ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Journal of Agronomy

ISSN 1812-5379
DOI: 10.3923/ja.2020.145.155

Research Article

Biofortification of Micronutrient in *Amaranthus cruentus* Using Time of Poultry Manure Incorporation in a Paleudult in Southeast Nigeria

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Abstract

Background and Objective: The quality of the plant products is critical for enhancing micronutrient balance in the soil-plant–animal-human food chain. This study evaluates the time of soil biofortification with Poultry Manure (PM) incorporation on the bioavailability of Cu, Fe, Mn, and Zn in edible plant parts of *Amaranthus cruentus* that will improve micronutrients and therefore, human nutrition. **Materials and Methods:** The experimental plots consisted of five intervals of biofortification using PM [1 and 2 WBT (weeks before transplanting), 0, 1 and 2WAT (weeks after transplanting)] arranged in RCBD with four replications. Data collected were: shoot height, shoot biomass yield, shoot micronutrient uptake, as well as the crude protein content of Amaranths. Data were statistically analyzed using ANOVA. **Results:** The results showed that the initial soil chemical properties were low to deficient in essential plant nutrients. Appropriate intervals of biofortification produced higher plant available micronutrients (Cu, Fe, Mn and Zn) content. Biofortification of soil with PM at 2 WBT significantly ($P<0.001$) increased shoot height, shoot biomass yields, micronutrient nutrition, as well as the crude protein content of Amaranths relative to other treatments. The maximum economic return obtained at 2WBT was adjudged to be in better synchrony between nutrient supply and crop demand. The yield difference can be economical on large scale *Amaranths cruentus* production. **Conclusion:** Timely intervals of biofortification using PM at two weeks before transplanting (2WBT) was recommended for improving micronutrients nutrition for human consumption of *Amaranths cruentus* cultivated in tropical rain forest ecological zone of southeastern Nigeria.

Key words: Amaranths, available micronutrients, biofortification, crude protein, micronutrient uptake

Citation: Eteng, E.U. and M.A.N. Anikwe, 2020. Biofortification of micronutrient in *Amaranthus cruentus* using time of poultry manure incorporation in a paleudult in southeast Nigeria. J. Agron., 19: 145-155.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Micronutrient deficiencies have persisted for decades because of the general reduction of the quality of less privileged people's diet, both in developed and developing countries[1]. Deficiencies of the micronutrients such as Cu, Fe, Mn and Zn, are a critical public health problem worldwide, with a negative impact on health, lifespan and productivity[2]. Human nutrition can be addressed either through mineral supplementation, food fortification, dietary diversification, or biofortification[1,3]. Biofortification is an agronomic way of improving human nutrition on a global scale[4-5]. Hence biofortification is considered as ideal biotechnology that can address mineral malnutrition along with yield enhancement of crops[1-2].

Plant foods such as staple food crops and vegetables comprise the most substantial part of the diet in Nigeria and the biofortification of both of these with micronutrients is an essential step in improving human nutrition and health[6]. The quality of the plant products is also critical for enhancing micronutrient balance in the soil–plant–animal–human food chain[7-8]. The soil-plant system, which is instrumental to human nutrition based on the "food chain" and improvements in this system resulting in better nutrient cycling, will contribute towards a better ecological environment[9]. This method has proved to be feasible, can be carried out at a relatively low cost, is efficient and has broad coverage, especially in the poor regions of the world[3,10].

Proper management of various organic manures is one way of improving the fertility status of agricultural soils[11]. Organic manures have been reported as essential sources of micronutrients of several farmlands[12] and contain a considerable amount of both macro and micronutrients for plants[13]. A long-term application of animal wastes (swine, poultry, or cattle manures) amendment[14,15] has been reported to increase Cu, Fe, Mn and Zn contents substantially in the surface soil (0-30 cm).

There are different types of poultry droppings, such as the deep litter manure, broiler manure, cage manure and high-rise manure[16]. The nutrient values of the poultry manures vary considerably depending upon several factors such as the source of manure, feed of animals, age and condition of animals, storage and handling and conditions under which it is processed[16-18]. The ratio of litter to manure and the moisture content causes considerable variation among manures from different poultry houses[16]. Accordingly, Amanullah *et al.*[16] and Ewulo *et al.*[19], reported that poultry manure contains about 3-5% nitrogen, 1.5-3.5 % phosphorous and 1.5-3.0 % potassium. In contrast, Eteng[11] reported that the poultry manure contains 14.60 mg kg^{-1} Cu, 15.02 mg kg^{-1} Fe, 21.07 mg kg^{-1} Mn and 13.78 mg kg^{-1} Zn mg kg^{-1} micronutrients. Poultry manure application improves soil retention, water movement and in fact, uptake of plant nutrients[20].

Generally, leafy vegetable crops are known to contain higher levels of micronutrients such as Fe and Mn whereas, the edible parts of roots/tuber crops and fruit vegetables typically contain lower levels of Zn and Cu[21,22]. However, vegetables such as *Amaranthus cruentus*, which belongs to the family Amaranthaceae, is one of the cheapest and widely cultivated vegetable crops in Nigeria[23]. It has a growing period of 5 to 6 weeks, thus making it an advantage for the rural and peri-urban farmers in Nigeria to keep cultivating it two or more times on the same piece of land in a year[24]. The world average yield of amaranth was estimated at 14.27 t ha^{-1} [25] while, the yield per hectare in Nigeria was reported to be as low as 7.60 t ha^{-1} compared to what obtains in the United States (77.27 t ha^{-1})[20]. Nigeria, for instance, ranks as the largest producer and consumer of *Amaranthus* spp. in Africa[23].

A significant constraint to intensive and extensive production of major vegetable crops in Nigeria is the low level of soil fertility, mostly characterized by acidic, sandy and pronounced soil degradation, strongly weathered and inherently weak in basic cations such as available micronutrients (Cu^{+2}, Fe^{+3}, Mn^{+4} and Zn^{+2}) for crops use[26]. To improve the soil fertility status, the use of organic manures as biofertilizers, which has proved to be a sound fertility management strategy in many countries of the world has been, advocated[25]. The objectives of this present study, therefore, was to determine the nutritional content of Cu, Fe, Mn and Zn in soils and shoots of *Amaranthus cruentus* vegetable and to compare the nutritional content of Cu, Fe, Mn and Zn in the plant shoots with results of the available content obtained in soils treated with timely biofortification.

MATERIALS AND METHODS

Soil characterization: The experiment was carried out for three successive cropping seasons (onset of the dry season in November 2017; dry season in early February 2018 and onset of raining season in late May 2018) at the Michael Okpara University of Agriculture, Umudike, Nigeria. The area lies between latitude 05° 9'N and longitude 07° 33'E with an altitude of 122 meters above sea level). The experimental area falls within the humid tropical zone of southeastern Nigeria, characterized by a tropical wet season from May to October

Table 1: Total and mean ten years meteorological data of the experimental site during the 2007-2018 cropping seasons*

Month	Rainfall (mm)	Temperature (°C)	Relative humidity (%)	Sunshine duration (hrs/day)	Wind speed (km/hr)	ETo (mm/day)
Mean total	2252.95	345.45	838.21	51.94	109.1	42.45
G. Mean	187.75	28.79	69.02	4.23	9.09	3.59

ETo = Reference crop evapotranspiration, Source: *NRCRI weather report[48]

and the dry season from November to April[27,28]. The mean annual rainfall in 2007-2018 (Table 1) was 2650 mm, characterized by an average rainfall of 187.75 mm, distributed in a bimodal pattern, starting in April, with peaks in July and in October. During the first two months of the rainy season up to the end of October, the rain comes as violet thunderstorms. The monthly temperatures were high and changed only slightly during each year, being generally highest in February to April. The monthly minimum and maximum temperatures ranged from 25 to 33°C, with a mean of 27.45°C. The temperature rarely falls below 21°C except during the 'harmattan' weather (December to January). The relative humidity is generally highest (89.07 %) at the peak of rains (July and October) and lowest (41.79 %) during the dry seasons (January to March) period. The sunshine duration of the experimental area in 2018 was highest in February, April, November and December, with a maximum sunshine duration, which ranged from 5.08 -5.97 hours/day. The lowest sunshine duration was recorded in July, August, September and October, with a minimum sunshine duration, which ranged from 2.27-3.3.48 hours/day. The study was conducted from a site previously cultivated with maize, assorted vegetables, cucumber, garden egg, carrot, pepper, cabbage and lettuce, from the 2007-2017 cropping seasons. The experimental soil at Umudike is an Ultisol and is classified as Typic Paleudult (Soil Survey Staff, 2010). Table 1 shows the mean total and geometric mean meteorological data of the experimental site during the 2017/2018 cropping seasons.

Field methods: The land was cleared, ploughed and harrowed with a Tractor, following which vegetable beds were prepared manually. The land measured 20 m x 20 m, given an experimental area of 400 m^2 (0.0400 ha). The total land area of 400 m^2 was partitioned into four blocks each measuring 4.0 × 20 m and each block was further divided into five experimental plots, with each plot (vegetable bed) measuring 4 × 4 m (16.0 m^2) and, 1.0 m paths within plots and between blocks were marked out. In all, there were a total of 20 experimental plots. The Amaranths seeds were obtained from the Department of Agronomy, Michael Okpara University of Agriculture, Umudike (MOUAU). The poultry manure used was obtained from the Animal Farm Unit of the University. Before field application, the poultry manure was air-dried and ground to prevent rapid decomposition, loss of nutrients and, to improve the physical characteristics of the organic manure.

The plots were laid out as a Randomized Complete Block Design (RCBD) with four replicates. There were five experimental treatments consisting of sequential time of poultry manure incorporation: (1) 2WBT (two weeks before transplanting), (2) 1WBT (two weeks before transplanting), (3) 0WAT (at transplanting), (4) 1WAT (two weeks after transplanting) and (5) 2WAT (four weeks after transplanting), on each plot to encourage rapid mineralization of the poultry manure before and after transplanting of the seedlings.

Fifteen tons per hectare (15.0 t ha^{-1}) of poultry manure was applied by broadcasting on the respective experimental plots and incorporated into the soil using a garden fork and spade at each sequential time (2WBT, 1WBT, 0WAT, 1WAT and 2WAT) application, respectively. Before the transplanting of the seedlings, the plots were irrigated twice; in the morning and evening with the use of a watering-can. The seedlings were transplanted to the test plots at a spacing of 10 cm within plots in a row and 20 cm between rows to give 15 rows per plot, 600 plants per plot and 100,000 plants per hectare. The poultry manure was applied only in the 1st cropping season and none was applied during the second and third cropping sequence.

Observation and data collection: The agronomic data collected were measured throughout the three cropping sequences and include the shoot height and shoot biomass yield of *Amaranthus cruentus* which, commenced at three weeks after transplanting of seedlings and these were carried out at two weeks intervals starting from 3WAT to 7WAT, respectively. Five plants were selected randomly and tagged with a label from each row for data on shoot height and total shoot biomass weight. Shoot height was determined using a meter rule as the height from the base of the crop to the tip of the plant. The shoot biomass weight was measured using an electrical weighing balance. A sample of the poultry manure was air-dried, crushed to pass through 2 mm sieve and analyzed for N, K, Ca, Mg, Na, Org C, Cu, Fe, Mn and Zn. Before the land preparation, five random soil samples were taken from the experimental site, using soil auguring equipment at a depth of 0-20 cm and designated initial soil samples. The initial soil samples were thoroughly mixed to make a composite sample. In contrast, the second soil samples were collected at the end of the 3rd cropping according to the biofortified poultry manure. Both the initial and the biofortified soil samples were air-dried, ground and sieved with 2 mm sieve to remove materials greater than 2 mm in diameters. The soil samples were subjected to chemical analysis using standard procedures. The plant micronutrients uptake in the shoot of *A. cruentus* were determined by multiplying the concentration of shoot micronutrients and the shoot dry matter yield (Nutrient concentration x DMY). The total N values in the shoot of *A. cruentus* were converted into crude protein by multiplying by a factor, 6.25. Separate samples were digested using the nitric-perchloric acid mixtures. All micronutrient concentrations were expressed in mg kg^{-1} DW.

Laboratory analysis: The soil samples of the experimental site were analyzed according to standard procedures. Soil pH was determined in a 1:2.5, using the soil: water and $CaCl_2$ suspension method[29]. Soil organic carbon was measured using the wet oxidation colorimetric method[30]. Organic carbon was converted to organic matter by multiplication, utilizing a factor of 1.724 (Van Bemmelen factor). Total nitrogen was analyzed using the modified macro-Kjeldahl digestion and distillation procedure described by Bremner[31]. Exchangeable bases were determined using the 1 M acidified ammonium acetate (NH_4OAc) methods[32]. The available micronutrients (Cu, Fe, Mn and Zn) were determined after extraction of the amended soil samples with 0.00MEDTA (disodium salt at pH 4.0) solution[33] after shaking for 120 min and the filtrate was read on UNICAM model SOLAAR 32: Astm D1691 AAS. The total elements were analyzed after digestion of dry and milled plant material with sulphuric acid, nitric acid and perchloric acid[34]. The Cu, Fe, Mn and Zn concentrations in the filtrate were determined by atomic absorption spectrophotometer (UNICAM model SOLAAR 32: Astm D1691).

Statistical analysis: The data collected were analyzed using the ANOVA (Analysis of variance) procedure, using the general linear model. Significant means were separated using F-LSD (Fisher's Least Significant Difference) at $P<0.05$ (two-tailed). Also, the Pearson correlation was used to evaluate the association between soil available micronutrients and micronutrients uptake in the shoot of *A. cruentus*. The statistical analyses were executed using the PASW Statistics software, version 18, for Windows 7.0.

RESULTS AND DISCUSSION

Properties of the soil and poultry manure used for the experiment: Table 2 shows the data on initial soil properties, which indicated that the soils were strongly acidic, low in organic matter (SOM) content and exchangeable cations (Ca^{+2}, K^{+1}, Mg^{+2}, Cu^{+2}, Fe^{+3}, Mn^{+4} and Zn^{+2}). It was evident from the data that the initial soil micronutrients were deficient while the final soils fortified with poultry manure had adequate plant nutrients (Table 2). Similar results were reported[11,13]. Also, the composition of the poultry manure suggests that the

Table 2: The initial, final soil properties and composition of the poultry manure used for the experiment

	Soil characteristics		Composition of poultry manure	
Properties	Initial soil test level	Soil test level after the third cropping	Composition	Manure test level
Chemical composition				
pH ($CaCl_2$)	4.68	5.02	pH ($CaCl_2$)	8.37
Organic matter (g kg^{-1})	8.25	12.11	Organic matter (%)	26.2
-	-	-	Moisture (%)	22.34
-	-	-	Ec (1:2.5)	36.54
Nutrient composition				
Total N (g kg^{-1})	1.14	1.32	Nitrogen (N %)	1.23
Cellulose	-	-	Cellulose	6.18
Exchangeable K (Cmol kg^{-1})	0.06	1.21	Potassium (%)	1.9
Exchangeable Ca (Cmol kg^{-1})	1.25	2.32	Calcium (%)	2.7
Exchangeable Mg (Cmol kg^{-1})	1.03	1.26	Magnesium (%)	1.37
Available Cu (mg kg^{-1})	0.29	13.31	Copper (%)	3.53
Available Fe (mg kg^{-1})	1.94	15.92	Iron (%)	4.54
Available Mn (mg kg^{-1})	0.62	16.49	Manganese (%)	3.75
Available Zn (mg kg^{-1})	0.23	9.49	Zinc (%)	2.93

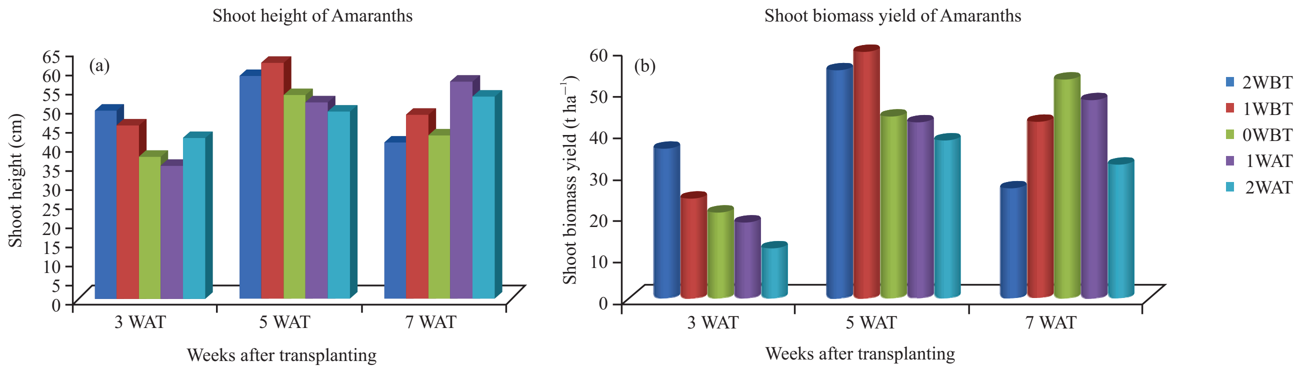

Fig. 1(a-b): Mean Effect of time of biofortification of poultry manure on (a) shoot height and (b) shoot biomass yield of *Amaranths cruentus* for three cropping seasons

Table 3: The relationship between soil bioavailable and uptake of micronutrients in plant shoot of *A. cruentus*

Soil and plant components	Soil OM	Soil micronutrients Cu	Fe	Mn	Zn	Crude protein	Micronutrients uptake in plant shoot Cu	Fe	Mn	Zn
Soil OM	1									
Soil Cu	0.427^{ns}	1								
Soil Fe	0.698*	0.535*	1							
Soil Mn	0.712*	0.983**	0.960**	1						
Soil Zn	0.664*	0.656*	0.852**	0.992**	1					
Crude Protein	0.756*	0.928**	0.983**	0.676*	0.990***	1				
Plant Cu	0.427^{ns}	1.000***	0.635*	0.983**	0.656*	0.928*	1			
Plant Fe	0.698*	0.735*	1.000***	0.960**	0.702*	0.802*	0.775*	1		
Plant Mn	0.712*	0.983**	0.960**	1.000***	0.992***	0.974**	0.983**	0.960**	1	
Plant Zn	0.754*	0.686*	0.852**	0.992***	1.000***	0.990***	0.696*	0.695*	0.992***	1

^{ns}: Not significant, *Correlation is significant at the 0.5% level (2 tailed), **Correlation is significant at the 0.01% level (2 tailed), ***Correlation is significant at the 0.001% level (2 tailed)

manure was alkaline and saline, with an adequate supplying factor of the essential nutrient minerals. The analysis confirmed the results reported by Aluko and Oyedele[35].

Effect of biofortification time on shoot height and shoot biomass yield of *Amaranthus cruentus*: The mean shoot height and shoot dry weights of amaranths for the three cropping seasons are presented in Fig. 1. The time of biofortification significantly influenced the shoot height of *Amaranths cruentus,* which increased from a growth stage of 3WAT to 7WAT. At a growth stage of 3WAT, the taller plants were found when poultry manure was biofortified at 2WBT whereas, the shorter plants were found at 1WAT, with a mean shoot height of 41.65 cm. At the growth stages of 5WAT, the plants were taller when poultry manure was biofortified at 1WBT while the application of poultry manure at 2WAT produced shorter plants. Similarly, at the growth stage of 7 WAT, taller plants (56.54 cm) were found at 1WAT and, shorter plants (40.75 cm) were found at 2WBT, with a mean of 48.06 cm. Similar results were reported[24].

The mean shoot biomass yield of the Amaranths for the three cropping seasons are also presented in Fig. 1. The values of shoot biomass yield varied significantly (p=0.05) and increased from growth stages 3WAT to 7WAT. The results followed the same trend as that of shoot height. At the growth stage of 3 WAT, the highest shoot biomass yield was highest (36.12 t ha^{-1}) when poultry manure was biofortified at 2WBT and was lowest (12.07 t ha^{-1}) at 2WAT, with a mean of 20.26 t ha^{-1}. At 5WAT, shoot biomass yields were highest (59.32 t ha^{-1}) when poultry manure was biofortified at 2WBT while the application of poultry manure at 2WAT gave lower shoot biomass yields (37.76 t ha^{-1}) with a mean of 47.70 t ha^{-1}. Also, at the growth stage of 7 WAT, the highest shoot biomass yields (50.62 t ha^{-1}) were determined at the 2WBT while the lowest shoot biomass yields (26.65 t ha^{-1}) was recorded at 2WAT with, a mean of 40.02 t ha^{-1}.

Generally, at 2WBT, higher values of shoot height and shoot biomass yield (Fig. 1) were obtained, whereas, at 2WAT, the least values were produced. The result is also consistent with the shoot nutrient uptake (Table 3) for these treatments. This result can be attributed to the synchrony in the time of availability of a sufficient amount of nutrients from poultry manure in the soil proportional to the demand of the plant for nutrient uptake. Thus, biofortifying poultry manure beyond 2WAT will perhaps be wastage as the Amaranths may not have the capacity to use the nutrients in any economical amount at this stage of its growth. Makus[21], Iren *et al.*[36] and Maerere *et al.*[37], reported similar trends on other major cations. Moreover, this result is corroborated by that of Adekiya and Agbede[6] and Ndukwe *et al.*[38], who reported that synchrony between crop demand and nutrient supply is necessary to improve nutrient use efficiency and better growth of plants. However, to obtain maximum economic values of the plant nutrients from biofortification, it should be fortified at the right time to meet the nutrient need of the crop[38].

Time of biofortification on soil organic matter and bioavailable micronutrients (Cu, Fe, Mn and Zn) in soil planted with *Amaranthus cruentus*

Time of biofortification on organic matter content in soils treated with poultry manure: A significant variation of the soil organic carbon as influenced by time of biofortification was observed and presented in Fig. 2. On the average, soil organic matter content decreased from the sequence of cropping from first cropping (21.09 g kg^{-1}) to the third cropping (9.33 g kg^{-1}) which is not visibly shown in the Fig. 2 presented. The decrease may be attributed to the rapid mineralization of poultry manure due to soil tillage and sequence of cropping[21, 37]. With regards to the intervals of biofortification, the content of soil organic matter was highly significant (P<0.01) and varied widely from 9.58 g kg^{-1} to 20.29 g kg^{-1} and these were obtained at 2WBT and 2WAT, respectively with an average content of 14.92 g kg^{-1}. A similar trend of the result was reported in[21, 38-40].

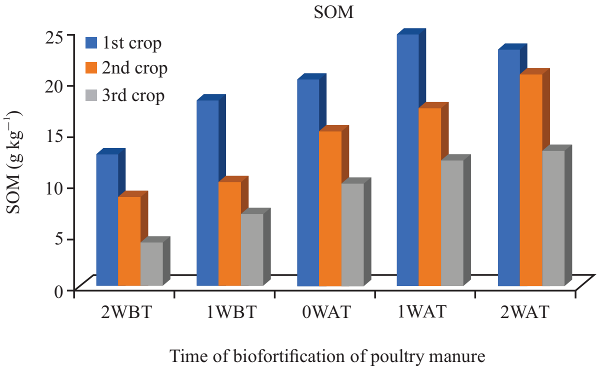

Fig. 2: Time of biofortification and cropping seasons on Soil Organic Matter (SOM) planted with *Amaranthus cruetus*

Time of biofortification on bioavailable micronutrients (Cu, Fe, Mn and Zn) in soil planted with *A. cruentus*: There was highly significant variation of the bioavailable micronutrients in the soil as influenced by the time of biofortification which decreased from the 1st to the 3rd cropping season, respectively (Fig. 3a-d). With regards to the time of biofortification, the content of bioavailable Cu varied significantly (P = <0.01) and ranged from 8.65 mg kg^{-1} at 2WBT to 19.53 mg kg^{-1} at 2WAT with an average of 13.63 mg kg^{-1}. The concentration of Cu in soil decreases from first cropping (16.49 mg kg^{-1}) to the third cropping (8.93 mg kg^{-1}). The content of bioavailable Fe obtained was equally significant ($P < 0.05$) and varied from 12.66 to 19.30 mg kg^{-1} at 2WBT and 1WAT, apiece, with a mean of 16.12 mg kg^{-1}. As indicated in Fig. 3b, bioavailable Fe content decreased from first cropping (19.13 mg kg^{-1}) to the 3rd cropping (11.92 mg kg^{-1}). Similarly, Fig. 3c, shows that levels of bioavailable Mn obtained varied significantly (P <0.01) and ranged between 12.71 and 19.45 mg kg^{-1} at 2WBT and 1WAT, respectively, with a mean of 18.70 mg kg^{-1}. The concentration decreased from first cropping (21.57 mg kg^{-1}) to the 3rd cropping (10.82 mg kg^{-1}).

Values of bioavailable Zn significantly differed (P <0.01) among the intervals of biofortification and varied widely between 7.12 and 12.35 mg kg^{-1}. These were determined from 2WBT and 2WAT, respectively, with an average of 10.67 mg kg^{-1}. According to the sequence of cropping, bioavailable Zn content decreased from 13.26 to 6.89 mg kg^{-1} in the first and third cropping, respectively. The values of bioavailable micronutrients determined in this study were within the range of values previously reported[12, 41-42].

The higher values of the soil micronutrients found at 2 WBT compared to other times of biofortification were probably because decomposition and mineralization of poultry manure led to early nutrients release and use by the Amaranths compared with biofortified plots at 2WAT. Since the Amaranths vegetables are short duration crops, the application of poultry manure at 2WAT may not be useful to the plant. It implies that the time of the release of the nutrients from the biofortification, the phonological stage for the need for the nutrients, would have passed. A similar trend of results was reported[6,43]. Generally, the order of soil bioavailable micronutrients obtained was: 2 WBT > 1 WBT > 0 WAT > 1 WAT > 2WAT in the decreasing order of time of biofortification. Which implied that biofortification at 2 WBT has the highest values of extractable micronutrients while at 2WAT, the least content of extractable micronutrients was determined.

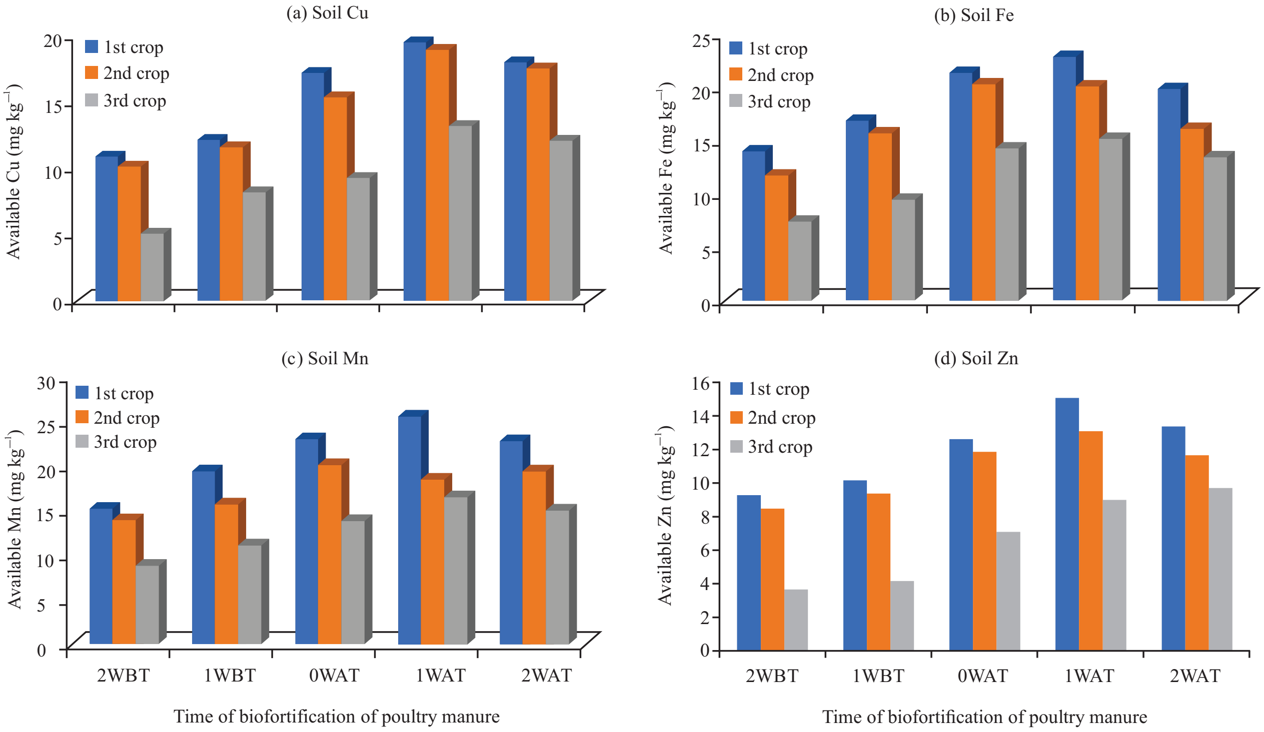

Fig. 3(a-d): Time of biofortification and cropping seasons on extractable (a) Cu, (b) Fe, (c) Mn and (d) Zn concentration planted with *Amaranthus cruetus*

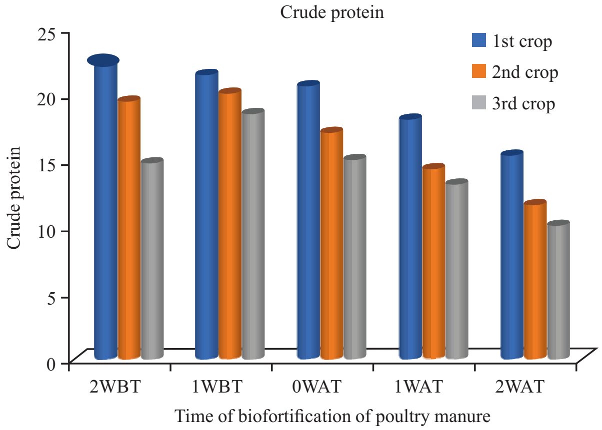

Fig. 4: Time of biofortification and cropping seasons on crude protein in shoots of *A. cruentus*

The observed decreases in the bioavailable micronutrients from first cropping to the third cropping according to intervals of biofortification with poultry manure were expected in this study. This phenomenon may be because the first cropping commenced during the onset of the dry season (November). The second was during the dry season (February), whereas the third was conducted during the onset of the rainy season (March). This suggests that, during the third cropping, there was a complete rapid mineralization rate of the poultry manure leading to the loss of the balance of nutrients from the first and second cropping coupled with, leaching of nutrients, uptake by plants and sorption mechanism[21,37,39,43].

Effect of time intervals of biofortification of poultry manure on crude protein and micronutrients uptake in plant shoot of *Amaranthus cruentus*

Crude protein in plant shoots of *Amaranthus cruentus*: Significant variations in crude protein in the leaf of amaranths were observed among the different time of biofortification for three cropping sequence (Fig. 4). In Fig. 4, the mean crude protein of amaranths for the three cropping seasons varied significantly ($P<0.01$), decreasing from 22.43 to 10.56 in the first and second cropping respectively, with an average of 15.51. According to the time of biofortification, the crude protein obtained in the shoot of *A. cruentus* varied from 13.71 to 17.68 at 2WAT and 1WBT, respectively.

Micronutrients (Cu, Fe, Mn and Zn) uptake in plant shoots of *Amaranthus cruentus*: Highly significant ($P<0.001$) variation of micronutrients (Cu, Fe, Mn and Zn) uptake in plant shoots of *A. cruentus* were affected by the time of biofortification for three sequences of cropping (Fig. 5a-d). The Cu content in shoots of *A. cruentus* varied widely but decreased from 2.36

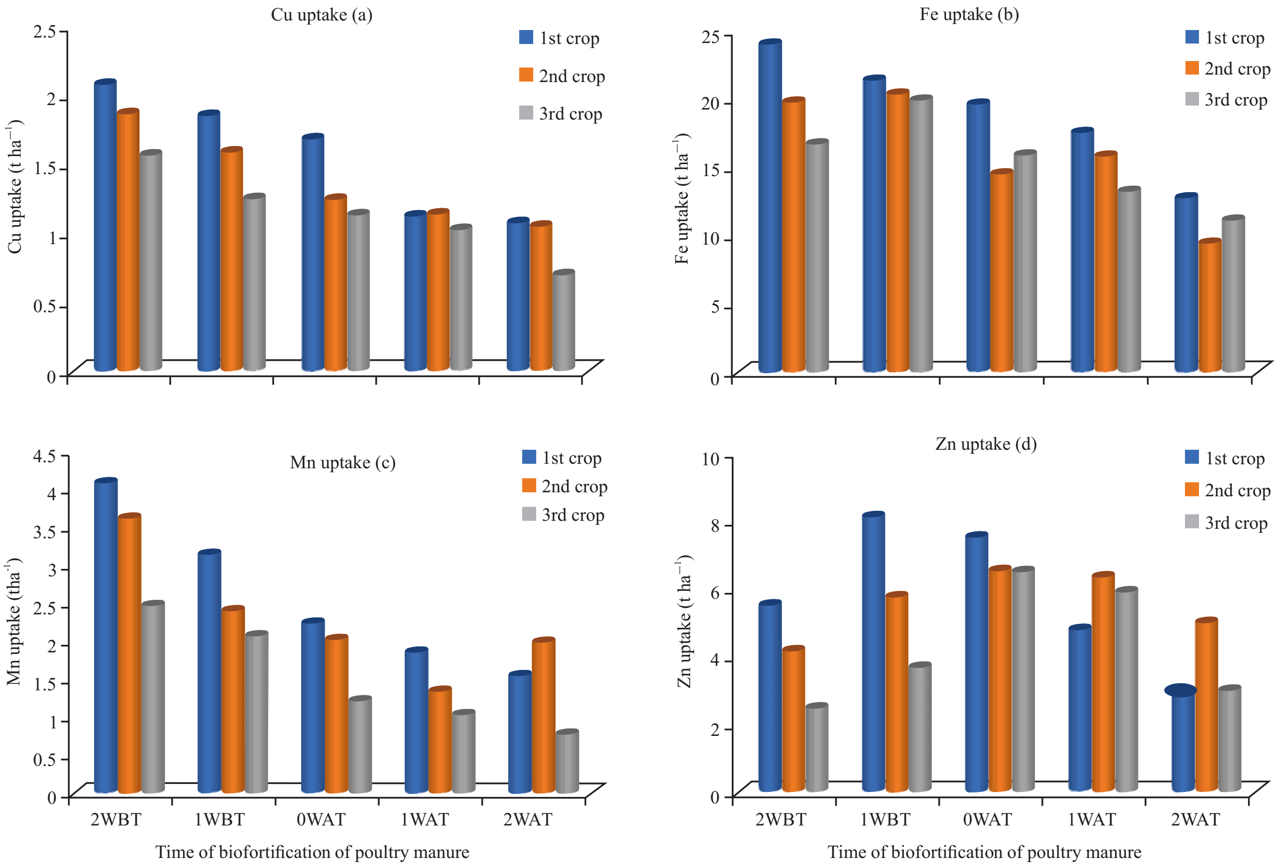

Fig. 5(a-d): Time of biofortification and cropping seasons on (a) Cu, (b) Fe, (c) Mn and (d) Zn uptake in shoots of *A. cruentus*

to 1.48 t ha^{-1} and these were obtained from the first and second cropping, respectively, with an average of 1.90 t ha^{-1}. According to the intervals of biofortification, Cu content in the shoot of *A. cruentus* varied from 1.76 to 2.07 t ha^{-1} and these were recorded from 1WBT and 1WAT, respectively. Iron uptake determined in the shoot of *A. cruentus* varied widely but decreased from 20.95 to 13.58 t ha^{-1} and these were determined from the first and second cropping, respectively, with a mean of 17.10 t ha^{-1}. Similarly, Fe uptake ranged from 14.14 to 20.19 t ha^{-1} and determined from 2WAT and 1WBT, respectively.

Concerning the cropping sequence, the highest mean value of Mn uptake (2.72 t ha^{-1}) was obtained at the 1st cropping, while the lowest Mn uptake (1.32 t ha^{-1}) was determined at the 3rd cropping with an average of 2.04 t ha^{-1}. According to the intervals of biofortification, the highest mean value of Mn uptake (2.51 t ha^{-1}) was recorded from 0WAT, while the lowest Mn uptake (1.45 t ha^{-1}) was determined at WAT.

Zinc uptake determined in shoots of *A. cruentus* varied widely but decreased from 9.34 to 5.31 t ha^{-1} and these were determined at the first crop and third crop, respectively, with an average of 7.33 t ha^{-1}. Accordingly, higher content of Zn uptake (8.34 kg ha^{-1}) was found at 1WAT, whereas, the lower content of Zn uptake (6.49 t ha^{-1}) was found at 1WBT.

Generally, significant higher crude protein and shoot micronutrient nutrition were observed at 2WBT. This was attributed to the fact that the nutrients in the poultry manure were mineralized first and nutrients were released to the amaranth's plants at the right time that the plant needs it for its growth and development. In this experiment, there was scarcely any synchronization between soil nutrients and crop demand at 2WAT treatment. This result is corroborated by that of Adekiya and Agbede[6], who reported that synchrony between crop demand and nutrient supply is necessary to improve nutrient use efficiency and better growth of plants. A similar trend of results and findings were reported[38, 43]. The result was also at par with Kolawole[44], who reported that biofortification at two weeks before planting improved grain yield and nutrient uptake compared with the biofortification at two weeks after planting.

Correlation matrix between soil available micronutrients and micronutrients nutrition in plant shoot: The correlation matrix between soil available micronutrients and micronutrients nutrition in *A. cruentus* is presented in Table 3. In these relationships, content of soil available Cu had significant positive relationship with Cu uptake ($r < 1.000^{***}$), Fe uptake ($r < 0.735^{*}$), Mn uptake ($r < 0.983^{**}$) and Zn uptake ($r < 0.686^{*}$). Content of soil available Fe had significant positive relationship with Cu uptake ($r < 0.635^{*}$), Fe uptake ($r < 1.000^{***}$), Mn uptake ($r < 0.960^{**}$) and Zn uptake ($r < 0.852^{**}$). Content of soil available Mn had significant positive relationship with Cu uptake ($r < 0.983^{**}$), Fe uptake ($r < 0.960^{**}$), Mn uptake ($r < 1.000^{***}$) and Zn uptake ($r < 0.992^{**}$). Content of soil available Fe had significant positive relationship with Cu uptake ($r < 0.656^{*}$), Fe uptake ($r < 0.702^{*}$), Mn uptake ($r < 0.992^{**}$) and Zn uptake ($r < 1.000^{***}$). The high significant positive correlations of the EDTA extractable micronutrients in biofortified amended soils with the micronutrient uptake may be explained by the fact that there occurred synergistic chemical interactions amongst them[1-2, 45].

The correlation among the metals (Table 3) equally may explain their relationships or interactions either as synergistic (positive Effect) or antagonistic (negative effect) effects in improving their nutritional status in plant tissue, which also showed that they were affected by similar factors. Many complex interactions among the micronutrients are observed within plant tissues, particularly in the uptake-transport mechanism. For instance, the interaction of soil available Cu with Zn nutrition indicates that the same mechanism absorbs these metals[46]. If there was any negative interaction among them, it unexpectedly shows their antagonistic interference, which further suggests that the uptake of one metal may competitively inhibit root absorption of the other[47]. For example, the Fe-Zn antagonism is widely known to adversely affect the translocation of each metal from roots to the top of crops. This type of mechanism is similar to the depressing effects of other metals on Fe uptake[48]. However, an excess of available Zn in soil may lead to a marked reduction of Fe concentration in plants. The observed synergism effects between the metals in this study, however, may be associated with the P supply[42]. Besides, there were no recorded negative interactions between soil micronutrients and their nutrient uptake in this study. Moreover, the synergisms among the micronutrients present both in plant and soil suggests that soil available nutrients from the biofortified amended soils have a direct effect on the nutrient's uptake accumulation in *A. cruentus*.

CONCLUSION

This study demonstrated the progress to date in delivering biofortified vegetable crops as well as the agronomic approaches and tools to improve *A. cruentus* yield and micronutrient content of the edible vegetable crop. The use of biofortification incorporated at two weeks before transplanting (2 WBT) had a significantly higher content of shoot micronutrients uptake, better shoot height and fresh biomass yields as well as crude protein in *A. cruentus* vegetable relative to the other time intervals of biofortification with poultry manure incorporation during the three sequences of cropping.

SIGNIFICANCE STATEMENT

This study shows that there was scarcely any synchrony between the soil available micronutrients and crop demand at 2WAT treatment for micronutrients uptake in shoots of *A. cruentus*. Though a significant loss of nutrients was observed during the third cropping, biofortification of poultry manure at 2 WBT for single cropping and 2WAT for 2 to 3 cropping is recommended for the cultivation of *A. cruentus* in the tropical rain forest southeastern Nigeria.

Thus, the increase in the bioavailable concentration of micronutrients in edible crop tissues (via biofortification) has become a promising strategy in modern organic agriculture, providing more nutritious foods, to more people, with the use of fewer land space. It is therefore recommended that timely application of poultry manure at least a week before transplanting of *Amaranthus* seedlings will enhance the mineralization of poultry manure and after that release plant nutrients at appropriate to meet the needed nutrient.

REFERENCES

1. White, P.J. and P.H. Brown, 2010. Plant nutrition for sustainable development and global health. Ann. Bot., 105: 1073-1080.
2. White, P.J. and M.R. Broadley, 2005. Biofortifying crops with essential mineral elements. Trends Plant Sci., 10: 586-593.
3. Graham, R.D., R.M. Welch and H.E. Bouis, 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. Adv. Agron., 70: 77-142.
4. Welch, R.M. and R.D. Graham, 1999. A new paradigm for world agriculture: Meeting human needs: Productive, sustainable, nutritious. Field Crops Res., 60: 1-10.

5. White, J.G. and R.J. Zasoski, 1999. Mapping soil micronutrients. Field Crops Res., 60: 11-26.
6. Adekiya, A.O. and T.M. Agbede, 2017. Effect of methods and time of poultry manure application on soil and leaf nutrient concentrations, growth and fruit yield of tomato (*Lycopersicon esculentum* Mill). J. Saudi Soc. Agric. Sci., 16: 383-388.
7. Welch, R.M. and L. Shuman, 1995. Micronutrient nutrition of plants. Crit. Rev. Plant Sci., 14: 49-82.
8. Welch, R.M., 2002. The impact of mineral nutrients in food crops on global human health. Plant Soil, 247: 83-90.
9. Rengel, Z., G.D. Batten and D.E. Crowley, 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res., 60: 27-40.
10. Poletti, S., W. Gruissem and C. Sautter, 2004. The nutritional fortification of cereals. Curr. Opin. Biotechnol., 15: 162-165.
11. Eteng, E.U., 2015. Temporal variations in micronutrients (Cu, Fe, Mn and Zn) mineralization as influenced by animal and plant manure-amended marginal soils, Southeastern Nigeria. Int. J. Plant Soil Sci., 8: 1-16.
12. Xu, Y., W. Yu, Q. Ma and M. Zhou, 2013. Accumulation of copper and zinc in soil and plant within ten-year application of different pig manure rates. Plant, Soil, Environ., 59: 492-499.
13. Eneji, A.E., M. Irshad, T. Honna, S. Yamamoto, T. Endo and T. Masuda, 2003. Potassium, calcium, and magnesium mineralization in manured-treated soils. Commun. Soil Sci. Plant Anal., 34: 1669-1679.
14. Benke, M.B., S.P. Indraratne, X. Hao, C. Chang and T.B. Goh, 2008. Trace element changes in soil after long-term cattle manure applications. J. Environ Qual., 37: 798-807.
15. Abu-Zahra, T.R., S.M. Abubaker, A.B. Tahboub and A.A. Al-Abbadi, 2010. Effect of organic matter sources on micronutrients and heavy metals accumulation in soil. J. Food Agric. Environ., 8: 1199-1202.
16. Amanullah, M.M., S. Sekar and P. Muthukrishnan, 2010. Prospects and potential of poultry manure. Asian J. Plant Sci., 9: 172-182.
17. Boateng, S.A., J. Zickermann and M. Kornahrens, 2006. Poultry manure effect on growth and yield of maize. West Afr. J. Applied Ecol., 9: 1-11.
18. Soremi, A.O., M.T. Adetunji, C.O. Adejuyigbe, J.G. Bodunde and J.O. Azeez, 2017. Effects of poultry manure on some soil chemical properties and nutrient bioavailability to soybean. J. Agric. Ecol. Res. Int., 11: 1-10.
19. Ewulo, B.S., S.O. Ojeniyi and D.A. Akanni, 2008. Effect of poultry manure on selected soil physical and chemical properties, growth, yield and nutrient status of tomato. Afr. J. Agric. Res., 3: 612-616.
20. Ojeniyi, S.O., O.A. Amusan, and A.O. Adekiya, 2013. Effect of poultry manure on soil physical properties, nutrient uptake, and yield of cocoyam (*Xanthosoma saggitifolium*) in southwest Nigeria. American-Eurasian J. Agric. Environ., 13: 121-125.
21. Makus, D.J., 1990. Composition and nutritional value of vegetable amaranth as affected by stage of growth, environment, and method of preparation. Proceedings of the Fourth Amaranth Symposium. Minnesota Extention Services. Minnesota Agriculture University St. Paul.
22. Broadley, M., P. Brown, I. Cakmak, Z. Rengel and F. Zhao, 2012. Function of nutrients. In: Marschner's Mineral Nutrition of Higher Plants, Marschner. P., Elsevier, Amsterdam, Netherlands, pp: 191-248.
23. Okoli, P.S.O. and I.A. Nweke, 2015. Effect of different rates of poultry manure on growth and yield of Amaranthus (*Amaranthus cruentus*). IOSR J. Agric. Vet. Sci., 8: 73-76.
24. Adewole, M.B. and O.A. Dedeke, 2012. Growth performance, yield and nutritional quality of *Amaranthus cruentus* L. under repeated applications of poultry manures. Ife J. Sci., 14: 345-355.
25. FAO, 2007. Food and agriculture organisation. FAOSTAT.FAO Statistic Division. Rome.
26. Ano, A.O. and J.A. Agwu, 2005. Effect of animal manures on selected soil chemical properties. Niger. J. Soil Sci., 15: 14-19.
27. Eteng, E.U. and M.O. Nwagbara, 2014. Estimating water needs of soybean (*Glycine max* (L.) Merr.) using the penman model method in Umudike South Eastern Nigeria. Int. J. Agric. Sci. Res., 4: 49-58.
28. Soil Survey Staff, 2010. Keys to Soil Taxonomy. 11th Edn., United States Department of Agriculture, Natural Resources Conservation Service, Washington DC., USA., Pages: 337.
29. Thomas, G.W., 1996. Soil pH and Soil Acidity. In: Methods of Soil Analysis: Chemical Methods, Part 3, Sparks, D.L. (Ed.). SSSA/ASA Inc., Madison, WI., pp: 475-490.
30. Nelson, D.W., and L.E. Sommers, 1996. Total carbon, organic carbon, and organic matter. In: Methods of Soil Analysis, Part 3, Sparks, D.L., Soil Science Society of America, Madison WI, USA, pp: 961–1010.
31. Bremner, J.M., 1996. Nitrogen-Total. In: Methods of Soils Analysis, Part 3: Chemical Methods, Sparks, D.L. (Ed.). Soil Science Society of America, Madison, WI., USA., pp: 1085-1121.
32. Thomas, G.W., 1982. Exchangeable Cations. In: Methods of Soil Analysis, Part 2, Page, A.L., A. Miller and D.R. Keeney (Eds.). 2nd Edn., ASA and SSSA, Madison, WI., pp: 159-166.
33. Norvell, W.A., 1991. Reactions of metal chelates in soils and nutrient solutions. In: Micronutrients in Agriculture, Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Welch, Soil Science Society of America, Madison, pp: 187-228.
34. Sims. J.T. and G.V. Johnson, 1991. Micronutrient Soil Tests. In: Micronutrients in Agriculture, Mortvedt, J.J., F.R. Cox, L.M. Shuman and R.M. Walch (Eds.). Soil Science Society of America, Inc., Madison, Wisconsin, USA., pp: 427-477.
35. Aluko, O.B. and D.J. Oyedele, 2005. Influence of organic waste incorporation on changes in selected soil physical properties during drying of a nigerian alfisol. J. Applied Sci., 5: 357-362.

36. Iren, O.B., D.O. Asawalam, and V.E. Osodeke, 2011. Effects of time and method of pig manure application on growth parameters and yield of *Amaranthus cruentus* in a rainforest ultisol in Nigeria. Prod. Agric. Technol. (PAT) J., 7: 103-116.
37. Maerere, A.P., G.C. Kimbi and D.L.M. Nonga, 2001. Comparative effectiveness of animal manures on soil chemical properties,yield and root growth of amaranthus (*Amaranthus cruentus* L.). Asian J. Sci. Technol., 1: 14-21.
38. Ndukwe, O.O., C.O. Muoneke and K.P. Baiyeri, 2011. Effect of the time of poultry manure application and cultivar on the growth, yield, and fruit quality of plantains (Musa spp. AAB). Trop. Subtrop. Agroecosyst., 14: 261-270.
39. Ozores-Hampton, M., 2012. Developing a vegetable fertility program using organic amendments and inorganic fertilizers. Hort. Tech., 22: 743-750.
40. Roy, S. and A. Kashem, 2010. Effects of organic manures in changes of some soil properties at different incubation periods. Open J. Soil Sci., 04: 81-86.
41. Escobar, M.E.O. and N.V. Hue, 2008. Temporal changes of selected chemical properties in three manure – amended soils of Hawaii. Bioresour. Technol., 99: 8649-8654.
42. Zang, Y., X. Wei and M. Hao, 2015. Long-term effect of crop rotation and fertilisation on bioavailability and fractionation of copper in soil on the loess plateau in northwest China. PLoS ONE, 10.1371/journal.pone.0145370
43. Yang, X.E., W.R. Chen and Y. Feng, 2007. Improving human micronutrient nutrition through biofortification in the soil-plant system: China as a case study. Environ. Geochem. Health, 29: 413-428.
44. Kolawole, G.O., 2014. Effect of time of poultry manure application on the performance of maize in Ogbomoso, Oyo State, Nigeria. J. Appl. Agric. Res., 6: 253-258.
45. White, P.J. and M.R. Broadley, 2009. Biofortification of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol., 182: 49-84.
46. Fageria, V.D., 2001. Nutrient interactions in crop plants. J. Plant Nutr., 24: 1269-1290.
47. Nabuloa, G., H. Oryem-Origa and M. Diamond, 2006. Assessment of lead, cadmium and zinc contamination of roadside soils, surface films and vegetables in Kampala city, Uganda. Environ. Res., 101: 42-52.
48. NRCRI, 2018. Federal ministry of agriculture and rural development. National Root Crops Research Institute, Umudike. Annual Report, 2018.