Special Conference Edition, April, 2022
http://dx.doi.org/10.4314/bajopas.v13i1.14S

Bayero Journal of Pure and Applied Sciences, 13(1): 80 - 86
ISSN 2006 – 6996

APPLICATION OF LINEAR DISCRIMINANT ANALYSIS FOR THE EVALUATION OF HEAVY METALS IN BROWN AND WHITE RICE (*Oryza sativa*) CULTIVATED IN KANO, NIGERIA

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ABSTRACT
Accumulation of toxic metals in locally harvested crops is of growing concern due to food safety and the associated health risks. This study evaluates the levels of some heavy metals (Pb, Cd, Fe, Cu and Mn) in white and brown rice samples locally cultivated in Kano using Atomic Absorption Spectrophotometry (AAS). Significant variations were observed (p<0.05), and found to be associated with the differences in rice type. Pb concentrations in all the rice samples analyzed were 0.291±0.024 to 0.699±0.152 mg/kg which surpassed the maximum limit set by FAO/WHO while other metals were below the safe limits, and Cd was not detected in all the rice samples analyzed. Linear discriminant analysis (LDA) revealed that white rice was much associated with Pb along canonical 1, while Fe and Mn were much associated with brown rice as confirmed by F- Ratio analysis. The presence of high levels of toxic metals beyond the maximum limits in the rice varieties might have originated from the fertilizer and other environmental sources which could be of health concern after consumption.
Keywords: Heavy Metal, LDA, Mineral, Pollution, Rice variety.

INTRODUCTION

Among the cereal crops cultivated, rice (*Oryza sativa*) is considered to be the third largest food harvested with an estimated global annual production of more than 2.0 billion tonnes (FAO/WHO, 2020). China is the world's leading producer of rice with an output of 142,000 kilotonnes (USDA, 2014). *Oryza glaberrima* (African rice) and *Oryza sativa* (Asian rice) are the two cultivated species of rice, and the most commonly available and grown specie in different parts of the world is the *Oryza sativa*. In Nigeria, rice is grown in all parts of the country but larger harvest comes from the northern parts. This is due to differences in soil type and fertility across the country in addition to rainfall (Sanni *et al.* 2005). Primarily, brown rice is the whole grain rice which has only the hull removed, but the layer that is rich in nutrients is retained which gives it the brown color and nutty flavor after chewing (Hansen *et al.* 2012). But in white rice, the bran, husk and germ are all removed and polished (Silva *et al.* 2013). That is basically the reason for the higher nutrients and minerals content in brown rice in comparable to the processed and polished white rice (Anjum *et al.,* 2007). Furthermore, Senadhira *et al.* (1998) highlighted that dissimilarity in the mineral contents depend upon the processing of rice, fertilizer used in rice cultivation and the quality of soil. In general, nutritionists and other researchers around the globe believed that people consumed higher amount of white rice in comparison to the brown rice. Rice contains various phytochemicals which are reported to have some health promoting potentials, including antioxidant and anticancer properties (Mao *et al.,* 2003; Min *et al.*, 2012). Minerals like calcium, magnesium, phosphorus are present along with some traces of iron, copper, zinc and manganese (Yousaf, 1992).

Apart from the essential mineral contents in rice, environmental pollution and poor agricultural practices introduces contaminants into the soil which are subsequently absorbed by the rice plant (Abrham and Gholap, 2021). Among the contaminants of concern are heavy metals which are toxic to both humans and plants (Koki and Jimoh, 2013; Rehman *et al.*, 2021). The contamination of agricultural soils with heavy metals is a growing concern to the researchers, government agencies, as well as the public due to the rising food safety issues and potential health risks (Yanez *et al.,* 2002).

Heavy metals such as cadmium, lead, and arsenic, are of primary concern in food particularly rice cropping system, because of their toxicity (Reeves and Chaney, 2001). These toxic elements accumulate in the soils, induce a potential contamination on food chain, and endanger the ecosystem safety and human health (Reynders *et al.,* 2008). Sources of heavy metals in soils are mostly from anthropogenic activities and in few cases from natural occurrence, derived from the parent materials. Anthropogenic inputs are associated with agricultural and industrial activities such as industrial effluents, vehicle exhaust, atmospheric deposition, urban effluent, fertilizer application, waste incineration, and the traditional application of sewage sludge as the fertilizer in agricultural land (Bilos *et al.,* 2001; Hlavay *et al.,* 2001; Koch and Rotard, 2001; Hammed and Koki, 2016). The dams, rivers and irrigation canals used for agriculture in the present study areas are linked to water sources across Kano state (Ahmad *et al*., 2018; Sangari, 2007). Plants are capable of absorbing substantial amount of heavy metals from the soil. After the adsorption process is saturated, more heavy metals are distributed in the aqueous phase thereby increasing the bioavailability (Mico *et al.,* 2006).

With the recent ban on the importation of foreign rice in to Nigeria, the production and consumption of locally produced rice has rapidly increased across the country. And considering the reported cases on the application of adulterated fertilizers, industrial wastes as manure, the environmental pollution across rivers and streams (Singh and Agrawal, 2010; Liu *et al*., 2011), and the increasing cases of heavy metal complications (Anyanwu *et al*., 2018), the present study focuses on the assessment of toxic heavy metals in locally produced white and brown rice varieties. The results obtained are further evaluated using multivariate statistical technique such as LDA to specifically identify the dominant heavy metals associated with each rice type.

The objectives of this study are to determine and evaluate the levels of heavy metals in white and brown rice cultivated in Kano, and to explore the relationship between the rice varieties and the associated heavy metals using chemometric approach.

MATERIALS AND METHODS

Sample collection and preparation

Locally cultivated rice samples were obtained from three geographical areas of Kano State (Table 1) in order to have a wider sample representation. Three samples of brown and white rice were obtained from Kura local government area (Kano south), Bagwai local government area (Kano north) and Yankura in Fagge local government area (Kano central) making a total of 18 samples. The samples obtained were stored in new polyethene bags and transported to laboratory for analysis (Zeng *et al.,* 2015).

Table 1: Sample locations and their coordinates

S/no	Sample code	Description	Coordinate
1	KBR	Kura brown rice	11°46′10.88″N, 8°25′19.15″E
2	KWT	Kura white rice	
3	BBR	Bagwai brown rice	12°09′42.61″N, 8°07′49.54″E
4	BWT	Bagwai white rice	
5	YBR	Yankura brown rice	12°00′42.72″N, 8°31′57.92″E
6	YWT	Yankura white rice	

Sample preparation

All the rice samples were dried in electric oven at 60 ℃ for 4 hr, followed by grinding into fine particles using mortar and pestle. Grinding was repeated until fine particles were obtained (Zeng *et al.,* 2015).

Sample digestion

About 0.5 g of the powdered rice samples was placed in Erlenmeyer flask and 10 ml of 65% HNO_3 (v/v) and 2 ml of 38% HCl (v/v) Analar were added. The samples were placed on hot plate and digested until a clear residue was obtained, diluted with deionized water and filtered using 0.45 micron Whatman filter paper. The resulting solution was analyzed for Fe, Mn, Pb, Cu and Cd using AAS. Blank solutions were prepared in triplicates using the acids and deionized water (Otitoju *et al.,* 2014).

Metal analysis

Atomic absorption spectrophotometer (Buck scientific world 210VGP) was used for the determination of heavy metal concentrations in the rice samples, blank solutions and certified reference materials.

Quality control

Quality control was practiced to ensure reliable results. All the plastic containers and glasswares were soaked in 10% HNO_3 (v/v), washed with detergent, and later rinsed twice with deionized water before drying in an oven. All the reagents used were of high analytical grade (Analar®), and deionized water was used in the analysis. CertiPUR® reference materials (Merck, Germany) were analyzed in triplicates for the validation of analytical results. The percentage recovery was calculated and compared with the certified values. Analyses of the results were carried out in triplicate, and the mean expressed at 95% confidence interval in mg/kg.

Data analysis

The results obtained in this study were processed using appropriate statistical techniques. The descriptive statistics, one-way ANOVA, and t-test were carried out using MS-Excel 2010, and statement reported at $p<0.05$. Multivariate statistical analysis was carried out using JMP Pro12. LDA was applied on the data set to discriminate the rice types based on the heavy metal concentrations (Idris *et al.*, 2017; Low *et al.*, 2011).

RESULTS

The analysis of certified reference material indicates good recoveries by agreement with the certified values in the range of 96 to 101% as shown in Table 2. The results of heavy metal analysis in white and brown rice are presented in Table 3 as mean and standard deviation.

Table 2: Analysis of certified reference material

	Cd	Pb	Mn	Fe	Cu
Certified (mg/l)	1002±2.0	1000±2.0	999±2.0	1000±2.0	1000±2.0
Measured (mg/l)	963.333	1005.080	992.160	1010.812	987.994
Recovery (%)	96.14	100.51	99.31	101.08	98.79

Table 3: Concentration of selected metals in local brown and white rice, mg/kg (wet weight)

Type	Cd	Pb	Mn	Fe	Cu
KBR	ND	0.314±0.156	0.332±0.029	0.359±0.097	0.318±0.023
BBR	ND	0.291±0.024	0.299±0.047	0.237±0.049	0.435±0.001
YBR	ND	0.566±0.099	0.250±0.006	0.302±0.157	0.358±0.044
KWT	ND	0.699±0.152	0.192±0.053	0.179±0.093	0.164±0.643
BWT	ND	0.595±0.206	0.287±0.038	0.293±0.039	0.132±0.038
YWT	ND	0.589±0.201	0.274±0.009	0.221±0.021	0.171±0.069
FAO/WHO (2004)	0.2	0.2	NA	NA	40

ND = Not detected, NA = Not available for rice

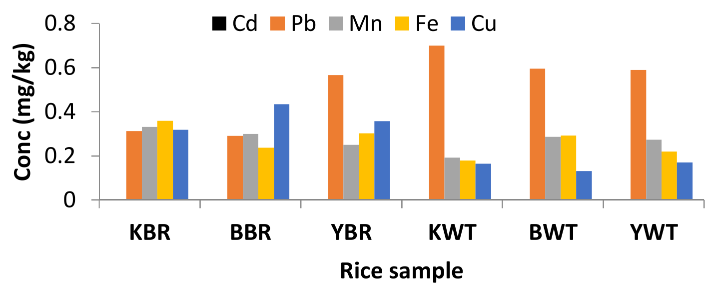

Fig. 1: Concentrations of heavy metals in brown and white rice

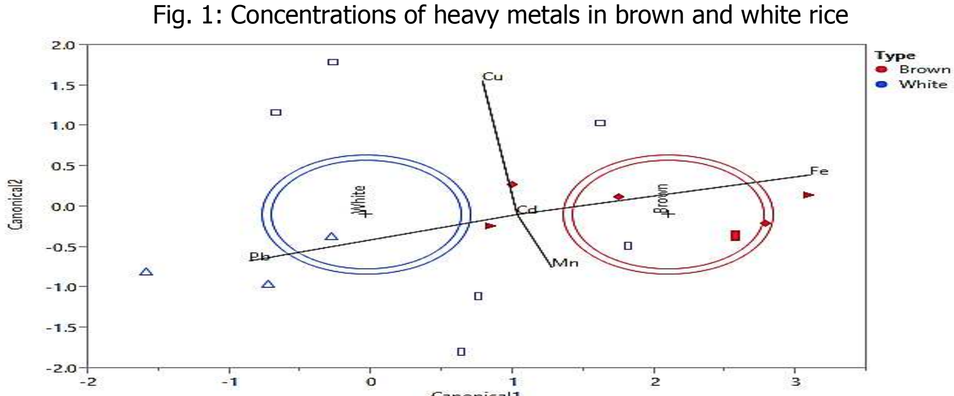

Fig. 2: Canonical plots for the discrimination of white and brown rice

Table 4: Analysis of discriminating variables in brown and white samples

Variable	F - Ratio	p - Value
Cd	0.000	1.000
Pb	5.986	0.037
Mn	0.096	0.765
Fe	7.460	0.023
Cu	0.112	0.746

DISCUSSSION

Lead

The lowest and highest mean concentrations of Pb in the rice samples analyzed were 0.291 and 0.699 mg/kg respectively for BBR and KWT as shown in Table 3. The concentrations of Pb in all the rice samples irrespective of its variety were found to be higher than maximum limit of 0.2 mg/kg (Fig 1) set by FAO/WHO (2004). Though findings on the Pb concentrations in rice varied greatly, Pb concentrations of 0.22 and 2.04 mg/kg were determined in rice grown in polluted area of China (Fu et al., 2008; Huang *et al*., 2013). Similarly, mean concentration of 0.62 mg/kg with a range of 0.26 - 1.73 mg/kg of Pb was detected in rice grown at sewage irrigation area in China (Wang *et al*., 2015). Elevated concentrations of Pb beyond the safe limit are threat to food safety of the consumers, and therefore of health risks to human health. The possible sources of Pb could be associated with contaminated agricultural soil, fertilizer, or industrial discharge into irrigation water among others (Huamain *et al*., 1999). FAO/WHO, 2017 further buttressed that occurrence of lead in food were mainly of environmental origin rather than associated with good management practice.

Manganese

The levels of Mn in brown rice were found to be in the range of 0.24 to 0.45 mg/kg with BBR having the highest mean concentration of 0.33 mg/kg as shown in Table 3. The lowest mean Mn concentration of 0.191 mg/kg was observed in KWT. It was reported that most of the minerals and vitamins in rice and other cereals are deposited in the husk and bran layers (Rathna Priya *et al.,* 2019). In similar study, variations in Mn concentrations were observed in four varieties of cereals (Tegegne, 2015), though no emphasis was given to Mn due to its importance in plants and human body, and high percent natural abundance. In this study, low Mn concentrations were determined in both white and brown rice samples.

Mn is an essential element required for various biochemical processes. The kidney and liver are the main storage places for Mn in the body. Mn is essential for the normal bone structure, reproduction and normal functioning of the central nervous system. Its deficiency causes reproductive failure in both male and female (Saraf and Samant, 2013).

Iron

The highest mean Fe concentration of 0.359 mg/kg with a range of 0.13 to 0.50 mg/kg was observed in KBR, while KWT was found to have the lowest mean concentration of 0.178 mg/kg. All the rice samples analyzed in this study were found to contain low Fe concentrations similar to a study carried out in Iran with 10% and 44% total Fe concentrations in brown rice and bran layer respectively (Ziarati and Azizi, 2013). Due to its importance in the human body, iron is required for a number of diverse cellular functions and metabolism. Fe is an essential element in humans and plays a vital role in the formation of haemoglobin, oxygen and electron transport in the body.

Copper

From the results presented in Table 3, it is clear that Cu concentrations varied with the variety of rice, brown rice contain higher Cu concentrations compared to white rice. The highest mean Cu concentration of 0.435 mg/kg was determined in BBR while the lowest mean Cu concentration of 0.132 mg/kg was detected in BWT. These results are much lower than maximum limit of 40 mg/kg set by FAO/WHO (2004). In a similar finding, 4.45 mg/kg of Cu was determined in rice samples grown in sewage irrigated soil (Wang et al., 2015). Xu *et al.* (2006) explained that sources of Cu in rice could be associated with contamination of soil from industries and other anthropogenic sources. Elevated levels of Cu beyond the safe limit could be of health concern when ingested.

Copper is one of the essential micronutrients and its adequate supply for growing plants should be ensured through artificial or organic fertilizers (Itanna, 2002). Cu is known to have a vital function in enzymatic activities and other metabolism in plant (Kabata-Pendıas and Pendias, 2001).

Cadmium

Cd was not detected in all the rice samples analyzed in this study. In similar finding by Otitoju *et al.* (2014), Cd was not detected in the rice samples. However, Shraim, (2014) reported lower Cd concentration in rice with a mean Cd concentration of 0.017 mg/kg and a range of 0.003 mg/kg to 0.046 mg/kg.

Furthermore, a study conducted in Iran by Ghazanfarirad *et al.* (2014) reported 0.022 mg/kg of Cd in rice. Comparably, Huang et al. (2013) detected 0.037 mg/kg of Cd in rice collected from Zhejiang, China which is below the safe limit of 0.2 mg/kg (FAO/WHO, 2004). Foods such as cereals, vegetables and starchy root tubers are known to contain lower cadmium concentrations, Cd levels detected in cereals may vary with cultivars or varieties due to differences in absorption rate and accumulation, and environmental influence of pollution sources (Rizwan *et al.,* 2016). Rice is known to accumulate high concentration of cadmium if grown on cadmium polluted soils, thus considered as one of the major sources of cadmium in humans (Wang *et al.,* 2015).

Cadmium exposure in humans has been linked with several adverse effects leading to illnesses notably amongst them being cancer (Ji *et al.,* 2012), it can also result to damages in gastrointestinal system in addition to bone, kidney, and lung disease (Jarup *et al.,* 1998).

Linear Discriminant Analysis (LDA)

Linear discriminant analysis was successfully applied on the data set which shows separation of the rice varieties based on the analyzed metals along the canonical 1 as shown in Fig 2.

The metals associated with brown rice are Fe and Mn, while Pb is very much associated with white rice samples. This indicates higher contamination of white rice samples with Pb and could reach toxic level with time after consumption. Elevated concentrations of Pb in rice samples could be associated with soil contamination due to anthropogenic activities (Wang *et al.,* 2003). Other possible source of heavy metals such as Pb in agricultural products has been linked to the use of inorganic fertilizer (TatahMentan *et al.,* 2020).

Similarly F-ratio values in Table 4 shows $p < 0.05$ for Pb (F- ratio = 5.986, P = 0.036), and Fe (F-ratio = 7.460, P= 0.023) indicating significant differences and dominance of the metals in the rice varieties analyzed. It is therefore clear that Fe and Pb make the largest contribution in the differences in the analyzed parameters between the white and brown rice.

CONCLUSION

The concentrations of Cu, Mn, and Fe in brown and white rice samples investigated in this study were low and not of concern or threat, with brown rice having the higher concentration of essentials elements (Mn, Fe and Cu) compared to white rice. Cd was not detected in all the rice samples, but concentrations of Pb above the maximum limit in all the rice samples under consideration indicates contamination of the locally cultivated rice samples which could reach toxic level with time. The higher association of Pb with the white rice is also of great health concern due to its increasing demand by the public consumers. It is obvious that Pb and Fe are the dominant variables that make the largest contribution in the differences between the white and brown rice. The agencies or institutions responsible for food regulations needs to periodically monitor the local food crops cultivated, the fertilizer and other chemicals used during farming activities for the safety of consumers.

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