Quantification of heavy metal levels in local rice (Oryza sativa) consumed in Anambra State, Nigeria

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Abstract. Rice is a little solid seed which is the most requested grain crop. Injection of this crop while contaminated can cause harm to the human organs. For this reason, the urge to study the Quantification of heavy metals in rice grain arises. Four towns in Anambra State (Nzam, Omor, Otuocha, Ufuma) were sampled. In the samples of the rice, analysis of heavy metals were conducted using Atomic Absorbance Spectrophotometer to determine the heavy metals present in the samples and the mean levels of heavy metals found in rice grains were; For lead: 0.0078mg/kg (Nzam), 0.1250mg/kg (Omor), 0.2070mg/kg (Otuocha). For Arsenic: 14.8889mg/kg (Nzam), 12.8333mg/kg (Omor), 12.8889mg/kg (Otuocha), and 12.3333 mg/kg (Ufuma). For Zinc: 0.7110mg/kg (Nzam), 0.7474mg/kg (Omor), 1.5930mg/kg (Otuocha), and 0.2518 mg/kg (Ufuma). For Iron: 0.6390mg/kg (Nzam), 0.5352mg/kg (Omor), 0.6194mg/kg (Otuocha), and 0.5709mg/kg (Ufuma). For Manganese: 0.0578mg/kg (Nzam), 0.0104mg/kg (Omor), and 0.714mg/kg (Ufuma). For Nickel: 0.0756mg/kg (Ufuma). There is no observed level of Cd, Co, Cu and Cr in the rice grown in these four towns (Nzam, Omor, Otuocha, Ufuma). With the results gotten from this Analysis, some rice samples were within the acceptable limit set by FAO/WHO while some were not within the permissible limit and it is therefore recommended that these four towns should be monitored continuously.

Keywords:

1 INTRODUCTION

Any little solid seed that is free from moisture, either with or without its outermost layer that can be peeled and can be eaten is known as a grain (Babcock, 1996). Plants that have the ability to produce grains are said to be grain crops and these crops are classified as either legumes or cereals. A cereal, also a grain look more like the caryopses are regarded as grains. We have the true cereal grains and the Pseudo cereal grains. True grains cereal such as rice and millet are from Poaceae while Pseudo grains such as buckwheat are not from the same family with the true cereal grains. Both of the cereal grains have the same nutritional value when compared to each other. There are also refined grains and whole grains. Refined grains are those grains that have only the Endosperm while the whole grains have the bran, the germ and the Endosperm. The Endosperm is the innermost part of the rice seed, the germ is the middle part of the seed while the Bran is the outermost part of the seed. *Oryza sativa* is a botanical name for Asian rice while *Oryza glaberrima* is a botanical name for African rice. One of the most important and valued crop in the world is rice and it is produced in large quantity of over 800 tons per year. Rice has the brown and white color type and it also comes in different sizes; rice can be extra long (greater than 7.5) , Long (6.6 - 7.5), Medium (5.51 - 6.6) and short (5.5)

There are numerous challenges associated with rice production worldwide depending on each geographical area. The common issues affecting most areas are; when the soil contained in the soil or seedling is so high, when it rains slow and steady, when pests such as stink bugs and diseases such as blast affect the crops, when there are lesser plants to plant on, when the price of the fertilizers to be used on the crops increases drastically, when there are no machines to make the farming easier, when there are no policies to guide the farmers, when the government and NGOs do not provide the farmers with quality information on increasing the agricultural production and when there are no handbooks to guide the farmers on how best to deal with insects, pests and issues of soil fertility.

Some cultural practices are used to increase Rice production by Farmers they are; Understanding the season which is the right time to plant i.e from fallowing to Preparing the land etc, Making use of the best type of rice in the best suitable Environment and using a seedling which has a high quality that has not been infected by pests and diseases, Preparing the land by removing any weed from the farm after making it a tabled (leveled) farm land, knowing the actual time to start planting so as to give a good yield, Spraying insecticide, herbicide, pesticide on the farm to make sure that insects and pests are killed in other for it not to affect the crops while putting on the protective clothing to avoid harm on the skin and inhalation of toxic chemicals, Applying fertilizers suitable for the plant like NPK 15:15:15 and Urea 49-100 kg per hectare, Making sure clean equipments and seeds are used, harvesting the crops when it is matured i.e. when 80 - 85% of the rice have changed their color to that of the straw, so that if it is harvested, it can still be used as seedling, storing the rice in husk and milling the rice to remove the husks and the brown layer covering it, to make it fit into an edible form.

Majority of the Nigerians are hopeful and believing that in years to come, it's economy will be stable and that has pushed the Nigerian government into banning the importation if foreign rice into her country due to the fact that they have visions of Nigerians becoming rice exporters and by this, the country is expected to produce more rice that will be enough for its citizen and also be enough to export. By so doing, rice is secured in Nigeria by being in excess to her citizens and there will be job opportunities created from rice production starting from the laborers to the people who manufacture the farm tools and machines to be used, to the fertilizer formulators down to the traders. Rice is processed by using hullers to remove outer layer of rice called the husk, it then becomes the popular brown rice and this process can continue till the middle layer of the rice is removed till it turns to white type of rice. As this process goes on, the nutrients of the rice decrease.

Heavy metals are elements from the periodic table that occupy the largest columns i.e. from 3 - 16 columns of the 4 - 6 periods. This includes all other metals such as the transition metals, the post transition metals, and also the lanthanides (Dufus, 2002). Heavy metals have elevated atomic number, atomic and density. When heavy metals are injected or inhaled into the body in a minute amount, it becomes very poisonous thereby causing severe health effects (Chem et al., 2005, Singh et al., 2004). A heavy metal has a density of at least $5gcm^{-3}$ and this can be differentiated from light metals. Some examples of heavy metals are Cobalt, Nickel, and Arsenic etc. Its atomic mass are usually higher than 23 while its atomic number is from 20 and above. Heavy metals cannot be destroyed neither can it be degraded; they have long biological half lives and are soluble in water. There are essential and non essential heavy metals and Manganese, Iron, Zinc are among the essential heavy metals. When heavy metals come in contact with the soil, the water and the atmosphere, it causes hazard to the humans living in the Environment and also the animals and plants. (Stankovic and Stankovic, 2003). Heavy metals can be obtained from the industries either by their effluent or by their discharge into the atmosphere, the mechanics through the emissions of Lead acid batteries from vehicles etc. (Bradl, 2005).

Intake of heavy metals at higher concentration is also very toxic and this leads to poisoning , this could result from the drinking water we take that are contaminated, through the food we eat or breathing air near emission sources. Heavy metals are very dangerous because when they are introduced into the biological organism, they are stored over time or bioaccumulated unlike other chemicals in the body that are broken down or excreted. High concentrations of heavy metals inform of pesticide or fertilizer in soils cause more harm than good to the Environment and all that is living in it, this is due to the fact that discharging heavy metals are hard and they don't decompose or decay in the soil, they tend to remain permanent in the soil in which it is disposed and this makes it possible for plants to uptake this heavy metals and causing possible health risk when eaten or consumed (Malidareh et al., 2014). Inhaling or injecting of foods contaminated with heavy metals consume the vital powers of some essential nutrients in the body thereby causing disabilities associated with malnutrition, impaired psycho-social behavior, gastro-intestinal cancer, growth retardation etc. (Arora et al., 2008). This causes some side effects by failing the kidney, destroying the Nervous system, failing the heart etc. due to the Environment contaminated by heavy metals from industries, fertilizers, broken lead pipes in water, chemicals, fumes from machines etc when crops are grown on the soil (Hario, 2003).

Heavy metals are quantified using techniques such as wet chemical methods (gravimetric, titrimetric, colorimetric etc), Atomic Absorption spectrophotometer (AAS). Another technique that can be used for determining heavy metal is diverse ion selective electrodes (Wang, 2005). Sensors like Lab-on-paper are used for determining metals like copper Nickel etc. and these sensors are operated by immobilized enzymes. In recent technology, florescent sensors that are genetically encoded, monitors the contained metal in the cell and can be used to determine Arsenic, Zinc, Mercury, Lead or Cadmium (Rasheed et al., 2018). Contamination of soil can be remedied traditionally by digging out the contaminated soil and disposing it in a landfill. This disposal strategy later causes hazard to the Environment because the contaminated soil was not treated rather it was shifted from one location to another and this also generates hazard while been transported. Alkaline lime precipitation is known to be a good method of testing heavily polluted waste water or effluent, but this Lime pollution can only treat effectively waste water with metal loads more than 1gL-1 asides this, others will still need ultimate disposal (Barakat, 2011). The modern method of treating this pollution is by using the Phytoremediation technique, ion exchange techniques, electro dialysis, and photo catalysis. Out of all these physiochemical techniques, the most commonly used methods for treating waste water polluted by heavy metals are by adsorption methods based on filtering the membrane (Premkumar et al., 2018). Using new adsorbent, heavy metals can be recovered by both inorganic materials (kaolinite, montmorillonite) (Bhattacharya and Gupta, 2008) and Organic materials (bio char, agricultural waste) (Poo et al., 2018).

Reflectivity, electrical, conductivity, strength, durability, density are all general characteristics of metals that help in classifying the used of heavy metals. Some of the uses of heavy metals are; Used in making steel balls in shot put and hammer throws (White, 2010), Making ballast for boats, motor vehicles and aero planes (Library, 2012), Used in making green bullets used by armies (Lacy et al., 2015), Neodymium, Bismuth etc are used in making magnets for power windows, pumps in fuel, locks for the doors of cars etc (Emsley, 2011). Tungsten is used in diagnostic imaging (Tisza, 2001), Lead, gold, silver, Zirconium, Tin, Cobalt, Chromium, Manganese are some heavy metals that produce colors in paints, plastics, glass, ceramics, glazes and pigments. Mercury is used in antiseptic formulations to control algae growth and are contained in agro chemicals (Nakbanpote et al., 2016). Tungsten, Lead, Zinc, Iron are used to manufacture ships, utensils, tools, appliances, machinery etc.

2 MATERIALS AND METHODS

All the materials that were used are: rice, weighing balance, mortar, pestle, grinder, 100ml beaker, distilled water, water bath, filter paper, atomic absorption spectrophotometer machine, HCl, HNO_3 and Volumetric flask, de-ionised water, plastic tray, polythene bags, Oven, brown envelope, sieve.

2.1 Selected Areas of Study

Omor, Otuocha Ufuma and Nzam were the four (4) selected towns in Anambra State in the Eastern parts of Nigeria for this research because it has been observed that these areas are known to produce food in large quantity yearly.

In Anambra State, rice samples were collected from four (4) towns Omor which is the first samples town is in Ayamelu, a Local Government Area in Anambra State which is located in the Southeast of Nigeria with its headquarters at Anaku and very close to Omambala River. Nzam is the second sampled town in Anambra West, a Local Government Area in Anambra State which is close to River Niger. Ufuma is the third sampled town in Orumba North, a Local Government Area in Anambra State, it's rice fields are seen near Ozi and Mamu River in Ufuma while Otuocha which is the fourth sampled town is a headquarter in Anambra East, a Local Government Area in Anambra state and it's rice fields are seen near Anam River in Otuocha.

2.2 Planting and harvesting of the rice seed

The rice seeds which have been soaked in water for 12 - 24 hours is scattered on the farm ground containing little acidic clay, as rain falls it immerses the seed into the soil. With the presence of sunlight and a dependable water source, the rice seed grows within 3 - 6 months. As the rice grains mature, they can go up to 17 inches high. Any extra water is removed or dried till it turns from green to gold color i.e. the stems are cut down and allowed for its moisture to be completely removed.

After about two (2) weeks, the rice must have dried and it would then change to change to gold color and be set. The stem which helps to carry the seed of the rice grain is cut off, there are small pockets over the stalk which are the hulls of the rice. When the stalks are cut down, they are covered in a news paper and kept to maintain dryness in sunlight for 2-3 weeks. The grains are baked form some time i.e. 60 minutes at a degree of 82o centigrade till they turn darker color of gold resulting to a gold brownish color. The brown covering which covers the Endosperm of the rice are then removed by buffing them either by the use of the two hands or by using a mortar and a pestle for a desired grain to be achieved.

2.3 Gathering and Preparation of the rice samples

Four (4) black nylon bags were used to simple randomly gather twenty (20) grams each of the rice samples from Omor, Otuocha, Ufuma, and Nzam. These four (4) rice grain samples were taken to the laboratory where it can be worked on, water which is de ionised was used to wash theses 20 grams each of the rice samples and then poured into a sieve and the water is allowed to sieve out. When the 20 grams each of the rice samples became dried, it was gathered and put in a paper which was made in a well covered cone-like shape and put in an oven to make sure that any other moisture trapped in the rice grains were removed. This was done for 48 hours at a temperature of 65 ^{o}C. After drying, the rice grains were pounded into a smooth powdered texture and using a 2mm sieve, it was sieved to make sure that whatever is used for the analysis was a fine textured form of the rice grain samples. It was then kept to continue drying until the weight of the rice was no longer changing (Otitoju et al., 2014).

2.4 Digestion of the rice samples using aqua Regia (Jarvis 1992)

One gram of the rice sample was added into a beaker, 10ml of distilled water and 10ml of Aqua Regia was added too. It was placed in a water bath and set at 80 ^{o}C and heated for 60 minutes. It was brought down and allowed to cool, and then a 10ml of distilled water was added, filtered and making up the filtrate to 100ml using distilled water. Then an elemental analysis was carried out on the samples using Atomic Absorption spectrophotometer (AAS) machine.

2.5 Preparation of Aqua Regia

Aqua Regia = 3HCl**:** 1HNO_3
To prepare 100ml of Aqua Regia
1/4 * 100 of HNO_3 = 25ml HNO_3 (It is then poured into a beaker),
3/4 * 100 of HCl =75ml HCl (it is Poured into the same beaker),
Then from the beaker 10ml of Aqua Regia is taken each for the digestion.

2.6 Quality Assurance and Statistical Analysis

The analysis was carried out for two sets each of the rice sample. The sample was grinded into a fine powdered texture. The breakers and Volumetric flasks used were washed properly and rinsed using water that is de ionised. These beakers and Volumetric flasks used were well caliberated, the chemicals and reagents used were puerto avoid given wrong results. Its mean and standard deviation were then determined.

2.7 Conversion of milligram/kilogram to microgram/kilogram

1 milligram/kilogram = 1000 microgram/kilogram
Formula: the mass/mass value is multiplied by 1000
Eg. 1mg/kg * 1000 = 1000 µg/kg

2.8 Conversion of microgram/kilogram to milligram/kilogram

1 microgram/kilogram = 0.001 milligram/kilogram
Formula: the mass or mass value is divided by 1000
Eg. 1 µg/kg / 1000 = 0.0001mg/kg

NB **PTWI:** Provincial Tolerable weekly intake
EDI: Estimated Daily Intake
MTWI: Maximum Tolerable weekly intake

A - Omor (Ayamelu L.G.A)

B - Nzam (Anambra West L.G.A)

C - Otuocha (Anambra East L.G.A)

D - Ufuma (Orumba North L.G.A)

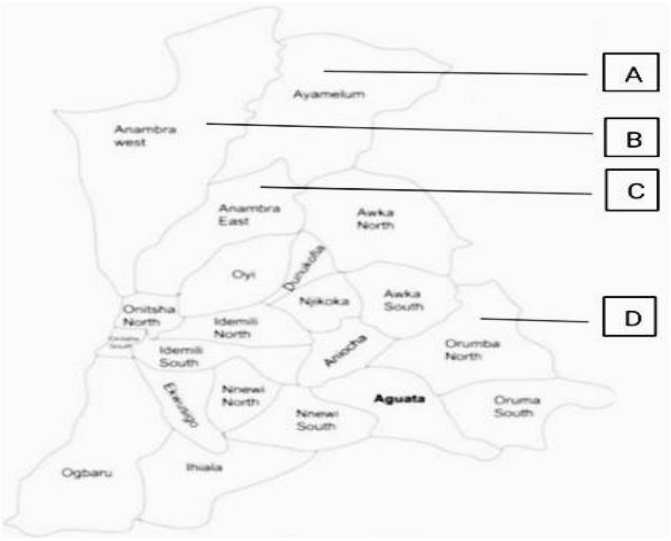

Figure 1: Map of Anambra State indicating the areas of study

3. RESULTS

Table 1. The results obtained after the analysis are presented below

Metals (mg/kg)	Nzam	Omor	Otuocha	Ufuma	PTWI (mg/kg)
Pb	0.0078 ± 0.0020	0.125 ± 0.0010	0.207 ± 0.0002	NO	0.025
Cu	NO	NO	NO	NO	3.5
Cr	NO	NO	NO	NO	0.0233
As	14.8889±0.0020	12.8333±0.0020	12.8889±0.0001	12.3333±0.0004	0.015
Zn	0.711 ± 0024	0.7474 ± 0.0003	1.593 ± 0.0013	0.2518 ± 0.0008	7
Fe	0.639 ± 0.0010	0.5352 ± 0.0001	0.6194 ± 0.0021	0.5709 ± 0.0010	EDI 1.042
Mn	0.0578 ± 0.0002	0.0104 ± 0.0003	NO	0.714 ± 0.0002	2.5
Cd	NO	NO	NO	NO	0.007
Co	NO	NO	NO	NO	MTWI 0.7
Ni	NO	NO	NO	0.0756± 0.0029	0.035

Lead (Pb)

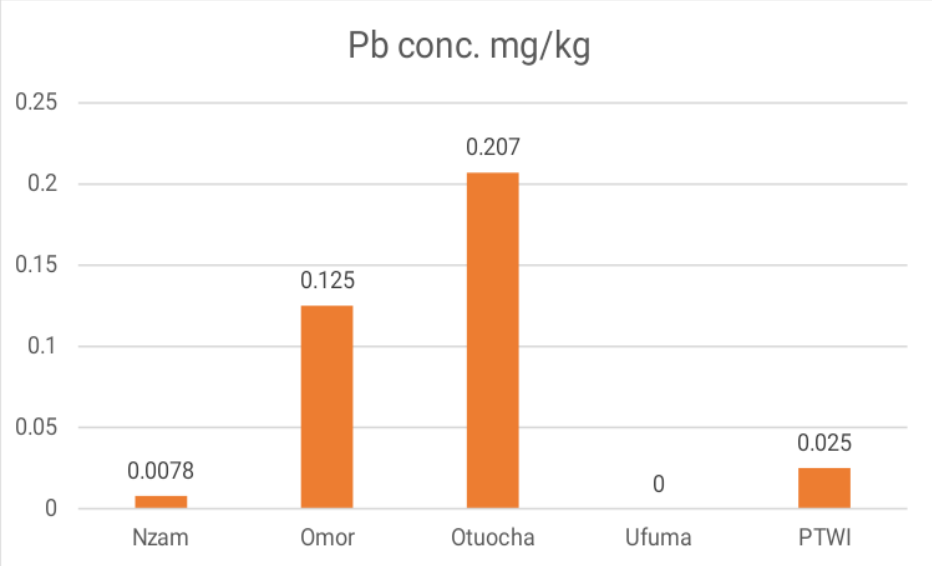

Figure 2: The concentration of Lead in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Lead in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Otuocha (0.207 mg/kg) has the most level of Lead in it, followed by rice grown in Omor (0.125 mg/kg), and finally that grow in Nzam 0.0078 mg/kg) as the smallest. There was no level of Lead in rice grown in Ufuma. Following PTWI, the standards given by the WHO/FAO which says that the allowable levels of Lead in rice sample is 0.025mg/kg (Cheng et al., 2006) and from the above table it is seen that only the rice grown in Nzam can be permitted by the WHO while the rice grown in

Otuocha and Omor cannot be accepted by the standards instructed by the WHO. (WHO/FAO, 1999).

Arsenic (As)

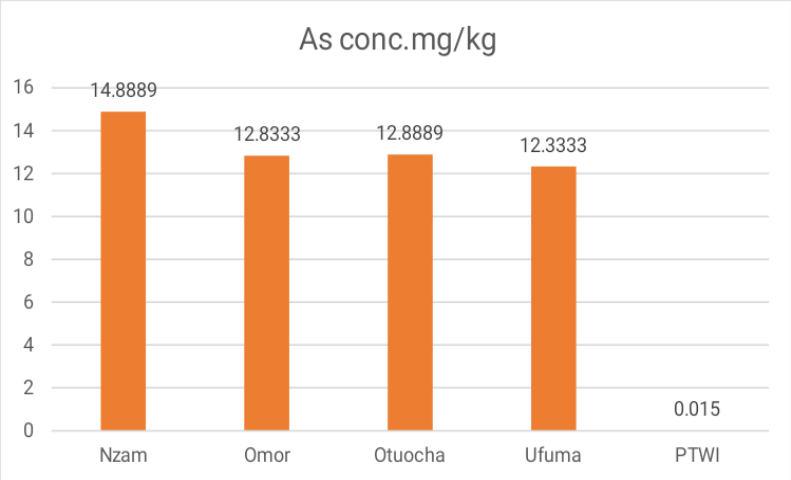

Figure 3: the concentration of Arsenic in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Arsenic in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Nzam (14.8889 mg/kg) has more levels of Arsenic observed in it, followed by rice grown in Otuocha (12.8889 mg/kg), that grown in Omor (12.8333 mg/kg) and finally rice grown in Ufuma (12.3333 mg/kg) as that which has the smallest levels of Arsenic in it. Arsenic was observed in all the rice samples gotten from the four towns. Following PTWI, the standards instructed by WHO/FAO which permits the levels of rice to be 0.015mg/kg (JECFA, 2011) and from the above table, it is seen that non of the rice grown in these four (4) towns can be permitted by the standard given by WHO.

Zinc (Zn)

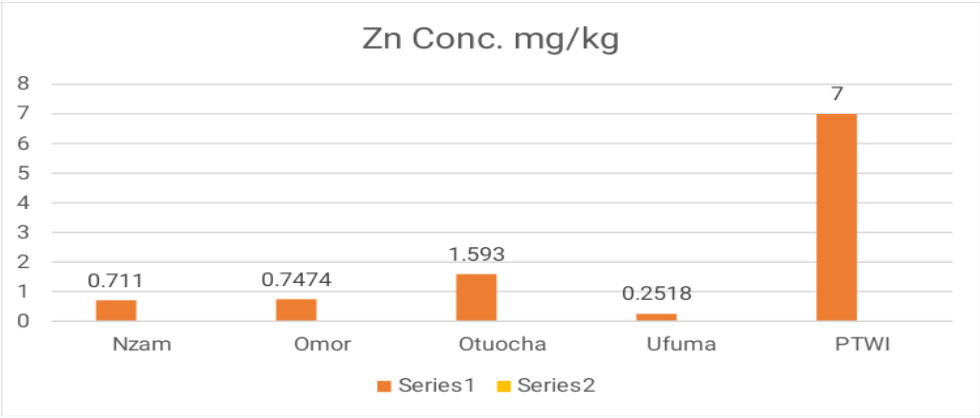

Figure 4: The concentration of Zinc in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Zinc in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Otuocha (1.5930 mg/kg) has more levels of Zinc observed in it, followed by rice grown in Omor (0.7474 mg/kg), that grown in Nzam (0.7110 mg/kg) and finally rice grown in Ufuma (0.2518 mg/kg) as that which has the smallest levels of Zinc in it. Zinc was observed in all the rice samples gotten from the four towns. Following PTWI, the standards instructed by WHO/FAO which permits the levels of rice to be 7 mg/kg (Machines, 2010) and from the above table, it is seen that all the rice grown in these four (4) towns can be permitted by the standard given by WHO.

Iron (Fe)

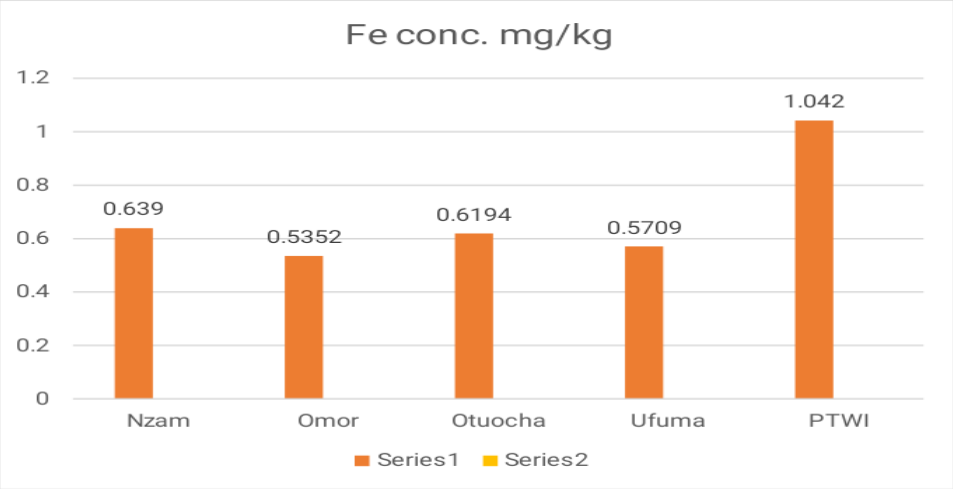

Figure 5: The concentration of Iron in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Iron in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Nzam (0.6930 mg/kg) has more levels of Iron observed in it, followed by rice grown in Otuocha (0.6194 mg/kg), that grown in Ufuma (0.5709 mg/kg) and finally rice grown in Omor (0.5352 mg/kg) as that which has the smallest levels of Iron in it. Iron was observed in all the rice samples gotten from the four towns. Following PTWI, the standards instructed by WHO/FAO which permits the levels of rice to be 1.042 mg/kg (Ezeofor et al., 2019) and from the above table, it is seen that all of the rice grown in these four (4) towns can be permitted by the standard given by WHO.

Manganese (Mn)

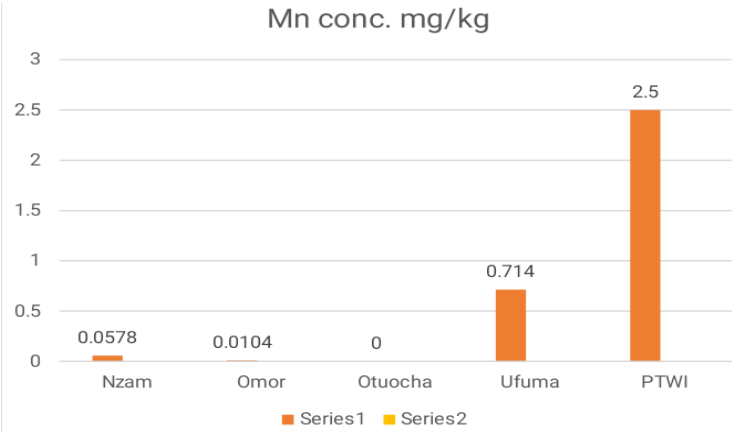

Figure 6: The concentration of Manganese in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Manganese in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Ufuma (0.714 mg/kg) has more levels of Arsenic observed in it, followed by rice grown in Nzam (0.058 mg/kg) and finally rice grown in Omor (0.0104 mg/kg) as that which has the smallest levels of Manganese in it. There was no observed level of Manganese in rice grown in Otuocha. Following PTWI, the standards instructed by WHO/FAO which permits the levels of Manganese in rice to be 2.5 mg/kg (Srinuttrakul et al., 2018) and from the above table, it is seen that all of the rice grown in these four (4) towns can be permitted by the standard given by WHO.

Nickel (Ni)

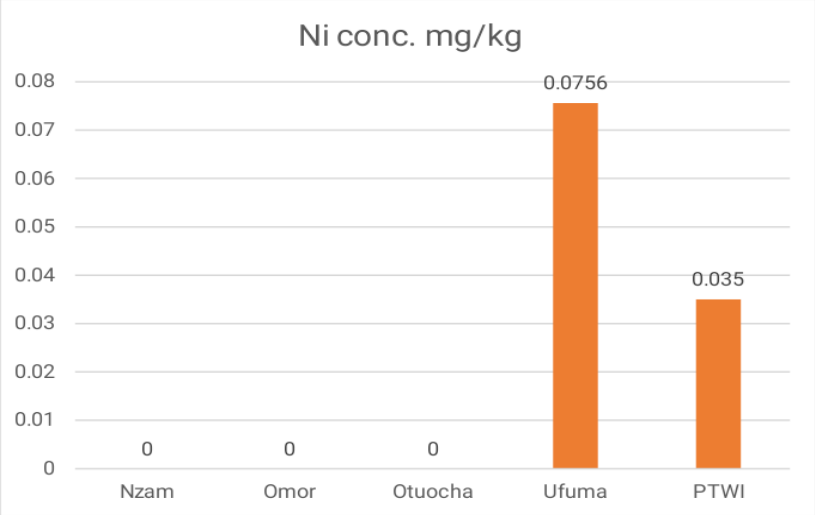

Figure 7: The concentration of Nickel in rice grain in Omor, Otuocha, Ufuma and Nzam of Anambra State.

From the chart above it explains the levels of Nickel in rice grown in Omor, Otuocha, Ufuma and Nzam. It also tells that the rice which is grown in Ufuma is the only rice that Nickel was observed in. Its level of Nickel is 0.0756 mg/kg as seen from the table and figure. Following PTWI, the standards instructed by WHO/FAO which permits the levels of rice to be 0.035 mg/kg (Naseri, 2014) and from the above table, it is seen that rice grown in Ufuma is not permitted by the standard given by WHO (1999).

Finally from the table above, it explains that there is no observed level of Cd, Cu, Cr, and Co in the rice grown in these four (4) towns (Nzam, Omor, Otuocha, Ufuma). Following the PTWI standards given by WHO/FAO which permits the level of Cadmium to be 0.007 mg/kg (Naseri et al., 2014), level of Copper to be 3.5 mg/kg (Srinuttrakul et al., 2018), level of Chromium to be 0.0233 mg/kg (Solidum et al., 2012) and Maximum Tolerable weekly Intake of Cobalt is 0.7 mg/kg (EVM, 2002).

4. CONCLUSION

The levels of some heavy metals (Co, Cu, Cr, Pb, Ni, Cd, Fe, Zn, Mn, As) in rice farm from Anambra State (Omor, Otuocha, Ufuma, and Nzam) in Eastern parts of Nigeria were investigated. And from the results, the Concentration level of heavy metals in the rice samples were not permitted by the WHO, we have come to a conclusion that the rice samples when eaten would cause harm to the human organs and this could lead to damages like the Kidney

failure, lungs failure, heart failure, cancer etc. So the study therefore encourages that these rice samples should always be monitored and the soil should be treated by using plants that have the ability to hyperaccumulate heavy metals that or crops uptake while growing. Also, the water supply used should be treated as well before use.

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