0.28	0.26,15.02	1.00,56.92	0.28,1.82	0.05,0.13	0.00,0.01	0.63,6.04	0.28,1.26	0.01,0.46	0.12,0.14
	(N=15)	CV	0.85	0.92	0.95	0.88	0.60	0.22	1.00	0.70	0.49	1.38	0.15
	ART3_7L	Mean± SD	0.89±0.90	0.14±0.14	4.40±4.69	17.60±18.47	0.88±0.58	0.08±0.02	0.00±0.00	1.43±0.95	0.47±0.23	0.05±0.02	0.14±0.02
	Variety 8	Min, Max	0.05,2.57	0.01,0.31	0.24,13.01	0.85,53.64	0.25,1.75	0.05,0.11	0.00,0.01	0.58,4.22	0.25,1.05	0.01,0.09	0.10,0.19
	(N=15)	CV	1.01	1.00	1.70	1.05	0.66	0.25	1.00	0.66	0.49	0.40	0.14
	ART_15	Mean± SD	0.85±1.04	0.13±0.16	4.48±4.86	18.26±19.27	0.82±0.56	0.08±0.01	0.00±0.00	1.56±0.95	0.53±0.24	0.06±0.04	0.13±0.02
	Variety 9	Min, Max	0.06,3.28	0.01,0.44	0.20,13.40	0.91,46.47	0.26,1.72	0.05,0.10	0.00,0.01	0.61,3.54	0.28,1.03	0.01,0.11	0.10,0.18
	(N=15)	CV	1.22	1.23	1.08	1.06	0.68	0.13	1.00	0.61	0.45	0.66	0.15
	BISALAYI	Mean± SD	0.95±0.95	0.14±0.14	4.23±4.36	18.09±18.88	0.85±0.53	0.09±0.03	0.00±0.00	1.70±1.11	0.56±0.28	0.07±0.03	0.13±0.02
	Variety 10	Min, Max	0.06,2.77	0.01,0.35	0.25,10.76	1.00,54.74	0.30,1.65	0.05,0.15	0.00,0.01	0.63,4.51	0.28,1.20	0.01,0.11	0.11,0.18
	(N=15)	CV	1.00	1.00	1.03	1.04	0.62	0.33	1.00	0.65	0.50	0.42	0.15
	Average	Mean± SD	0.96±0.95	0.14±0.14	4.96±5.03	20.58±20.43	0.84±0.53	0.08±0.02	0.00±0.00	1.74±1.15	0.56±0.27	0.07±0.06	0.13±0.02
	(N=150)	Min, Max	0.03,3.28	0.00,0.44	0.10,15.12	0.50,70.02	0.24,2.54	0.03,0.15	0.00,0.01	0.29,6.25	0.13,1.32	0.01,0.52	0.05,0.19
		CV	0.99	1.00	1.01	0.99	0.63	0.25	1.00	0.66		0.86	0.15

SD=Standard Deviation, CV= Coefficient of Variance, Max = Maximum, Min = Minimum, N= Number of samples, CR = Concentration Ratio

In the result of the field experiment generally, the inter-varietal variation (IVV) of Ca among the 10 selected rice varieties was 1.3 folds, Fe was 4 folds, K was 1.3 folds, Mg was 1.2 folds, Zn was 1 fold, Mn was 1.2 folds, Co was 1.9 folds and Cu was 1.3 folds.

For the unflooded area, the inter-varietal variation (IVV) among the rice varieties for Ca was 1.2 folds, Fe was 1 fold, K was 1.4 folds, Mg was 1.4 folds, Zn was 1.9 folds, Mn was 1.9 folds, Co was 2.2 folds and Cu was 2.1 fold (Table 6.5). At the flooded side of the field, Ca was 1.1 folds, Fe was 1 fold, K was 1.6 folds, Mg was 1.4 folds, Zn was 1 folds, Mn was 1.2 folds, Co was 1.9 folds and Cu was 1.3 fold. As explained previously, the result shows no significant difference among the two set of data for the flooded and the unflooded area of the field. Table 7.7 compares the CR among the 10 rice varieties for the unflooded and the flooded area of the field with their inter-varietal variations while Table 7.8 presents same for the general field.

Table 7. 7: Comparing the essential elements' CR for both the unflooded and the flooded area of the field and their IVV.

	Rice Varieties	N	Mn-CR	Co-CR	Cu-CR	Zn-CR	Se-CR	Pb-CR	Ca-CR	Fe-CR	K-CR	Mg-CR	Cs-CR	Sr-CR
	1	15	a	a	a	a	a	a	abc	b	cd	d	b	a
	2	15	a	a	a	a	a	a	c	b	abcd	bcd	b	ab
	3	15	a	a	a	a	a	a	ab	b	abcd	abcd	b	b
Unflooded	4	15	a	a	a	a	a	a	abc	b	cd	cd	b	ab
	5	15	a	a	a	a	a	a	a	b	abcd	abc	b	ab
	6	15	a	a	a	a	a	a	c	b	bcd	cd	ab	ab
	7	15	a	a	a	a	a	a	abc	b	abc	abc	ab	ab
	8	15	a	a	a	a	a	a	abc	b	a	a	ab	b
	9	15	a	a	a	a	a	a	abc	b	ab	ab	ab	b
	10	15	a	a	a	a	a	a	bc	a	d	bcd	a	ab
	IVV	150	**1.9**	**2.2**	**2.1**	**1.9**	**1.2**	**7.5**	**1.2**	**1**	**1.4**	**1.4**	**2.8**	**1.1**
		N	Mn-CR	Co-CR	Cu-CR	Zn-CR	Se-CR	Pb-CR	Ca-CR	Fe-CR	K-CR	Mg-CR	Cs-CR	Sr-CR
	1	15	a	a	a	a	a	a	a	b	a	a	a	a
	2	15	a	a	a	a	a	a	a	b	a	a	a	a
	3	15	a	a	a	a	a	a	a	ab	a	a	a	a
Flooded	4	15	a	a	a	a	a	a	a	a	a	a	a	a
	5	15	a	a	a	a	a	a	a	b	a	a	a	a
	6	15	a	a	a	a	a	a	a	b	a	a	a	a
	7	15	a	a	a	a	a	a	a	b	a	a	a	a
	8	15	a	a	a	a	a	a	a	b	a	a	a	a
	9	15	a	a	a	a	a	a	a	ab	a	a	a	a
	10	15	a	a	a	a	a	a	a	b	a	a	a	a
	IVV	150	**1.4**	**5.7**	**1.4**	**1.2**	**1.2**	**2.2**	**1.1**	**1**	**1.6**	**1.4**	**1.6**	**1.1**

IVV = Inter-varietal variation. N=sample number. CR=Concentration Ratio. Different letters = significant difference. Same letters = no significant difference.

Table 7. 8: Comparing the essential elements' uptake using CR and the IVV for the rice varieties.

Rice Varieties	N	Ca-CR	Fe-CR	K-CR	Mg-CR	Zn-CR	Se-CR	Mn-CR	Co-CR	Cu-CR
IRAT-170	30	ab	b	de	c	a	ab	d	f	a
SIPI-692033	30	d	b	a	a	a	ab	bc	a	abc
ITA-315	30	a	b	cd	b	bcd	b	abc	de	abc
WITA-4	30	b	b	ef	c	d	ab	d	cd	cd
NERICA-L19	30	a	b	bc	b	bc	a	c	de	abc
NERICA-L34	30	d	b	ef	c	cd	b	d	cde	d
NCRO-49	30	a	b	ab	b	ab	b	abc	de	cd
ART3-7L	30	ab	b	a	a	a	b	abc	b	bcd
ART-15	30	bc	b	a	a	a	b	a	e	ab
BISALAYI	30	cd	a	f	b	cd	ab	ab	bc	abcd
Inter-Varietal Variation (IVV)	300	1.25	4.00	1.34	1.20	1.04	1.03	1.18	1.86	1.28

Human growth and general development, normal functioning of the body systems, mental balances and healthy living depend on the availability of the required nutrients and the essential elements in the body to set them running (Whitney, DeBruyne, Pinna, & Rolfes, 2010). Deficiency can lead to health challenges (Whitney & Rolfes, 2018).

The result of the field samples shows that the 10 rice varieties were different based on the uptake of the essential elements. For the purpose of the varietal selection, the rice varieties were ranked and the result of the rankings is presented in Table 7.9. Art-15 rice was observed to uptake the highest Cu, Zn, K, Se and Mn and was ranked the best to be a good source of these elements. Bisalayi rice which is the local rice in the region appeared to be a good source of Fe more than other nine rice varieties. Ncro appeared to be the best for Ca more than the other rice varieties.

Table 7. 9: Varietal selection and ranking for the essential elements in the field samples

Rice Varieties	**Highest in the Essential Elements**								
	Ca	**Fe**	**K**	**Mg**	**Zn**	**Se**	**Mn**	**Co**	**Cu**
IRAT-170*									
SIPI-692033									
ITA-315									
WITA-4									
NERICA-L19									
NERICA-L34*								✓	
NCRO-49*	✓								
ART3-7L				✓					
ART-15***			✓		✓	✓	✓		✓
BISALAYI**		✓							

✓ = uptake highest in the field experiment, *** = highest ranked, no star = no rank.

7.2.2 Inter-varietal variation of the essential elements in rice (pot experiment)

The mean concentration of Ca was 141.38±53.29 mg/kg, Fe was 30.92±161.19 mg/kg, Cu was 4.90±1.14 mg/kg, Mg was 958.78±158.06mg/kg, K was 2218.55±458.91 mg/kg, Zn was 28.76±5.09 mg/kg, Mn was 18.39±3.27 mg/kg, Co was 0.03±0.05 mg/kg, and Se was 0.05±0.05 mg/kg in rice samples in the pot experiment respectively and the ranges (mg/kg) were from 0.03 to 855.79 for Ca, 0.05 to 2802.62 for Fe, 0.01 to 11.20 for Cu, 0.03 to 1412.97 for Mg, 0.05 to 3279.95 for K, 0.01 to 54.31 for Zn, 0.05 to 27.10 for Mn, 0.01 to 0.48 for Co, and 0.01 to 0.69 for Se respectively.

In the soil, the mean concentration of Ca was 1789.81±304.93 mg/kg (ranges from 0.03 mg/kg to 3191.93 mg/kg), Fe was 9371.08±1231.17 mg/kg (ranges from 0.01 mg/kg to 15185.55 mg/kg), Cu was 6.42±0.93 mg/kg (ranges from 0.01 mg/kg to 13.33 mg/kg), Mg was 2129.36±248.05 mg/kg (ranges from 0.03 mg/kg to 2889.50 mg/kg), K was 2015.39±230.46 mg/kg (ranges from 0.05 mg/kg to 2662.48 mg/kg), Zn was 11.37±1.65 mg/kg (ranges from 0.01 mg/kg to 18.76 mg/kg), Mn was 172.69±30.29 mg/kg (ranges from 0.01 mg/kg to 435.77 mg/kg), Co was 3.56±0.90 mg/kg (ranges from 0.03 mg/kg to 11.28 mg/kg), and Se was 0.97±0.26 mg/kg (ranges from 0.10 mg/kg to 1.40 mg/kg) respectively. The soil physico-chemical parameters of the clean soil before and after the pot experiment is presented in Table 7.10. Statistical summary of the result of the pot experiment is presented in Table 7.11.

Table 7. 10: Result of the soil physico-chemical analysis before and after the pot experiment.

	Soil (n=60) (n=300)	pH (H_2O) 1:1	^{a}EC (dS/m)	%Organic Carbon	Av-P_2O_2	Exchangeable cations (cmol /kg)				Exchangeable Acidity	CEC	Particle size			N
						Ca	K	Mg	Na			%Clay	%Silt	%Sand	
Pre	Mean	5.75	0.17	2.65	8.85	7.69	0.08	2.37	0.04	1.38	11.58	5.96	12.77	81.23	3.50
Planting	SD	0.49	0.19	0.72	7.08	1.61	0.03	0.50	0.01	0.32	2.14	2.30	3.56	4.50	0.52
Soil	Min	4.17	0.05	1.38	1.33	5.00	0.10	1.50	0.02	1.06	7.81	3.36	3.94	68.68	0.08
Analysis	Max	6.29	1.12	6.01	26.32	12.00	0.12	4.00	0.06	2.72	18.83	15.46	19.94	90.72	8.50
Post	Mean	5.76	0.98	2.63	7.94	1789.81	2015.39	2129.36	26.50	0.66	32.55	7.35	20.29	72.36	2.49
Planting	SD	0.70	0.52	0.72	4.17	304.93	230.46	248.05	36.27	0.17	3.21	2.72	5.90	6.66	1.84
Soil	Min	3.98	0.05	1.35	0.62	0.03	0.05	0.03	0.05	0.36	23.03	3.36	3.94	55.62	0.06
Analysis	Max	7.38	2.30	6.23	26.32	3191.93	2662.48	2889.50	278.75	2.37	44.88	16.49	37.98	90.72	7.25

SD = Standard Deviation, Max = Maximum, Min = Minimum, n= Number of samples, EC= Electrical Conductivity, Av-P_2O_2 = Available Phosphorus, N = Nitrogen

Table 7. 11: Result summary of mean concentrations (mg/kg) of the essential elements in rice and their corresponding soil samples

		Parameters	**Mn**	**Co**	**Cu**	**Zn**	**Se**		**Fe**	**K**	**Mg**
IRAT_170 Variety 1	RICE (N=30)	Mean± SD	16.46±1.04	0.02±0.01	4.41±0.72	26.67±2.77	0.05±0.04	168.00±17.257	17.51±4.88	2149.12±144.00	938.70±75.21
		Min, Max	14.37, 18.58	0.01, 0.03	3.76, 7.29	22.61, 35.39	0.00, 0.10	142.88, 208.76	10.67, 32.62	1970.47, 2523.09	848.96, 1102.74
		CV	0.06	0.23	0.16	0.10	0.85	0.103	0.28	0.07	0.08
	SOIL (N=30)	Mean± SD	169.46±38.98	3.53±1.08	6.29±1.13	11.14±2.06	0.95±0.29	1711.97±227.71	9190.47±985.48	1981.59±178.03	2100.72±187.17
		Min, Max	44.77, 311.45	0.95, 7.93	1.83, 8.50	2.85, 14.14	0.36, 1.39	1291.10, 2198.84	7722.84, 12279.840	1609.08, 2325.57	1730.12,2396.37
		CV	0.23	0.31	0.18	0.19	0.30	0.13	0.11	0.09	0.09
SIPI_692033 Variety 2	RICE (N=30)	Mean ±SD	17.26±1.47	0.02±0.01	5.91±0.97	30.51±2.40	0.05±0.03	113.27±16.49	21.76±3.61	2152.02±169.20	853.26±90.39
		Min, Max	14.88, 21.99	0.02, 0.03	5.29, 10.74	26.87, 36.73	0.01, 0.10	84.28, 149.65	14.4, 29.96	1869.71, 2543.68	702.50, 1059.25
		CV	0.09	0.19	0.16	0.08	0.73	0.15	0.17	0.08	0.11
	SOIL (N=30)	Mean ±SD	172.89±19.29	3.56±0.53	6.32±0.56	11.42±1.42	0.98±0.26	1736.69±231.90	9288.99±828.05	1989.30±167.85	2112.70±182.06
		Min, Max	136.67, 231.31	2.72, 4.95	5.20, 7.11	9.51, 14.68	0.59, 1.31	1410.12, 2356.36	8081.23, 11034.890	1718.70, 2415.53	1797.97, 2484.30
		CV	0.11	0.15	0.09	0.12	0.26	0.13	0.09	0.08	0.09
ITA_315 Variety 3	RICE (N=30)	Mean ±SD	17.42±1.08	0.01±0.01	5.04±1.09	28.23±2.86	0.06±0.04	143.65±17.81	15.07±2.24	2254.21±176.27	1009.25±90.44
		Min, Max	15.24, 20.52	0.01, 0.02	4.30, 9.65	23.88, 37.29	0.00, 0.10	115.55, 194.76	11.82, 20.43	2014.36, 2818.97	876.04, 1294.53
		CV	0.06	0.36	0.22	0.10	0.69	0.12	0.15	0.08	0.09
	SOIL (N=30)	Mean	165.67±41.62	3.34±0.99	6.14±1.32	11.36±2.91	0.94±0.30	1801.82±262.61	9329.43±1286.52	2024.44±247.10	2140.66±275.42
		Min, Max	0.01, 293.23	0.03, 6.73	0.01, 7.64	0.01, 18.76	0.10, 1.30	1323.15, 2444.80	6292.72, 12399.26	1492.18, 2662.48	1496.31, 2767.96
		CV	0.25	0.30	0.22	0.26	0.32	0.15	0.14	0.12	0.13
WITA_4 Variety 4	RICE (N=30)	Mean± SD	17.36±1.20	0.02±0.00	4.49±0.88	26.12±2.11	0.05±0.04	158.88±25.44	16.08±3.08	2179.18±190.88	970.99±105.03
		Min, Max	14.70, 19.78	0.02, 0.03	3.83, 8.99	23.85, 33.05	0.00, 0.10	126.45, 240.66	11.89, 24.31	1874.81, 2830.48	810.63, 1310.24
		CV	0.07	0.09	0.20	0.08	0.78	0.16	0.19	0.09	0.11
	SOIL	Mean± SD	169.27±17.26	3.39±0.38	6.51±0.87	11.63±1.47	0.97±0.25	1854.96±242.82	9455.92±1261.33	2044.49±178.06	2148.95±186.53

	(N=30)	Min, Max	140.38, 202.17	2.80, 4.14	5.29, 9.20	9.64, 15.06	0.58, 1.35	1496.68, 2389.68	7915.11, 14519.68	1638.94, 2336.37	1746.65, 2566.92
		CV	0.10	0.11	0.13	0.13	0.26	0.13	0.13	0.09	0.09
NERICA_L19	RICE	Mean± SD	16.14±1.10	0.01±0.01	5.19±1.21	29.86±2.69	0.04±0.04	131.20±18.45	15.54±2.68	2261.55±157.32	1014.80±88.16
Variety 5	(N=30)	Min, Max	14.09, 17.88	0.01, 0.02	4.45, 10.66	26.02, 37.65	0.01, 0.10	89.78, 167.96	11.64, 20.80	1889.03, 2571.50	835.07, 1187.54
		CV	0.07	0.36	0.23	0.09	0.93	0.14	0.17	0.07	0.09
	SOIL	Mean± SD	171.85±16.82	3.48±0.44	6.41±0.65	11.42±1.27	0.99±0.26	1881.04±361.50	9432.80±958.41	2046.90±189.24	2160.31±224.79
	(N=30)	Min, Max	138.50, 212.44	2.70, 4.65	5.19, 7.31	9.50, 13.98	0.59, 1.33	1455.97, 3191.93	7593.45, 11967.70	1698.57, 2485.37	1822.07, 2889.50
		CV	0.10	0.13	0.10	0.11	0.26	0.19	0.10	0.09	0.10
NERICA_L34	RICE	Mean± SD	16.89±3.55	0.03±0.03	4.75±1.01	29.22±7.23	0.05±0.04	134.88±35.84	22.33±8.87	2127.20±433.63	949.09±198.61
Variety 6	(N=30)	Min, Max	0.05, 23.87	0.02, 0.16	0.01, 6.21	0.01, 51.18	0.01, 0.10	0.03, 211.38	0.05, 45. 17	0.05, 2662.45	0.03, 1199.42
		CV	0.21	0.97	0.21	0.25	0.83	0.27	0.40	0.20	0.21
	SOIL	Mean± SD	173.05±22.27	3.68±1.02	6.70±1.41	11.38±1.38	0.98±0.26	1789.77±463.47	9360.01±2368.85	1971.19±402.04	2071.70±433.93
	(N=30)	Min, Max	139.19, 228.28	2.80, 7.85	5.31, 13.33	9.30, 15.07	0.61, 1.31	0.03,2853.97	0.01,15185.55	0.05, 2334.12	0.03, 2553.62
		CV	0.13	0.28	0.21	0.12	0.26	0.26	0.25	0.20	0.21
NCRO_49	RICE	Mean± SD	16.30±1.14	0.01±0.01	4.68±1.25	27.49±2.49	0.05±0.04	162.81±19.65	15.14±2.91	2243.18±152.11	978.39±87.05
Variety 7	(N=30)	Min, Max	14.27, 18.98	0.01, 0.02	3.91, 9.52	23.54, 35.87	0.00, 0.10	131.27, 206.04	11.32, 22.19	2017.12, 2542.29	839.04, 1170.93
		CV	0.07	0.36	0.27	0.09	0.77	0.12	0.19	0.07	0.09
	SOIL	Mean± SD	179.94±31.28	3.65±0.75	6.64±0.79	11.68±1.37	0.98±0.25	1802.04±342.71	9673.99±1244.90	2041.85±237.59	2141.42±219.12
	(N=30)	Min, Max	141.23, 272.47	2.83, 6.81	5.44, 8.96	9.66, 15.12	0.61, 1.36	1406.01, 2925.32	7661.16, 13041.36	1651.75, 2481.12	1689.43, 2801.51
		CV	0.17	0.21	0.12	0.12	0.26	0.19	0.13	0.12	0.10
ART3_7L	RICE	Mean± SD	23.17±1.11	0.02±0.00	5.23±1.15	33.31±2.30	0.04±0.03	158.20±133.18	19.86±9.01	2649.93±181.67	1119.22±90.05
Variety 8	(N=30)	Min, Max	21.38, 25.85	0.02, 0.03	4.61, 11.20	28.74, 40.98	0.00, 0.10	105.81, 855.79	13.80, 61.53	2362.83, 3263.36	969.06, 1412.97
		CV	0.05	0.09	0.22	0.07	0.77	0.84	0.45	0.07	0.08
	SOIL	Mean± SD	170.34±14.09	3.60±0.80	6.36±0.76	11.13±1.41	0.98±0.26	1828.33±312.65	9471.93±912.31	2074.33±234.10	2211.95±249.58
	(N=30)	Min, Max	138.34, 194.99	2.81, 7.49	4.84, 8.22	8.73, 14.69	0.59, 1.40	1120.25,2426.58	7077.66, 11027.69	1487.17, 2587.77	1558.50, 2719.44
		CV	0.08	0.22	0.12	0.13	0.26	0.17	0.10	0.11	0.11
ART_15	RICE	Mean± SD	24.39±1.42	0.02±0.00	5.42±0.97	35.00±4.73	0.06±0.12	137.01±21.31	20.68±5.54	2931.63±146.88	1088.03±71.10
Variety 9	(N=30)	Min, Max	20.66, 26.91	0.01, 0.03	4.78, 10.26	30.73, 54.31	0.00, 0.69	106.46, 203.47	14.35, 43.06	2730.07, 3279.95	974.71, 1226.39
		CV	0.06	0.23	0.18	0.14	2.11	0.16	0.27	0.05	0.07
	SOIL	Mean± SD	173.67±27.70	3.67±0.95	6.42±0.65	11.31±1.18	0.98±0.27	1706.024±218.417	9195.29±898.50	1976.09±155.93	2083.30±169.25
	(N=30)	Min, Max	143.70, 296.37	2.77, 7.39	5.23, 7.79	9.47, 14.03	0.57, 1.35	1391.52, 2164.39	7702.93, 11628.02	1704.25, 2338.29	1869.00, 2576.95
		CV	0.16	0.26	0.10	0.10	0.27	0.13	0.10	0.08	0.08
BISALAYI	RICE	Mean± SD	18.56±2.43	0.15±0.08	3.85±0.74	21.16±2.87	0.06±0.02	105.93±58.57	145.21±502.54	1237.43±161.22	666.11±74.79
Variety 10	(N=30)	Min, Max	15.16, 27.10	0.06, 0.48	3.14, 7.46	16.74, 29.36	0.01, 0.15	68.28, 394.67	30.81, 2802.62	1093.73, 1946.09	575.41, 930.14
		CV	0.13	0.53	0.19	0.14	0.45	0.55	3.46	0.13	0.11
	SOIL	Mean± SD	180.73±51.10	3.73±1.52	6.43±0.73	11.27±1.48	0.98±0.28	1785.48±292.31	9311.92±900.43	2003.69±223.64	2121.84±249.40
	(N=30)	Min, Max	141.87, 435.77	2.82, 11.28	5.12, 8.25	9.25, 15.36	0.53, 1.36	1312.49, 2418.88	7751.43, 11600.56	1607.70, 2488.77	1701.36, 2805.68
		CV	0.28	0.41	0.11	0.13	0.29	0.16	0.10	0.11	0.12
Total	RICE	Mean± SD	18.39±3.27	0.03±0.05	4.90±1.14	28.76±5.09	0.05±0.05	141.38±53.29	30.92±161.19	2218.55±458.91	958.78±158.06
	(N=300)	Min, Max	0.05, 27.10	0.01, 0.48	0.01, 11.20	0.01, 54.31	0.01, 0.69	0.03, 855.79	0.05, 2802.62	0.05, 3279.95	0.03, 1412.97
		CV	0.18	1.48	0.23	0.18	1.06	0.38	5.21	0.21	0.17
	SOIL	Mean± SD	172.69±30.29	3.56±0.90	6.42±0.93	11.37±1.65	0.97±0.26	1789.81±304.93	9371.08±1231.17	2015.39±230.46	2129.36±248.05
	(N=300)	Min, Max	0.01, 435.77	0.03, 11.28	0.01, 13.33	0.01, 18.76	0.10, 1.40	0.03, 3191.93	0.01, 15185.55	0.05, 2662.48	0.03, 2889.50
		CV	0.18	0.25	0.15	0.15	0.27	0.17	0.13	0.11	0.12

SD = Standard Deviation, Max = Maximum, Min = Minimum, N= Number of samples, CV= Coefficient of Variance

The mean CR for the pot experiment (Table 7.12) shows that the uptake in all the essential elements in the 10 selected rice varieties was lower than that of the field experiment. Ca (soil n=300, rice n=300) was 0.08±0.03 (min 0.03, max 0.05). The lowest of 0.06±0.03 was found in bisalayi rice (variety 10) and the highest of 0.10±0.02 was found in irat-170 (variety 1) which is similar to ita-315 (variety 3), wita-4 (variety 4), and nerica-l34 (variety 6) (Table 7.13), the Duncan multiple range test shows. The concentration ratio (CR) of the nine essential elements in the rice samples for the 10 varieties in the pot is presented in Table 7.12.

Iron (Fe) accumulation in both the field experiment and the pot experiment were significantly similar (Figure 7.3). In both experiments bisalayi had highest iron uptake, art-15 rice had the highest content of potassium (K) and bisalayi rice had the lowest potassium content. art-15 rice also had the highest Manganese (Mn) in both experiments. Others were different in both sides of the trials and this was used with the concentration ratio to calculate inter-varietal variation (IVV) among the 10 varieties. IVV is the mean CR in the high accumulated variety divided by mean CR in the lowest accumulated variety. The variety of rice with the highest and the lowest uptake for all the nine essential element is revealed in Figure 7.2 (a-i) for both the field and the pot experiment. While Figure 7.1 (a-i) compares the mean concentrations (mg/kg) of the essential elements in the rice samples for both the field and the pot experiments. Table 7.13 presents the Duncan multiple range post ANOVA test and the inter-varietal variation for both the field and pot experiments.

Table 7. 12: Result of the concentration ratio (CR) for the pot samples.

		Mn-CR	Co-CR	Cu-CR	Zn-CR	Se-CR	Ca-CR	Fe-CR	K-CR	Mg-CR
IRAT-170	Mean± SD	0.1058±0.0502	0.0055±0.0025	0.7413±0.2864	2.6011±1.4133	0.0520±0.0603	0.0997±0.0164	0.0019±0.0006	1.0935±0.1296	0.4507±0.0582
Variety 1	Min, Max	0.0503, 0.3610	0.0024, 0.0174	0.5466, 2.1011	1.9522, 9.9508	0.0018, 0.2796	0.0769, 0.1339	0.0012, 0.0033	0.9126, 1.4621	0.3794, 0.5946
	CV	0.4742	0.4502	0.3863	0.5434	1.1606	0.1644	0.3038	0.1185	0.1291
SIPI-692033	Mean± SD	0.1011±0.0150	0.0068±0.0011	0.9386±0.1454	2.7016±0.3102	0.0473±0.0390	0.0665±0.0133	0.0024±0.0004	1.0871±0.1038	0.4063±0.0514
Variety 2	Min, Max	0.0700, 0.1488	0.0044, 0.0094	0.7838, 1.5735	2.0916, 3.3716	0.0111, 0.1693	0.0378, 0.0938	0.0017, 0.0031	0.8740, 1.2852	0.3284, 0.5247
(N=30)	CV	0.1480	0.1580	0.1549	0.1148	0.8241	0.2008	0.1611	0.0955	0.1265
ITA-315	Mean± SD	0.1030±0.0150	0.0054±0.0066	0.7904±0.1693	2.4111±0.4929	0.0619±0.0544	0.0818±0.0174	0.0017±0.0004	1.1333±0.2007	0.4818±0.0981
Variety 3	Min, Max	0.0559, 0.1392	0.0019, 0.0400	0.5000, 1.4428	1.0000, 3.4025	0.0002, 0.1835	0.0491, 0.1231	0.0012, 0.0029	0.8336, 1.8892	0.3475, 0.8651
	CV	0.1454	1.2268	0.2142	0.2044	0.8802	0.2128	0.2288	0.1771	0.2036
WITA-4	Mean± SD	0.1034±0.0115	0.0062±0.0008	0.6993±0.1523	2.2738±0.2791	0.0459±0.0309	0.0871±0.0178	0.0017±0.0004	1.0719±0.1172	0.4547±0.0618
Variety 4	Min, Max	0.0846, 0.1265	0.0048, 0.0081	0.4851, 1.3654	1.7461, 2.8009	0.0031, 0.1092	0.0601, 0.1302	0.0010, 0.0027	0.8307, 1.5009	0.3629, 0.6875
	CV	0.1111	0.1244	0.2178	0.1227	0.6736	0.2042	0.2182	0.1094	0.1359
NERICA-L19	Mean± SD	0.0948±0.0111	0.0042±0.0007	0.8130±0.1665	2.6384±0.3050	0.0381±0.0355	0.0723±0.0175	0.0017±0.0003	1.1136±0.1228	0.4742±0.0606
Variety 5	Min, Max	0.0729, 0.1193	0.0027, 0.0061	0.6639, 1.5209	2.0775, 3.2537	0.0095, 0.1707	0.0410, 0.1069	0.0011, 0.0023	0.8482, 1.3774	0.3569, 0.5860
	CV	0.1166	0.1645	0.2048	0.1156	0.9316	0.2425	0.1984	0.1103	0.1278
NERICA-L34	Mean± SD	0.0993±0.0240	0.0075±0.0068	0.7268±0.1770	2.6047±0.7011	0.0489±0.0442	0.0766±0.0194	0.0024±0.0010	1.0795±0.0809	0.4786±0.1092
Variety 6	Min, Max	0.0003, 0.1366	0.0023, 0.0428	0.0015, 0.9145	0.0004, 4.6187	0.0050, 0.1561	0.0383, 0.1237	0.0010, 0.0053	0.9004, 1.2203	0.3886, 1.0000
	CV	0.2416	0.9091	0.2435	0.2692	0.9031	0.2536	0.3984	0.0749	0.2281
NCRO-49	Mean± SD	0.0928±0.0146	0.0041±0.0008	0.7134±0.2025	2.3723±0.2468	0.0507±0.0418	0.0933±0.0196	0.0016±0.0004	1.1117±0.1406	0.4613±0.0632
Variety 7	Min, Max	0.0562, 0.1145	0.0024, 0.0054	0.4926, 1.4898	1.7731, 2.8672	0.0012, 0.1560	0.0490, 0.1292	0.0009, 0.0024	0.8981, 1.5032	0.3538, 0.6662
	CV	0.1575	0.1842	0.2839	0.1040	0.8247	0.2101	0.2630	0.1265	0.1371
ART3-7L	Mean± SD	0.1369±0.0131	0.0057±0.0009	0.8298±0.1740	3.0295±0.3669	0.0412±0.0251	0.0887±0.0722	0.0021±0.0010	1.2930±0.1699	0.5127±0.0756
Variety 8	Min, Max	0.1136, 0.1662	0.0025, 0.0082	0.6617, 1.6070	2.3562, 3.9001	0.0008, 0.0850	0.0480, 0.4580	0.0014, 0.0071	1.0024, 1.8814	0.4037, 0.7467
	CV	0.0954	0.1532	0.2097	0.1211	0.6088	0.8141	0.4916	0.1314	0.1475
ART-15	Mean± SD	0.1425±0.0156	0.0049±0.0009	0.8459±0.1207	3.1226±0.4953	0.0356±0.0272	0.0818±0.0172	0.0023±0.0006	1.4901±0.1063	0.5256±0.0547
Variety 9	Min, Max	0.0885, 0.1665	0.0026, 0.0076	0.6958, 1.3483	2.3562, 4.9092	0.0015, 0.0863	0.0529, 0.1259	0.0014, 0.0045	1.2474, 1.6638	0.4070, 0.6276
	CV	0.1097	0.1916	0.1427	0.1586	0.7658	0.2107	0.2792	0.0713	0.1041
BISALAYI	Mean± SD	0.1070±0.0226	0.0422±0.0239	0.6046±0.1277	1.9023±0.3201	0.0691±0.0363	0.0616±0.0361	0.0161±0.0570	0.6243±0.1009	0.3184±0.0547
Variety 10	Min, Max	0.0404, 0.1730	0.0116, 0.1343	0.4707, 1.1593	1.3901, 2.5947	0.0087, 0.1654	0.0292, 0.2311	0.0033, 0.3175	0.4984, 0.8979	0.2336, 0.4801
	CV	0.2111	0.5656	0.2113	0.1683	0.5259	0.5865	3.5317	0.1616	0.1719
Average	Mean± SD	0.1087±0.0272	0.0092±0.0137	0.7703±0.1963	2.5657±0.6766	0.0491±0.0415	0.0809±0.0316	0.0034±0.0183	1.1098±0.2423	0.4564±0.0896
(N=300)	Min, Max	0.0003, 0.3610	0.0019, 0.1343	0.0015, 2.1011	0.0004, 9.9508	0.0095, 0.2796	0.0292, 0.4580	0.0009, 0.3175	0.4984, 1.8892	0.2336, 1.0000
	CV	0.2503	1.4794	0.2548	0.2637	0.8449	0.3900	5.3973	0.2183	0.1964

SD = Standard Deviation, Max = Maximum, Min = Minimum, N= Number of samples, CV= Coefficient of Variance

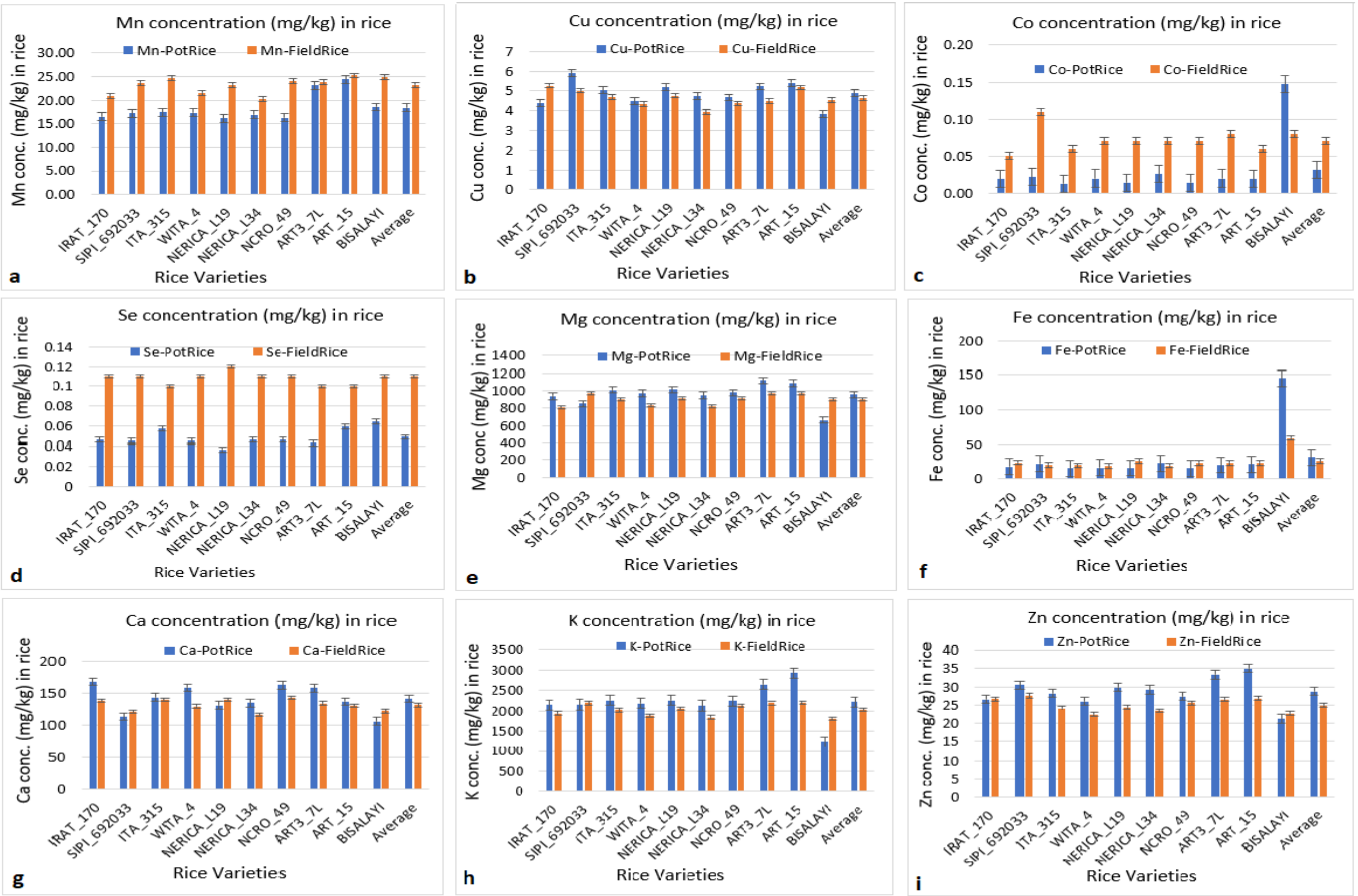


Figure 7. 1: Comparison of the concentrations (mg/kg) of the essential elements in the rice samples for both the field and the pot experiments.

Figure 7.2: Essential elements concentration ratio (CR) in the 10 studied rice varieties for both the pot and the field experiment.

Table 7. 13: Result of the Duncan multiple range test and the inter-varietal variation for both the field and pot experiments.

	Rice Varieties	**N**	**Ca-CR**	**Fe-CR**	**K-CR**	**Mg-CR**	**Zn-CR**	**Se-CR**	**Mn-CR**	**Co-CR**	**Cu-CR**
Field trial	1. IRAT-170	30	ab	b	de	c	a	ab	d	f	a
	2. SIPI-692033	30	d	b	a	a	a	ab	bc	a	abc
	3. ITA-315	30	a	b	cd	b	bcd	b	abc	de	abc
	4. WITA-4	30	b	b	ef	c	d	ab	d	cd	cd
	5. NERICA-L19	30	a	b	bc	b	bc	a	c	de	abc
	6. NERICA-L34	30	d	b	ef	c	cd	b	d	cde	d
	7. NCRO-49	30	a	b	ab	b	ab	b	abc	de	cd
	8. ART3-7L	30	ab	b	a	a	a	b	abc	b	bcd
	9. ART-15	30	bc	b	a	a	a	b	a	e	ab
	10. BISALAYI	30	cd	a	f	b	cd	ab	ab	bc	abcd
	Inter-Varietal Variation	300	**1.25**	**4.00**	**1.34**	**1.20**	**1.04**	**1.03**	**1.18**	**1.86**	**1.28**
Pot trial	1. IRAT-170	30	a	b	cd	c	ef	a	de	b	e
	2. SIPI-692033	30	de	b	cd	d	b	a	d	b	a
	3. ITA-315	30	abc	b	c	b	cde	a	d	b	bcd
	4. WITA-4	30	abc	b	cd	bc	f	a	d	b	de
	5. NERICA-L19	30	cde	b	c	b	bc	a	e	b	bc
	6. NERICA-L34	30	bcd	b	d	c	bcd	a	de	b	cde
	7. NCRO-49	30	ab	b	cd	bc	def	a	e	b	cde
	8. ART3-7L	30	abc	b	b	a	a	a	b	b	bc
	9. ART-15	30	bcd	b	a	a	a	a	a	b	ab
	10. BISALAYI	30	e	a	e	e	g	a	c	a	f
	Inter-varietal Variation	300	**1.62**	**9.47**	**2.39**	**2.07**	**1.64**	**1.82**	**1.50**	**7.82**	**1.55**

Different letters indicate significant differences in the elemental concentration ratio in rice among the rice varieties and same letters indicates no significant difference.

The result for both the field and pot experiment show that some of the 10 rice varieties were significantly different while some are similar based on the uptake of the essential elements. This is revealed in Table 7.13. The inter-varietal variations among the 10 selected rice varieties in both the field and the pot experiment also revealed on the same table (Table 7.13).

For the purpose of the varietal selection, the rice varieties were ranked based on the variety of rice that uptake the highest (highest CR) essential elements, the result of the rankings is presented in Table 7.14. Art-15 rice was observed to uptake the highest Mg, Zn, K, Se and Mn and was ranked the best to be a good source of these elements. Bisalayi rice which is the local rice in the region appeared to be a good source of Fe more than other nine rice varieties. Irat-170 appeared to be the best for Ca more than the other rice varieties.

Table 7. 14: Varietal selection and ranking for the essential elements in the field samples

	Highest in the Essential Elements								
Rice Varieties	**Ca**	**Fe**	**K**	**Mg**	**Zn**	**Se**	**Mn**	**Co**	**Cu**
IRAT-170*	Δ								
SIPI-692033									
ITA-315									
WITA-4									
NERICA-L19									
NERICA-L34*								✓	
NCRO-49*	✓								
ART3-7L				✓					
ART-15***			Δ ✓	Δ	Δ ✓	Δ ✓	Δ ✓		Δ ✓
BISALAYI**		Δ ✓						Δ	

Δ = highest uptake (pot), ✓ = highest uptake (field), *** = highest ranked, no star = no rank

7.2.3 Estimated Daily Intake (EDI) and Percentage Contribution to Recommended Daily Intake (RDI) for the essential elements

The Estimated Daily Intake (EDI) of the essential elements for the 10 selected rice varieties was calculated (Table 7.15) using the equation 6 (chapter 3, section 3.9.2) and this was used to calculate the percentage contribution to recommended daily intake (RDI) from the equation 7 (chapter 3, section 3.9.3). The EDI for the essential elements were all significant at $P<0.01$ with 95% confidence interval. The findings of this study demonstrated that some of the understudied rice varieties significantly added to the RDI of some essential elements while some rice varieties appeared not to be a good source of a few essential elements. The elemental mean concentration of the nine essential elements, Estimated Daily Intake (EDI) of the essential elements for Children (<18years old) and adult (>18yeays) is presented in Table 7.15 and the result for the percentage contribution to recommended daily intake (RDI) based on the age and sex is presented in Table 7.16 for the field experiment. While that of the pot experiment is presented in Table 7.17 and Table 7.18 respectively.

Table 7. 15: Mean concentration (mg/kg) of the essential elements in rice, Estimated Daily Intake for Children (<18years old) and adult (>18yeays), for the field experiment.

	Rice Varieties	**Rice-Mn**	**Rice-Co**	**Rice-Cu**	**Rice-Zn**	**Rice-Se**	**Rice-Ca**	**Rice-Fe**	**Rice-K**	**Mg-Rice**
Essential elements mean concentration (mg/kg)	**Variety 1 (**IRAT-170)	20.918	0.046	5.266	26.793	0.108	138.028	23.409	1938.472	809.916
	Variety 2 (SIPI-692033)	23.682	0.106	5.020	27.618	0.113	120.793	20.212	2189.191	971.762
	Variety 3 (ITA-315)	24.626	0.064	4.676	24.208	0.103	139.621	19.188	2021.439	900.907
	Variety 4 (WITA-4)	21.595	0.073	4.354	22.296	0.109	129.605	17.948	1879.768	828.984
	Variety 5 (NERICA-L19)	23.264	0.067	4.770	24.485	0.117	139.807	25.291	2049.161	919.174
	Variety 6 (NERICA-L34)	20.270	0.071	3.917	23.331	0.106	116.090	19.037	1842.318	821.256
	Variety 7 (NCRO-49**)**	24.026	0.066	4.373	25.678	0.107	143.395	23.348	2129.671	914.757
	Variety 8 (ART3-7L)	23.835	0.084	4.495	26.663	0.105	134.450	23.236	2189.197	974.408
	Variety 9 (ART-15)	25.261	0.060	5.181	26.858	0.105	130.497	23.082	2206.373	976.535
	Variety 10 (BISALAYI)	24.952	0.078	4.560	22.620	0.112	122.221	59.514	1813.934	902.160
		Mn-EDI	**Co-EDI**	**Cu-EDI**	**Zn-EDI**	**Se-EDI**	**Ca-EDI**	**Fe-EDI**	**K-EDI**	**Mg-EDI**
Children (EDI)	**Variety 1 (**IRAT-170)	2.092	0.005	0.527	2.679	0.011	13.803	2.341	193.847	80.992
	Variety 2 (SIPI-692033)	2.368	0.011	0.502	2.762	0.011	12.079	2.021	218.919	97.176
	Variety 3 (ITA-315)	2.463	0.006	0.468	2.421	0.010	13.962	1.919	202.144	90.091
	Variety 4 (WITA-4)	2.159	0.007	0.435	2.230	0.011	12.961	1.795	187.977	82.898
	Variety 5 (NERICA-L19)	2.326	0.007	0.477	2.449	0.012	13.981	2.529	204.916	91.917
	Variety 6 (NERICA-L34)	2.027	0.007	0.392	2.333	0.011	11.609	1.904	184.232	82.126
	Variety 7 (NCRO-49**)**	2.403	0.007	0.437	2.568	0.011	14.339	2.335	212.967	91.476
	Variety 8 (ART3-7L)	2.384	0.008	0.450	2.666	0.010	13.445	2.324	218.920	97.441
	Variety 9 (ART-15)	2.526	0.006	0.518	2.686	0.010	13.050	2.308	220.637	97.653
	Variety 10 (BISALAYI)	2.495	0.008	0.456	2.262	0.011	12.222	5.951	181.393	90.216
		Mn-EDI	**Co-EDI**	**Cu-EDI**	**Zn-EDI**	**Se-EDI**	**Ca-EDI**	**Fe-EDI**	**K-EDI**	**Mg-EDI**
Adult (EDI)	**Variety 1 (**IRAT-170)	4.184	0.009	1.053	5.359	0.022	27.606	4.682	387.694	161.983
	Variety 2 (SIPI-692033)	4.736	0.021	1.004	5.524	0.023	24.159	4.042	437.838	194.352
	Variety 3 (ITA-315)	4.925	0.013	0.935	4.842	0.021	27.924	3.838	404.288	180.181
	Variety 4 (WITA-4)	4.319	0.015	0.871	4.459	0.022	25.921	3.590	375.954	165.797
	Variety 5 (NERICA-L19)	4.653	0.013	0.954	4.897	0.023	27.961	5.058	409.832	183.835
	Variety 6 (NERICA-L34)	4.054	0.014	0.783	4.666	0.021	23.218	3.807	368.464	164.251
	Variety 7 (NCRO-49**)**	4.805	0.013	0.875	5.136	0.021	28.679	4.670	425.934	182.951
	Variety 8 (ART3-7L)	4.767	0.017	0.899	5.333	0.021	26.890	4.647	437.839	194.882
	Variety 9 (ART-15)	5.052	0.012	1.036	5.372	0.021	26.099	4.616	441.275	195.307
	Variety 10 (BISALAYI)	4.990	0.016	0.912	4.524	0.022	24.444	11.903	362.787	180.432

Table 7. 16: Percentage contribution to the RDI based on age and sex groups for the field experiment

	Sex/Age group	**RDI**	**% Contribution to Recommended Daily Intake (RDI)**									
		(mg/day)	**Variety 1**	**Variety 2**	**Variety 3**	**Variety 4**	**Variety 5**	**Variety 6**	**Variety 7**	**Variety 8**	**Variety 9**	**Variety 10**
[1]Ca	Infant (0-12m)	400	3.5	3.0	3.5	3.2	3.5	2.9	3.6	3.4	3.3	3.1
	Children (1-9)	700	2.0	1.7	2.0	1.9	2.0	1.7	2.0	1.9	1.9	1.7
	Male (10-18)	1300	1.1	0.9	1.1	1.0	1.1	0.9	1.1	1.0	1.0	0.9
	Female (10-18)	1300	1.1	0.9	1.1	1.0	1.1	0.9	1.1	1.0	1.0	0.9
	Male (>19)	1000	2.8	2.4	2.8	2.6	2.8	2.3	2.9	2.7	2.6	2.4
	Female (>19)	1200	2.3	2.0	2.3	2.2	2.3	1.9	2.4	2.2	2.2	2.0
	Pregnant W	1200	2.3	2.0	2.3	2.2	2.3	1.9	2.4	2.2	2.2	2.0
	Lactating W	1000	2.8	2.4	2.8	2.6	2.8	2.3	2.9	2.7	2.6	2.4
[2]Mn	Infant (0-12m)	0.60	348.6	394.7	410.4	359.9	387.7	337.8	400.4	397.3	421.0	415.9
	Children (1-9)	1.50	139.5	157.9	164.2	144.0	155.1	135.1	160.2	158.9	168.4	166.3
	Male (10-18)	2.20	95.1	107.6	111.9	98.2	105.7	92.1	109.2	108.3	114.8	113.4
	Female (10-18)	1.60	130.7	148.0	153.9	135.0	145.4	126.7	150.2	149.0	157.9	156.0
	Male (>19)	2.30	181.9	205.9	214.1	187.8	202.3	176.3	208.9	207.3	219.7	217.0
	Female (>19)	1.80	232.4	263.1	273.6	239.9	258.5	225.2	267.0	264.8	280.7	277.2
	Pregnant W	2.00	209.2	236.8	246.3	215.9	232.6	202.7	240.3	238.4	252.6	249.5
	Lactating W	2.60	160.9	182.2	189.4	166.1	179.0	155.9	184.8	183.3	194.3	191.9
[3]Co	Infant (0-12m)	0.005	92.8	212.9	128.0	146.4	133.1	142.4	132.3	167.9	120.8	155.1
	Children (1-9)	0.005	92.8	212.9	128.0	146.4	133.1	142.4	132.3	167.9	120.8	155.1
	Male (10-18)	0.05	9.3	21.3	12.8	14.6	13.3	14.2	13.2	16.8	12.1	15.5
	Female (10-18)	0.05	9.3	21.3	12.8	14.6	13.3	14.2	13.2	16.8	12.1	15.5
	Male (>19)	0.05	18.6	42.6	25.6	29.3	26.6	28.5	26.5	33.6	24.2	31.0
	Female (>19)	0.05	18.6	42.6	25.6	29.3	26.6	28.5	26.5	33.6	24.2	31.0
	Pregnant W	0.05	18.6	42.6	25.6	29.3	26.6	28.5	26.5	33.6	24.2	31.0
	Lactating W	0.05	18.6	42.6	25.6	29.3	26.6	28.5	26.5	33.6	24.2	31.0
[4]Cu	Infant (0-12m)	0.22	239.3	228.2	212.6	197.9	216.8	178.0	198.8	204.3	235.5	207.3
	Children (1-9)	0.44	119.7	114.1	106.3	98.9	108.4	89.0	99.4	102.2	117.8	103.6
	Male (10-18)	0.89	59.2	56.4	52.5	48.9	53.6	44.0	49.1	50.5	58.2	51.2
	Female (10-18)	0.89	59.2	56.4	52.5	48.9	53.6	44.0	49.1	50.5	58.2	51.2
	Male (>19)	0.90	117.0	111.6	103.9	96.7	106.0	87.0	97.2	99.9	115.1	101.3
	Female (>19)	0.90	117.0	111.6	103.9	96.7	106.0	87.0	97.2	99.9	115.1	101.3
	Pregnant W	1.00	105.3	100.4	93.5	87.1	95.4	78.3	87.5	89.9	103.6	91.2
	Lactating W	1.30	81.0	77.2	71.9	67.0	73.4	60.3	67.3	69.2	79.7	70.2
[5]Zn	Infant (0-12m)	8.40	31.9	32.9	28.8	26.5	29.1	27.8	30.6	31.7	32.0	26.9
	Children (1-9)	11.20	23.9	24.7	21.6	19.9	21.9	20.8	22.9	23.8	24.0	20.2
	Male (10-18)	17.10	15.7	16.2	14.2	13.0	14.3	13.6	15.0	15.6	15.7	13.2
	Female (10-18)	14.40	18.6	19.2	16.8	15.5	17.0	16.2	17.8	18.5	18.7	15.7
	Male (>19)	14.00	38.3	39.5	34.6	31.9	35.0	33.3	36.7	38.1	38.4	32.3
	Female (>19)	9.80	54.7	56.4	49.4	45.5	50.0	47.6	52.4	54.4	54.8	46.2
	Pregnant W	20.00	26.8	27.6	24.2	22.3	24.5	23.3	25.7	26.7	26.9	22.6

	Lactating W	19.00	28.2	29.1	25.5	23.5	25.8	24.6	27.0	28.1	28.3	23.8
[6]Se	Infant (0-12m)	0.02	54.0	56.5	51.6	54.3	58.6	53.2	53.6	52.3	52.4	56.2
	Children (1-9)	0.03	36.0	37.7	34.4	36.2	39.0	35.4	35.7	34.9	34.9	37.5
	Male (10-18)	0.04	27.0	28.3	25.8	27.1	29.3	26.6	26.8	26.2	26.2	28.1
	Female (10-18)	0.04	27.0	28.3	25.8	27.1	29.3	26.6	26.8	26.2	26.2	28.1
	Male (>19)	0.055	39.3	41.1	37.5	39.5	42.6	38.7	39.0	38.0	38.1	40.9
	Female (>19)	0.055	39.3	41.1	37.5	39.5	42.6	38.7	39.0	38.0	38.1	40.9
	Pregnant W	0.06	36.0	37.7	34.4	36.2	39.0	35.4	35.7	34.9	34.9	37.5
	Lactating W	0.07	30.8	32.3	29.5	31.0	33.5	30.4	30.6	29.9	29.9	32.1
[7]Fe	Infant (0-12m)	40.0	5.9	5.1	4.8	4.5	6.3	4.8	5.8	5.8	5.8	14.9
	Children (1-9)	40.0	5.9	5.1	4.8	4.5	6.3	4.8	5.8	5.8	5.8	14.9
	Male (10-18)	40.0	5.9	5.1	4.8	4.5	6.3	4.8	5.8	5.8	5.8	14.9
	Female (10-18)	40.0	5.9	5.1	4.8	4.5	6.3	4.8	5.8	5.8	5.8	14.9
	Male (>19)	45.0	10.4	9.0	8.5	8.0	11.2	8.5	10.4	10.3	10.3	26.5
	Female (>19)	45.0	10.4	9.0	8.5	8.0	11.2	8.5	10.4	10.3	10.3	26.5
	Pregnant W	45.0	10.4	9.0	8.5	8.0	11.2	8.5	10.4	10.3	10.3	26.5
	Lactating W	45.0	10.4	9.0	8.5	8.0	11.2	8.5	10.4	10.3	10.3	26.5
[8]K	Infant (0-12m)	3000	6.5	7.3	6.7	6.3	6.8	6.1	7.1	7.3	7.4	6.0
	Children (1-9)	3800	5.1	5.8	5.3	4.9	5.4	4.8	5.6	5.8	5.8	4.8
	Male (10-18)	4500	4.3	4.9	4.5	4.2	4.6	4.1	4.7	4.9	4.9	4.0
	Female (10-18)	4500	4.3	4.9	4.5	4.2	4.6	4.1	4.7	4.9	4.9	4.0
	Male (>19)	4700	8.2	9.3	8.6	8.0	8.7	7.8	9.1	9.3	9.4	7.7
	Female (>19)	4700	8.2	9.3	8.6	8.0	8.7	7.8	9.1	9.3	9.4	7.7
	Pregnant W	4700	8.2	9.3	8.6	8.0	8.7	7.8	9.1	9.3	9.4	7.7
	Lactating W	4700	8.2	9.3	8.6	8.0	8.7	7.8	9.1	9.3	9.4	7.7
[9]Mg	Infant (0-12m)	75	108.0	129.6	120.1	110.5	122.6	109.5	122.0	129.9	130.2	120.3
	Children (1-9)	130	62.3	74.8	69.3	63.8	70.7	63.2	70.4	75.0	75.1	69.4
	Male (10-18)	410	19.8	23.7	22.0	20.2	22.4	20.0	22.3	23.8	23.8	22.0
	Female (10-18)	360	22.5	27.0	25.0	23.0	25.5	22.8	25.4	27.1	27.1	25.1
	Male (>19)	420	38.6	46.3	42.9	39.5	43.8	39.1	43.6	46.4	46.5	43.0
	Female (>19)	320	50.6	60.7	56.3	51.8	57.4	51.3	57.2	60.9	61.0	56.4
	Pregnant W	400	40.5	48.6	45.0	41.4	46.0	41.1	45.7	48.7	48.8	45.1
	Lactating W	360	45.0	54.0	50.1	46.1	51.1	45.6	50.8	54.1	54.3	50.1

V=Varieties, 1= Khan et al. (2015) and Mwale et al. (2018), 2=MedlinePlus (2019), 3=Lison (2015) and University of Rochester (2019), 4=Winchester Hospital (2019), 5=Khan et al. (2015), 6= WebMed (2019), 7=NIH (2018), 8=USDA (2005), 9=NIH (2016; EFSA, 2010; FDA, 2018).

Table 7. 17: Mean concentration (mg/kg) of the essential elements in rice, Estimated Daily Intake for Children (<18years old) and adult (>18yeays), for the pot experiment.

	Rice Varieties	**Mn-Rice**	**Co-Rice**	**Cu-Rice**	**Zn-Rice**	**Se-Rice**	**Ca-Rice**	**Fe-Rice**	**K-Rice**	**Mg-Rice**
Essential elements mean concentration (mg/kg)	**Variety 1 (**IRAT-170)	16.46	0.019	4.407	26.669	0.046	168	17.505	2149.121	938.699
	Variety 2 (SIPI-692033)	17.256	0.022	5.905	30.507	0.045	113.265	21.761	2152.029	853.264
	Variety 3 (ITA-315)	17.421	0.013	5.041	28.231	0.057	143.645	15.07	2254.209	1009.245
	Variety 4 (WITA-4)	17.355	0.02	4.49	26.123	0.045	158.877	16.075	2179.18	970.987
	Variety 5 (NERICA-L19)	16.142	0.014	5.192	29.864	0.037	131.197	15.542	2261.545	1014.793
	Variety 6 (NERICA-L34)	16.893	0.026	4.746	29.222	0.046	134.88	22.33	2127.203	949.086
	Variety 7 (NCRO-49**)**	16.295	0.014	4.68	27.487	0.046	162.806	15.144	2243.175	978.389
	Variety 8 (ART3-7L)	23.166	0.02	5.234	33.305	0.043	158.201	19.864	2649.929	1119.216
	Variety 9 (ART-15)	24.387	0.019	5.415	35.001	0.059	137.007	20.681	2931.626	1088.034
	Variety 10 (BISALAYI)	18.564	0.147	3.845	21.161	0.064	105.929	145.205	1237.43	666.112
		Mn-EDI	**EDI-Co**	**EDI-Cu**	**EDI-Zn**	**EDI-Se**	**EDI-Ca**	**EDI-Fe**	**EDI-K**	**EDI-Mg**
Children (EDI)	**Variety 1 (**IRAT-170)	1.646	0.0019	0.4407	2.6669	0.0046	16.8	1.7505	214.9121	93.8699
	Variety 2 (SIPI-692033)	1.7256	0.0022	0.5905	3.0507	0.0045	11.3265	2.1761	215.2029	85.3264
	Variety 3 (ITA-315)	1.7421	0.0013	0.5041	2.8231	0.0057	14.3645	1.507	225.4209	100.9245
	Variety 4 (WITA-4)	1.7355	0.002	0.449	2.6123	0.0045	15.8877	1.6075	217.918	97.0987
	Variety 5 (NERICA-L19)	1.6142	0.0014	0.5192	2.9864	0.0037	13.1197	1.5542	226.1545	101.4793
	Variety 6 (NERICA-L34)	1.6893	0.0026	0.4746	2.9222	0.0046	13.488	2.233	212.7203	94.9086
	Variety 7 (NCRO-49**)**	1.6295	0.0014	0.468	2.7487	0.0046	16.2806	1.5144	224.3175	97.8389
	Variety 8 (ART3-7L)	2.3166	0.002	0.5234	3.3305	0.0043	15.8201	1.9864	264.9929	111.9216
	Variety 9 (ART-15)	2.4387	0.0019	0.5415	3.5001	0.0059	13.7007	2.0681	293.1626	108.8034
	Variety 10 (BISALAYI)	1.8564	0.0147	0.3845	2.1161	0.0064	10.5929	14.5205	123.743	66.6112
		EDI-Mn	**EDI-Co**	**EDI-Cu**	**EDI-Zn**	**EDI-Se**	**EDI-Ca**	**EDI-Fe**	**EDI-K**	**EDI-Mg**
Adult (EDI)	**Variety 1 (**IRAT-170)	3.292	0.0038	0.8814	5.3338	0.0092	33.6	3.501	429.8242	187.7398
	Variety 2 (SIPI-692033)	3.4512	0.0044	1.181	6.1014	0.009	22.653	4.3522	430.4058	170.6528
	Variety 3 (ITA-315)	3.4842	0.0026	1.0082	5.6462	0.0114	28.729	3.014	450.8418	201.849
	Variety 4 (WITA-4)	3.471	0.004	0.898	5.2246	0.009	31.7754	3.215	435.836	194.1974
	Variety 5 (NERICA-L19)	3.2284	0.0028	1.0384	5.9728	0.0074	26.2394	3.1084	452.309	202.9586
	Variety 6 (NERICA-L34)	3.3786	0.0052	0.9492	5.8444	0.0092	26.976	4.466	425.4406	189.8172
	Variety 7 (NCRO-49**)**	3.259	0.0028	0.936	5.4974	0.0092	32.5612	3.0288	448.635	195.6778
	Variety 8 (ART3-7L)	4.6332	0.004	1.0468	6.661	0.0086	31.6402	3.9728	529.9858	223.8432
	Variety 9 (ART-15)	4.8774	0.0038	1.083	7.0002	0.0118	27.4014	4.1362	586.3252	217.6068
	Variety 10 (BISALAYI)	3.7128	0.0294	0.769	4.2322	0.0128	21.1858	29.041	247.486	133.2224

Table 7. 18: Percentage contribution to recommended daily Intake (RDI) of the essential elements based on sex and age group, for the pot experiment.

	Sex/Age group	RDI	% Contribution to Recommended Daily Intake (RDI) by each Variety of Rice									
		mg/day	Variety 1	Variety 2	Variety 3	Variety 4	Variety 5	Variety 6	Variety 7	Variety 8	Variety 9	Variety 10
[1]Ca	Infant (0-12m)	400	4.2	2.8	3.6	4.0	3.3	3.4	4.1	4.0	3.4	2.6
	Children (1-9)	700	2.4	1.6	2.1	2.3	1.9	1.9	2.3	2.3	2.0	1.5
	Male (10-18)	1300	1.3	0.9	1.1	1.2	1.0	1.0	1.3	1.2	1.1	0.8
	Female (10-18)	1300	1.3	0.9	1.1	1.2	1.0	1.0	1.3	1.2	1.1	0.8
	Male (>19)	1000	3.4	2.3	2.9	3.2	2.6	2.7	3.3	3.2	2.7	2.1
	Female (>19)	1200	2.8	1.9	2.4	2.6	2.2	2.2	2.7	2.6	2.3	1.8
	Pregnant W	1200	2.8	1.9	2.4	2.6	2.2	2.2	2.7	2.6	2.3	1.8
	Lactating W	1000	3.4	2.3	2.9	3.2	2.6	2.7	3.3	3.2	2.7	2.1
[2]Mn	Infant (0-12m)	0.6	274.3	287.6	290.4	289.3	269.0	281.6	271.6	386.1	406.5	309.4
	Children (1-9)	1.5	109.7	115.0	116.1	115.7	107.6	112.6	108.6	154.4	162.6	123.8
	Male (10-18)	2.2	74.8	78.4	79.2	78.9	73.4	76.8	74.1	105.3	110.9	84.4
	Female (10-18)	1.6	102.9	107.9	108.9	108.5	100.9	105.6	101.8	144.8	152.4	116.0
	Male (>19)	2.3	143.1	150.1	151.5	150.9	140.4	146.9	141.7	201.4	212.1	161.4
	Female (>19)	1.8	182.9	191.7	193.6	192.8	179.4	187.7	181.1	257.4	271.0	206.3
	Pregnant W	2	164.6	172.6	174.2	173.6	161.4	168.9	163.0	231.7	243.9	185.6
	Lactating W	2.6	126.6	132.7	134.0	133.5	124.2	129.9	125.3	178.2	187.6	142.8
[3]Co	Infant (0-12m)	0.005	38.0	44.0	26.0	40.0	28.0	52.0	28.0	40.0	38.0	294.0
	Children (1-9)	0.005	38.0	44.0	26.0	40.0	28.0	52.0	28.0	40.0	38.0	294.0
	Male (10-18)	0.05	3.8	4.4	2.6	4.0	2.8	5.2	2.8	4.0	3.8	29.4
	Female (10-18)	0.05	3.8	4.4	2.6	4.0	2.8	5.2	2.8	4.0	3.8	29.4
	Male (>19)	0.05	7.6	8.8	8.8	8.0	5.6	10.4	5.6	8.0	7.6	58.8
	Female (>19)	0.05	7.6	8.8	8.8	8.0	5.6	10.4	5.6	8.0	7.6	58.8
	Pregnant W	0.05	7.6	8.8	8.8	8.0	5.6	10.4	5.6	8.0	7.6	58.8
	Lactating W	0.05	7.6	8.8	8.8	8.0	5.6	10.4	5.6	8.0	7.6	58.8
[4]Cu	Infant (0-12m)	0.22	200.3	268.4	229.1	204.1	236.0	215.7	212.7	237.9	246.1	174.8
	Children (1-9)	0.44	100.2	134.2	114.6	102.0	118.0	107.9	106.4	119.0	123.1	87.4
	Male (10-18)	0.89	49.5	66.3	56.6	50.4	58.3	53.3	52.6	58.8	60.8	43.2
	Female (10-18)	0.89	49.5	66.3	56.6	50.4	58.3	53.3	52.6	58.8	60.8	43.2
	Male (>19)	0.9	97.9	131.2	112.0	99.8	115.4	105.5	104.0	116.3	120.3	85.4
	Female (>19)	0.9	97.9	131.2	112.0	99.8	115.4	105.5	104.0	116.3	120.3	85.4
	Pregnant W	1	88.1	118.1	100.8	89.8	103.8	94.9	93.6	104.7	108.3	76.9
	Lactating W	1.3	67.8	90.8	77.6	69.1	79.9	73.0	72.0	80.5	83.3	59.2
[5]Zn	Infant (0-12m)	8.4	31.7	36.3	33.6	31.1	35.6	34.8	32.7	39.6	41.7	25.2
	Children (1-9)	11.2	23.8	27.2	25.2	23.3	26.7	26.1	24.5	29.7	31.3	18.9
	Male (10-18)	17.1	15.6	17.8	16.5	15.3	17.5	17.1	16.1	19.5	20.5	12.4
	Female (10-18)	14.4	18.5	21.2	19.6	18.1	20.7	20.3	19.1	23.1	24.3	14.7
	Male (>19)	14	38.1	43.6	40.3	37.3	42.7	41.7	39.3	47.6	50.0	30.2
	Female (>19)	9.8	54.4	62.3	57.6	53.3	60.9	59.6	56.1	68.0	71.4	43.2
	Pregnant W	20	26.7	30.5	28.2	26.1	29.9	29.2	27.5	33.3	35.0	21.2

	Lactating W	19	28.1	32.1	29.7	27.5	31.4	30.8	28.9	35.1	36.8	22.3
[6]Se	Infant (0-12m)	0.02	23.0	22.5	28.5	22.5	18.5	23.0	23.0	21.5	29.5	32.0
	Children (1-9)	0.03	15.3	15.0	19.0	15.0	12.3	15.3	15.3	14.3	19.7	21.3
	Male (10-18)	0.04	11.5	11.3	14.3	11.3	9.3	11.5	11.5	10.8	14.8	16.0
	Female (10-18)	0.04	11.5	11.3	14.3	11.3	9.3	11.5	11.5	10.8	14.8	16.0
	Male (>19)	0.055	16.7	16.4	20.7	16.4	13.5	16.7	16.7	15.6	21.5	23.3
	Female (>19)	0.055	16.7	16.4	20.7	16.4	13.5	16.7	16.7	15.6	21.5	23.3
	Pregnant W	0.06	15.3	15.0	19.0	15.0	12.3	15.3	15.3	14.3	19.7	21.3
	Lactating W	0.07	13.1	12.9	16.3	12.9	10.6	13.1	13.1	12.3	16.9	18.3
[7]Fe	Infant (0-12m)	40	4.4	5.4	3.8	4.0	3.9	5.6	3.8	5.0	5.2	36.3
	Children (1-9)	40	4.4	5.4	3.8	4.0	3.9	5.6	3.8	5.0	5.2	36.3
	Male (10-18)	40	4.4	5.4	3.8	4.0	3.9	5.6	3.8	5.0	5.2	36.3
	Female (10-18)	40	4.4	5.4	3.8	4.0	3.9	5.6	3.8	5.0	5.2	36.3
	Male (>19)	45	7.8	9.7	6.7	7.1	6.9	9.9	6.7	8.8	9.2	64.5
	Female (>19)	45	7.8	9.7	6.7	7.1	6.9	9.9	6.7	8.8	9.2	64.5
	Pregnant W	45	7.8	9.7	6.7	7.1	6.9	9.9	6.7	8.8	9.2	64.5
	Lactating W	45	7.8	9.7	6.7	7.1	6.9	9.9	6.7	8.8	9.2	64.5
[8]K	Infant (0-12m)	3000	7.2	7.2	7.5	7.3	7.5	7.1	7.5	8.8	9.8	4.1
	Children (1-9)	3800	5.7	5.7	5.9	5.7	6.0	5.6	5.9	7.0	7.7	3.3
	Male (10-18)	4500	4.8	4.8	5.0	4.8	5.0	4.7	5.0	5.9	6.5	2.7
	Female (10-18)	4500	4.8	4.8	5.0	4.8	5.0	4.7	5.0	5.9	6.5	2.7
	Male (>19)	4700	9.1	9.2	9.6	9.3	9.6	9.1	9.5	11.3	12.5	5.3
	Female (>19)	4700	9.1	9.2	9.6	9.3	9.6	9.1	9.5	11.3	12.5	5.3
	Pregnant W	4700	9.1	9.2	9.6	9.3	9.6	9.1	9.5	11.3	12.5	5.3
	Lactating W	4700	9.1	9.2	9.6	9.3	9.6	9.1	9.5	11.3	12.5	5.3
[9]Mg	Infant (0-12m)	75	125.2	113.8	134.6	129.5	135.3	126.5	130.5	149.2	145.1	88.8
	Children (1-9)	130	72.2	65.6	77.6	74.7	78.1	73.0	75.3	86.1	83.7	51.2
	Male (10-18)	410	22.9	20.8	24.6	23.7	24.8	23.1	23.9	27.3	26.5	16.2
	Female (10-18)	360	26.1	23.7	28.0	27.0	28.2	26.4	27.2	31.1	30.2	18.5
	Male (>19)	420	44.7	40.6	48.1	46.2	48.3	45.2	46.6	53.3	51.8	31.7
	Female (>19)	320	58.7	53.3	63.1	60.7	63.4	59.3	61.1	70.0	68.0	41.6
	Pregnant W	400	46.9	42.7	50.5	48.5	50.7	47.5	48.9	56.0	54.4	33.3
	Lactating W	360	52.1	47.4	56.1	53.9	56.4	52.7	54.4	62.2	60.4	37.0

V=Varieties, 1= Khan, Khan, Khan, Qamar, and Waqas (2015) and Mwale, Rahman, and Mondal (2018), 2=MedlinePlus (2019), 3=Lison (2015) and University of Rochester (2019), 4=Winchester Hospital (2019), 5=Khan et al. (2015), 6= WebMed (2019), 7=NIH (2018), 8=USDA (2005), 9=NIH (2016), 10= % contribution to Pb maximum ingestible daily allowable limit (Pb-MIDAL) (EFSA, 2010; FDA, 2018)

7.2.3.1 Calcium

The contribution of the rice varieties to recommended daily intake (RDI) of the essential elements was measured based on different age categories as the rice ingestion rate used was different with age. From the field experiment, the rice variety 7 (ncro-49) had the highest percentage contribution (1.1% to 3.6%) to the daily required calcium across all ages including the pregnant women while variety 6 (nerica-L34) had the lowest contribution (0.9% to 2.9%). The contribution to Ca RDI in infants (age 0-12), variety 9 (art-15) was the highest (3.3%), while variety 6 (nerica-L34) was the lowest (2.9%). Variety 7 (ncro-49) was the highest (2%) and variety 6 (nerica-L34) was the lowest (1.7%) for children age 1 to 9. Variety 7 (ncro-49) appeared to be highest (1.1%) while variety 6 (nerica-L34) appeared to be the lowest (0.9%) for male and female adolescent (age 10-18). Variety 7 (ncro-49) observed to be the highest (2.4%) and variety 6 (nerica-L34) observed to be the lowest (1.9%) for male and female adult (age >19). Variety 7 (ncro-49) appeared to be the highest contributor (2.9% and 2.4%) to percentage Ca RDI for lactating and pregnant women respectively. None of the rice varieties contributes more than 4% to the Ca RDI across all the age groups (Figure 7.3).

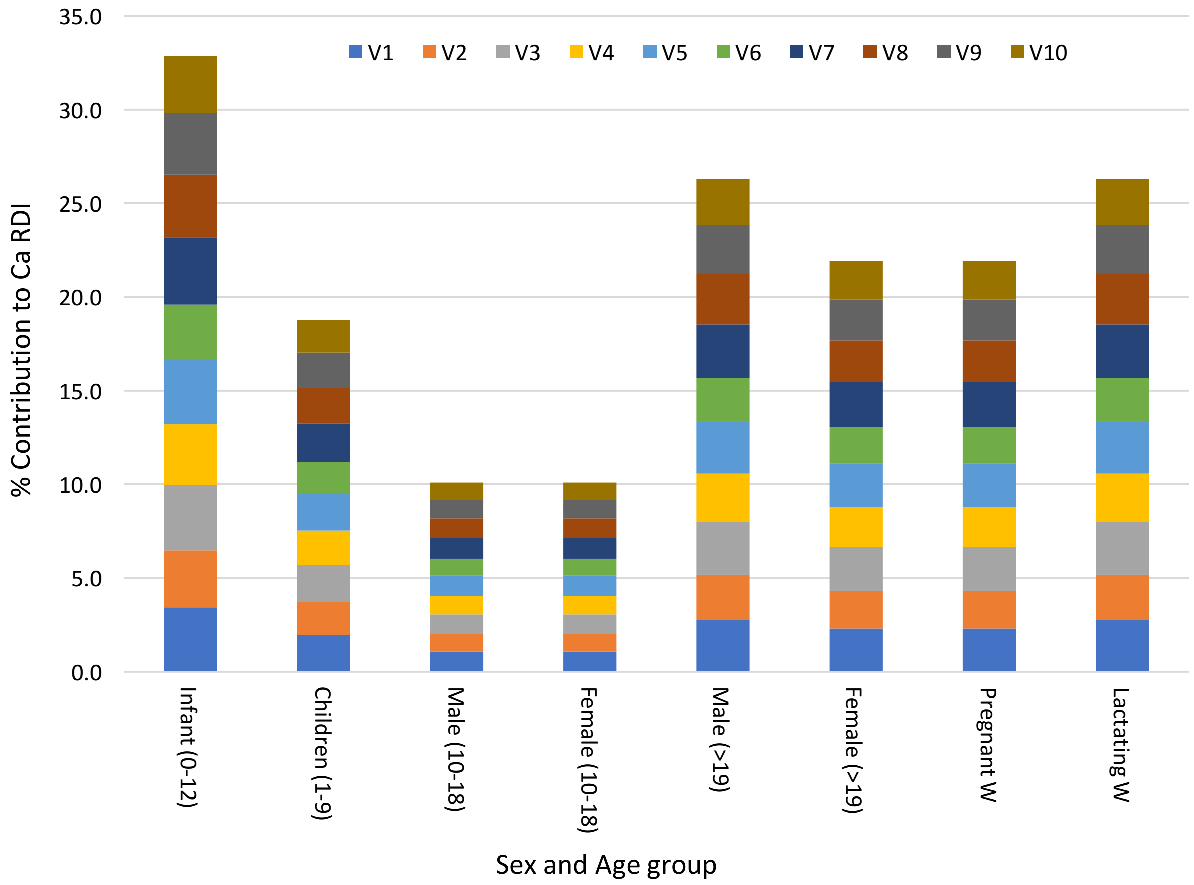


Figure 7.3: % Contribution of rice varieties to Ca RDI (v=varieties).

From the pot experiment, the rice variety 1 (irat-170) had the highest percentage contribution (1.3% to 4.2%) to the daily required calcium across all ages including the pregnant women while variety 10 (bisalayi rice) had the lowest contribution (0.8% to 2.6%). On average, in all the age groups and sex, rice variety 1 (irat-170) contributes the highest (3.4%) to Ca RDI, variety 10 (bisalayi) contributes the lowest (2.1%) to Ca RDI. Details has been presented previously in Table 7.18.

Calcium (Ca) is essential in teeth formation and it is an element that forms a great component of bone in the human body (Dorozhkin, 2007). Blood-clothing when there is a bleeding injury cannot happen when calcium is lacking (Fridman et al., 2006). It is the calcium that stimulates the release of thromboplastin from the blood platelets (Heemskerk, Bevers, & Lindhout, 2002). Calcium is vital in energy metabolism and also the formation of cartilages (Nguyen & Jafri, 2005). Calcium can also be toxic when it is above the recommended level and this condition is called hypercalcemia (Lorenzen et al., 2007).

7.2.3.2 Manganese

The rice variety 10 (bisalayi) had the highest percentage contribution (113.4% to 415.9%) to the daily required Manganese (Mn) across all ages including the pregnant women while V6 (nerica-L34) had the lowest contribution (92.1% to 337.8%) in the field experiment and this is revealed in Figure 7.4. The contribution to percentage Mn RDI in all age groups (except male age 10 – 18) was at the extreme. The percentage contribution was too high, and this may result in manganese poisoning. Manganese is a micro-element in human, it can be toxic at higher concentration and accumulation in the body and when this happens, it results in various challenges in human body physiology such as neurodegenerative diseases and blood brain barrier disorders (Hesketh, Sassoon, Knight, & Brown, 2008). The contribution was highest by the variety 10 (bisalayi) while the lowest was by the variety 6 (nerica-L34) for infants, and highest by the variety 7 (ncro-49), lowest by the variety 6 (nerica-L34) for children (age 1-9). Variety 7 (ncro-49) had the highest percentage contribution while variety 6 (nerica-L34) had the lowest for male and female adolescent (age 10-18). Variety 7 (ncro-49) had the highest percentage contribution while variety 6 (nerica-L34) had the lowest percentage contribution for male adult (age >19). All the rice varieties contribute about 100% to the Mn RDI in the male and female category between the age of 10 and 18. Apart from that age group, others were observed to be too high comparing with recommended daily intake (RDI) of Mn (Table 7.16 and Table 7.18).

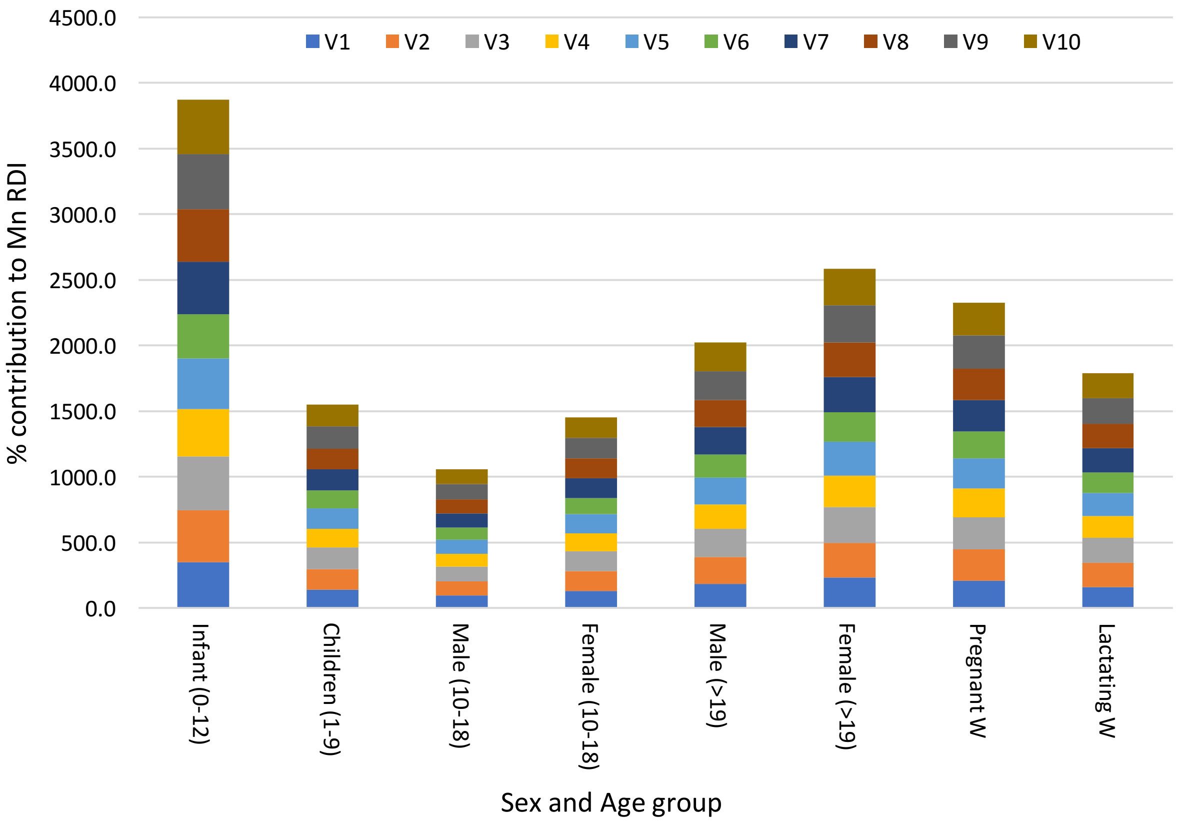


Figure 7.4: % contribution of rice varieties to manganese RDI (v=varieties).

In the pot experiment, variety 9 (art3-7L) presented the highest percentage contribution (110.9% to 406.5%) to the Mn RDI while variety 5 (nerica-L19) was the lowest (73.4% to 269%) for Mn across all the ages and sex groups. Also as we had in the field samples, all the rice varieties contribute about 100% to the Mn RDI in the male and female category between the age of 10 and 18 and apart from this age groups, others appeared to be too high compared to recommended daily intake (RDI) of Mn.

Manganese (Mn) is a vital component of the body enzymes such as hexokinase, superoxide dismutase, and alkaline phosphate (Adedire et al., 2015). Both the children and the adults require Mn for bone formation, formation of carbohydrate, and regeneration of the red blood cells in the body (Hernandez-Gil, Gracia, del Canto Pingarrón, & Jerez, 2006). Deficiency of Mn may lead to an inflammation of the skin, (dermatitis), and some other skin rashes, abnormal low level of cholesterol in the blood (hypocholesterolemia), vomiting, nausea, and some other physiological disorderliness.

7.2.3.3 Cobalt

In the field samples, the rice variety 2 (sipi-692033) had the highest percentage contribution (21.3% to 212.9%) to cobalt RDI across all sex and the age groups while variety 1 (irat-170) had the lowest (9.3% to 92.8%). All the rice varieties demonstrated to be good sources of cobalt as the least of 9.3% observed was among the male and female 10 to 18-year age groups. The varietal percentage contribution to Co RDI across all age and sex groups is shown in Figure 7.5.

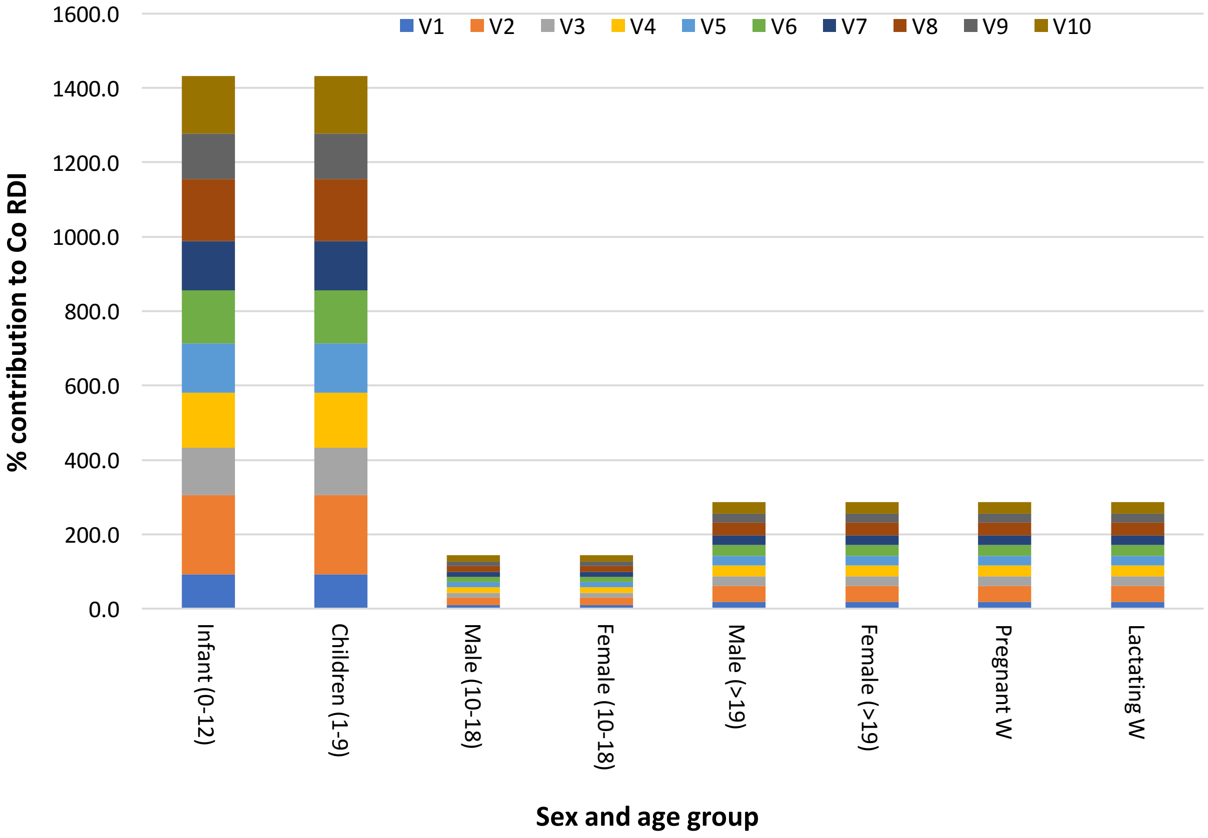


Figure 7.5: % contribution of rice varieties to cobalt RDI (v=varieties).

In the pot experiment, variety 10 (bisalayi rice) presented the highest percentage contribution (29.4% to 294%) to the Co RDI while variety 3 (ita-315) was the lowest (2.6% to 26%) for Co across all the age groups.

Cobalt is the centre and vital component of vitamin B12 and it aids formation of t6he red blood cells (haemoglobin) ((Davenport, 2015). This means is very important to life. It supports cells in the synthesis of DNA and helps in the normal functioning of the CNS (Lindsay & Kerr, 2011). Cobalt is used in the treatment of diseases such as infections and anaemia (Van Saun, 2014) and also helps in myelin repair (MacPherson & Dixon, 2003). Myelin is a protective membrane that protect the nerve cells (Lison, 2015). One can be exposed to overdose of cobalt causing its toxicity and this can produce a toxic effect on the body systems such as renal failure and it can as well result to cancer (Luz, Wu, & Tokar, 2018). Anaemia, impaired CNS, body weakness and more are resulted when there is deficiency of cobalt (Hackbart et al., 2010) though it is less common (Mertz, 2012).

7.2.3.4 Copper

From the field experiment, the rice variety 1 (Irat-170) had the highest percentage contribution (59.2% to 239.3%) to Cu RDI across all sex and the age groups while variety 6 (NERICA-L34) had the lowest (44.0% to 178.0%) though the percentage contribution to Cu RDI in variety 6 was not poor too. The varietal percentage contribution to Cu RDI is revealed in Figure 7.6. All the rice varieties demonstrated to be good sources of copper.

In the pot experiment, variety 2 (sipi rice) presented the highest percentage contribution (66.3% to 268.4%) to the Cu RDI while variety 10 (bisalayi rice) was the lowest contributor (2.6% to 26%) for Cu across all the age and sex groups. Just as recorded in the field samples, all the rice varieties contribute about 200% to the Cu RDI for the infants (age 0 to 12 months) and all the rice varieties also contribute about 50% to the Cu RDI for the male and female adolescents (10 to 18 years) while adult (>19 years) male, female, pregnant and lactating women are likely to get 100% of their daily need of Cu from any of the rice variety among the 10 selected rice examined. They are all rich with copper in both experiments (field and the pot).

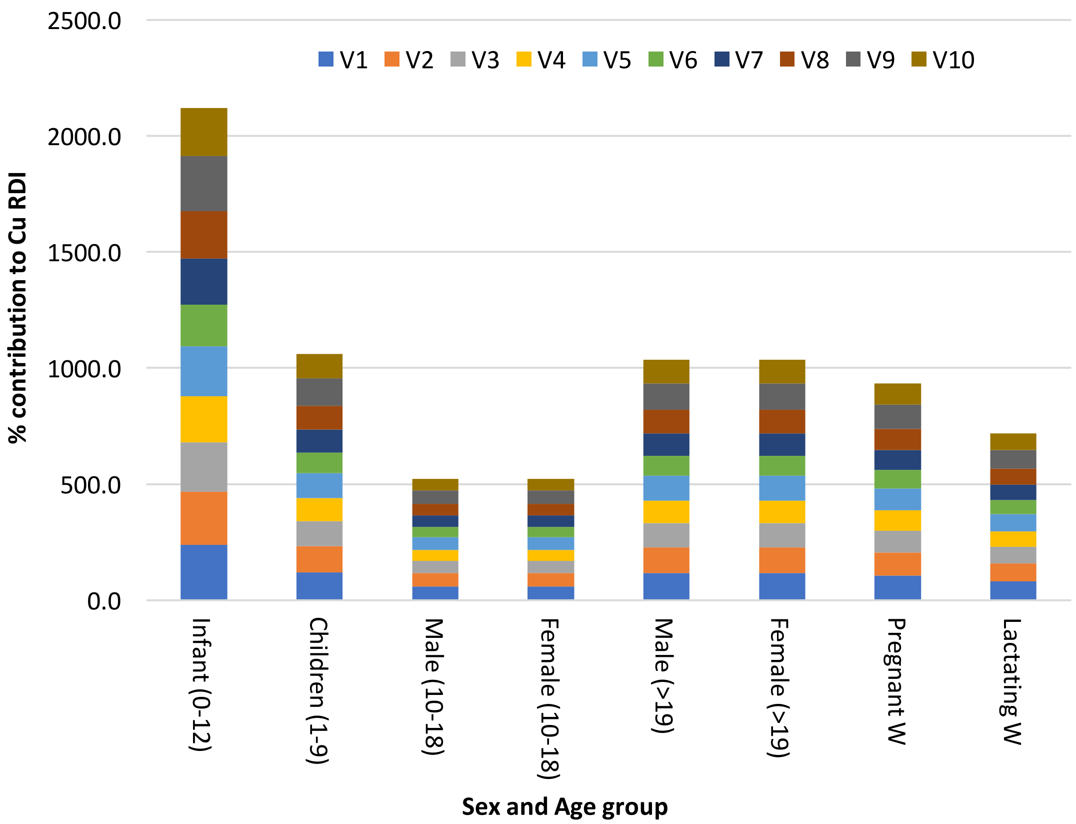


Figure 7.6: % contribution of rice varieties to copper RDI (v=varieties).

Copper is notable as a vital part of enzymes, hormones and some essential part of the body cells (Iakovidis, Delimaris, & Piperakis, 2011). Copper aids iron metabolism and therefore has a direct link to red blood cell formation and screening (Arredondo & Núñez, 2005). It keeps the brain active, helps the CNS to function appropriately and it maintains the CNS (Scheiber, Mercer, & Dringen, 2014). Copper functions to support both extra and intracellular activities with oxidases such as cytochrome oxidase (Jomova & Valko, 2011). That is the electron transport chain system in the body cells (Belyaeva, Sokolova, Emelyanova, & Zakharova, 2012).

The lysyl oxidase enzyme as well is made of copper protein which is responsible for hydroxylation of lysine in collagen and elastin within the system (Cromwell, 1997; Solomon et al., 2014). Inadequate or deficiency of copper do result in arterial vessel's rupture, achromotrichia (depigmentation of skin and hair), low immunity among others (Jaiser & Winston, 2010). Copper toxicity effects are blood vomit (hematemesis), psychiatric symptoms, low blood pressure (hypotension), black faeces (melena), yellowish pigmentation on the skin among others (Bandmann, Weiss, & Kaler, 2015).

7.2.3.5 Zinc (Zn)

In the field samples, the 10 selected rice varieties appeared to be good sources of zinc. The rice variety 2 (sipi-692033) had the highest percentage contribution (16.2% to 56.4%) to Zn RDI across all sex and the age groups while variety 4 (wita-4) had the lowest (13% to 45.5%) though the percentage contribution to Zn RDI in variety 4 was not poor. The varietal percentage contribution to Zn RDI across all ages and sex groups is revealed in Figure 7.7. All the rice varieties demonstrated to be good sources of Zn in both experiments.

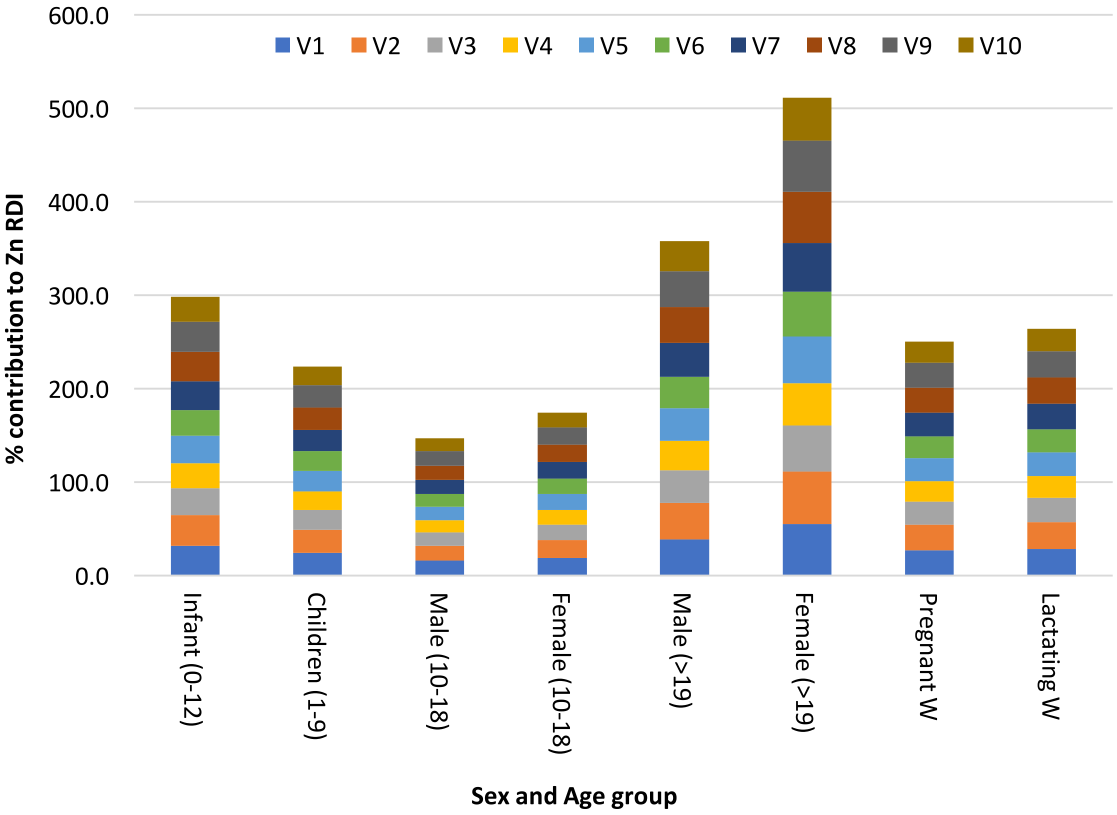


Figure 7.7: % contribution of rice varieties to zinc RDI (v=varieties).

In the pot experiment, variety 9 (art-15) presented the highest percentage contribution (20.5% to 50%) to the Zn RDI while variety 10 (bisalayi rice) was the lowest contributor (12.4% to 43.2%) for zinc across all the age and sex groups. Just as recorded in the field samples, all the rice varieties contribute at least 30% to the Zn RDI for the infants (age 0 to 12 months) and all the rice varieties also observed to contribute more than 16% to the Zn RDI for the male and female adolescents (10 to 18 years) while adult (>19 years) male, female, pregnant and lactating women are likely to get more than 40% of their daily need of Zn from any of the rice variety among the 10 selected rice examined.

The body metallo-enzymes such as glutamic dehydrogenase, carbonic anhydrase, and alkaline phosphatase rely on Zn to function (Rink & Gabriel, 2001). Zinc significantly involves in regulating the intracellular signalling and gene expression (Jansen et al., 2012). All the body cells contain Zn and it is in the second position after Iron in terms of its abundance in the human body (Chasapis, Loutsidou, Spiliopoulou, & Stefanidou, 2012). It helps the immune system to function appropriately (Caballero, Finglas, & Toldrá, 2015). The organs of taste and smell cannot work without Zn (Gupta & Gupta, 2014).

In human development, cell division needs Zn to perform this function and the hormone insulin needs Zn to work correctly (Roohani, Hurrell, Kelishadi, & Schulin, 2013). Many organs system such as central nervous system (CNS), Skeletal, immune, digestive, and reproductive systems will be affected by deficiency of zinc (Kambe, Tsuji, Hashimoto, & Itsumura, 2015). Zinc toxicity (accumulation more than necessary) results in body pain, vomiting, nausea, cramps, diarrhoea, abnormal growth rate, (Fosmire, 1990; Osredkar & Sustar, 2011). It also affects the absorption of copper and Iron from food into the body as their pathways are blocked (Osredkar & Sustar, 2011).

7.2.3.6 Selenium

In the field samples, the rice variety 5 (nerica-L19) had the highest percentage contribution (29.3% to 58.6%) to Se RDI across all sex and the age groups while variety 3 (ita-315) had the lowest (25.8% to 51.6%). This percentage contribution to Se RDI in variety 3 was not seen to be poor. The rice varietal percentage contribution to Se RDI across all ages and sex groups is revealed in Figure 7.8. All the rice varieties appeared to be good sources of Se.

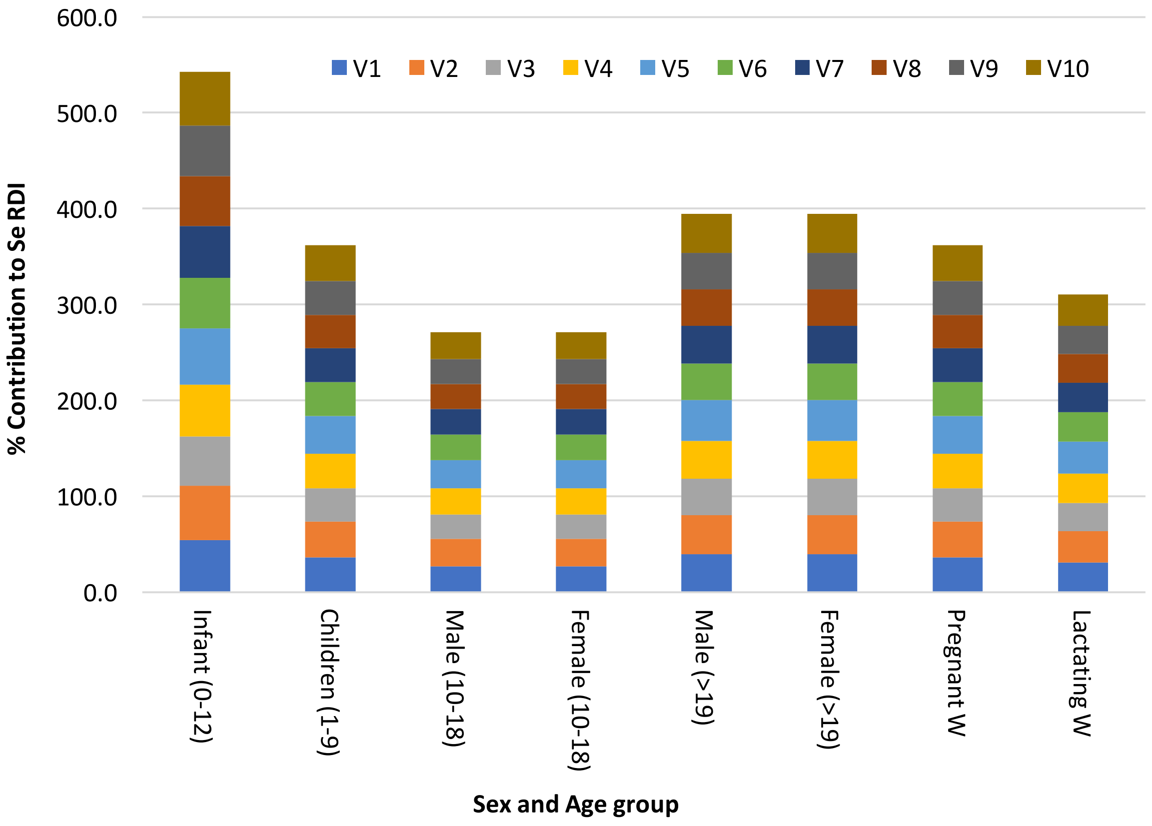


Figure 7.8: % contribution of rice varieties to selenium RDI (field samples) (v=varieties).

In the pot experiment, variety 10 (bisalayi rice) presented the highest percentage contribution (16% to 32%) to the Se RDI while variety 5 (nerica-L19) was the lowest contributor (9.3% to 18.5%) for selenium across all the age and sex groups. Just as recorded in the field samples though the percentage was a bit higher in the field samples, all the rice varieties contribute at least more than 20% to the Se RDI for the infants (age 0 to 12 months) and all the rice varieties also seen to contribute more than 13% to the Se RDI for the male and female adolescents (10 to 18 years) while adult (>19 years) male, female, pregnant and lactating women are likely to get more than 10% of their daily need of Se from any of the rice variety among the 10 selected rice examined.

Selenium is an essential component of the body cells that deals with the cell's protection against an oxidative destruction (Stone, Kawai, Kupka, & Fawzi, 2010). It is an important component of an enzyme called glutathione peroxidase which is responsible for reduction of peroxide radicals in the body cells to alcohol and oxygen (Mulgund, Doshi, & Agarwal, 2015). The ubiquinone (electron transport cellular co-enzyme) synthesis also involves selenium (Dasgupta & Klein, 2014). This important co-enzyme can also be referred to as Q10 which also helps to protect against the heart failure and fibromyalgia (Machado, Ambrosano, Lage, Abdalla, & Costa, 2017). Accumulation of selenium in the body system (selenium toxicity) result in hair loss, foul breath odour (garlic breath), nail decolouration, irritability and body weakness (MacFarquhar et al., 2010). The recommended daily intake (RDI) of selenium is 55 µg/day.

7.2.3.7 Iron

In the field samples, the rice variety 10 (bisalayi) had the highest percentage contribution (14.9% to 26.5%) to Fe RDI across all sex and the age groups while variety 4 (wita-4) had the lowest (4.5% to 8%). This percentage contribution to Fe RDI in variety 4 was observed to be lower. The varietal percentage contribution to Fe RDI is revealed in Figure 7.9. Variety 10 appeared to be a good variety in supplying more Iron to the adult especially the adult females, pregnant and lactating women.

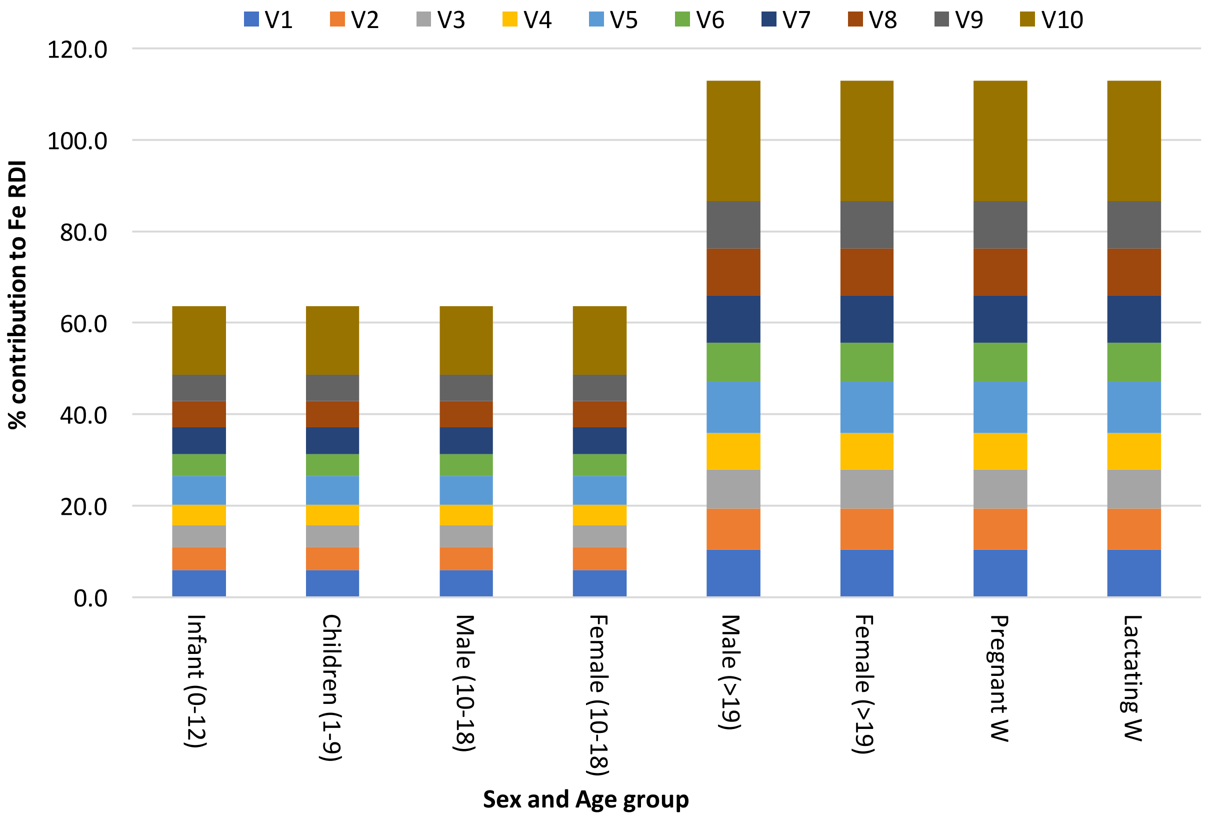


Figure 7.9: % contribution of rice varieties to iron RDI (v=varieties).

In the pot experiment, variety 10 (bisalayi rice) also presented the highest percentage contribution (36.3% to 64.5%) to the Fe RDI just as it was recorded in the field samples while variety 3 (ita-315) and variety 7 (ncro-49) were both appeared to be the lowest contributors (3.8% to 6.7%) for iron across all the age and sex groups. The percentage was higher in both experiment for variety 10 (bisalayi rice). It contributes more than 36% to the Fe RDI for the infants (age 0 to 12 months) and contributes more than 36% as well to the Fe RDI for the male and female adolescents (10 to 18 years) while adult (>19 years) male, female, pregnant and lactating women are likely to get more than 64% of their daily need of Fe from any of the rice variety among the 10 selected rice examined.

Iron (Fe) is an important component of the blood cells which helps in haem synthesis (haemoglobin) that assist the body in transporting oxygen (respiration) to all body parts (NHS, 2019). Iron is used in the bone marrow for blood production (Hughes, Stuart-Smith, & Bain, 2004). Red blood cell houses about 70% of the body iron (Camaschella, 2015). There is an important process of energy generation for the body that also involves iron and that is why one of the resulted effects of iron deficiency in the body is fatigue (Brown, 2019). Iron helps in maintaining a functional immune system and contribute to normal cognitive functions (Spatone, 2019). Female adults require more iron due to their monthly menstrual cycle which involves blood loss, and this is greater when they are pregnant (Pasricha et al., 2010).

Fe RDI of an adult female increases by about 20 µg/day in addition to 45 µg/day Fe RDI recommended when she is pregnant (Nestle, 2019). There are two groups of iron from the food sources; haem and non-haem iron. Haem iron is originated from animal-based foods such as poultry, fish and red-meat while non-haem iron is originated from plant-based foods such as legumes, vegetables and cereals which includes rice (Nestle, 2019). Anaemia, unusual tiredness, abnormal breathing, dizziness and headache, heart malfunctioning, dry skin, soreness and swelling of the tongue and mouth generally, spoon-shaped fingernails among others are the result of iron deficiency in the body (Zimmermann & Hurrell, 2007).

7.2.3.8 Potassium

Potassium (K), the rice variety 9 (Art-15) had the highest percentage contribution (4.9% to 9.4%) to K RDI across all sex and the age groups while variety 10 (bisalayi rice) had the lowest (4.0% to 7.7%). The varietal percentage contribution to K RDI is revealed in Figure 7.10. To get the required potassium that is needed daily in adult, that requires eating higher quantity of rice daily as they require more than the children and the adolescents. Our result suggests that variety 9 is the best for potassium supplies and there still need to supplement from other food that may be rich in potassium which include the food sources mentioned in the next two paragraphs.

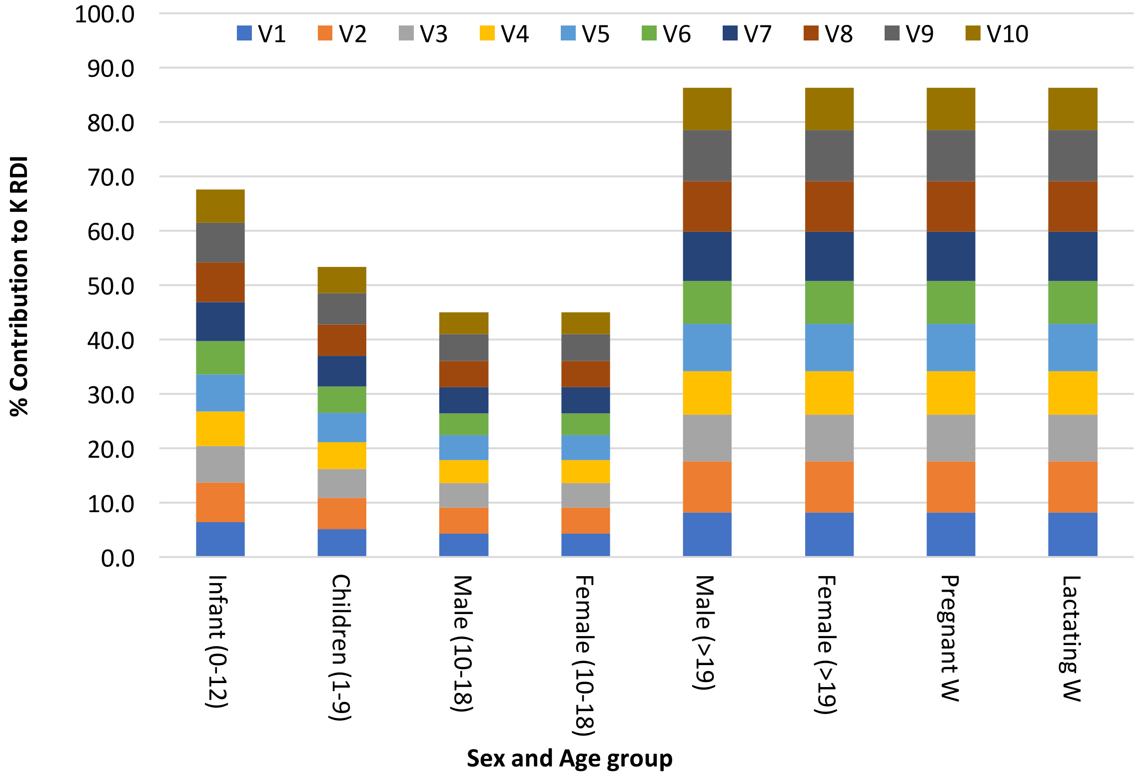


Figure 7.10: % contribution of rice varieties to potassium RDI (v=varieties).

In the pot experiment, variety 9 (art-15) also presented the highest percentage contribution (4.9% to 9.4%) to the K RDI just as it was recorded for the field samples and variety 10 (bisalayi rice) also appeared to be the lowest contributor (4% to 7.7%) for K across all the age and sex groups.

Potassium is an integral component of minerals in the body which supports the electrical and cellular functions (Akhter, Ashraf, Mohammad, Orfi, & Ahmad, 2003; Gropper & Smith, 2012). The work of potassium helps in muscle building and normal growth maintenance, muscle contraction, steady heartbeat, and more importantly the nutrient transfer in the cells is regulated by the potassium (Gropper & Smith, 2012). It maintains normal functions of the muscle, nerves, kidney, skeletal and the stomach dietary secretions (Whitney & Rolfes, 2018). Muscle weakness, low blood pressure (hypotension), bone fragility, abnormal functioning of CNS and death may be the result of potassium deficiency (Binia, Jaeger, Hu, Singh, & Zimmermann, 2015). Cooked spinach, cooked broccoli, potatoes, sweet potatoes, mushrooms, eggplant, peas, zucchini, cucumber, grape, oranges, apricot, tomatoes, are rich in potassium (WebMed, 2019). High potassium (potassium toxicity) always results to high blood pressure (hypertension), renal failure and heart failure (Weaver, 2013).

7.2.3.9 Magnesium

For the field experiment, variety 9 (ART-15) had the highest percentage contribution (23.8% to 130.2%) to Mg RDI across all sex and the age groups from while variety 1 (IRAT-170) had the lowest (19.8% to 108.0%). This is shown in Figure 7.11. All the rice varieties appear to contribute more than 100% of what is required in infants (0-12m age group) and between 39% to more than 50% in adults including the women groups. This study suggests that Mg supplement is required from other food sources for age 1 year and above. In the pot experiment, variety 8 (art3-7L) presented the highest percentage contribution (27.3% to 149.2%) to the Mg RDI unlike the field samples, variety 10 (bisalayi rice) appeared to be the lowest contributor (18.5% to 88.8%) for Mg across all the age and sex groups. All the rice varieties contribute significantly to the Mg RDI for the infants and the adults.

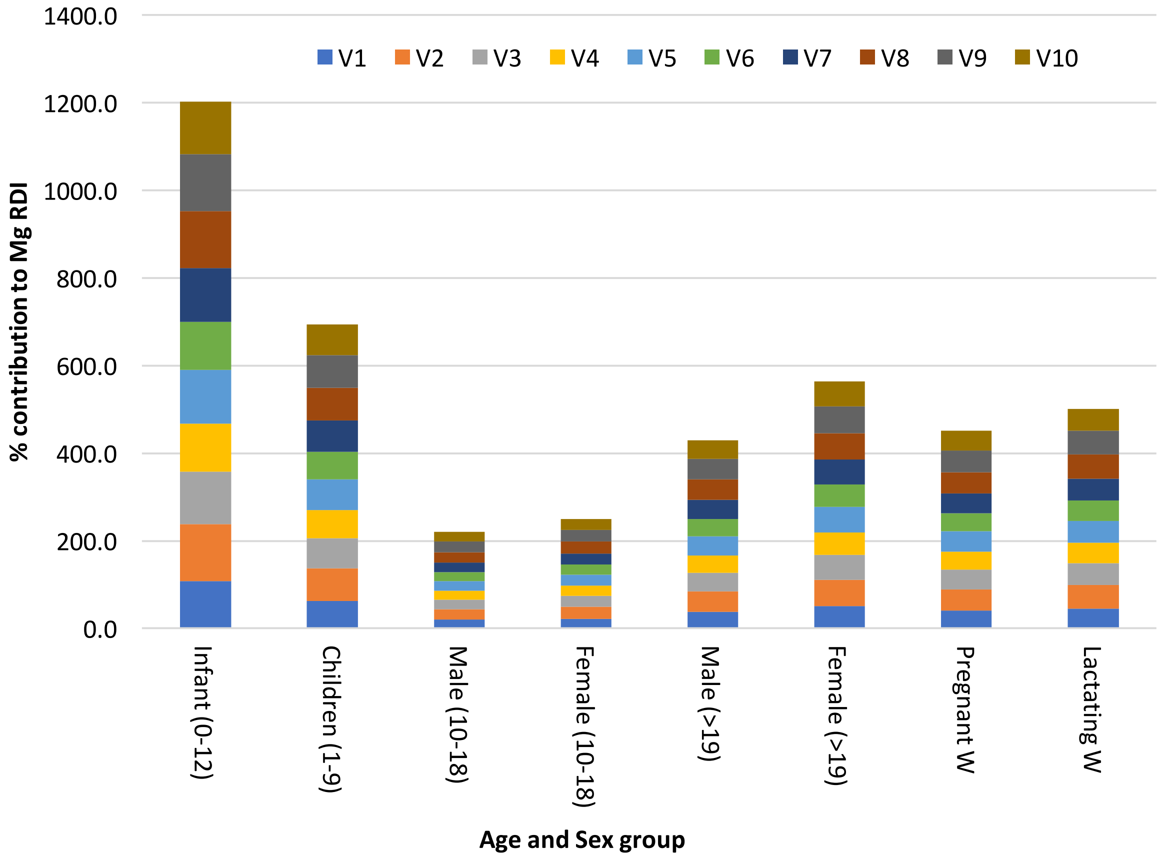


Figure 6. 2: % contribution of rice varieties to magnesium RDI (v=varieties).

Magnesium is a vital component of human body that is needed in about 300 bio-chemical reactions (Vahčić, Hruškar, Marković, Banović, & Barić, 2010). Without magnesium, normal muscle and nerve functions (exercise functions) cannot be achieved (Faryadi, 2012). Immune system maintenance, strong bones, regulation of blood glucose and heartbeat, energy and protein production, gene maintenance, brain function among others are the benefits of magnesium in the body (Gröber, Schmidt, & Kisters, 2015). Foods that are rich in magnesium are; quinoa, black beans, whole wheat, avocado, dark chocolate, peanuts, cashew nuts, almond nuts and some other nuts, cultured yoghurt etc. Magnesium deficiency may result in body weakness, type II diabetes, joints and body pains, depression weak immunity, hypertension migraine, insulin resistance among others (Jahnen-Dechent & Ketteler, 2012).

7.3 Conclusion

The contribution of the 10 selected rice varieties to recommended daily intake (RDI) of the essential elements was measured based on sex and different age categories as the rice ingestion rate used was different with age. There were no significant differences (p>0.05) between the percentage contribution to recommended daily intake (RDI) of the essential elements in the field samples and the percentage contribution to recommended daily intake (RDI) of the essential elements in the pot samples. Based on the varietal ranking (Table 7.14) and the % contribution to essential elements' recommended daily intake (RDI), variety 1 (irat-170) appeared to be the richest for Ca among the pot samples and variety 7 (ncro-49) among the field samples and variety 7 (ncro-49) also appeared to be the richest for Co among the field samples. Variety 10 (bisalayi) was the richest for Fe in both the pot and the field experiment. Variety 9 (art-15) appeared to be the richest for K, Zn, Se, Mn, and Cu in both experiments. Also, the richest for Mg among the field samples was variety 9 (art-15). Good numbers of the rice varieties demonstrated to be good sources of all the essential elements required by the human body.

In conclusion, it is worthy to note that the RDI of any of the essential elements are not expected to be met from only the rice as a food, other foods eaten in the day will also contribute. If the RDI of a particular essential element is low from rice, it is expected to be supplemented from another food source or fruit. Water also contributes if it is not de-mineralised or de-ionised (Dinelli et al., 2010; Maraver et al., 2015).

7.4 Limitation

The method employed in this study has provided a simple way to assess the rate at which the rice varieties could contribute the essential elements recommended daily intake (RDI). However, there are limitations in the applications of the method employed which has to do with accuracy. More information is required on the rice ingestion rate for different age groups and cooking methods in preparing the rice before consumption. These may have impacts on the result. 100g of rice per day ingestion rate that was suggested and used for children (0 to 18 years) and 200g that was used for adults (18 and above) as previously used by AfricaRice (2005) and Norton et al. (2014) seem not realistic looking at the rate at which rice is been consumed in Africa generally especially Zamfara state.

CHAPTER EIGHT

Stable Caesium and Strontium: Assessing the Inter-varietal Variation (Field Experiment)

8.0 Materials and methods

The method involved and the materials used including the sample collection, preparation and analysis have been previously discussed in chapter 3, section 3.7.3. This study selected 10 most grown rice varieties across Nigeria. The selected rice was germinated, grown on clean soil and then transplanted onto a contaminated soil (a selected field in Dareta village Zamfara previously discussed in chapter 3, section 3.2) using a Randomized Complete Block Design (RCBD) planting pattern. This was to make the result more precise and block multiple source of variations that may arise from other factors such as topography of the farm, weather and soil conditions etc. RCBD as explained in chapter 3, has blocked every other sources of variation in this experiment leaving variety of rice as the only source of variation.

8.1 Data Analysis

All data analysis was done with SPSS version 23.0 (SPSS Inc, Chicago, IL, USA) and Excel for windows 2016. The significance level was set at $p<0.05$ and 0.01 to present the result (more details in chapter 3, section 3.9). The concentration ratio (CR) was calculated as the concentration of the stable element in the rice sample (mg/kg) divided by the concentration of the stable element (mg/kg) in the soil of the respected rice sample. During the data cleaning and formatting, below detection limit (BDL) in the data was replaced by limit of detection (LOD)/2 values (Norton et al., 2014; Płotka-Wasylka, Frankowski, Simeonov, Polkowska, & Namieśnik, 2018). This was recommended because below detection level (BDL) values are not zeros and if it is left blank, the mean values and some other calculations will be affected (Shrivastava & Gupta, 2011). Correlation analysis was conducted using a Pearson test (2-tailed) to check for both possible positive or negative relationships among variables. The data was presented as mean and standard deviation. Analysis of variance was carried out to detect significant statistical differences in the inter-varietal variation that exist among the 10 rice varieties for stable caesium and strontium uptake. Multiple comparison was done by Least Significant Difference (LSD) test at $p<0.05$.

8.2 Result and Discussion

8.2.1 Quality Control Analysis

To guarantee analytical quality, NIST 1568b Rice Flour Standard Reference Material from the National Institute of Standards and Technology, USA was used to validate (quality control) the method of analysis. The detection limit for both Cs and Sr were 0.016 ug/L and 0.214 ug/L respectively. The calculated limits of quantification were 0.706 ug/L and 0.052 ug/L for Sr and Cs and since there was no data for both elements (Sr & Cs) in the SRM (NIST 1568b) used. The analysis was spiked with Sr and Cs and their recoveries agreed with the techniques as Sr and Cs were within ±15% acceptable limits. The spiked concentration for Cs was 0.25 mg/kg and the recovery was 96.7 ± 2.5% while the spiked concentration for Sr was 2.1 mg/kg and the recovery was 96.5 ± 2.2%. A linear response was generated from the calibration standard solution measurement with R^2 (correlation coefficient) of 1.00.

8.2.2 Soil Characteristics

The characteristics of the experimental soil are presented in Table 8.1. The soil was slightly acidic as the pH ranged between 4.51 and 8.51. The values recorded for the soil parameters in this study are similar to those obtained in previous studies by Mohammed & Abdu, (2014), Udiba et al. (2012), UNICEF (2011), and Uriah, Kenneth, Gusikit, & Ayuba, (2013) in this area.

Table 8. 1: Result for the Soil physico-chemical characteristics.

Soil (n=300)	pH (H_2O) 1:1	[a]EC (dS/m)	%Organic Carbon	Av-P_2O_2	Exchangeable cations (cmol /kg)				Exchangeable Acidity	CEC	Particle size			N
					Ca	K	Mg	Na			%Clay	%Silt	%Sand	
Mean	6.58	1.19	4.00	7.77	1458.58	1400.28	1759.41	27.12	0.65	26.10	46.63	39.74	45.63	3.12
SD	0.86	0.36	0.90	2.84	307.33	707.97	586.80	46.37	0.19	7.89	7.26	14.64	16.84	1.59
Min	4.51	0.3	1.47	0.620	971.64	318.55	684.68	0.05	0.34	12.85	2.00	2.00	11.50	1.32
Max	8.51	2.5	7.67	14.00	2505.62	3046.49	3015.34	296.82	2.37	43.32	37.4	69.1	94.00	7.25

SD = Standard Deviation, Max = Maximum, Min = Minimum, n= Number of samples, EC= Electrical Conductivity, Av-P_2O_2 = Available Phosphorus, N = Nitrogen

8.2.3 Inter-varietal variation of Stable Strontium and Caesium in rice

Summary of the result for stable strontium (Sr) and caesium (Cs) concentrations in the 10 selected rice varieties and their corresponding soil samples is presented in Table 8.2. In all the rice varieties and the soil samples, the arithmetic mean concentration (mg/kg) of Sr was higher than that of the Cs likewise the geometric mean of Sr was as well, higher. A strong positive relationship between the Cs concentration (mg/kg) in rice samples and Cs concentration (mg/kg) in the soil samples with correlation coefficient (R^2) of approximately 1.00 (Figure 8.1). This was similar in the regression analysis for the concentrations of the Sr in the rice samples and the concentration of the Sr in the soil samples (Figure 8.2). The arithmetic mean concentration ratio (CR) across the 300 samples for Sr was 0.135 ranges from 0.130 to 0.140 across the 10 rice varieties. This was far higher above the CR obtained for Cs in this study. The arithmetic mean concentration ratio across the 300 samples recorded for Cs was 0.065 and it ranged between 0.051 and 0.086 (Table 8.2).

The soil was majorly dark yellowish brown, and sandy loam based on the Munsel colour chart used (Munsell, 2000) and textural test using texture triangle (Reuter, Peverill, & Sparrow, 1999; US NIFA, 2017). Details on soil test was discussed in chapter 3, section 3.7. Each rice variety (n=30) vary in their CRs. For Sr, it was 0.14 for irat-170, 0.136 for sipi-692033, 0.131 for ita-315, 0.135 for wita-4, 0.138 for nerica L19, 0.133 for nerica L34, 0.129 for ncro-49, 0.133 for art-7L, 0.135 for art-15 and 0.140 for bisalayi rice. For Cs, 0.63 was recorded for irat-170, 0.85 for sipi-692033, 0.74 for ita-315, 0.57 for wita-4, 0.51 for nerica L19, 0.70 for nerica L34, 0.68 for ncro-49, 0.56 for art-7L, 0.66 for art-15 and 0.69 for bisalayi rice (Table 8.2).

Table 8. 2: Statistical summary of the result of stable Sr and Cs concentrations in the 10 rice varieties, their corresponding soil samples and CR.

		Rice Varieties ->	**IRAT_170**	**SIPI_692033**	**ITA_315**	**WITA_4**	**NERICA_L19**	**NERICA_L34**	**NCRO_49**	**ART3_7L**	**ART_15**	**BISALAYI**	**Total (Average)**
Strontium	**Rice**	N	30	30	30	30	30	30	30	30	30	30	300
		Arithmetic Mean± SD	1.37±0.47	1.350±0.41	1.21±0.09	1.29±0.35	1.35±0.33	1.28±0.34	1.18±0.26	1.25±0.31	1.28±0.30	1.41±0.46	1.30±0.35
		Geo-Mean ± SD	1.29± 0.47	1.31±0.41	1.21±0.09	1.26±0.35	1.32±0.33	1.25±0.34	1.16±0.26	1.22±0.31	1.25±0.30	1.35±0.46	1.26±0.35
		Min, Max,	0.31, 2.30	0.92, 2.38	0.97, 1.34	0.76, 2.32	1.02, 2.19	0.86, 2.3	0.63, 2.32	0.82, 2.31	0.95, 2.32	0.89, 2.32	0.31, 2.38
		Min/max, CV	7.47, 0.34	2.60, 0.30	1.38, 0.07	3.07, 0.27	2.15, 0.24	2.67, 0.26	3.67, 0.22	2.81, 0.24	2.44, 0.23	2.60, 0.32	7.73, 0.27
	Soil	N	30	30	30	30	30	30	30	30	30	30	300
		Arithmetic Mean ± SD	9.75±2.07	9.76±1.63	9.20±0.14	9.38±1.05	9.61±1.08	9.47±1.00	9.06±1.22	9.30±0.86	9.39±1.00	9.87±1.53	9.48±1.26
		Geo-Mean ± SD	9.51±2.07	9.66±1.63	9.20±0.14	9.33±1.05	9.55±1.08	9.43±1.00	8.97±1.22	9.27±0.86	9.34±1.00	9.768	9.40±1.26
		Min, Max,	3.79, 13.51	8.52, 15.34	8.57, 9.34	7.76, 12.32	8.62, 12.68	8.26, 12.30	4.28, 13.32	8.02, 12.30	8.55, 13.52	8.53, 14.32	3.79, 15.34
		Min/max, CV.	3.56, 0.21	1.80, 0.17	1.09, 0.02	1.59, 0.11	1.47, 0.11	1.49, 0.11	3.11, 0.14	1.53, 0.09	1.58, 0.11	1.68, 0.16	4.05, 0.13
	CR	Arithmetic Mean	0.14003	0.13617	0.13155	0.13589	0.13866	0.13329	0.12958	0.1325	0.13498	0.1402	0.13529
		Standard Deviation	0.035045	0.017802	0.008068	0.019467	0.016294	0.019231	0.013467	0.017674	0.014918	0.022779	0.01965
		Geometric Mean	0.13562	0.13516	0.13131	0.13463	0.13782	0.13207	0.12894	0.13145	0.13426	0.1385	0.13394
		Geometric SD	0.035045	0.017802	0.008068	0.019467	0.016294	0.019231	0.013467	0.017674	0.014918	0.022779	0.01965
		Minimum	0.049	0.107	0.113	0.097	0.118	0.098	0.107	0.102	0.111	0.104	0.049
		Maximum	0.261	0.189	0.144	0.188	0.179	0.187	0.174	0.187	0.188	0.185	0.261
		Max/Min	5.33	1.77	1.27	1.94	1.52	1.91	1.63	1.83	1.69	1.78	5.33
		CV	0.25	0.13	0.06	0.14	0.12	0.14	0.10	0.13	0.11	0.16	0.15
Caesium	**Rice**	N	30	30	30	30	30	30	30	30	30	30	300
		Arithmetic Mean ± SD,	0.06±0.04	0.10±0.18	0.07±0.07	0.05±0.03	0.05±0.03	0.07±0.03	0.06±0.03	0.05±0.03	0.08±0.17	0.06±0.04	0.06±0.09
		Geo-Mean ± SD	0.04±0.04	0.06±0.18	0.04±0.07	0.04±0.03	0.04±0.03	0.05±0.03	0.05±0.03	0.04±0.03	0.04±0.17	0.05±0.04	0.04±0.09
		Min, Max,	0.01, 0.21	0.01, 1.02	0.01, 0.41	0.01, 0.10	0.01, 0.10	0.01, 0.11	0.01, 0.11	0.01, 0.10	0.01, 0.95	0.01, 0.10	0.01, 1.02
		Min/max, CV	42.00, 0.75	145.71, 1.84	58.57, 1.09	17.17, 0.58	17.33, 0.64	13.13, 0.48	18.33, 0.54	17.17, 0.63	158.83,2.21	14.86, 0.55	204.00, 1.33
	Soil	N	30	30	30	30	30	30	30	30	30	30	300
		Arithmetic Mean ± SD,	0.90±0.05	0.95±0.19	0.90±0.05	0.90±0.04	0.90±0.05	0.92±0.05	0.91±0.05	0.90±0.05	0.93±0.18	0.91±0.04	0.91±0.09
		Geo-Mean ± SD	0.90±0.05	0.94±0.19	0.90±0.05	0.90±0.04	0.90±0.05	0.92±0.05	0.91±0.05	0.90±0.05	0.92±0.18	0.91±0.04	0.91±0.09
		Min, Max,	0.82, 1.01	0.82, 1.92	0.82, 0.99	0.83, 1.01	0.82, 0.98	0.82, 0.99	0.83, 1.00	0.82, 0.99	0.82, 1.84	0.83, 0.99	0.82, 1.92
		Min/max, CV.	1.23, 0.06	2.33, 0.20	1.22, 0.05	1.21, 0.04	1.20, 0.06	1.20, 0.05	1.21, 0.06	1.21, 0.06	2.24, 0.19	1.20, 0.04	2.35, 0.10
	CR	Arithmetic Mean	0.0628	0.08562	0.07409	0.05685	0.05128	0.07033	0.06786	0.0556	0.06611	0.06889	0.06594
		Standard Deviation	0.049706	0.091373	0.081083	0.032238	0.030862	0.032734	0.03494	0.033928	0.091443	0.036778	0.05698
		Geometric Mean	0.04545	0.06117	0.04889	0.04427	0.04038	0.05914	0.05587	0.04122	0.04112	0.05378	0.04858
		Geometric SD	0.049706	0.091373	0.081083	0.032238	0.030862	0.032734	0.03494	0.033928	0.091443	0.036778	0.05698
		Minimum	0.006	0.008	0.008	0.007	0.007	0.009	0.007	0.007	0.007	0.008	0.006
		Maximum	0.257	0.532	0.457	0.113	0.107	0.115	0.112	0.111	0.519	0.112	0.532
		Max/Min	42.83	66.50	57.13	16.14	15.29	12.78	16.00	15.86	74.14	14.00	88.67
		CV	0.79	1.07	1.09	0.57	0.60	0.47	0.51	0.61	1.38	0.53	0.86

SD = Standard Deviation, Max = Maximum, Min = Minimum, N= Number of samples, CV= Coefficient of Variance, CR = Concentration Ratio

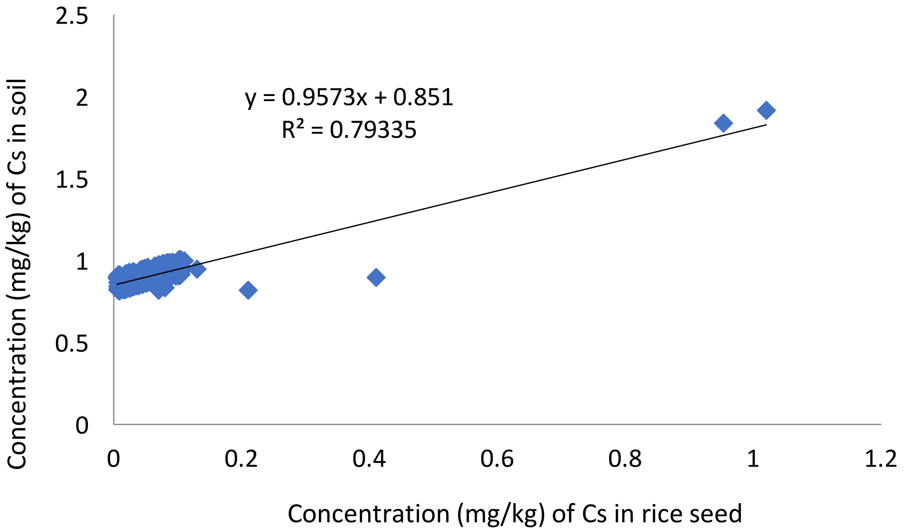


Figure 8. 1: Positive relationship between the Cs in both the rice and the soil samples.

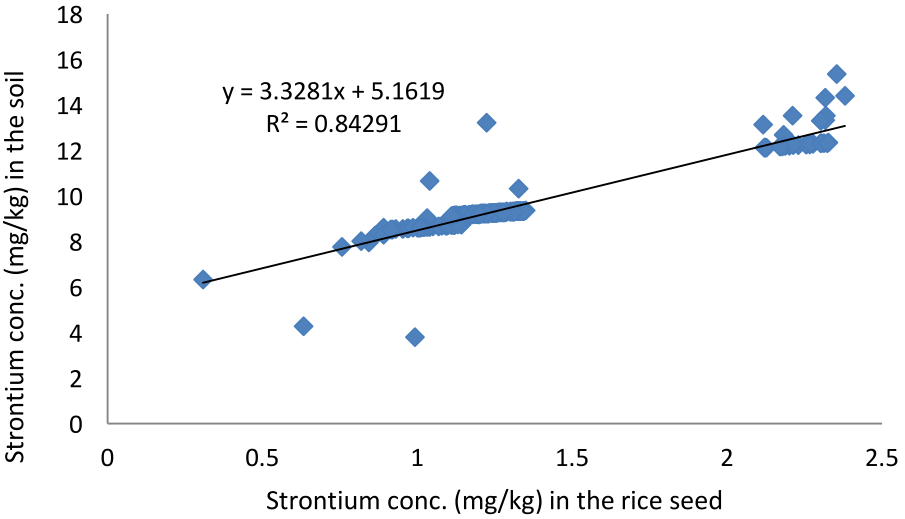


Figure 8. 2: Positive relationship between the Sr in both the rice and the soil samples.

This positive relationship between the Sr in rice and Sr in the soil samples likewise the positive relationship between the Cs in rice and the Cs in the soil samples (Figures 8.1 and 8.2) indicate that the Sr and the Cs in the rice samples were from the soil. The CR obtained was the highest in bisalayi (variety 10) and lowest in ncro-49 (variety 7) for Sr while sipi-692033 (variety 2) shows the highest and nerica-L19 shows the lowest for Cs in terms of the concentration ratio. The Duncan multiple range post ANOVA test result of the concentration ratio (CR) reveals that all the 10 rice varieties were not significantly different for Sr and Cs uptake (Figures 8.3 and 8.4). The inter-varietal variation recorded among the 10 selected varieties for Sr was 1.10 folds and that of the Cs was 2 (1.96) folds.

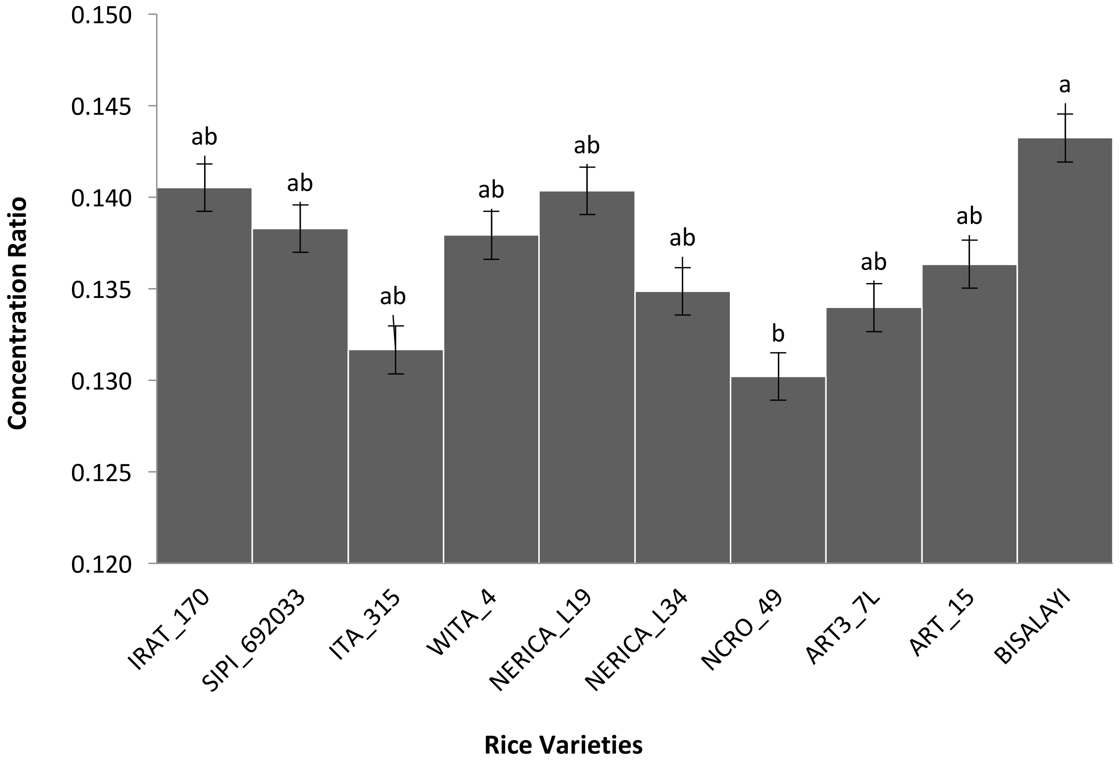


Figure 8. 3: Strontium Concentration Ratio.

Same letter in the data labels shows they are not significantly different statistically and vice-versa. Same letters in the data labels shows they are not significantly different statistically (result of the Duncan multiple range test at $p<0.05$ confidence level). And different letters indicate significant difference.

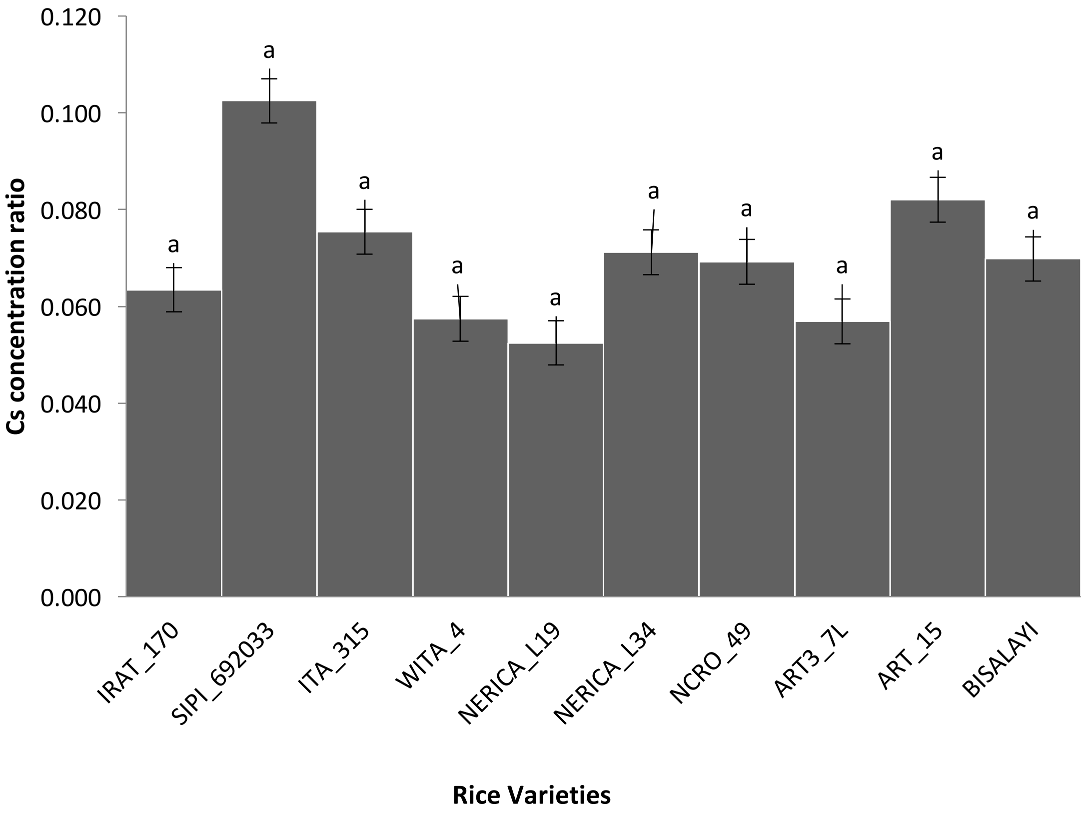


Figure 8. 4: Caesium Concentration Ratio.

Same letters in the data labels shows they are not significantly different statistically (result of the Duncan multiple range test at p<0.05 confidence level). And different letters indicate significant difference.

In terms of the Sr accumulation, ncro-49 (Variety 7) demonstrated lowest accumulation with 1.18 mg/kg ranges from 0.632 – 2.316 mg/kg while bisalayi (variety 10) demonstrated the highest accumulation with 1.41 mg/kg ranges from 0.892 – 2.313 mg/kg (Figure 8.5). The accumulation was in the series of ncro-49 < ita-315 < art3-7l < nerica-L34 < art-15 < wita-4 < nerica-L19 < sipi-692033 < irat-170 < bisalayi.

In terms of the Cs accumulation nerica-L19 (variety 5) demonstrated the lowest accumulation with 0.05 mg/kg ranges from 0.01 – 0.10 mg/kg while sipi-692033 (variety 2) demonstrated the highest accumulation with 0.10 mg/kg ranges from 0.01 – 1.02 mg/kg (Figure 8.6) and it was in the series of nerica-L19 < art3-7L < wita-4 < irat-170 < ncro-49 < bisalayi < nerica-L34 < ita-315 < art-15 < sipi-692033. The result of the Duncan multiple range test at p<0.05

confidence level revealed no significant difference in the accumulation of Sr and Cs in all the rice varieties (Figures 8.6 and 8.7).

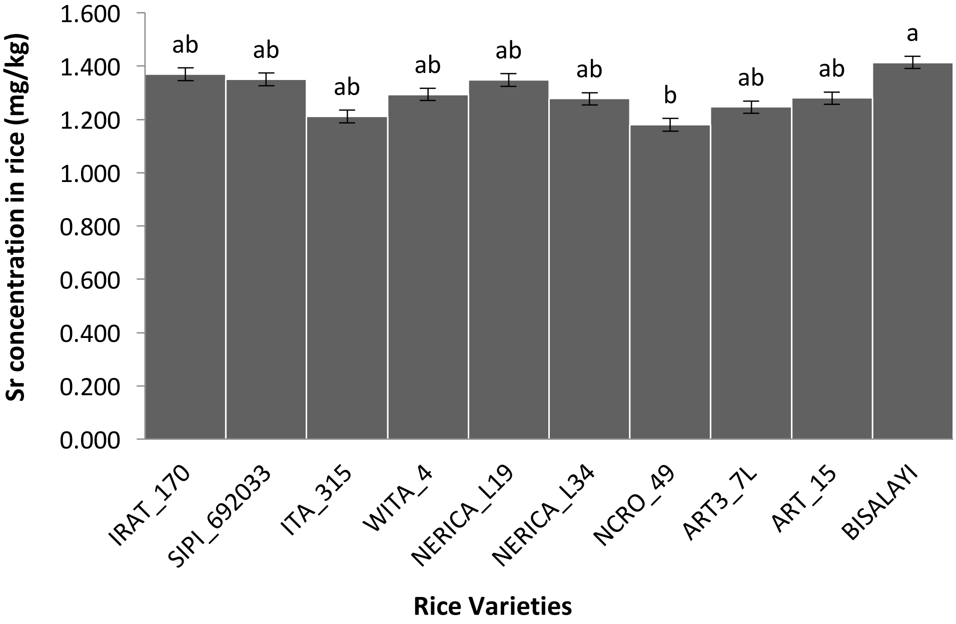


Figure 8. 5: Accumulation of stable Sr across the 10 varieties of rice.

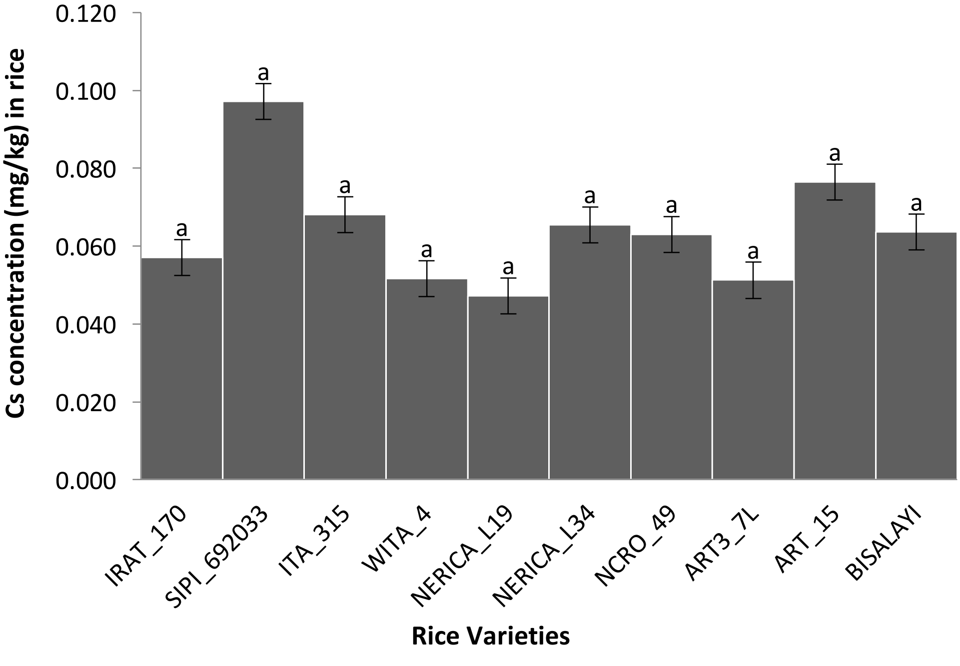


Figure 8. 6: Accumulation of stable Cs across the 10 varieties of rice.

The maximum/minimum Sr ratio was below 10 in both the rice and the soil samples which signifies a narrow concentration distribution range across all the varieties. Whereas, this was very wide in Cs as the maximum/minimum Cs ratio were all greater than 10 with the lowest value of 13.125 mg/kg was observed in nerica-L34 (variety 6) and the highest value of 158.8 mg/kg was observed in art-15 (variety 9).

The mean concentration of the stable Sr recorded for rice samples for individual variety and in all the 10 varieties (1.297± 0.3 mg/kg) were higher than the previous study by David et al. (2019) that reported <0.04±0.00 mg/kg for Nigerian rice. There are limited studies for Nigeria. Srinuttrakul and Yoshida (2017) reported 0.33 ± 0.1 mg/kg, ranges between 0.107 and 0.825 for Thai rice. Study by Uchida, Tagami, and Hirai (2007) reported 0.07 ± 0.01 mg/kg, ranges between 0.014 and 0.14 for Japanese rice and Gonzálvez, Armenta, and De La Guardia (2011) reported 0.9 ± 0.02 mg/kg, ranges between 0.2 and 3.7 for Spanish rice and these are all lower than the result of this study. Our result is lower than the stable Sr of 3.1±01 mg/kg reported for Chinese rice by Lu et al. (2006), 44±0.1 mg/kg reported for Japanese rice by Tsukada, Hasegawa, Takeda, and Hisamatsu (2007) and 3.16 mg/kg reported for Jamaican rice by Huang et al. (2016).

For the stable Cs in the rice varieties, the mean concentration recorded for the 10 varieties (0.064±0.085 mg/kg, n=300) in this study was in the same range with the stable Cs (0.07±0.01 mg/kg) reported for Nigerian rice by David et al. (2019) but higher than the Cs concentration (0.0017±0.002 mg/kg) reported for Japanese rice by Tsukada et al. (2007) and 0.004 mg/kg reported for Jamaican rice by Antoine, Fung, Grant, Dennis, and Lalor (2012). All these variations are likely to be due to differences in soil characteristics where the rice varieties were grown including the soil Sr and Cs content and the varieties of rice involved. Different rice varieties behave differently (Liu, Ma, Wang, & Sun, 2013; Norton et al., 2014).

The mean concentration of the stable Sr recorded for soil samples collected with the 10 varieties in this study (9.48±1.26 mg/kg) was lower than the previous study by Tsukada et al. (2005) that reported 41±0.1 mg/kg for soil samples from Japanese rice farm. Takeda, Tsukada, Takaku, Akata, and Hisamatsu (2008) also reported 121 mg/kg for rice farm in Japan. This may be due to the history of nuclear accident in Japan (Holt, Campbell, & Nikitin, 2012). Also, our result is higher than the stable Sr of 1.6 mg/kg reported by Dyer, Chow, and Umar (2000) for the UK arable soil.

It has been difficult to find previous study in Nigeria that presented data for stable caesium in soil to compare with the result of this study. Most of the previous studies found from other region were higher than our result. This study presented soil mean concentration of 0.912±0.09 mg/kg for stable Cs, Tsukada, Hasegawa, Hisamatsu, and Yamasaki (2002) reported 2.5 mg/kg and Ogasawara et al. (2019) reported between 2.53 mg/kg and 3.89 mg/kg for Japanese soil (farms) respectively and Cook, Inouye, McGonigle, and White (2007) reported 2.03 and 6.29 mg/kg for United States (Idaho) soil.

7.2.4 Spatial distribution of stable Sr and Cs Uptake on the field.

It is obvious from the result that the uptake of Sr and Cs by the rice varieties across the experimental field was not uniform and this seems to depend on the varieties using the spatial distribution imagery. The high Sr concentration in the soil does not appear to show high Sr concentration in the rice seeds likewise the Cs. Figures 8.7 and 8.8 reveal the spatial distribution for both stable Sr and Cs in the samples.

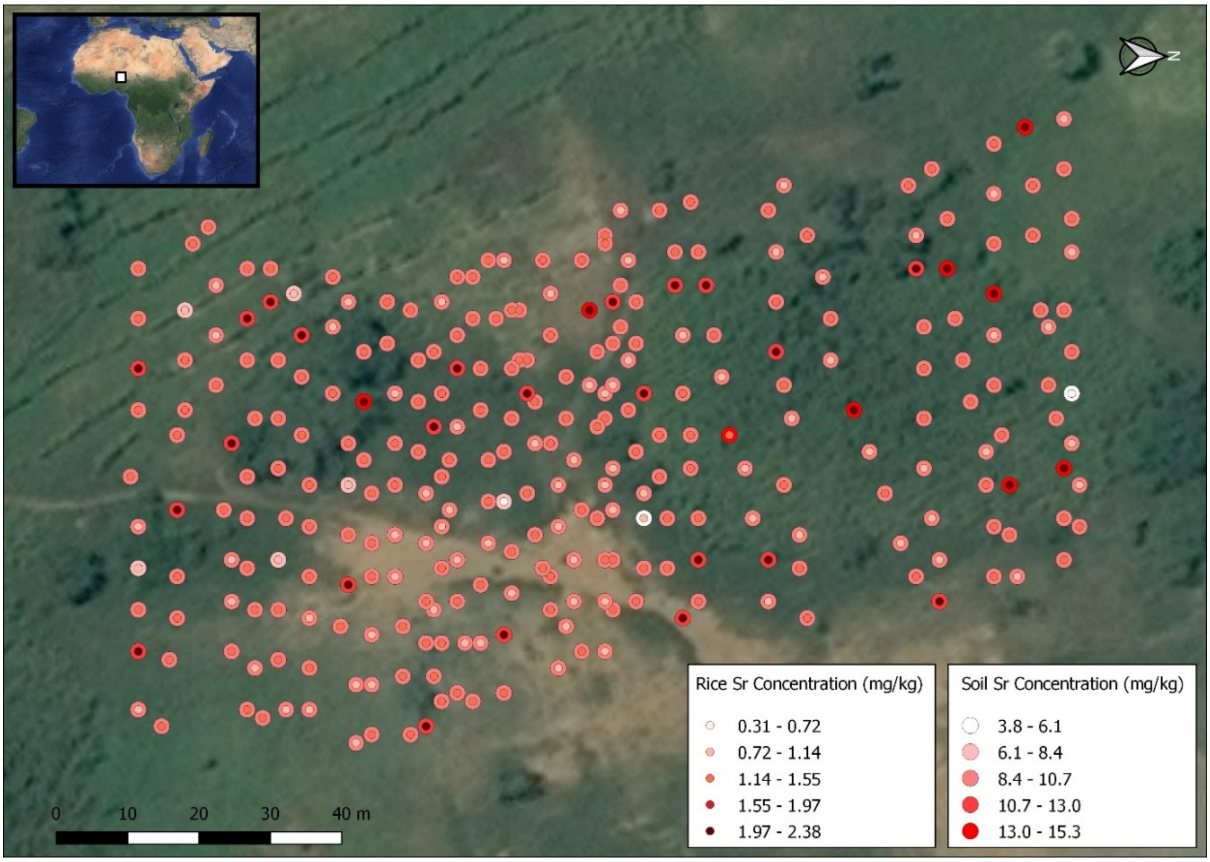


Figure 8. 7: Spatial distribution of rice and soil for stable Sr in the field experiment.

The outer circle represents the Sr concentration in the soil samples (n=300) while the inner circle stands for the concentration of Sr in the rice samples (seeds, n=300).

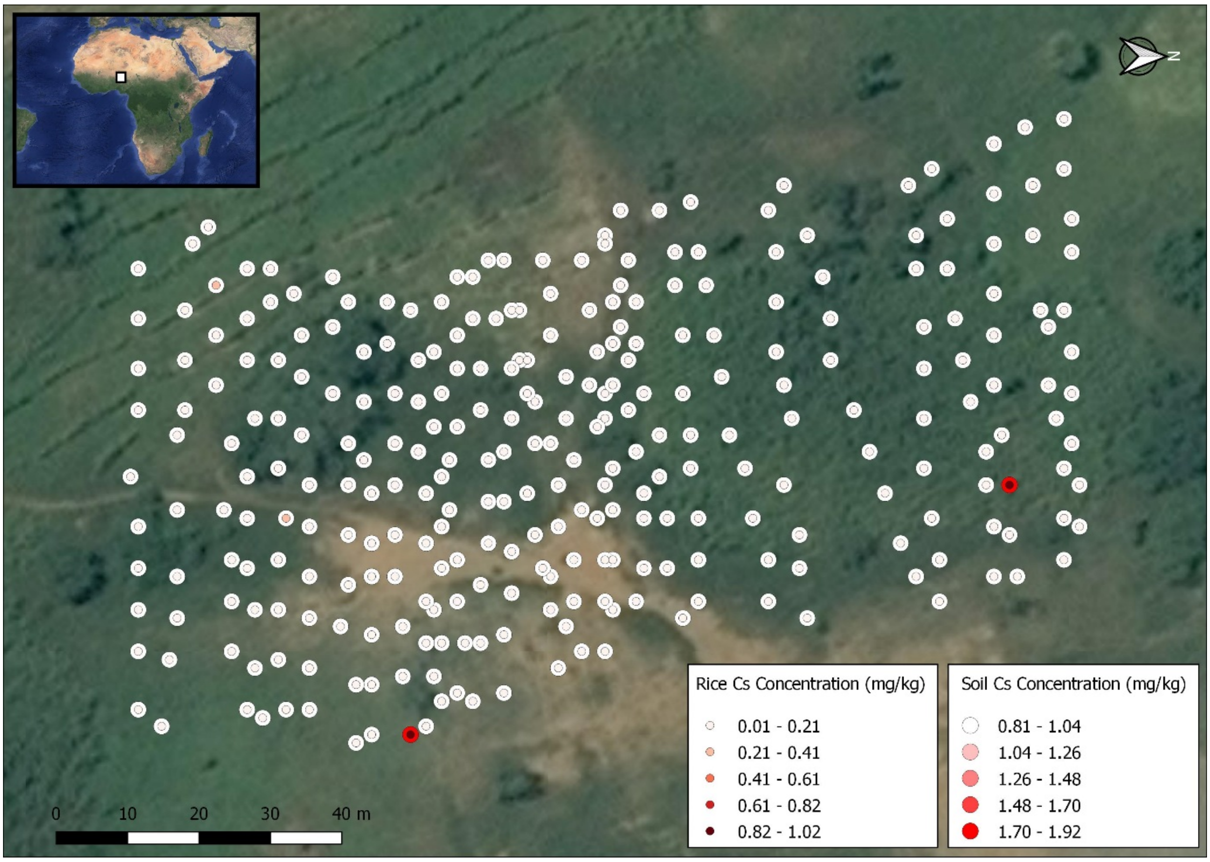


Figure 8. 8: Spatial distribution of rice and soil for stable Cs in the field experiment.

The outer circle represents the Cs concentration in the soil samples (n=300) while the inner circle stands for the concentration of Cs in the rice samples (seeds, n=300).

7.3 Conclusion and Recommendation

This study appears to be the first to provide data for the stable Cs in Nigerian soil and the first to present data on the inter-varietal variation among 10 Nigerian rice varieties regarding uptake and accumulation of stable strontium (Sr) and caesium (Cs) to the best of our knowledge. The study suggests more studies on varietal selection through inter-varietal variation assessment. As discussed previously (chapter 1, section 1.5), varietal selection is one of the recommended remediation techniques to reduce human exposure to Pb and one of the options to reduce transfer of Sr and Cs to human body through rice consumption. This study has provided a baseline data for the anthropogenic radionuclides in rice and soil for Zamfara state given that the state has few of the allocated proposed sites for the construction of the Nigerian nuclear power stations. Our data will help the policy makers in prospective dose assessments and emergency planning before the commencement of the project. Current radionuclide transfer datasets have been derived based on a combination of data from radionuclides and their stable elements. Two radionuclides of importance in both operational discharges and emergency (accident) situations are likely to be radio-caesium and radio-strontium.

It is acknowledged in this study that the behaviour among the 10 selected rice varieties in terms of the uptake of Sr and Cs using the CR, a further investigation is required. There was no significant difference statistically observed in the uptake and accumulation of Sr and Cs among the 10 rice varieties. The inter-varietal variation (IVV) among them for Strontium (Sr) was 1.1 folds and 1.95 folds for Cs. It is concluded that a further study is required to explore the rice behaviour not only in Zamfara state but in different location in Nigeria in order to have a wide range of judgement regarding the varietal selection for the accumulation of stable caesium and strontium.

CHAPTER NINE

General Discussion, Conclusion and Recommendations

9.0 General Discussion

Lead (Pb) poisoning epidemic in Zamfara state Nigeria has been one of the major public health issues for more than a decade (Orisakwe et al., 2017). Many people, including children, have died and dietary Pb intake through rice consumption has been identified as one of the major exposure routes (Greig et al., 2014). The overarching aim of this research is to evaluate the potential for rice variety selection to reduce the exposure to Pb in rice. The ten most grown rice varieties in Nigeria were grown in a field experiment in a mining-polluted farms in Dareta Village, Zamfara State and in a pot experiment using three-hundred pots of Pb-contaminated soil at the University of Abuja, Abuja, Nigeria. The rice varieties used in this research include: bisalayi, nerica-L34, wita-4, sipi-692033, nerica-L19, art-15, ita-315, art3-7L, ncro-49 and irat-170. These include the local varieties (bisalayi) and the new improved ones. The new improved varieties are called NERICA which means "New Rice for Africa" (AfricaRice, 2011). These are rice varieties derived from successful genetic crossing of the African rice (Oryza glaberima) with the Asian rice (Oryza sativa) to produce the best traits of both parents (Samado, Guei, & Nguyen, 2008). Over 60 NERICA varieties exist in Nigeria but few are being grown due to economic value and acceptability (National Cereals Research Institute, 2017). The rice selected for use within this research included both lowland and uplands varieties as described (National Cereals Research Institute, 2017). Detailed information about the rice varieties is presented in Appendix A, Table I.

Site characterisation was conducted prior to rice planting in Dareta whereby four rice farms were selected from four different areas within the village (details in Chapter 3, section 3.1, paragraph 5). Samples of soil from 0-10 cm, 10-20 cm and 20-30 cm depth were collected alongside their rice plants and rice samples collected were dissected into root, stem, husk and seeds. X-ray fluorescent spectrometer (XRF) was also used to scan the top soil on the four selected rice farms. Both the rice planting (field and the pot) and the site characterisation were to address the following objectives:

1. To evaluate the influence of soil physico-chemical properties on Pb uptake in rice
2. To assess the localisation of Pb in different parts of rice (root, shoot, husk and seed).
3. To establish the inter-varietal variation in the uptake of Pb, Cs, Sr and nine essential elements uptake among the varieties of rice grown in Nigeria.
4. To examine rice varieties contribution to the recommended dietary intake of nine essential elements via rice consumption.

The first and second objectives were addressed with the site characterisation (the first research work) conducted in Dareta Village. This work was also directed to selection of an appropriate site that would suit the varietal trial experiment (rice growing) and the results for the site characterisation were presented in chapters 4 and 5. From analysis of the 10 selected rice varieties grown on the field (field experiment) and in the screen-house (pot experiment), an evaluation was made of inter-varietal variation in Pb uptake (chapter 6) and in essential elements' uptake (chapter 7). The evaluation of inter-varietal variation of Cs and Sr uptake among the 10 selected rice varieties (chapter 8) was based on results from the field experiment alone. In combination, these chapters addressed the third and the fourth research objectives. This chapter presents the discussion of the findings based on these objectives and the emerging recommendations for practice, policy and research relating to reduction of rice-associated Pb poisoning in mining polluted environment. It also considers the research limitations.

9.1 Summary of Findings

9.1.1 Influence of soil physico-chemical properties on lead uptake in Rice

The soil characterisation with only bisalayi rice variety, the popular local rice already grown by the farmers in the area prior to the commencement of the research revealed that physico-chemical characteristics of soil including pH, available phosphorus, organic carbon, soil nitrogen, and zinc content influence Pb uptake in rice. Inverse association was found between bisalayi rice and some of the characteristics of the soil which include pH, organic carbon, available phosphorus, zinc, and nitrogen.

The concentration of Pb in rice was above both the EU and FAO/WHO permissible limit of 0.2 mg/kg and 0.3 mg/kg. This result indicates that consumption of bisalayi rice grown in this area could possibly have health impact on the population and may lead to Pb poisoning since rice is a staple food consumed in the area frequently. Previous studies have reported that the principal source of Pb to plant is the soil (Ross, 1994; Bi et al., 2010; Kovalchuk, Titov, Hohn, & Kovalchuk, 2005; Pourrut, Shahid, Dumat, Winterton, & Pinelli, 2011; Norton et al., 2014). Therefore, it was not surprising that soil-Pb concentration of the farms also exceeded both the EU and FAO/WHO permissible limits 100 mg/kg. The high soil-Pb concentration could be explained by the contamination of the farmlands with mining activities as previously discussed in chapter 1, section 1.2.

In addition, this study demonstrated that Pb accumulation in rice was facilitated by acidity of the soil. The result shows that the pH significantly influenced the Pb in rice. The soil pH was negatively correlated with the Pb concentrations in rice (chapter 4, Figure 4.3). The mean pH across all the four selected farms in the initial study was 6.7±0.6 and ranged from 4.11 to 7.92. As the soil acidity increases (decreasing pH scale), the Pb in rice increases. Same behaviour was seen in the organic carbon and the soil nitrogen that also increase alongside the decreased pH scale. The findings of the present study are in agreement with some previous studies outside Nigeria; Prasad (1999), Nigam, Srivastava, Prakash, and Srivastava (2001), Basta, Ryan, and Chaney (2005), Amini, Khademi, Afyuni, and Abbaspour (2005) and Khan, Khan, Khan, Qamar, and Waqas (2015). It is confirmed by Tsadilas, Karaivazoglou, Tsotsolis, Stamatiadis, and Samaras (2005) and Alloway (2012) that at low pH, the bioavailability and mobility of some metals including Pb increases. Soil pH is one of the factors that influences not only the mobility and availability of metals but also the availability of soil nutrient to plant (Peng, Song, Yuan, Cui, & Qiu, 2009). At low pH, the plants access more nutrients (Smith & Smith, 2011). Lofts, Spurgeon, Svendsen, and Tipping (2004) added that soil pH has a direct influence on toxicological effects of metal ions on plants.

The soil organic carbon ranged from 0.71 mg/kg to 2.82 mg/kg, 0.70 mg/kg to 2.81 mg/kg and 0.68 mg/kg to 2.80 mg/kg with mean value of 1.57±0.6 mg/kg, 1.57±0.6 mg/kg and 1.54±0.6 mg/kg across the three soil sampling depths; 10 cm, 20 cm and 30 cm respectively. Soil Organic carbon showed a negative correlation with the Pb concentration in rice (chapter 4, Figure 4.4e). This means as the organic carbon in the soil decreases, the Pb concentration in the rice increases. Research also shows that the organic carbon component of the soil can keep the soil

Pb immobile (Alloway, 2012; Khan et al., 2015). Research shows that Pb bioavailability decreases when there is an increased Organic carbon (Shaheen et al., 2016). Organic carbon determines the amount of the organic matter in the soil. According to Udo et al., (2009), about 60% of the organic carbon comes from the organic matter. Higher the organic matter, the higher the organic carbon. The major thing to note is that the organic matter is a sorbent for Pb and other metals (Park et al., 2011).

Availability of metals is induced also by the soil texture (Kashem & Singh, 2001). High clay content (clay soil) can make the metal ions unavailable in the soil (Vega et al., 2010) but with mild organic matter content, it forms humous together with the clay content. Retention of Pb may occur when Pb ions are adsorbed onto clay particles, making it unavailable (Sammut et al., 2010; Uzu, Sobanska, Aliouane, Pradere, & Dumat, 2009). The result of the particle size and the soil colour are shown in Appendix D, Table III and IV. The four farms demonstrated wide distribution of same soil colour. It was found that the soil condition in some farms favour higher Pb mobility compare to others. For instance, the farm B that has the lowest % clay shows the highest Pb in both the soil and the rice.

The concentration of Pb was highest in rice root and decreases upwardly (root > shoot > husk > seed) towards the rice seed. The mean soil-Pb concentration was highest at the 0-10 cm soil sampling depth (598.15±782.69 mg/kg) and lowest at 21-30 cm soil sampling depth (495.55±738.04 mg/kg). Results from both the initial experiment (site characterisation) and the varietal trial (field and the pot experiments) confirm the fact that the rice rooting system is domicile within 0 – 30 cm soil depth as against Khan et al., (2010) that says the rice root is predominantly domicile within 0 to 15 cm soil depth. During the sample collection, Bisalayi rice (rice collected during the site characterisation) had its root system domiciled within 0 – 30 cm soil depth as this was seen clearly during the sample collection at 30 cm soil depth. Although the mean soil Pb concentration was significantly different across the four selected rice farms and ranges from 39.66 mg/kg to 6147.55 mg/kg, while there was no significant difference in the concentration ratio using the Pb concentration in the soil and the Pb concentration in rice across all the four farms. This result means that the behaviour of the rice plants in terms of the Pb uptake across the four selected rice farms was the same (more in chapter 4).

9.1.2 Pb accumulation and distribution in the parts of rice

Pb mobility from soil to the root was higher than the Pb mobility within the rice parts using the index of bioaccumulation and translocation factor (Bonano, 2011). The Pb distribution and accumulation varied among the four parts of the rice plant and this was in the order of root > shoot > husk > seed. Based on the sampling depth in all samples, the soil-Pb concentration (mg/kg) was highest (598.15±782.69) at the 0-10 cm sampling depth and lowest (495.55±738.04) at 20-30 cm sampling depth. Farm B had the highest soil Pb across the sampled soil depths and distribution across the farm and farm D had the lowest. The highest CR was also found at the last sub soil (20 – 30 cm sampling depth). In all the four selected rice farms (characterised farms), the Pb mobility from the soil to the root was highest, follow by the root and the shoot, the root and the husk, while the root to the seed was the least (Table 5.1). There was no significant difference in the Pb mobility between the shoot and across the other rice parts in all the selected four rice farms. No significant difference was observed in the CR across the sampling depth ($p>0.05$) among all the four farms (Table 5.2).

Regression analysis indicated a positive relationship ($R^2 = 0.6912$) between the Pb concentration (mg/kg) in the whole rice plants (root + shoot + husk + seed) and the Pb concentration (mg/kg) in the soil (Figure 5.1) in all the four selected rice farms. This shows that the Pb uptake by rice in this study was predominantly from the soil. There may be other sources of Pb in plant as speculated by Choi et al., (1998) cited in IAEA-TRS 472, (2010) which states that the Pb in plant could also be through the foliar uptake. Pb uptake through the rice leaves cannot be ignored as the atmosphere is confirmed to have particles of Pb (Udiba et al., 2013). The Pb aerosols and dry air (dust) that contains Pb could deposit on the rice leaves and these could get absorbed through the leaves (Feng et al., 2011; Schreck et al., 2014). If at all in this study there were folia uptake and leaves absorption of Pb through the rice leaves, using the soil samples has accounted for all the uptake because the concentration ratio was used to calculate the rate of uptake. The concentration ratio represents what is in the rice (whole rice) and what is in the soil. The soil may not be the only source but the amount of Pb concentration in the soil is a good representative of the amount of Pb that has been deposited from all sources.

No significant difference was found in the concentration ratios (CR) among all the selected rice farms (Table 5.7). This indicated that the Pb uptake and the accumulation in the rice plants were similar in all the farms in Dareta village. The Pb concentration in the root was the highest and it agreed with the previous studies that confirms more than 50% of the Pb taken by rice plants remains in the rice roots due to the retention capacity of the root (Lee et al., 2016; Kibria et al., 2006). Looking at the variation and the differences in the Pb concentration accumulated in the various rice parts, it may be that there is a regulatory metabolic control Pb in the rice plant. This may be to prevent toxicity in the rice plant according to MacFarlane (2007). The mean Pb concentration (mg/kg) accumulated in both the shoot (vegetative part) and seeds together in this study was more than the FAO/WHO international permissible limits of 10 mg/kg which may not be healthy to use as animal feed. Having observed that some farmers in the area use the shoot to feed their farm animals (goats, sheeps, camel, donkeys and cows)

9.1.3 Inter-varietal variation of Pb uptake in rice (pot and the field experiment)

The overall mean Pb concentration in the rice from the field experiment was 0.74±0.33 mg/kg, with the mean concentrations in each of the ten different varieties rangeing from 0.03 mg/kg to 2.51 mg/kg. The overall mean Pb concentration in the rice from the pot experiment was 1.56 mg/kg. Therefore, in the case of both the field and the pot experiments, the overall mean Pb concentration in the rice exceeded the European Union (EU) standard permissible limit of 0.2 mg/kg (EC, 2001, 2006) and the joint World Health Organization (WHO) and Food and Agriculture Organisation (FAO) of the United Nations permissible limit of 0.3 mg/kg (FAO/WHO, 2001, 2011).

In terms of Pb accumulation, the highest Pb accumulated rice variety in the varietal trial (field and pot experiment) was Irat-170 with 1.12±0.010 mg/kg (ranging from 0.97 mg/kg to 1.34 mg/kg) in the field experiment while the highest in the pot experiment was Nerica-l34 with 2.33±1.25 mg/kg (ranging from 0.00 mg/kg to 7.93 mg/kg). A Duncan post ANOVA test showed these two rice varieties to be significantly different in the field experiment but not in the pot experiment in terms of concentration ratio. The lowest Pb accumulation in the field experiment was recorded from bisalayi rice with 0.38 (0.03 mg/kg – 1.13 mg/kg) while the lowest Pb accumulated rice variety in the pot experiment was Art-15 rice with 0.98 (0.84 mg/kg – 1.90 mg/kg) but there was no significant difference found among these two rice varieties using concentration ratio (CR) values. The soil Pb concentration was similar across all the soil samples in both the field and the pot experiments. Meanwhile, the Pb concentration (accumulation) in rice across the 10 selected varieties was significantly different ($p < 0.05$) in both experiments but similar when comparing the two set of data of the two experiments together.

A wide variation in Pb accumulation (0.03 - 1.13 mg/kg) was found in the local variety, bisalayi rice (variety 10) and the minimum Pb (mean concentration) was recorded for the same variety. The mean Pb concentration in rice across all the 10 selected rice varieties (0.74±0.33mg/kg) of this study is similar to 0.73 mg/kg Pb concentration in local rice in Zamfara reported by Simba et al. (2018) for the same local rice collected from Bagaega village. Bagega is a neighbouring village to Dareta village and our result also similar to mean Pb concentration (0.68±0.80 mg/kg) reported for Chinese rice by Norton et al. (2014). It was lower than the mean Pb concentration in market rice (2.70 mg/kg) reported in Nigeria by Adedire et al. (2015). Our result was higher than the one reported for Ghana rice (0.007±0.01 mg/kg), USA rice (0.021±0.31 mg/kg) by Norton et al. (2014) and Brazilian (0.185±0.05mg/kg), Thailand (0.383±0.01 mg/kg), and Vietnamese rice (0.308±0.01mg/kg) reported by Otitoju, Otitoju, and Igwe (2014). The previous studies are compared with results from the present study in section 9.2, Table 9.1. It was deduced that the consumption of rice varieties grown in Dareta village may pose a public health risk to consumers in terms of Pb uptake.

The Pb uptake based on the recorded mean value of CR was 0.13±0.26 (ranges from 0.07 to 1.73) for the field experiment. The lowest mean CR of 0.07±0.14 was found in sipi_692033 (variety 2) and bisalayi (variety 10) respectively as the two varieties provided same CR while the highest mean CR of 0.22 ± 0.39 was observed in art-15 (variety 9). sipi-692033 was originally from Taiwan and it was improved upon and adopted in Nigeria (Appendix A, Table I) and bisalayi is the most popular local rice variety in the northern states of Nigeria and it has been with farmers for many years. The CR was 0.0009±0.0004 (range 0.0001 - 0.0048) for the pot experiment, which was lower than the CRs estimated from the field experiment. The highest of 0.0012±0.0006 was found in nerica-L34 rice for the pot experiment while the lowest CR was found in Art-15 as recorded in the field experiment. The inter-varietal variation (IVV) of Pb among the 10 selected rice varieties was 2.7 folds for the field experiment while it was 2.4 folds for the pot experiment. It was found that acidic soil support Pb mobility and uptake more as it was observed between the field and the pot experiments. Soil analysis (Chapter 7,

Table 7.2 and 7.10) for both experiment (field and pot) shows that the soil used in the pot experiment was more acidic than that of those obtained from the field.

Regarding the hazard index (HI) which indicates the risk of developing health challenges from rice consumption (USEPA, 1989; Dai et al., 2016). The HI value obtained for the 10 selected rice varieties in this study were less than 1 which indicate that the people who consume the rice varieties are not likely to develop non-cancer health risks except for only one rice, Irat-170 which recorded HI value above 1. In Irat-170, the HI was greater than 1 indicating that the consumers of this variety may be exposed to the associated health risks previously discussed in chapter 2 (Figure 2.3). The lowest HI among the 10 selected rice varieties in this study was bisalayi rice (HI = 0.35) which indicates that bisalayi is the safest rice among the understudied 10 selected rice varieties in this work.

Concerning the carcinogenicity of consuming the 10 selected rice varieties, the chances was between one in hundred thousand population (minimum) and three in hundred thousand population (maximum) over a lifetime of 60.5 years. The CR_k (cancer risk) was 1.03×10^{-5} for bisalayi rice (minimum), 1.70×10^{-5} for sipi-692033 rice, 2.37×10^{-5} for tta-315 rice, 1.65×10^{-5} for wita-4 rice, 1.94×10^{-5} for nerica-L19 rice, 1.53×10^{-5} for nerica-L34 rice, 2.50×10^{-5} for ncro-49 rice, 2.38×10^{-5} for art3-7l rice, 2.0×10^{-5} for art-15 rice and 3.04×10^{-5} for irat-170 rice (maximum).

9.1.4 Inter-varietal variation of essential elements uptake in rice (pot and the field experiment)

The mean concentration (mg/kg) of Ca, Fe, Cu, Mg, K, Zn, Mn, Co, and Se in rice was 131.45, 25.43, 4.66, 901.99, 2025.95, 25.05, 23.24, 0.07 and 0.11 respectively. The mean concentration of these essential elements varies across different varieties of rice. The inter-varietal variation (IVV) of the essential elements was 1.3 folds for Ca, 4 folds for Fe, 1.3 folds for K, 1.2 folds for Mg, 1 fold for Zn, 1 fold for Se, 1.2 folds Mn, 1.9 folds for Co and the inter-varietal difference among the 10 varieties of rice for Cu was 1.3 folds for the field experiment. Chapter 7, Table 7.3 and Table 7.4 present more information. For the purpose of the varietal selection for the field samples based on the uptake of the essential elements, bisalayi (the local rice) had the highest accumulation of the essential elements among the 10 selected rice varieties as the good source of Fe. Ncro-49 had the highest Ca, art3-7L for Mg, nerica-L34 appeared for Co and art-15 rice had the highest Cu, Zn, K, Se and Mn and was ranked the best considering the concentration of the essential elements it can provide more than other rice varieties (chapter 7, Table 7.9).

For the pot experiment, the mean concentration (mg/kg) of Ca, Fe, Cu, Mg, K, Zn, Mn, Co, and Se in rice was 141.38, 30.92, 4.90, 958.78, 2218.55, 28.76, 18.39, 0.03 and 0.05 respectively (chapter 7, section 7.22). As was observed for the field experiment, the mean concentration of these essential elements varies across the different rice varieties. The IVV of the essential elements was 1.6 folds for Ca, 9.5 folds for Fe, 2.4 folds for K, 2.1 folds for Mg, 1.6 fold for Zn, 1.8 folds for Se, 1.5 folds Mn, 7.8 folds for Co and 1.6 folds for Cu. Chapter 7, Table 7.13 compares the CR of the field and the pot experiment. For the purpose of the varietal selection for the pot samples based on the uptake of the essential elements, just as it appears in the field experiment, bisalayi (the local rice) was the highest among the 10 selected rice varieties as the good source of Fe and unlike the field, it was also the highest for Co. Irat-170 was the highest for Ca, Art-15 was the highest for Mg and similar to the field experiment. Meanwhile, art-15 rice was also the highest for Cu, Zn, K, Se and Mn and was ranked the highest considering the number of the essential elements it can provide more than other rice varieties (chapter 7, Table 7.14).

Based on the percentage contribution to the daily recommended intake (RDI) of the essential elements by the 10 selected rice varieties as it was calculated (chapter 7, section 7.2.3), all the rice varieties demonstrated to be good source of all the essential elements to the recommended daily intake (RDI) in male and female and across all the age groups in both the field and the pot experiments considering the ingestion rate used.

9.1.5 Inter-varietal variation of Cs and Sr uptake in rice (field experiment only)

In both the rice and the soil samples, the mean concentration of Sr was higher than that of the Cs. The mean concentration of Sr was 1.30 mg/kg in rice and 9.48 mg/kg in soil. The mean concentration of Cs was 0.06 mg/kg in rice and 0.91 mg/kg in soil. A positive relationship was found between the concentration of these two stable isotopes of radionuclides in rice and soil samples. There was no significant difference in the accumulation of Sr across all the rice varieties. No significant difference was observed also in the accumulation of Cs across the 10 rice varieties. The mean concentration ratio in the rice samples for Sr was 0.135±0.02 while that of the Cs was 0.066±0.06. IVV among the 10 selected Nigeria rice varieties for Sr was 1.1 folds and 2 folds for Cs.

The positive relationship between the Sr in rice and Sr in the soil samples likewise the positive relationship between the Cs in rice and the Cs in the soil samples (chapter 8, Figures 8.1 and 8.2) indicate that the Sr and the Cs in the rice samples were from the soil. The Duncan multiple range post ANOVA test result of the concentration ratio (CR) revealed that all the 10 rice varieties were not significantly different for Sr and Cs uptake (chapter 8, Figures 8.3 and 8.4).

This study appears to be the first to provide data for the stable Cs in Nigerian soil and the first to present data on the inter-varietal variation among Nigerian rice varieties regarding uptake and accumulation of stable strontium (Sr) and caesium (Cs) to the best of our knowledge. Inter-varietal assessment for varietal selection has been proposed as a remediation strategy to minimise human exposure to environmental contaminants, especially those entering the food chain via plant uptake (Penrose et al., 2017). There is little known globally in this study area on rice (Akinwale et al., 2011), so the present study findings make a significant contribution to the available knowledgebase.

9.2 Relationships between the Pb and the essential elements

In both the pot and the field experiments, the result revealed a positive correlation between Pb concentration (mg/kg) in rice and some essential elements such as Ca, Co, Cu, K, Mg but Pb was negatively correlated with Fe in both experiments (Appendix E, Table V). In the rice samples, there was also a positive correlation between Pb and Co, K, and Ca. This means that the more the Pb in the rice, the less the Manganese and vice-versa. On the other hand, the positive correlation means the more the Pb in the rice, the more the Co, K and Ca. There was also a negative correlation between the Pb and the Fe in the rice samples. This is in agreement with the common symptom of anaemia in the Pb poisoning patients (Shah et al., 2010; Kersey, Chi and Cutts, 2011). Anaemia is a condition in which there is a deficiency of red cells or of haemoglobin in the human blood which as a result cause weariness and pallor (Diaz-Catro et al., 2012). Within the essential elements, there was a positive correlation between the Cu concentration (mg/kg) in rice and Cu concentration (mg/kg) in the soil samples, Se concentration (mg/kg) in rice samples and the Se concentration (mg/kg) in the soil samples, K concentration (mg/kg) in rice samples and the K concentration (mg/kg) in the soil samples.

There were some correlations found within the concentration ratio. Pb concentration ratio (Pb-CR) was positively correlated with all the concentration ratio of the essential elements (Co-CR, Cu-CR, Zn-CR, Mn-CR, K-CR, Fe-CR, Se-CR, Mg-CR) at 0.01 level except that of the calcium (Ca) that shows no correlation. There was a negative correlation between the Pb concentration ratio (Pb-CR) and the clay content of the soil, which is in line with previous research that suggest Pb ions may be immobilised through adsorption onto soil clay (Alloway, 2013; Khan et al., 2015). The Pb in the soil would not be available for plant uptake unless it is been released from soil components such as the clay. The higher the clay content of the soil, the lesser the available Pb ions in the soil. The correlation results of the elemental concentration ratio and some soil parameters obtained are presented in the appendix E, Table V, VI, VII, VIII. The results are compared with the previous studies across other regions of the world and this is presented in Table 9.1. Overall, the rice varieties are within the acceptable limit of the concentration of the essential element compare with the concentration these elements in rice in the previous studies from other regions of the world.

Table 9. 1: Comparison of Pb and essential elements in rice between this study and studies from other regions of the world.

Research	Mean Pb in rice (mg/kg)	Mean Essential Elements in rice (mg/kg)									References
		Ca	Fe	K	Mg	Zn	Se	Mn	Co	Cu	
This Study (Field)	0.740±0.330	131.45±18.38	25.43±17.33	2025.95±242.99	901.99±104.31	25.05±3.99	0.11±0.02	23.24±2.99	0.07±0.03	4.66±1.28	This Study (field experiment)
This Study (pot)	1.56±0.60	141.38±53.29	30.92±161.19	2218.55±458.91	958.78±158.06	28.76±5.09	0.05±0.05	18.39±3.27	0.03±0.05	4.90±1.14	This study (pot experiment)
^{a}Nigerian rice	0.05±0.01	87.00±24.40	59.33±0.0005*	183.33±0.001*	23.67±0.0052*	12.63±0.64	0.10±0.03	9.40±0.34	0.03±0.01	3.70±0.44	Adedire et al. (2015) Abdulrahman and Omoniyi (2016)*
^{b}Nigerian rice		45.63±10.22	10.69±12,64	1438±408	247±87	8.21±2.71	0.06±0.02	6.03±2.34	0.04±0.04	3.51±0.63	Mwale et al. (2018)
^{c} Nigerian rice	0.73±026*	538.66±1.90	119.70±0.00	149.63±3.35	179.55±0.07	107.73±0.38	0.15±0.01	82.87±3.01	1.45±0.00	0.94±0.08	David et al. (2019)
^{d}Nigerian rice	0.314*	94.33±0.30	25.00±0.11	102.94±0.16	306.67±0.10	25.00±0.25	-	5.00±0.13	-	6.00±0.17	Mohammed and Ahmad (2014), Ogbuagu, Ezenwankwo, Ekpunobi, Ofora, and Onyema (2015)*
Brazilian rice	0.005±4.1	42.70±12.80	2.50±1.7	0.06±0.01	-	-	-	-	-	-	Batista et al. (2012)
South Korean	0.206	94.80	10.20	2200.00	985.00	17.00	-	-	0.006	1.85	Jung, Yun, Lee, and Lee (2005)
Chinese rice	0.400±0.200	116.00*	10.42*	3246.00*	1718.00*	17.76*	-	28.64±5.57	0.39±0.13	4.26±0.83	Liu et al. (2015) Fu et al. (2008) Huang et al. (2016) *
Japanese rice	-	102 ± 16	10.4±2.5	2480±211	1270±153	22.3±2.9	-	28.1±6.8	-	3.33±1.35	
Iranian rice	0.110±0.080	6.20±2.70	13.50±8.60	-	-	28.6±11.9	-	-	-	22.80±9.50	Falahi, Hedaiati, and Ghiasvand (2010)
Macedonian	0.201±0.030	-	-	-	-	27.86±13.68	-	-	-	3.00±1.62	Rogan, Serafimovski, Dolenec, Tasev, and Dolenec (2009)
USA rice	0.014±0.001	310±17.60	10.00±1.40	3400±19.3	140±27.30	2.6±47.6	-	1.5±62.3	-	0.56±40.80	Otitoju et al. (2014), Mohammed and Spyrou (2009)
Tanzanian	-	62.00±10.00	23.00±3.00	1169.00±87.00	626.00±43.00	30.00±2.00	-	19.00±1.00	-	3.40±0.70	
Thailand	0.383±0.002*	342.99±1.43	76.22±5.35	95.28±3.85	114.33±0.10	68.60±1.98	0.10±0.00	52.77±3.15	0.92±0.11	0.87±0.04	Otitoju et al. (2014) * David et al. (2019)

a, b, and c collected rice samples from the southern part (another region different from where this study was carried out) of Nigeria and also from the market, d sampled rice from Kano State (a neighbouring state to Zamfara).

9.3 Conclusion

This study has established the inter-varietal variation in the uptake and accumulation Pb, Cs, Sr and nine essential elements for 10 varieties (cultivars) of rice currently grown in Nigeria. The results provide a knowledgebase to inform evaluation of varietal selection as a public health intervention to reduce human exposure to Pb, radio-strontium and radio-caesium (the latter two contaminants are likely to be discharged directly to the Nigerian environment in the future from planned nuclear power plants. The medium and the low accumulators (rice varieties) in both the field and the pot experiments appears similar. The essential element concentration data, especially the percentage contribution to the recommended dietary intake (RDI), could be used to inform identification of varieties that both meet RDI requirments and contribute to reducing the exposure to Pb, Cs and Sr.

The most popular rice variety which is widely grown among the farmers in Zamfara (bisalayi rice) is one of the rice varieties identified with low Pb uptake. This variety also demonstrated good yield among the ten selected varieties on the field and also during the pot experiment. Therefore, it is concluded that there is no action needed to be taken regarding the variety currently grown in Dareta village Zamfara State.

It is worthy to note that some people among the exposed population may be exposed to additional doses of Pb through drinking of contaminated water, consumption of other contaminated foods, inhalation of contaminated air and domestic use of contaminated water which may include bathing because skin absorption has been identified as one of the exposed routes (chapter 2, section 2.5.2, paragraph 2). Therefore, the result of this study is not absolute for total Pb dose of the affected individuals in this area. Also, there are other toxic elements that are carcinogenic in nature (IARC, 2011a) such as Arsenic (As) and Cadmium (Cd) which may be present and were not included in this study considering Zamfara population that is on subsistence rice diet.

This study has provided an estimate of the transfer of anthropogenic radionuclides (raddiocaesium and radiostrontium) to rice, based on stable element data. These data provide a valuable resource for underpinning safety cases associated with the proposed construction of nuclear power stations in Nigeria. These data will help the policy makers in prospective dose assessments and emergency planning before the commencement of the project.

9.4 Recommendations

9.4.1 Recommendations for Policy

This study recommends that the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC), Standard Organisation of Nigeria (SON) and other related agencies monitor Pb in both the Nigerian rice and the imported rice regularly. Rice and other food that is produced from a confirmed contaminated area needs to be tested to guarantee food safety before such food is sold in the market. It is necessary for the stakeholders in health and environmental matters in Nigeria to include the Ministry of agriculture and rural development in the implementation of Pb poisoning intervention plans in Zamfara State and across Nigeria to be able to tackle the challenge of Pb poisoning in a holistic manner. As far as gold price continue to increase, mining activities will continue, and the soil contamination will continue.

It is no doubt that Pb contaminated soil will continue to be used for food production in Zamfara State and some other states in Nigeria. An increased education and awareness creation about the dangers of Pb poisoning is needed for the exposed population of Zamfara State and across Nigeria from the Federal and Zamfara State government in collaboration with relevant agencies and civil society organisations. Government should intensify effort to make sure the miners are not only licensed but trained to comply with the regulation of safe mining by WHO to ensure the safety of themselves, their families and the community. All the suggested further studies of this work (section 9.5) are highly recommended to government of Nigeria, Civil Societies organisations, health and food safety stakeholders for funding.

9.4.2 Recommendations for Practice

XRF field screening is recommended for all the rice farms prior to rice planting to provide contaminants estimated data before the rice is grown on any soil in this area and other areas in the country that shares similar condition. Previous studies confirm high level of Pb accumulation in rice that were grown in industrial area (Fu et al., 2008; Nordberg, Fowler, & Nordberg, 2014; Williams et al., 2009) and gold mining environment is categorised as an industrial area (Ono et al., 2012). This study suggests a temporary ban of rice growing in industrial area until a thorough contamination assessment is conducted before such land could be used for agricultural purpose.

9.5 Future Research

This study is not an exhaustive investigation. Many of the Nigerian rice were not included in the study because they were not currently grown. This work selected only the rice varieties that were currently grown in Nigeria as at the time the study commences to check the inter-varietal variation. The findings may change when more rice varieties are involved in the study. Hence, larger experimental survey is however required. This study used different soil types for the two conducted experiments (pot and the field varietal trial) which was suspected to be responsible for different result in the varietal selection generally. It is recommended that the same study should be conducted in more than 2 places on the same soil type to guarantee the same soil conditions, parameters and this would make identification of consistently lower accumulating rice variety easier. Any decision made thereafter will be justified.

It is recommended that a complete diet assessment is conducted over a specific period to ascertain the actual complete dose of Pb that is been consumed over time in a population. As part of the further studies on this work, it is important to look at different cooking methods on these 10 selected rice varieties to see if methods of cooking can reduce the Pb content of the rice varieties as it was recently studied for Arsenic content in rice by Mwale et al. (2018). In addition, the rice ingestion rate of 100g and 200g used for the assessment is not realistic based on the researcher's experience during his fieldwork in Zamfara. 100g rice ingestion rate was used for children (0 to 18 years) and 200g was used for adults (18 and above) as previously used by AfricaRice (2005) and Norton et al. (2014). The rate seems too high for the children and low for adults.

We are suggesting a thorough research on the exposed population to gather and document specific information about rice ingestion rate as it affects different age groups e.g. 1month to 12month, 1year to 5years, 5years to 10years etc. to ascertain the actual dose of Pb through rice. More information is also required on the perception of the people in this area about the issue of Pb poisoning. This will provide a reality on the issue and guide the policy makers on the gaps.

Further study should look at how rice can be modified genetically to reduce the uptake of Pb and other environmental contaminants from the soil, whilst maintaining/enhancing the essential elements. Research suggests that gene modification could be used to change the behaviour of a plant (Chen & Ni, 2006).

The inter-varietal variation assessment supposed to use the CR data that is derived from the elemental concentration of the whole plant and the elemental concentration of the soil. The Inter-varietal variation assessment in this study due to limited resources considered the elemental concentration in only the rice seed (grain) and the soil, and not the entire rice plant. The whole rice plants are used as animal feed and therefore the whole plant concentration needs to be considered for this indirect transfer route into the human foodchain.

Overall, the present study has significantly enhanced the knowledgebase on contaminant and essential element transfer to rice in Nigeria. It also provides the first data on the influence of varietal slelection on rice uptake in Zamfara State. The findings provide a strong basis for the formulation of public health intervention strategies and the evaluation of plans for nuclear power plant development. The future research directions proposed will compliment and further enhance the contribution of this research.

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Appendixes

A. Rice varieties that exist in Nigeria
B. Complete Randomise Block Design used for this study
C. Approval letters
D. The result of the particle size and the soil colour for the four selected rice farms during the soil characterisation
E. Relationships between Pb and the essential elements in rice

A. All the rice varieties in Nigeria currently

Table I: Morphological Characteristics of Rice Varieties in Nigeria, 1954 –to date (NCRI, 2017).

NAMES OF VARIETY	*COMMON NAMES*	*CROSSES*	*ORIGIN*	*HABITAT*	*PLANT HEIGHT (CM)*	*TILLER-RING CAPA-CITY*	*STEM BASE*	*LEAVES*	*LEAF SHEATH*	*PANICLE EXERTION*	*FLAG LEAF*	*HUSK COLOUR, UNRIPE TO MATURED SEED*	*APICULUS COLOUR, UNRIPE TO MATURED SEED*	*STIGMA*	*AWNS*	*LIGULE*	*MATU-RITY (DAYS)*	*POTEN-TIAL YIELD (T/HA)*	*GRAIN TYPE*
FARO 1	**BG 79**	**Unknown**	**British Guinea**	**Lowland Shallow Swamp**	**105-120**	**12-18**	**Purple**	**Long Broad and lax**	**Red/ Purple**	**Fully Exerted**	**Drooping**	**Dark green to brown**	**Purple to brown**	**Black**	**None**	**Medium**	**135-174**	**3.0-5.0**	**B**
2	**D 114**	**-do-**	**British**	**-do-**	**100-115**	**10-15**	**Purple**	**-do-**	**Purple**	**Exerted Erect**	**-do-**	**Green to straw colour**	**Purple to brown**	**Black**	**None**	**Medium**	**135-176**	**3.0-4.5**	**B**
3	**AGBEDE 16/56**	‘	**Nigeria**	**Upland**	**95-100**	**6-10**	**Green**	“	**Green**	**Fully Exerted** and **drooping**	**Erect**	**-do-**	**Green to straw colour**	**Colour-less**	**None**	**Medium**	**95-120**	**1.5-2.5**	**B**
4	**KAV-12**	‘	**Madras**	**Lowland Deep swamp**	**145-150**	**18-20**	**Purple**	“	**Purple**	**Fully Exerted**	**Drooping**	**Green to straw colour**	**Purple to brown**	**Black**	**Very short**	**Medium**	**189-220**	**2.0-4.0**	**B**
5	**MAKALIOKA 823**	‘	**Madagascar**	**Lowland**	**111-115**	**18-20**	**Green**	“	**-do-**	**Erect Drooping**	Long Drooping	**Green to brown**	**Purple to brown**	**Purple**	**Short**	**Medium**	**135-154**	**2.0-4.5**	**B**
6	**INDO CHINA BLANC**	‘	**Thailand Via Bamako**	**Lowland Shallow Swamp**	**156-160**	**10-15**	**Green and elongated internode**	“	**Green**	**Fully Exerted**	**Drooping**	Greenish-dark-brown to brown	**Green to brown**	**Colour-less**	**None**	**Long**	**176-189**	**2.0-3.0**	**B**
7	**MALIONG**	‘	**Thailand**	**Lowland Deep floods**	**160-165**	**9-15**	**-do-**	“	**-do-**	**-do-**	**-do-**	Greenish-dark-brown to straw colour	**Green to straw colour**	**-do-**	**None**	**Long**	**160-217**	**2.5-3.5**	**A**
8	**MAS 2401**	‘	**Indonesia**	**Lowland Shallow Swamp**	**120-125**	**9-15**	**Green and Stiff**	“	“	“	“	Green to straw colour	**-do-**	“	**None**	**Medium**	**155-160**	**3.5-4.5**	**A**
9	**SIAM 29**	‘	**Malaya**	**-do-**	**120-125**	**-do-**	**-do-**	“	**Purple**	**Fully Exerted compact**	**Lax**	Dark-green to brown-yellow	**Purple to straw colour**	“	**Long**	**Medium**	**189-220**	**2.5-3.0**	**A**
10	**SINDANO**	‘	**Lowland Shallow Swamp**	**-do**	**125-130**	**10-15**	“	**Long semi-broad Lax**	**Green**	**Fully Exerted**	**Drooping**	**Green to straw colour**	**Green to straw colour**	“	**None**	**Medium**	**115-145**	**2.5-3.5**	**A**
11	**OS-6**	‘	**Belgian Congo**	**Upland**	**103-110**	**9-12**	“	**Long broad and Lax**	**-do-**	**-do-**	**-do-**	**Green to straw colour**	**Pink to straw colour**	**Black**	**None**	**Medium**	**110-122**	**1.5-2.5**	**B**
12	**SML 140/ 10**	‘	**Suriname**	**Lowland Shallow Swamp**	**135-140**	**9-15**	**Purple and Stiff**	**Narrow Long Needle Erect**	**Purple**	**Semi-Exerted and drooping**	**Erect**	**Green to straw colour**	**Purple to brown**	**Purple**	**Occa-sional**	**Medium**	**145-155**	**2.0-3.5**	**A**

13	**IR-8**	**PETA/ DGWG**	**Philippines**	**-do-**	**90-100**	**18-20**	**Green and Stiff**	**Short broad and Erect**	**Green**	**Short and Dense**	**Erect**	**-do-**	**Green to straw colour**	**Colour-less**	**None**	**Medium**	**135-140**	**2.5-4.5**	**A**
14	**FRSS 43/3**	**Chanyza 133 x 1 CB**	**Nigeria (NCRI)**	**Lowland Deep Flooded**	**150-160**	**9-15**	**Green and Red prostrate**	**Long broad and Lax**	**Green and pigmented**	**Fully Exerted Dense**	**Long broad**	Greenish-yellow and dark-brown to yellow	**Greenish purple to brown**	**Purple**	Occasional	**Medium**	**170-196**	**1.5-2.5**	**B**
15	**FRRS-162 B 111-1**	**BG79 X IR 8**	**Nigeria (NCRI)**	**Lowland Shallow Swamp**	**150-160**	**20-25**	**Green and Stiff**	**Short Erect broad**	**Green**	**Fully Exerted Dense**	**Erect**	**Green Straw**	**Green to straw colour**	**Colourless**	**None**	**-do-**	**145-160**	**3.0-4.0**	**A**
16	**FRRS-168 B 111-2**	**Mas 2401 x SML 140/10**	**Nigeria (NCRI)**	**-do-**	**90-100**	**12-18**		**Short Narrow Erect**	**Green**	**Fully Exerted Loose drooping**	**Erect**	**Green to straw colour**	**Green to straw colour**	**Colourless**	Occasional	“	**140-160**	**2.0-4.0**	**B**
17	**FRRS-148 B 111-3**	**Mas 2401 x TJINA**	**Nigeria (NCRI)**	**-do-**	**110-120**	**12-18**	**Green and Stiff**	**Long heavy & Exerted**	**Purple**	**Long Semi broad and Lax**	**Long Broad & Lax**	**Purple to Brown**	**Purple to Brown**	**Colourless**	**-do-**	**Long**	**145-160**	**2.5-4.0**	**B**
18	**TJINA**	**Unknown**	**Indonesia**	**-do-**	**145-150**	**15-16**	**Purple**	**Medium Long & Lax**	**Green**	**Fully Exerted**	**Erect**	**Green to straw colour**	**Green to straw colour**	**Black**	**None**	**-do-**	**167-179**	**3.5-4.5**	**B**
19	**IR-20**	**(Peta-3x T(N)1)xTKM6**	**Philippines**	**-do-**	**90-100**	**18-20**	**Green**	**Short Broad and Lax**	**Purple**	**-do-**	**Drooping**	**Purple to Brown**	**Purple to straw colour**	**Colourless**	**None**	“	**135-140**	**2.5-3.5**	**B**
20	**BP1-79 (B1Col)**	**Unknown**	**Philippines**	**-do-**	**90-100**	**18-20**	**Purple**	Short Semi Broad Erect	**Green**	**Short Erect and Dense**	Erect Short Painted Apex	**Green to straw colour**	**Green to straw colour**	**Purple**	Occasional	**Medium**	**125-130**	**2.5-3.5**	**B**

NAMES OF VARIETY	***COMMON NAMES***	***CROSSES***	***ORIGIN***	***HABITAT***	***PLANT HEIGHT (CM)***	***TILLER-RING CAPA-CITY***	***STEM BASE***	***LEAVES***	***LEAF SHEATH***	***PANICLE EXERTION***	***FLAG LEAF***	***HUSK COLOUR UNRIPE TO MATURED SEED***	***APICULUS COLOUR, UNRIPE TO MATURED SEED***	***STIGMA***	***AWNS***	***LIGULE***	***MATU-RITY (DAYS)***	***POTEN-TIAL YIELD (T/HA.)***	***GRAIN TYPE***
FARO 21	**TN-1**	**DEE GEO WOO GEN/TSA 1-YUAH-CHAN**	**Philippine s**	**Lowland Shallow Swamp**	**80-90**	**15-18**	Green and Stiff	Short Semi Broad and Lax	Green	Fully Exerted and drooping	**Drooping**	**Green to straw colour**	**Green to straw colour**	**Colourless**	**None**	**Medium**	**90-110**	**2.0-3.0**	**C**
22	**IR-627-1-3-1-4-3-7**	**-do-**	**Philippine s**	**-do-**	**90-110**	**18-20**	Green and Stiff	Erect long and lax	-do-	Fully Exerted long drooping	**Erect**	**Greenish yellow to straw colour**	**-do-**	**-do-**	**None**	**Medium**	**145-150**	**2.5-4.0**	**B**
23	**IR-5-47-2**	**“**	**Philippine s**	**-do-**	**90-100**	**18-20**	Green	Long narrow and lax	-do-	**Exerted**	**Drooping**	**Green to straw colour**	**-do-**	**Colourless**	**None**	**Long**	**145-150**	**2.5-4.0**	**B**
24	**DE-GAULLE**	**“**	**Vietnan**	**-do-**	**135-145**	**15-18**	-do-	-do-	‘	Fully Exerted and drooping	**Erect**	**-do-**	**Pink to straw colour**	**Colourless**	**None**	**Long**	**135-145**	**3.0-4.0**	**A**
25	**FAROX 56/30**	Jete x TJINA	**Nigeria (NCRI)**	**Upland**	**105-110**	**12-15**	“	“	‘	Fully Exerted	**-do-**	**-do-**	**Green to straw colour**	**Colourless**	**None**	**Medium**	**120-125**	**2.0-3.0**	**B**
26	**TOS 78**	**Unknown**	**Nigeria**	**Lowland Shallow Swamp**	**105-110**	**11-15**	“	Long Broad and Lax	‘	-do-	‘	‘	**-do-**	**-do-**	**None**	**Medium**	**130-365**	**2.0-3.0**	**B**

27	TOS 103	IR400-15-12-10-2 x IR662	Nigeria	-do	90-100	15-18	Green and Stiff	-do-	‘	‘	“	Pink to purple colour	Pink to purple	“	None	Medium	110-120	2.5-3.5	A
28	FAROX 188A	TJINA x IR 8	Nigeria	“	125-130	15-18	Purple	Long Medium and Lax	Purple	‘	Inter-miolate	Green to straw colour	Green to straw	Black	None	Inter-mediate	130-140	2.5-3.5	A
29	BG 90/2	Peta*3//TN1/ Remadja	Nigeria	“	100-115	12-18	Green and Stiff	-do-	Green	‘	-do-	-do-	-do-	Colourless	None	Medium	120-130	3.0-4.0	B
30	FAROX 228-2-1-1	FARO 15/ IR 28	Nigeria (NCRI)	“	120-125	15-18	Green and Stiff	“	-do-	“	“	‘	-do-	Colourless	Awned	Medium	110-115	5.0-8.0	B
31	FAROX 228-3-1-1	FARO 15/ IR 28	-do-	“	120-125	15-18	Green	“	‘	‘	Erect	‘	“	Colourless	None	Medium	110-115	5.0-8.0	B
32	FAROX 228-4-1-1	FARO 15/ IR 28	“	“	110-120	15-18	-do-	“	‘	‘	-do-	‘	“	Colourless	Awned	Medium	110-115	4.0-7.0	B
33	FAROX 232-1-1-1	FARO 12/ IR 28	“	“	115-125	10-15	“	Narrow Long and Lax	Purple	‘	Erect	‘	“	Colourless	-do-	Medium	110-115	4.0-7.0	A
34	FAROX 239-2-1-1	IR 28/ FARO 12	“	“	115-120	12-15	-do-	Long Semi Broad Lax	Green	‘	-do-	‘	“	Colourless	“	Medium	105-115	4.0-7.5	A
35	ITA 212	BG90-2 x TETEP	IITA (Nigeria)	Lowland Irrigated Swamp	100-115	15-18	“	-do-	Green	Fully Exerted	‘	Green to straw colour	Straw	Colourless	Occa-Sional	Medium	120-135	5.0-8.0	B
36	ITA 222	Mashuri x 1 ET 1444	-do-	-do-	100-115	15-18	“	“	-do-	-do-	‘	-do-	-do-	Colourless	Short	Medium	120-135	5.0-8.0	B
37	ITA 306	TOX 494-3696/TOX 711/BG6812	“	-do-	100-115	15-20	“	“	‘	‘	‘	‘	“	Colourless	Awned	Medium	125-140	5.0-8.0	A
38	IRAT 133	IRAT 13/ IRAT 10	“	Upland	100-110	10-12	“	“	‘	‘	‘	‘	Pale yellow	Colourless	-do-	-do-	100-105	1.0-4.0	C
39	IRAT 144	IRAT 13/ IRAT 10	IAR&T (Nigeria)	Upland	95-105	10-12	“	“	“	‘	‘	‘	-do-	Colourless	None	“	100-105	1.0-4.0	C
40	FAROX 299	Unknown	NCRI (Nigeria)	Upland	115-120	10-12	‘	‘	‘	“	Erect	‘	‘	-do-	Awned	‘	115-120	1.0-4.0	B
41	IRAT 170	IRAT 13/ Palawan	NCRI (Nigeria)	Upland	80-90	10-15	‘	‘	‘	“	-do-	‘	‘	“	Awned	‘	115-120	1.0-4.0	B

NAMES OF VARIETY	COMMON NAMES	CROSSES	ORIGIN	HABITAT	PLANT HEIGHT (CM)	TILLER-RING CAPA-CITY	STEM BASE	LEAVES	LEAF SHEATH	PANICLE EXERTION	FLAG LEAF	HUSK COLOUR UNRIPE TO MATURED SEED	APICULUS COLOUR, UNRIPE TO MATURED SEED	STIGMA	AWNS	LIGULE	MATU-RITY (DAYS)	POTEN-TIAL YIELD (T/HA)	GRAIN TYPE
FARO 42	ART 12	TOX 475/ IR154/056	IAR&T (Nigeria)	Upland	110-115	8-12	Green	Long Semi Broad and Lax	Green	Fully Exerted	Erect	Green to straw colour	Pale Yellow	Colourless	Awned	Medium	115-120	1.0-4.0	B

43	ITA 128	63-83/(Iguape Cateto/IET 1444/IR 1416-131/ LITA 506	IITA (Nigeria)	Upland	110-115	10-15	Purple	-do-	Purple	-do-	-do-	Green to Purple	-do-	Black	-do-	Medium	115-120	1.0-4.0	B
44	SiPi 692033	Sipi 661044/ Sipi 651020	Taiwan	Irrigated Swamp	110-120	15-20	Green	“	Green	“	‘	Green to straw colour	Straw	Colourless	Short awn	-do-	110-120	4.0-8.0	A
45	ITA 257	IRAT 13/ (Dourado Precoce # 689//TOX 490-1)	IITA (Nigeria)	Upland	90-100	10-12	Purple	“	Purple	“	Acute	Purple	Grey	Black	Awnless	‘	95-100	2.0-3.0	B
46	ITA 150	63-83/ (Dourado Precoce/ ROK1/ SE363G	IITA (Nigeria)	Upland	80-90	10-15	Green	“	Green	“	-do-	Green to straw colour	-do-	Colourless	-do-	Medium	115-120	2.0-3.0	B
47	ITA 117	134-18-3-1-3/TOX 7-4-2-5-2	IITA (Nigeria)	Upland	77-89	12-18	-do-	Long	-do-	Moderately weakly exerted	Erect	Straw	Straw	-do-	“	Short	115-120	2.0-3.5	A
48	ITA 301	IRAT 13/Dourado Precoce 689// Padipayak	IITA (Nigeria)	Upland	80-95	10-12	‘	Long, broad	Green (external) and pink (internal)	Fully exerted	-do-	-do-	Straw	“	‘	-do-	123	2.0-3.5	B
49	ITA 315	IR 43/Iguape Cateto	IITA (Nigeria)	Upland	77-89	12-18	‘	Long	Green	Moderately well exerted	‘	“	Straw	“	‘	‘	120	1.5-3.5	B
50	ITA 230	BG 90-2*4/Tetep	IITA (Nigeria)	Lowland Irrigated Swamp	100-110		‘	-do-	-do-	Fully exerted	‘	“		“	‘	Medium	125	3.0-10	B
51	Cisadane	Pelita-1//IR 789-98-2-3/IR 2157-3	Indonesia	Lowland Shallow Swamp	100-110	10-15	‘	Broad	“	-do-	‘	“	Grey	“	‘	Short	145-150	3.0-6.0	B
52	WITA 4	Unknown	IITA (Nigeria)	Lowland irrigated and shallow swamp	95-105	12-18	‘	Long	“	‘	‘	“	Grey	“	‘	Medium	125-130	3.0-7.0	A
53	ITA 321	TOX 1525F2 DWARF/ NGOVIE 20 SLR	IITA (Nigeria)	Upland	102-105	6-12	‘	-do-	“	Fully drooping	‘	“	Straw	“	‘	-do-	115-120	2.0-3.0	B
54	WAB 189-B-B-B-8-HB	IRAT 104/ITA 257	WARDA	Upland	110-130	4-8	‘	“	“	Fully	Drooping	“	Straw	“	‘	“	100-105	2.5-3.0	B

55	NERICA 1	WAB 56-104/CG 14	WARDA	Upland	110-120	4-6	Light Purple	-do-	Purple	-do-	Erect	Golden brown	Purple	Purple	‘	Short	100-105	2.0-3.0	B
56	NERICA 2	WAB 450-11-1-P31-4B	WARDA	Upland	97-105	4-5	Light Purple	Short	Light Purple	“	-do-	-do-	Purple	Light Purple	Short Awns	-do-	100-110	2.0-3.0	B

NAMES OF VARIETY	*COMMON NAMES*	*CROSSES*	*ORIGIN*	*HABITAT*	*PLANT HEIGHT (CM)*	*TILLER-RING CAPA-CITY*	*STEM BASE*	*LEAVES*	*LEAF SHEATH*	*PANICLE EXERTION*	*FLAG LEAF*	*HUSK COLOUR OF UNRIPE TO MATURED SEED*	*APICULUS COLOUR, UNRIPE TO MATURED SEED*	*STIGMA*	*AWNS*	*LIGULE*	*MATU-RITY (DAYS)*	*POTEN-TIAL YIELD (T/HA)*	*GRAIN TYPE*
57	TOX 4004-43-1-2-1		WARDA	Lowland irrigated and shallow swamp	115-125	10-12	Green	Long	Green	Fully exerted	Erect	Green to Straw colour	colourless	Colourless	Awnless	Medium	120-135	6.0-8.0	B
FARO 58	NERICA 7		AfricaRICE	Upland	100-115	5-8	green	long	Green	Fully exerted	Erect	Grenn to straw	Colourless	Cream	Awnless	Medium and pointed	95-100		B
FARO 59	NERICA 8		AfricaRICE	Upland	100-115	5-8	Light purple	long	Light purple	Fully exerted	Erect	Green to Golden colour	colourless	Cream	Awnless	Medium and pointed	95-100		B
FARO 60	NERICA L-19	WAS 122-IDS WAS-6-1/Tog5681/3*I	AfricaRICE	Irrigated Lowland	100-115	15-20	Green	long	Green	Fully exerted	Erect	Green to straw colour	Colourless	Cream	Awnless	Long and pointed	95-100	8 t/ha	B+
FARO 61	NERIC L-34	WAS 161-B-6-1 TOg5681/4* IR64	AfricaRICE	Irrigated Lowland	90-100	18-25	Green	Narrow and long	Green	Well exerted	Erect	Brown	Brown	Colourless	Awnless	Long and pointed	95-100	7 t/ha	A-
FARO 62	NCRO 49	FAROX 501-B 2 FARO 44 /FA	NCRI	Shallow Swamp	110-115	16-20	Green	Short and broad	Green	Well exerted	Erect	Brown	Brown	Colorless	Awnless	Long/pointed	120-125	6 t/ha	B
FARO 63	ART3-7L9P8-3-B-B-2-1	WAB56-104/CG14//WA 104)/MOROB N	AfricaRice	Upland	140-147	8-10	Green	Long	Green	Well exerted	Erect	Straw	straw	Cream	Present	Long/Bifurcated	95-100	6t/ha	B-
FARO64	ART15-7-16-38-1-B-B-2	WAB56-104/IRGC1061 B56-104	AfricaRice	Upland	130-135	6-10	Green	Long	Green	Well exerted	Erect	Straw	straw	Cream	Aawnless	Long/pointed	110-115	5t/ha	B-
FARO65	ART16-5-9-22-B-B-2	WAB56-104/IRGC1061 B56-104///WA	AfricaRice	Upland	105-165	8-12	Green	Long	Green	Well exerted	Erect	Straw	straw	Cream	Awnless	Long/pointed	100-110	6t/ha	B--
Bisalayi	Bisalayi/ Ofada	-	Nigeria	Upland/ Lowland	110-120	15-20	Green	“	Green	“	‘	Green to straw colour	Straw	Colourless	Short awn	-do-	110-120	4.0-8.0	A

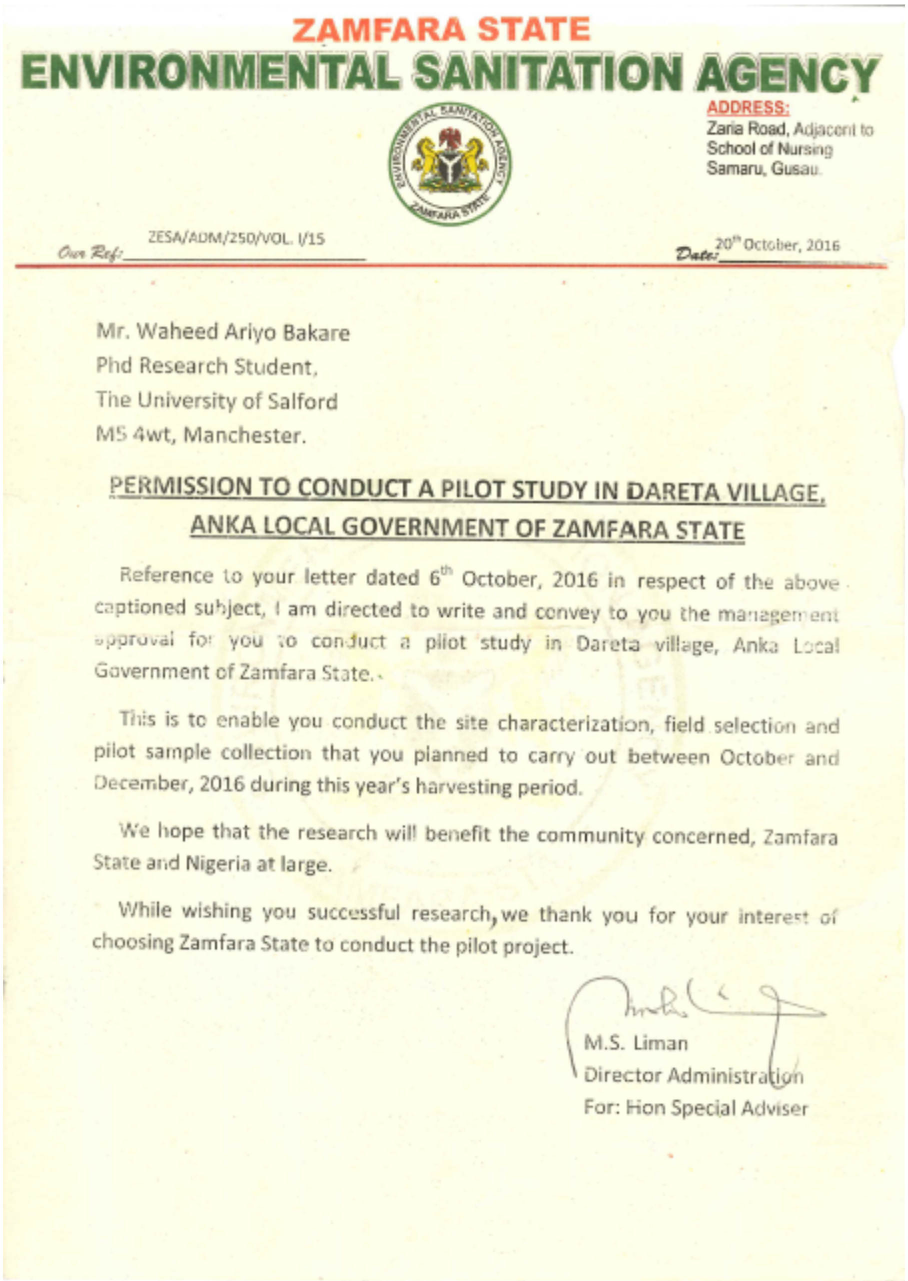
B. RANDOMIZED COMPLETE BLOCK DESIGN (RCBD)

Table II: Randomized Complete Block Design used for rice planting in this study

	REPLICATES																														VARIETIES
START	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**	**R11**	**R12**	**R13**	**R14**	**R15**	**R16**	**R17**	**R18**	**R19**	**R20**	**R21**	**R22**	**R23**	**R24**	**R25**	**R26**	**R27**	**R28**	**R29**	**R30**	
1	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V2	V3	V4	V5	V6	V7	V8	V9	V10	V1	V3	V4	V5	V6	V7	V8	V9	V10	V10	V2	
2	V4	V5	V6	V7	V8	V9	V10	V1	V2	V3	V5	V6	V7	V8	V9	V10	V1	V2	V3	V4	V6	V7	V8	V9	V10	V1	V2	V3	V4	V5	
3	V7	V8	V9	V10	V1	V2	V3	V4	V5	V6	V8	V9	V10	V1	V2	V3	V4	V5	V6	V7	V9	V10	V1	V2	V3	V4	V5	V6	V7	V8	
4	V9	V10	V1	V2	V3	V4	V5	V6	V7	V8	V10	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V1	V2	V3	V4	V5	V6	V7	V8	V9	
5	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V2	V3	V4	V5	V6	V7	V8	V9	V10	V1	V3	V4	V5	V6	V7	V8	V9	V10	V10	V2	
6	V4	V5	V6	V7	V8	V9	V10	V1	V2	V3	V5	V6	V7	V8	V9	V10	V1	V2	V3	V4	V6	V7	V8	V9	V10	V1	V2	V3	V4	V5	
7	V7	V8	V9	V10	V1	V2	V3	V4	V5	V6	V8	V9	V10	V1	V2	V3	V4	V5	V6	V7	V9	V10	V1	V2	V3	V4	V5	V6	V7	V8	
8	V10	V1	V2	V3	V4	V5	V6	V7	V8	V9	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V2	V3	V4	V5	V6	V7	V8	V9	V10	V1	
9	V3	V4	V5	V6	V7	V8	V9	V10	V1	V2	V4	V5	V6	V7	V8	V9	V10	V1	V2	V3	V5	V6	V7	V8	V9	V10	V1	V2	V3	V4	
10	V6	V7	V8	V9	V10	V1	V2	V3	V4	V5	V7	V8	V9	V10	V1	V2	V3	V4	V5	V6	V8	V9	V10	V1	V2	V3	V4	V5	V6	V7	

*V = Variety, R = Replicate, 3blocks in different colour

C. Approval letters for the permissions to carry out the Fieldwork



ZAMFARA STATE
ENVIRONMENTAL SANITATION AGENCY

ADDRESS:
Zaria Road, Adjacent to
School of Nursing
Samaru, Gusau.

Our Ref: ZESA/ADM/250/VOL. I/15

Date: 20th October, 2016

Mr. Waheed Ariyo Bakare
Phd Research Student,
The University of Salford
M5 4wt, Manchester.

PERMISSION TO CONDUCT A PILOT STUDY IN DARETA VILLAGE, ANKA LOCAL GOVERNMENT OF ZAMFARA STATE

Reference to your letter dated 6th October, 2016 in respect of the above captioned subject, I am directed to write and convey to you the management approval for you to conduct a pilot study in Dareta village, Anka Local Government of Zamfara State.

This is to enable you conduct the site characterization, field selection and pilot sample collection that you planned to carry out between October and December, 2016 during this year's harvesting period.

We hope that the research will benefit the community concerned, Zamfara State and Nigeria at large.

While wishing you successful research, we thank you for your interest of choosing Zamfara State to conduct the pilot project.

M.S. Liman
Director Administration
For: Hon Special Adviser

UNIVERSITY OF ABUJA
Department of Agricultural Economic and Soil Science
P.M.B 117
Km 23 Along, Airport Road,
Abuja - FCT, Nigeria.

12th October, 2016

Mr Waheed Ariyo Bakare
PhD research Student
The University of Salford
M5 4WT, Manchester
United Kingdom.

RE: LETTER OF INTRODUCTION AND REQUEST FOR SUPPORT FOR ON-GOING PHD RESEARCH BEING CONDUCTED BY WAHEED ARIYO BAKARE, STUDENT ID:@00407819

Reference to your letter dated 6th October, 2016 in respect of the above; I am writing you as directed by the Vice Chancellor, the University of Abuja to notify you that your research (pot experiment) has been approved to take place within the faculty of Agriculture under the Soil Science Department, University of Abuja in 2017. We are pleased to support you technically to make-sure you succeed on this academic project.

Please let me know if you need anything further from the University of Abuja. You may also get in touch with me on phone anytime or via my email addresses.

As you proceed on your research project, I wish you success in your career.

Regards;

Dr Effiom Essien Oku
Head,
Soil Science Department
The University of Abuja.
Tel: +234(0)8025325056
Email: e.essienoku@uniabuja.edu.ng
Alt email: eessienoku@gmail.com

D. The result of the particle size and the soil colour for the four selected rice farms during the soil characterisation.

Table III: Particles size and the soil texture using soil textural triangle for the 80 soil samples

SN	SAMPLE POINT	% Clay	% Silt	%Sand	Texture	SN	SAMPLE POINT	% Clay	% Silt	%Sand	Texture
1	A1	12.4	51.9	35.7	Loam	21	B1	12.8	51.4	35.8	Clay loam
2	A2	11.8	48.5	39.7	Loam	22	B2	10.8	49.4	39.8	Loam
3	A3	16.8	23.4	59.8	Loam	23	B3	16.8	23.4	59.8	Loam
4	A4	12.8	39.4	47.8	Loam	24	B4	12.8	39.4	47.8	Loam
5	A5	12.8	33.4	53.8	Sandy loam	25	B5	12.8	33.4	53.8	Sandy clay
6	A6	16.8	39.4	43.8	Sandy loam	26	B6	16.8	39.4	43.8	Loam
7	A7	12.8	27.4	59.8	Sandy loam	27	B7	12.8	27.4	59.8	Clay
8	A8	10	15.4	74.6	Sandy clay loam	28	B8	10	15.4	74.6	Silt loam
9	A9	14	43.4	42.6	Sandy loam	29	B9	14	43.4	42.6	Silt loam
10	A10	22	32.6	45.4	Loam	30	B10	10	29.4	60.6	Silt loam
11	A11	26	21.4	52.6	Loam	31	B11	16	31.4	52.6	Loam
12	A12	18	19.4	62.6	Sandy loam	32	B12	21	29.6	49.4	Loam
13	A13	18.8	51.4	29.8	Sandy loam	33	B13	18.8	51.4	29.8	Loam
14	A14	24.8	37.4	37.8	Sandy loam	34	B14	24.8	37.4	37.8	Clay loam
15	A15	19.4	38.8	41.8	Sandy loam	35	B15	19.4	38.8	41.8	Loam
16	A16	37.4	6.8	55.8	Loam	36	B16	17.4	26.8	55.8	Sandy clay loam
17	A17	21.4	54.8	23.8	Loam	37	B17	21.4	54.8	23.8	Sandy clay loam
18	A18	21.4	30.8	47.8	Loam	38	B18	21.4	30.8	47.8	Sandy loam
19	A19	27.4	34.8	37.8	Sandy loam	39	B19	17.4	34.8	47.8	Sandy clay loam
20	A20	27.4	25.4	47.2	Sandy loam	40	B20	17.4	25.4	57.2	Loam
41	C1	12.8	51.4	35.8	Sandy loam	61	D1	12.8	51.4	35.8	Sandy clay loam
42	C2	10.8	49.4	39.8	Loam	62	D2	10.8	49.4	39.8	Sandy loam
43	C3	16.8	23.4	59.8	Loam	63	D3	16.8	23.4	59.8	Loam
44	C4	12.8	39.4	47.8	Loam	64	D4	12.8	39.4	47.8	Loam
45	C5	12.8	33.4	53.8	Loam	65	D5	12.8	33.4	53.8	Sandy loam
46	C6	16.8	39.4	43.8	Loam	66	D6	16.8	39.4	43.8	Sandy loam
47	C7	12.8	27.4	59.8	Clay loam	67	D7	12.8	27.4	59.8	Silty clay loam
48	C8	10	15.4	74.6	Clay loam	68	D8	10	15.4	74.6	Sandy loam
49	C9	14	43.4	42.6	Loam	69	D9	14	43.4	42.6	Loam
50	C10	20	39.4	40.6	Sandy clay loam	70	D10	24.2	39.3	36.5	Loam
51	C11	26	21.4	52.6	Sandy clay loam	71	D11	26	21.4	52.6	Silt loam
52	C12	18	19.4	62.6	Silty clay loam	72	D12	18	19.4	62.6	Loam
53	C13	18.8	51.4	29.8	Sandy loam	73	D13	18.8	51.4	29.8	Silty clay loam

54	C14	24.8	37.4	37.8	Loam	74	D14	24.8	37.4	37.8	Sandy loam
55	C15	19.4	38.8	41.8	Loam	75	D15	19.4	38.8	41.8	Loam
56	C16	37.4	6.8	55.8	Silt loam	76	D16	37.4	6.8	55.8	Loam
57	C17	21.4	54.8	23.8	Loam	77	D17	21.4	54.8	23.8	Silt loam
58	C18	21.4	30.8	47.8	Loam	78	D18	21.4	30.8	47.8	Loam
59	C19	27.4	34.8	37.8	Loam	79	D19	27.4	34.8	37.8	Loam
60	C20	27.4	25.4	47.2	Clay loam	80	D20	27.4	25.4	47.2	Clay loam

ABCD represents the sampling location and their numbers represent the sampling points.

Table IV: Result of the soil colour test for the four selected rice farms.

SN	SAMPLE POINT	0–10 cm DEPTH (matched codes)	10–20 cm DEPTH (matched codes)	20–30 cm DEPTH (matched codes)	SN	SAMPLE POINT	0–10cm DEPTH (matched codes)	10–20cm DEPTH (matched codes)	20–30 cm DEPTH (matched codes)
1	A1	10YR ¾	10YR ¾	10YR ¾	21	B1	10YR ¾	10YR ¾	10YR ¾
2	A2	10YR ¾	10YR ¾	10YR ¾	22	B2	10YR ¾	10YR ¾	10YR ¾
3	A3	10YR 3/6	10YR 3/6	10YR 3/6	23	B3	10YR 3/6	10YR 3/6	10YR 3/6
4	A4	10YR ¾	10YR 2/2	10YR 2/2	24	B4	10YR ¾	10YR 2/2	10YR 2/2
5	A5	10YR ¾	10YR ¾	10YR ¾	25	B5	10YR ¾	10YR ¾	10YR ¾
6	A6	10YR ¾	10YR ¾	10YR ¾	26	B6	10YR ¾	10YR ¾	10YR ¾
7	A7	10YR 4/3	10YR 4/3	10YR 4/3	27	B7	10YR 4/3	10YR 4/3	10YR 4/3
8	A8	10YR 2/2	10YR 2/2	10YR 2/2	28	B8	10YR 2/2	10YR 2/2	10YR 2/2
9	A9	10YR 2/2	10YR 2/2	10YR 2/2	29	B9	10YR 2/2	10YR 2/2	10YR 2/2
10	A10	10YR 3/6	10YR 3/6	10YR 3/6	30	B10	10YR 3/6	10YR 3/6	10YR 3/6
11	A11	10YR 4/4	10YR 4/4	10YR 4/4	31	B11	10YR 4/4	10YR 4/4	10YR 4/4
12	A12	10YR 4/3	10YR 4/3	10YR 4/3	32	B12	10YR 4/3	10YR 4/3	10YR 4/3
13	A13	10YR 2/2	10YR 2/2	10YR 2/2	33	B13	10YR 2/2	10YR 2/2	10YR 2/2
14	A14	10YR 3/6	10YR 3/6	10YR 3/6	34	B14	10YR 3/6	10YR 3/6	10YR 3/6
15	A15	10YR ¾	10YR ¾	10YR ¾	35	B15	10YR ¾	10YR ¾	10YR ¾
16	A16	10YR 4/3	10YR 4/3	10YR 3/3	36	B16	10YR 4/3	10YR 4/3	10YR 4/3
17	A17	10YR 2/2	10YR 2/2	10YR 2/2	37	B17	10YR 2/2	10YR 2/2	10YR 2/2
18	A18	10YR 2/2	10YR 2/2	10YR 2/2	38	B18	10YR 2/2	10YR 2/2	10YR 2/2
19	A19	10YR 3/6	10YR 3/6	10YR 3/6	39	B19	10YR 3/6	10YR 3/6	10YR 3/6
20	A20	10YR 4/4	10YR 4/4	10YR 4/4	40	B20	10YR 4/4	10YR 4/4	10YR 4/4
41	C1	10YR ¾	10YR 2/2	10YR 2/2	61	D1	10YR 3/6	10YR 3/6	10YR 3/6

42	C2	10YR ¾	10YR ¾	10YR ¾	62	D2	10YR ¾	10YR 2/2	10YR 2/2
43	C3	10YR ¾	10YR ¾	10YR ¾	63	D3	10YR ¾	10YR ¾	10YR ¾
44	C4	10YR 4/3	10YR 4/3	10YR 4/3	64	D4	10YR ¾	10YR ¾	10YR ¾
45	C5	10YR 2/2	10YR 2/2	10YR 2/2	65	D5	10YR 4/3	10YR 4/3	10YR 4/3
46	C6	10YR 2/2	10YR 2/2	10YR 2/2	66	D6	10YR 3/6	10YR 3/6	10YR 3/6
47	C7	10YR 3/6	10YR 3/6	10YR 3/6	67	D7	10YR ¾	10YR 2/2	10YR 2/2
48	C8	10YR 4/4	10YR 4/4	10YR 4/4	68	D8	10YR ¾	10YR ¾	10YR ¾
49	C9	10YR 4/3	10YR 4/3	10YR 4/3	69	D9	10YR ¾	10YR ¾	10YR ¾
50	C10	10YR ¾	10YR 2/2	10YR 2/2	70	D10	10YR 4/3	10YR 4/3	10YR 4/3
51	C11	10YR ¾	10YR 2/2	10YR 2/2	71	D11	10YR 2/2	10YR 2/2	10YR 2/2
52	C12	10YR ¾	10YR ¾	10YR ¾	72	D12	10YR 2/2	10YR 2/2	10YR 2/2
53	C13	10YR ¾	10YR ¾	10YR ¾	73	D13	10YR 6/6	10YR 6/6	10YR 6/6
54	C14	10YR 4/3	10YR 4/3	10YR 4/3	74	D14	10YR 4/4	10YR 4/4	10YR 4/4
55	C15	10YR 2/2	10YR 2/2	10YR 2/2	75	D15	10YR 4/3	10YR 4/3	10YR 4/3
56	C16	10YR 2/2	10YR 2/2	10YR 2/2	76	D16	10YR ¾	10YR ¾	10YR ¾
57	C17	10YR 3/6	10YR 3/6	10YR 3/6	77	D17	10YR ¾	10YR ¾	10YR ¾
58	C18	10YR 4/4	10YR 4/4	10YR 5/4	78	D18	10YR 4/3	10YR 4/3	10YR 4/3
59	C19	10YR 4/3	10YR 4/3	10YR 4/3	79	D19	10YR 3/6	10YR 3/6	10YR 3/6
60	C20	10YR ¾	10YR 2/2	10YR 2/2	80	D20	10YR ¾	10YR 2/2	10YR 2/2

ABCD represents the sampling location and their numbers represent the sampling points.

Munsell Matched Colour Codes	10YR ¾	10YR 3/6	10YR 4/4	10YR 4/6	10YR 2/2	10YR 4/3	10YR 3/3	10YR 6/6	10YR 5/4
Colour Code Interpretation on the Colour Chart	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown	Very Dark	Brown	Dark brown	Brownish yellow	Yellowish brown

E. Relationship between the Pb and the essential elements in both the field and the pot experiment

Table V: Pearson correlations among the elemental concentrations (mg/kg) of Pb and the essential elements in the field experiment

	Pb-Rice	Pb-Soil	Ca-Rice	Ca-Soil	Fe-Rice	Fe-Soil	K-Rice	K-Soil	Mg-Rice	Mg-Soil ->
Pb-Rice	1	0.176**	0.636**	-0.193**	-0.195**	0.055	0.516**	-0.134*	0.243**	-0.106
Pb-Soil		1	-0.275**	0.603**	0.04	-0.640**	-0.198**	0.930**	-0.223**	0.849**
Ca-Rice			1	-0.221**	-0.02	0.176**	0.549**	-0.237**	0.453**	-0.196**
Ca-soil				1	0.027	0.021	- 0.241**	0.712**	-0.164**	0.738**
Fe-Rice					1	-0.02	-0.104	0.026	0.102	0.025
Fe-soil						1	0.078	-0.519**	0.151**	-0.414**
K-Rice							1	-0.176**	0.887**	-0.153**
K-Soil								1	-0.214**	0.977**
Mg Rice									1	-0.203**
Mg-Soil										1
->	**Zn-Rice**	**Zn-Soil**	**Se-Rice**	**Se-Soil**	**Mn-Rice**	**Mn-Soil**	**Co-Rice**	**Co-Soil**	**Cu-Rice**	**Cu-Soil**
	0.545**	0.069	-0.242**	-0.041	-0.087	0.05	0.555**	0.083	0.421**	0.125*
	-0.091	0.514**	0.291**	0.615**	-0.104	0.494**	-0.076	0.423**	-0.197**	0.295**
	0.407**	-0.022	-0.208**	-0.148*	0.190**	-0.055	-0.419**	-0.011	0.354**	0.089
	-0.174**	-0.041	0.258**	0.031	-0.132*	-0.009	0.047	-0.108	-0.071	-0.251**
	-0.130*	0.033	0.029	0.021	0.244**	-0.001	0.067	-0.004	0.024	0.021
	0.024	-0.581**	-0.151**	-0.655**	0.067	-0.504**	0.07	-0.492**	0.183**	-0.348**
	0.737**	0.089	-0.167**	-0.07	0.506**	0.067	-0.093	0.119*	0.430**	0.184**
	-0.042	0.508**	0.241**	0.574**	-0.108	0.478**	-0.092	0.408**	-0.117*	0.272**
	0.534**	-0.042	-0.047	-0.163**	0.641**	-0.039	0.127*	-0.004	0.304**	0.009
	-0.01	0.466**	0.181**	0.508**	-0.096	0.434**	-0.096	0.368**	-0.055	0.282**
Zn-Rice	1	0.218**	-0.111	0.018	0.347**	0.217**	-0.208**	0.259**	0.575**	0.288**
Zn-Soil		1	-0.002	0.784**	0.092	0.745**	-0.190**	0.773**	0.037	0.683**
Se-Rice			1	0.094	-0.033	0.042	0.162**	-0.007	-0.148*	-0.179**
Se-Soil				1	-0.055	0.721**	-0.146*	0.736**	-0.117*	0.584**
Mn-Rice					1	0.055	0.368**	0.102	0.208**	0.164**
Mn-Soil						1	-0.095	0.964**	0.006	0.592**
Co-Rice							1	-0.112	-0.267**	-0.224**
Co-Soil								1	0.044	0.656**
Cu-Rice									1	0.154**
Cu-Soil										1

** Correlation is significant at $p < 0.01$ (2-tailed).

* Correlation is significant at $p < 0.05$ (2-tailed). -> = Joint table

Table VI: Pearson correlations (2-tailed) among the concentration ratio (CR) for Pb and the essential elements in the field experiment

	Pb-CR	Ca-CR	Fe-CR	K-CR	Mg-CR	Zn-CR	Se-CR	Mn-CR	Co-CR	Cu-CR
Pb-CR	1	0.119*	-0.127*	0.738**	0.636**	0.712**	0.566**	0.684**	0.524**	0.661**
Ca-CR		1	-0.046	0.517**	0.551**	-0.098	-0.136*	-0.093	-0.182**	-0.152**
Fe-CR			1	-0.149**	-0.098	-0.198**	-0.208**	-0.174**	-0.168**	-0.185**
K-CR				1	0.963**	0.588**	0.460**	0.561**	0.449**	0.469**
Mg-CR					1	0.508**	0.401**	0.496**	0.400**	0.401**
Zn-CR						1	0.908**	0.964**	0.898**	0.896**
Se-CR							1	0.907**	0.879**	0.836**
Mn-CR								1	0.933**	0.874**
Co-CR									1	0.804**
Cu-CR										1

** Correlation is significant at $p < 0.01$ (2-tailed).

* Correlation is significant at $p < 0.05$ (2-tailed). CR = Concentration Ratio

Table VII: Pearson correlation for Pb and the essential elements in rice and soil samples for the pot experiment

	Mn in Rice	Co in Rice	Cu in Rice	Zn in Rice	Se in Rice	Pb in Rice	Ca in Rice	Fe in Rice	K in Rice	Mg in Rice	Pb in Soil	Ca in Soil	K in Soil	Mg in Soil	Fe in Soil	Mn in Soil	Co in Soil	Cu in Soil	Zn in Soil	Se in Soil
Mn in Rice	1																			
Co in Rice	0.387**	1																		
Cu in Rice	0.403**	-0.036	1																	
Zn in Rice	0.508**	-0.099	0.780**	1																
Se in Rice	-0.096	0.011	-.242**	-.190**	1															
Pb in Rice	-.217**	.260**	.297**	.406**	-0.021	1														
Ca in Rice	-.186**	-.395**	-.233**	-.141*	0.102	.209**	1													
Fe in Rice	.375**	.604**	.128*	0.056	.163**	-.126*	-.212**	1												
K in Rice	.544**	-.268**	.494**	.656**	-0.053	.470**	.295**	0.01	1											
Mg in Rice	.426**	-.313**	.370**	.528**	-0.012	.322**	.417**	-0.041	.932**	1										
Pb in Soil	-0.055	-0.062	0.033	0.032	-0.006	-0.002	0.066	0.035	-0.016	-0.032	1									
Ca in Soil	0.103	0.078	0.086	0.052	-0.044	0.037	-0.08	0.012	0.109	.156**	-.239**	1								
K in Soil	-0.032	-0.004	-0.09	0.036	0.075	-0.058	0.065	-0.014	.128*	.206**	-0.063	.465**	1							
Mg in Soil	.118*	.118*	.119*	0.091	-.208**	0.037	-.129*	-0.069	0.052	0.087	-.138*	.553**	.664**	1						
Fe in Soil	.138*	0.092	.161**	.133*	-.255**	0.081	-.175**	-0.046	0.038	0.065	-.123*	.506**	.431**	.729**	1					
Mn in Soil	0.02	.117*	.139*	0.085	0.096	0.027	-0.048	.182**	.126*	.189**	0.06	.195**	.196**	.131*	.324**	1				
Co in Soil	0.08	.146*	.194**	.126*	-0.08	-0.011	-.143*	.133*	0.104	.135*	-0.017	.283**	.194**	.269**	.460**	.834**	1			
Cu in Soil	0.062	.159**	.130*	0.084	-0.007	0.041	-0.095	.135*	0.1	.170**	-.124*	.427**	.340**	.383**	.532**	.690**	.741**	1		
Zn in Soil	0.1	.163**	.229**	0.113	-.118*	0.059	-.141*	0.082	0.096	.150**	-.243**	.520**	.227**	.420**	.502**	.570**	.598**	.784**	1	
Se in Soil	-0.056	0.02	-0.006	0.062	.246**	-0.052	0.071	.192**	.196**	.275**	-0.052	.254**	.507**	0.079	.150**	.574**	.443**	.594**	.416**	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table VIII: Pearson correlation for the concentration ratio (CR) for Pb and the essential elements and some selected soil parameters in the pot experiment

	Pb-CR	**Mn-CR**	**Co-CR**	**Cu-CR**	**Zn-CR**	**Se-CR**	**Ca-CR**	**Fe-CR**	**K-CR**	**Mg-CR**	**Clay**	**Silt**	**Sand**	**pH-Soil**	**N-Soil**	**Phos-Soil**	**EC-Soil**	**OrgC**	**ExAc**	**CEC**
Pb-CR	1																			
Mn-CR	.187**	1																		
Co-CR	.238**	.130*	1																	
Cu-CR	.233**	.468**	-.200**	1																
Zn-CR	.212**	.683**	.266**	.631**	1															
Se-CR	.185**	.127*	.203**	0.009	.164**	1														
Ca-CR	0.019	0.077	.179**	0.058	.150**	0.103	1													
Fe-CR	.257**	0.04	.582**	-0.051	-.114*	.173**	-0.061	1												
K-CR	.262**	.329**	.577**	.350**	.415**	-0.1	.297**	-.156**	1											
Mg-CR	.216**	0.091	.458**	0.088	.236**	0.07	.324**	-.129*	.779**	1										
Clay	-.119*	-0.015	-0.069	0.054	0.08	0.088	0.061	-0.006	0.084	0.098	1									
Silt	-0.034	0.047	-0.042	-0.01	.147*	.121*	0.064	-0.053	0.015	0.071	0.069	1								
Sand	0.079	-0.036	0.066	-0.013	.163**	-.143*	-0.081	0.05	-0.048	-0.103	-.470**	-.913**	1							
pH-Soil	0.074	-0.004	0.083	0.011	-0.036	0.07	-0.012	-0.002	-0.077	-0.042	-0.111	-0.026	0.068	1						
N-Soil	-0.045	-0.062	-0.056	-0.06	0.093	0.111	.130*	-0.074	-0.004	.157**	0.069	.384**	-.368**	0.1	1					
Phos-Soil	0.085	0.033	0.033	.123*	0.029	0.017	0.089	-0.057	-0.04	-0.051	-.115*	-0.109	.144*	0.087	.433**	1				
EC-Soil	0.02	.124*	-0.068	0.091	.315**	.235**	0.075	-0.097	-0.001	.129*	.212**	.487**	-.517**	-0.02	.507**	-.147*	1			
OrgC	0.033	-0.049	0.047	-0.058	-0.04	-0.023	0.063	0.022	-0.079	-0.073	0.021	0.08	-0.079	-0.013	-0.019	-0.033	-0.055	1		
ExAc	-0.014	0.064	0.018	0.029	-0.021	-0.06	-0.021	-0.007	0.07	0.02	-0.026	0.001	0.01	0.041	-0.045	0.086	-0.017	0.055	1	
CEC	0.096	-.137*	-0.022	-.149**	.283**	.278**	-.280**	-0.025	-.239**	.321**	-.139*	.255**	.283**	0.038	.331**	0.071	.334**	0.036	0.037	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).