

Impact of the growth stages and seasons of harvesting cassava (*Manihot esculenta* Crantz) leaves on heavy trace elements and antioxidant activity

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**Impact of the growth stages and seasons of harvesting cassava (*Manihot esculenta*
Crantz) leaves on chemicals composition**

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Abstract

The study assessed effects of plant growth and seasons of harvesting cassava (*M. esculenta*) leaves (CL) on antioxidants and heavy metals (HMs). The CL of five varieties include *TMS92/0326*, *TMS96/1414*, *IRAD4115*, *EN* and *AD* were collected at different months after planting (MAP) and seasons. Heavy metals were assessed by Microwave Plasma Atomic Emission Spectroscopy. Results showed significant ($p < 0.05$) effect of harvesting month and season on HMs. However Pb and Co did not detected at 6 MAP and 9 MAP. Cd was also not detected in *IRAD4115*, *EN* and *AD* at 15 MAP. Besides, higher content of HMs was observed in dry than in rainy season. All these variations were visualized by principal component analysis (PC1 x PC2: 58.15%). Concerning the antioxidant, it was also varied significantly through seasons and within varieties. This study revealed that CL are moderate in HMs and rich in antioxidants compounds at different growths and seasons.

Keywords: Antioxidant; Cassava; Health risk; Metals; Seasons.

1. Introduction

Plants constitute the major food chain for animal and human in the world. Certain nutritional elements contained in plants in high concentration can cause some concerns about the possibility of their toxicity after high consumption. The tubers and aerial organs of plants such as leaves, fruits, roots and flowers can absorb the heavy metals (HMs) at different concentrations dependent of the contaminations of soil by fertilizer, environmental factors or others such as through precipitations. These metals can be absorbed in plant leaves through many routes including foliar transfer [1,2]. Some amongst them have unclear function in human organism and are considered as non-essential because they are sometimes toxic in certain concentrations which can induce severe toxicity to living organisms. These metals include arsenic (As), lead (Pb), cadmium (Cd), cobalt (Co) and nickel (Ni) [2]. The levels of these HMs can vary in respect with seasonal conditions, age of plant, age of leaves as well as the harvest times of plant leaves. Example, it was reported that the concentrations of As, Cd, Ni, Cr (chromium) and Pb were above the critical toxic levels in plant leaves in both dry and wet seasons, while, Zn and Cu occurred at toxic levels only in the dry season [3]. It was also reported that some plants are potentially hyperaccumulators than others and their contents varied between and within species [4]. However, the translocation quantities of heavy metal in the plants from contaminated soil was higher than that of non-contaminated soil [5]. Furthermore, it was reported that leafy vegetables tend to accumulate higher concentrations of metals in edible tissues compared to fruits [6]. In addition, Souri et al. [7] found that young plant leaves accumulate higher Cd, Pb and As than mature plant leaves. In this line, the vegetables and edible leaves of some plants may be generally the most route to be exposed to health risk of toxic metals for human. Thus, this can be dangerous for human health by entering in the food chain of human by consuming these vegetables, leafy vegetables, fruits or their processed products. Fact, the toxicity of HMs cause many diseases such as cancers include prostate, kidney and pancreas [8,9], cardiovascular disease, kidney disease, lung disease and other numerous concerns [10]. Especially, the chronic exposure to Pb can induce neurodegenerative disease [11]. Therefore, investigate the toxic heavy metals level in these food gain great attention in order to avoid the health risk. Because of the expensive price of foods, people use the leafy vegetables, vegetables and other plant leaves to prepare various dishes rich in essential amino-acids, minerals, vitamins and other bioactive compounds in order to maintain good health.

Cassava is a shrub which plays an important role in human and livestock nutrition in many countries across the world. It is used as a staple food in most countries of Africa or Asia. The cassava storage roots are used to produce flour, gari and many other products. Their leaves are rich in proteins, minerals, vitamins, phenolics, carotenoids and fibres [12]. These leaves also are used for medicinal values to treat some diseases such as headache, fever, anaemia and so on because of its richness in antioxidants and polyphenolics [12]. The shoots or cassava leaves (CL) of this shrub are used in preparation of several delicacies in Cameroon such as *magani*, soup, salad and other dishes after boiling. Besides, these CL are consumed throughout the seasons as well as at any age of cassava plants. Several previous studies regarding CL were determined the levels of heavy metals, however majority of these studies did not take into account the effect of seasonal variation, age of plant or leaf, month or time of the harvests, effect of variety as well as health risk assessment. In addition, the instruments used to analyze HMs may affect also the contents of HMs in edible plant leaves. Various instruments are used to determine HMs in edible plant leaves such as inductively coupled plasma optical emission spectrometer (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrophotometry (AAS), while used Microwave Plasma Atomic Emission Spectroscopy (MP-AES) is very scarce in the literature. Whereas, the last instrument may be more accurate than others.

Hence, the present study aims to assess HMs in CL from five varieties grown (growing season of 2015-2017) from Far North region (10°54'0.0'' North latitude and 14°12'0.0'' East longitude) of Cameroon which were collected at 6 months after planting (MAP), 9 MAP, 12 MAP and 15 MAP during dry and rainy seasons in order to know times and seasons where the optimal antioxidants and the lowest level of HMs content. Among the HMs as the most toxic when exceeding the average limit, the contents of Pb, Cd, As, Cr, Co, Ni, and Al were analyzed in the CL by Microwave Plasma Atomic Emission Spectroscopy (MP-AES). This contribute to avoid the health risk in CL consumed daily by the populations.

2. Materials and Methods

2.1. Leaves collection

Cassava (*M. esculenta*) leaves (CL) of different varieties include EN and AD (local varieties), TMS92/0326 and TMS96/1414 (improved varieties from International Institute of Tropical Agriculture, Nigeria) and IRAD4115 (improved variety from Institute of Agricultural Research for Development, Cameroon) were utilized. Plant materials were collected with local

guidelines from the laboratory of the University of Maroua, Cameroon. The leaves were collected during different growth stages and months at two seasons including dry and rainy. CL were washed three times with distilled water, then cut and dried in an air oven (UN75 Memmert, 30-750, Germany) at 60 °C for 24 h. The dried samples were powdered, sieved and stored in polyethylene bag (Handgards®, Texas, USA) prior to analysis.

2.2. Chemicals and reagents

The authentic compounds include Trolox (purity > 98%), was purchased via Sigma-Aldrich (Mumbai, India). minerals (purity > 98%); Na, Fe, Zn, Cu, K, Mn, Se, Hg, Pb, Cd, As, Cr, Co, Ni, Al, etc.) were from Agilent Technologies (Japan). Milli-Q-Water (Millipore, Bedford, MA, USA) was provided by Engineering Department of the Institute (Central Food Technological Research Institute, CFTRI; India). N, N-dimethyl-p-phenylenediamine (purity >97%), sulphanilamide (purity >98%), phosphoric acid (purity >85%), naphthylethylene diamine dihydrochloride (purity >98%), perchloric acid (purity >65%), nitric (69%), sulfuric (purity >98%), sodium nitroprusside (purity > 99%) used were of analytical grade provided by Sisco Research Laboratory (SRL) (Bengaluru, India).

2.3. Condition of samples digestion for heavy metals

Cassava ash was obtained after reducing CL in furnace at 550 ± 10 °C for 9 hrs. An amount of 0.3 g of ash was mixed with 2 mL of acid (69% nitric, 98% sulfuric and 65% perchloric acid as in ratio of 8:1:1). This solution was kept overnight before adding 4 mL of the same acid. The mixture was incubated in a water bath for 3 hrs. After cooling the solution, it was heated on a hot plate until complete digestion. Once again, 10 mL of Milli-Q water was added before filtering in flask (25 mL). Finally, the filtrate solution obtained was evaporated and kept at -20°C.

2.3. Condition for analysis by Microwave Plasma Atomic Emission Spectroscopy

The residue was dissolved in 100 mL of Milli-Q water and filtered with 0.22 µm (Millipore, USA). Then, sample was injected automatically into HPLC system. The authentic compounds were also prepared in 5% nitric acid in Milli-Q water, however blank was constituted with aqueous solution containing 5% nitric acid. Determination and quantification of heavy metals

were analyzed by MP-AES. The plasma of the 4210 MP-AES operated at 5000K. 4210 IDL and the nebulizer flow rate (L/min) was kept at range of 0.45-0.95 for a total time of 3 mins.

2.4. Polyphenolic compounds extraction

Phenolics were extracted following the method of Chen et al. [13] with some modifications. Volume of 20 mL of methanol was mixed with powder (300 mg) in triplicate and the mixture obtained was centrifuged at 10000 rpm at 4 °C for 20 min. Then, solution was filtered with Whatman paper no.1, and the residue was washed with 5 mL of methanol. The solution, was centrifuged again as described above. The residue obtained was mixed with 10 mL NaOH (2N) solution and boiled in water bath at 50 rpm for 30 min before adding 5 mL HCl (2M) and incubated for 60 min at 60 °C. The mixture was cooled before adding 10 mL of ethyl acetate and the supernatants were centrifuged in the same conditions as described above and filtered again. After this step, the solvent was evaporated at 45 °C. The residue obtained was dissolved in methanol and stored at -20 °C for analysis.

2.5. Total anthocyanins extraction and quantification

The pH differential method proposed by Lee et al. [14] was used to quantify the total anthocyanin content (TAC) in leaf samples. Two buffers of pH1 and pH4.5 made from 0.025 potassium chloride and 0.4 M sodium acetate were used, respectively. In brief, 200 µL of extract sample or standard were mixed in 1.8 mL of either 25mM HCl/KCl, pH1.0 or 0.4 M acetic acid/sodium acetate, pH 4.5 solutions. Then, the mixture was incubated for 30 min before taking absorbance against a blank (Milli-Q-Water) at 520 nm and 700 nm. Finally, the Absorbance (A) of the diluted sample was calculated according to the following equation:

$$A = [(A_{520nm} - A_{700nm})_{pH1.0}] - [(A_{520nm} - A_{700nm})_{pH4.5}]$$

where $A_{520nm\ pH\ 1.0}$ and $A_{700nm\ pH1.0}$ are the absorbances of the samples at the dilution pH 1.0 at 520nm and 700nm, respectively. $A_{520nm\ pH\ 4.5}$ and $A_{700nm\ pH4.5}$ are the absorbances of the samples at the dilution pH 1.0 at 520nm and 700nm, respectively. TAC was calculated according to the following equation:

$$TAC = (A \times MW \times df \times 1000) / (\epsilon \times lm)$$

where MW is the molecular weight of cyaniding-3-O-glucoside (cyd-3-glu) (449.2 g mol⁻¹), ϵ its molar extinction coefficient (26900 L cm⁻¹ mol⁻¹) of standard, df the dilution factor, 1000 factor for conversion from g to mg, m the masse of sample and l path length in cm.

2.6. Antioxidant activity

2.6.1. Nitric Oxide Radical Scavenging Assay (NO)

Griess reagent was prepared by mixing 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid [15] with some modifications. A volume of 150 μ L of 10 mM sodium nitroprusside in phosphate buffered saline (PBS) (0.1 mM) was mixed with 150 μ L of the sample in a 96-well plate and incubated in the dark place. The absorbance was taken at 546 nm using multimode reader (Tecan SPARK 10M, V1.2.20). The results were evaluated from Trolox standard.

2.6.2. N, N-dimethyl-p-phenylendiamine assay (DMPD)

The assay is based on decolorization of the purple-colored radical DMPD (N, N-dimethyl-p-phenylendiamine) in the presence of antioxidant [16] with some modification using microplate. The reagent was the mixture of 100 mM DMPD and 0.05 M ferric chloride solution in 0.1 M acetate buffer till was equilibrated. Then, 50 μ L of reagent was mixed with 150 μ L of the sample in a 96-well plate and incubated for 40 mins in the dark place before the absorbance was measured at 505 nm. The results were also evaluated from Trolox standard.

2.7. Statistical analysis

One and two-way ANOVA were done by Statgraphics software (version. 16). Tukey's test was utilized and significance was accepted at level $p < 0.05$ and $p < 0.01$. Principal component analysis were determined the relationship between parameters and the total variance of traits via XLSTAT (version 16). Results were expressed as means $\pm$ standard deviation. Experiments were done in quadruple with the exception of heavy metals which were in triplicate.

3. Results and discussion

3.1. Effect of growth stage and harvesting time of cassava (*M. esculenta*) leaves on polyphenolics

The results presented in table 1 showed significantly ($p < 0.05$) high variation of anthocyanins in respect with varieties and the collection times. The variety TMS96/1414 had higher anthocyanins content than others, however, AD variety showed low value at 6MAP as shown in Table 1. This variation of anthocyanins content was in agreement with Koponen et al.[17], who found that anthocyanins content varied significantly in respect with three strawberry varieties. Then, an increasing concentration of anthocyanins level is significant at 9MAP with the values range from 12.00 to 16.57 mg C3G E/100g in IRAD4115 and AD varieties, respectively suggesting an effects of season on anthocyanins as found previously by Koponen et al. [17]. Laya et al. [12] reported also in their study the effects of seasons on polyphenolics compounds in CL collected at different times across seasons. A significant increase of anthocyanins at this harvest time may due to early water deficit during the dry season which characterized by favorable temperature may be in favor of anthocyanins synthesis pathway. Similar result was reported by Bucchetti et al. [18], who found that anthocyanins content increased in *V. Vinifera* during water deficit. At 12MAP, low content of anthocyanin was observed in TMS96/1414 variety which had showed high value at 6MAP, while a significant content was observed in EN variety (Table 1). A general trend was that a decrease of anthocyanthins in all varieties was observed except the EN and AD varieties which their contents were slightly increased. This decrease could be due to high temperature at the collection time of leaves which reduced anthocyanins accumulation in plants [19]. The TMS92/0326 variety showed significantly high concentration (18.63 mg C3G E/100g) as an increase in TMS92/0326 and TMS96/1414 at 15MAP and AD variety had the lowest content (5.38 mg C3G E/100g) suggesting the effects of seasons depending on the variety. In comparison with the results found by Koponen et al. [17] from 9 vegetables (3.50-75.10 mg/100gfw), leaves harvested in all seasons can be classified as rich in anthocyanins. Hence, the results are suggesting the use of these leaves as leafy vegetables which can be benefit for consumers by reducing an incidence of cardiovascular disease, cancer, hyperlipidemias and other chronic diseases through the intake of anthocyanin rich foods [7].

For phytic acid contents as presented in table 1, values range from 0.89 to 1.82 mgPAE/g at 6MAP in EN and TMS96/1414 varieties, respectively. The present values found were lower than the values (0.58-0.66mg/100g) found in five cassava varieties reported by Sebiomo and Banjo [20]. Then, contents of phytic acids increased at 9MAP in all varieties with the exception

of EN variety which its content decreased slightly suggesting the effect of variety. Continually, the contents of phytic acids increased at 12MAP in all varieties with the exception of TMS92/0326 and TMS96/1414 varieties which their contents decreased at this harvest time (Table 1). This variation of phytic acids content may be due to the seasonal variation already reported by Umezuruike and Nwabueze [21] when they found significant variation of phytic acid (0.80 mg/100g, 1.60 mg/100g and 2.30 mg/100g) in *T. africana*, a legume during different seasons. Lastly, the contents of phytic acids increased in all varieties from 12MAP to 15MAP. In comparison with the results (0.19 g/100g) found by Madalla et al. [22] in some CL leaves, the present leaves were lower in phytic acids content. The difference can be due to the methods used for analysis, age leaf, location are among others.

Saponnin contents varied also significantly with respect of the harvest times and varieties showed in table 1. However, the contents of saponnins increased from 6MAP to 12MAP in all varieties with the exception of EN variety which its content decreased slightly at 9MAP suggesting again the effects of age plant and varieties as reported by Wobeto et al. [23], who found a significant variation of saponins content in five cassava varieties at different time of the harvests. Compared to the values obtained in the present study, the contents (1.74-4.73 g/100g) observed in five cassava varieties by Wobeto et al. [23] were higher. Sebiomo and Banjo [20] were also reported values of saponins ranged from 3.14 to 3.63 mg/100g in CL which were higher than the values observed in the present study. These results may be due to the agronomic practices and varieties as well as environmental variations where the plant leaves were collected. From 12MAP to 15MAP, the contents of saponins continually increased in all varieties except EN and AD varieties which their contents were decreased (Table 1). This increase in most leaves may be due to the plant maturity and the influence of variety. Similar trends were observed by Wobeto et al. [23] during three harvests stage of plants (12, 15 and 17 months) of CL. This significant variation of saponins throughout the seasons was also reported by Umezuruike and Nwabueze [21] who found significant variation of saponins (1.65 mg/100g, 1.90 mg/100g and 2.56 mg/100g) in *T. africana*, a legume during different seasons. Seal et al. [24] were also reported the values (3.70-8.50 mg/100g) of saponins in wild edible leaves of five plants significantly higher than those found in the present study. These results suggest that CL can be used as safe leafy vegetables for the population.

3.2. Heavy metals content in cassava leaves impacted by harvesting month and variety

Two-way analysis of data performed showed significant effects of variety (P-value: 0.000), harvest time (p-value: 0.000) as well as Variety x harvest (P-value: 0.001) on heavy metals (HMs) analyzed in the present study. The HMs content of CL harvested at 6 month after planting (MAP) varied with a significant ($p < 0.05$) difference as presented in Table 2. However, except Pb and Co which are not detected in leaves, other metals are found in all varieties with the exception of AD variety which As is not detected, while the contents of Ni are range from 0.00 to 0.5 $\mu\text{g/g}$. The contents of Ni (0.12-9.23 $\mu\text{g/g}$) found in CL are lower than those found in eight medicinal plants [25]. As shown in table 2, TMS92/0326 variety showed significantly higher values of As (149.15 $\mu\text{g/g}$) and Cr (3.42 $\mu\text{g/g}$) when compared to others. For the contents of Cd (19.37 $\mu\text{g/g}$) and Al (469.55 $\mu\text{g/g}$), significant values are observed in IRAD4115 and EN varieties, respectively.

The contents of HMs in leaves sampled at 9 MAP were varied also with a significant difference among varieties (Table 3), with the exception of Pb and Co which were not detected in all varieties at 6 months. Similar result was abserved by Oluyemi et al. [3] who found that Co was not detected in CL suggesting the effects of seasonal variation of the accumulation of HMs in leaves. Laya et al. [26] reported also that seasons affect significantly the minerals composition in CL harvested in different periods. In addition, Tshilongo et al. [27] had reported the effects of harvesting stages on phtytomutrients. The maximum Cd (41.15 $\mu\text{g/g}$) and As (71.6 $\mu\text{g/g}$) concentrations were found in EN and IRAD4115 varieties, respectively, while the minimum (4.09 and 1.25 $\mu\text{g/g}$, respectively) concentrations were found in TMS96/1414. The TMS92/0326 variety had significantly higher content of Al (1186.50 $\mu\text{g/g}$) in comparaisn with others, however EN showed low value (246.41 $\mu\text{g/g}$) and high Cr (5.05 $\mu\text{g/g}$) concentration as well suggesting that variety affects signifcantly the accumulation of toxic HMs in leaves (P-value: 0.000). These effects of variety on metals composition in CL were already reported by Alamu et al. [28].The values of all these minerals are significantly higher in leaves compared to 6MAP. These results may be due to seasonal variations as well as genetic factors. Furthermore, this difference may be due to age plant leaves as reported by Souri et al. [7], who found that the contents of Cd and As are higher in young plant leaves than mature plants leaves, while, Pb and Ni are high in young plant leaves.The contents of Cd (0.03-0.49 $\mu\text{g/g}$) found in eight plants reported by Kirmani et al. [25] are lower compared to the values obtained in CL at 9 months. These results may linked with species and soil of the geographical location. The results are in agreements with Alamu et al. [28] who found that the agroecological affected significantly the minerals in CL. Toxic HMs such as Pb, Cr and Ni were not detected at 12 MAP in all varieties except Ni (0.67 $\mu\text{g/g}$) which was found in TMS92/0326 variety (Table 4),

and the same variety showed with a significant difference high concentration of Co (9.72 µg/g) and Al (590.70 µg/g), while TMS96/1414 and IRAD4115 varieties had the highest values of As (32.63 µg/g), and Cd (19.69 µg/g), respectively. Kirmani et al. [25] reported values varied from 0.19 to 1.83µg/g of Co in eight medicinal plants which are lower compared to the values obtained at 12 months in the present study suggesting the effects of species, maturity and ecological conditions as well. Whereas, AD and TMS96/1414 varieties showed low values of Al and As, respectively (Table 4). Similar results were reported by Ahiakwo et al. [29] who found that Cd, Pb and Cr were concentrated in high levels in dry than rainy season in leaves of *T. occidentalis*. However, Nwadinigwe et al. ([6] found in the same leaves of *T. occidentalis* Co and Cd were higher in dry season, while Pb and Ni were higher during rainy season suggesting that the toxic HMs contents may depend on geographical loaction, soil and climatic conditions. For example, Qadir et al. [30] reported also a significant effect of season, plant species, anthrogenic activities and location on HMs (Cd, Cr, Pb and Ni) accumulation in six plants.

A significant difference was observed for contents of HMs in all varieties at 15 MAP, however, once again Pb, Cr and Cd were not detected with the exception of Cd which was found in TMS92/0326 and TMS96/1414 (Table 5). The results suggest the effects of seasons (P-value: 0.000) and variety (P-value: 0.000) as well. The results were consistent to Oluyemi et al. [3] who found that Cr was not detected in CL during dry season. This result also is in agreement with Sharma et al. [31] who reported the effects of soil and variety on accumulation of metals in plants. The Cd (16.53 µg/g), Al (263.53 µg/g) and As (45.85 µg/g) as well as Co (3.14 µg/g) were significantly higher in TMS92/0326 and IRAD4115 varieties, respectively. The seasonal variation of the contents of HMs was also observed by Qadir et al. [30] who found that except some HMs (Cu, Mn), all metals are affected by season and plants x season. Pb was not detected in leaves at different harvests suggest that CL can be consumed without human health risks exists. Fact, Pb was reported to be a leukemic metals, linked also with brain and kinedy diseases, gastrointestinal colitis and thromboembolic venous diseases [32]. Similar, Co was not detected from 6MAP to 9MAP as well as Cr from 12MAP to 15MAP, whereas all these metals were known to induce cancer if consumed in food, in large amount suggesting safety consumption of the present CL [33]. The results can be due to the soil which may be not rich in Pb, Co and Cr, however, anthropogenic activities and atmospheric deposition may be responsible of a little accumulation of these metals in leaves detected at some harvests [31 34]. The Cr is highly toxic to humans, which may lead to severe health problems like lung cancer (Alloway, 2013) [35]. The levels of As were relatively high in leaves at different harvests which can be toxic for

consumer at high quantities. As is known to induce cancer of the bladder, lung, and skin when consumed at high level through food, however, the level of this metal in all CL collected at different months and seasons is within safe limit as recommended by WHO. A general trend showed that some toxic HMs content were slightly higher than the limit values given in some vegetables by China [36], and other author include Gebeyehu and Bayissa [37] with some discrepancies due to seasonal variations and plant ages. The moderate contents of Co is important for human since its role in vitamin B12.

3.3. Antioxidant potential of cassava leaves impacted by harvesting time and variety

The results of radical scavenging activity (RSA) of leaves are presented in supplementary material (Fig. S1 and Fig. S2). For nitric oxide (NO), the radical scavenging effect was affected by variety throughout the harvests time (Fig. S1). At 6MAP, NO radical scavenging effects varied with a significant difference between 169.37 and 249.25 $\mu\text{gTE/g}$ in AD and IRAD4115 varieties, respectively. Then, the values of NO radical scavenging were increased in all varieties with the range of 267.54 to 449.85 $\mu\text{gTE/g}$ in TMS92/0326 and AD varieties, respectively suggesting the lowest activity in comparison with the leaves collected at 6MAP. While, the values of NO radical scavenging decreased significantly (4.76 to 78.00 $\mu\text{gTE/g}$ in EN and TMS96/1414 varieties, respectively) indicating the highest antioxidant activity in dry season. The seasonal variations of the radical scavenging activity was also found by Sivaci and Duman [38] in their study on leaves of almond varieties (Nonpareil, Texas and Ferragnes) which radical scavenging activity evaluated by DPPH methods varied with a significant difference throughout the seasonal variations. This may be the case of the results of the present study. However, the activity decreased at 15MAP in all varieties except TMS96/1414 (16.73 $\mu\text{gTE/g}$) and IRAD4115 (8.38 $\mu\text{gTE/g}$) varieties when compared to 12MAP (Fig. S2). Similarly, Bahukhand et al. [39] found significant difference variations of radical scavenging activity of *A. calamus* during different months and seasons in their study.

DMPD radical scavenging effect was also significantly varied among varieties at 6MAP (Fig. S2) with the values range from 13.12 to 113.56 $\mu\text{gTE/g}$ in AD and TMS92/0326 varieties, respectively. However, DMPD radical scavenging ranges from 13.10 to 117.69 $\mu\text{gTE/g}$ in IRAD4115 and TMS92/0326 varieties at 9MAP shown in fig. 2S. At 12MAP, DMPD radical scavenging ranges from 6.93 to 78.00 $\mu\text{gTE/g}$ in EN and TMS96/1414 varieties, respectively. This high radical scavenging activity observed at this harvest time may be due to the dry season when the plants may be more in stress. Many other researchers reported that radical scavenging

activities evaluated by DPPH and ABTS methods were higher during dry season than rainy season in *S. floribunda* or during winter and spring season in *C. althaeoides* leaves which may be the case of the results of the present study [39]. While, the radical scavenging varied significantly from 8.38 to 162.08 $\mu\text{gTE/g}$ in IRAD4115 and TMS92/0326 varieties, respectively at 15MAP. These variations of antioxidant activity are linked to the seasonal variations which are in agreement with Ahmed et al. [40], who found that radical scavenging activity of *P. atlantica* leaves, vegetables varied significantly in respect with spring, summer and autumn. The season, variety, month of the harvest time as well as age plant may affect significantly the antioxidant activity.

3.4. Principal component analysis

A principal component analysis (PCA) was done in order to establish any relationship amongst variables. The first and second main components showed a total explained variance of 58.15%. PC1 explained 30.26% and PC2 explained 22.35% of the variance, respectively (Fig. 1). The contents of Ni and Al had high relationship with PC2 (Fig. 1a), however Cr, Co and Cd were correlated mostly with PC1. While, As was placed alone in the negative side due to its high content and high effect of the harvest month. As presented in figure 1, PCA shows three groups of HMs. Fig. 1b showed that the contribution of observations percentages and variables were highly correlated with the dry seasons, which corresponded to higher amounts of HMs contents in most samples. However, both dry and wet seasons had significant effects on the HMs composition (Fig. 1b). The result therefore suggested that HMs are dependent on seasonal variation and cassava variety as well.

4. Conclusions

The study was carried out to evaluate the potential of cassava leaves (CL) at different growth stages and seasons regarding an antioxidant potential and heavy metals (HMs) accumulation in their leaves for safe human consumption. The results showed that the antioxidant activity evaluated by nitric oxide radical scavenging activity is high at 6MAP with the values varied significantly between 169.37 and 249.25 $\mu\text{gTE/g}$ in AD and IRAD4115 varieties, respectively while, for DMPD radical scavenging the highest activity was observed at 12 MAP (6.93 to 78.00 $\mu\text{gTE/g}$ in EN and TMS96/1414 varieties, respectively). The levels of HMs varied also significantly among varieties through the different stages of harvests across rainy and dry

seasons. Season and variety influence the rate of assimilation of HMs in CL explained by principal component analysis (PCA). Pb and Co were not detected in leaves at different harvests (6 MAP and 9 MAP) suggesting that CL can be consumed without human health risks exists. The CL are rich in antioxidant compounds through the stages of harvest months and seasons. Considering the current levels of HMs, it is obvious that CL have concentrations within safe limit, however with some discrepancies at different harvest stages. The levels of these elements may not contribute to metal toxicity and the human health risks. While, the future study will investigate *in vivo* study the toxicity of HMs of CL.

Author Contribution

L.A .and S.B.F. Investigated, wrote the main manuscript text and prepared figures and tables. D.R. and K.B.B. wrote and supervised. All authors reviewed the manuscript.

Data availability

Data that support the findings of this study are not openly available but are available from the corresponding author upon reasonable request

Plant materials collection

Plant materials were collected with local guidelines, University of Maroua, Cameroon.

Ethics declaration

Not applicable

Ethical Statement

The authors declare that there is no conflicting interest

Consent of publication

Not applicable

Concent to participate declaration

Not applicable

Clinical trial

Not applicable

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Figures

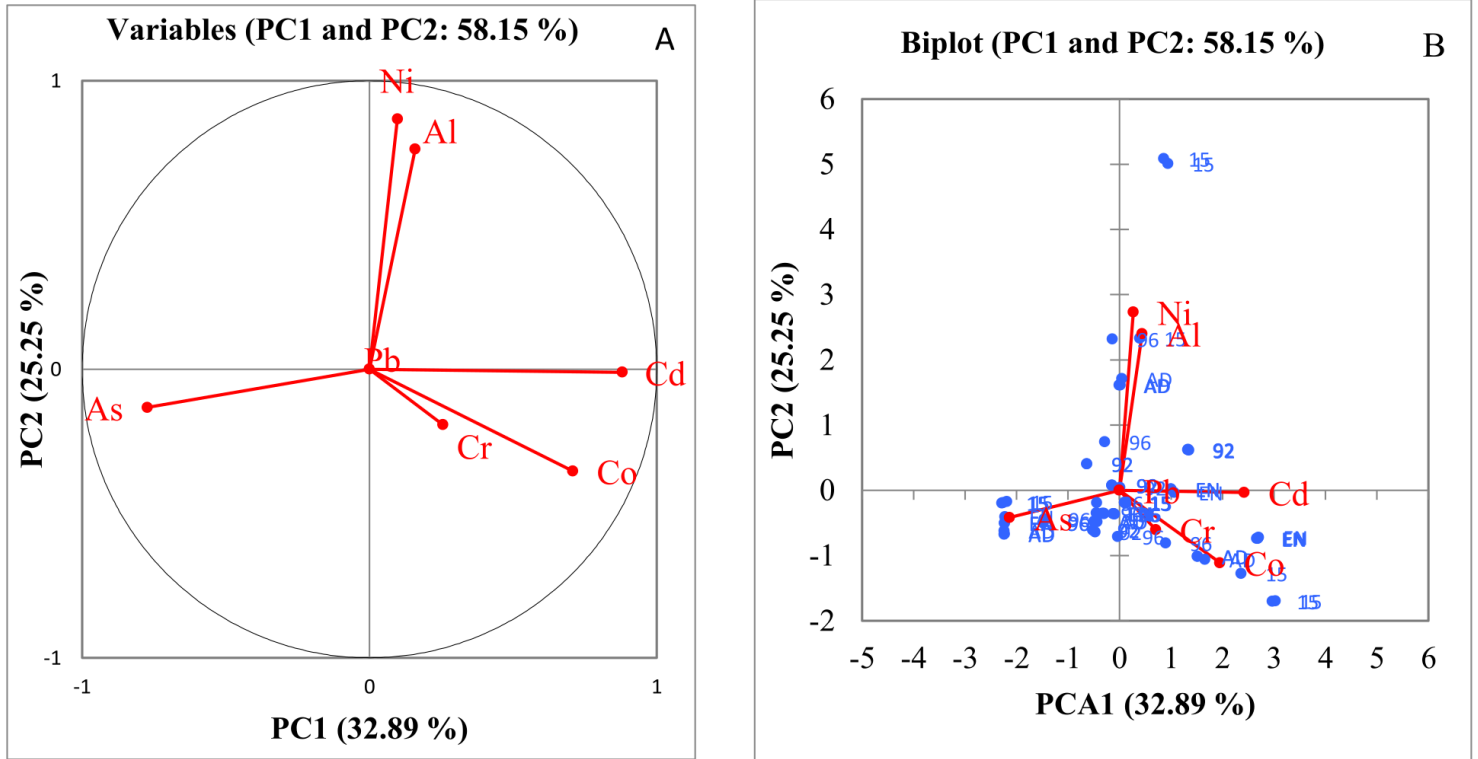


Fig 1. Principal component analysis (PCA) shows the relationship among heavy metals content affected by harvest season and variety. Correlation (a) between variables and factors and biplot (b).

Figure 1

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