Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.4, No.16, 2014 www.iiste.org

Effect of Gas Flaring on Some Phytochemicals and Trace Metals of Fluted Pumpkin (Telferia Occidentalis)

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Abstract
The study determined the impact of gas flaring on some phytochemical and trace metal compositions of *Telferia occidentalis* in Obrikom, a gas flaring community. Plants for the study were obtained from farmlands in the gas flaring community in Rivers State, Nigeria and values obtained were compared with those from non gas flaring community (Rumualogu). The phytochemical composition (mg/100g) of *Telferia occidentalis* leaves grown both in gas flaring and non gas flaring sites was measured. The results obtained from non-gas flaring community are alkaloid (3.34 ± 0.006), flavonoid (6.67±0.009), saponins (8.21±0.020) and tannins (0.01±0.001) while the values from the gas flaring community are alkaloid (2.18±0.004), flavonoid (0.83±0.001), saponins (2.22±0.009) and tannins (0.46±0.012). The findings showed that plants in gas flaring community had reduced phytochemicals except tannins which increased significantly (P>0.05). There was significant increase (P>0.05) in the levels of Fe (2.78±0.01 to 3.51±0.02), Zn (0.90±0.06 to 1.30±0.02), Pb (0.21±0.01 to 0.54±0.01) and Cd (0.00±0.00 to 0.07±0.01) when the leaves grown in a non gas flaring site were compared with the gas flaring site samples. However, there was no significant difference in Cr concentration of the vegetable from both sites; Cr (0.2±0.01 to 0.06±0.01). All these may have possible implication on the nutritional and medicinal values of *Telferia occidentalis.*
Keywords: Gas flaring, Phytochemicals,Trace metals.

1. INTRODUCTION

Nigeria is a nation highly endowed with natural resources among which are oil and gas. In the year 2000, Nigeria's total crude oil and condensate production stood at 2.3million barrels per day, this shows an increase of 9.1% over the previous year (Ugbana, 2004).

Gas flaring is the burning off of gas, which sends a cocktail of poisons into the atmosphere. It is necessary to have an understanding of the adverse impact of chronic exposure from multiple flaring discharges on the health of people who live and work in proximity to the industry. Proximity has been defined as any distance between 0.2 to 35 km from the flare stack (Argo, 2002).

Nigeria is said to have emitted more than 3,438 metric tons of gas in 2002. While flaring gas in the western countries has been minimized, in Nigeria it has grown proportionately with oil production (Friends of the Earth Nigeria, 2008). Due to poor infrastructure and unsustainable practices among oil companies, only 19% of the total gas flared is recovered (Ibhade 2001, Evoh 2002). Recent studies have investigated the impact of gas flaring on micro-climate and vegetation (Efe, 2003) soil, air and water quality (Ekanem, 2001), human health (Obajimi 1998; Oniero and Aboribo 2001) and on national economy (Oghifo 2001). Other studies associated gas flaring with increasing poverty among rural women (Obadina 2000, Gabriel 2004), climate change (Emerole 2008), and increase in political activism in the Niger Delta Region (Akingbade 2001).

Telfairia occidentalis is a tropical plant. Its habitat is the wet part of Nigeria and Africa in general. It belongs to the family cucurbitaceae. The fruit is fluted as it can be seen from the shape of the gourd. It is from this morphology it derives its name-fluted pumpkin. The plant found use mostly in Ethno medicine for the treatment of anaemia. There is hardly any home in Nigeria where Ugu is not consumed in daily meals due to its health restoration value. The young leaves are sliced and stored in a bottle to which coconut water and salt are added. This is used for the treatment of convulsion in ethno medicine (Gbile, 1986). The roots are used as rodenticide and ordeal poison (Gill, 1992). The leaves contain essential oils, vitamins; root contains cucubitacine, sesquiterpene, lactones (Iwu, 1983).

A phytochemical is a natural bioactive plant component that works with nutrients and dietary fibre to protect against diseases. Research suggests that phytochemicals working with nutrients found in fruits, vegetables and nuts may help slow the aging process and reduce the risk of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infection (Akah *et al.,* 2002). Heavy metals can be poisonous for macro- and micro-organisms through direct influence on the biochemical and physiological procedures, reducing growth, deteriorating cell organelles, and preventing photosynthesis. Regarding the transportation of metals from roots to the aerial parts of the plants, some metals (especially Pb) tend to be accumulated in roots more than in aerial parts, because of some barriers that prevent their movement. However, other metals, such as Cd, moves easily in plants (Garbisu and Alkorta, 2001). The consequence of trace metals in foods such as vegetables and tubers have been a considerable interest because of their toxicity effect which are important in human beings (Asaolu, 1995).

The aim of this study is to investigate the possible effect of gas flaring on phytochemical and heavy metal composition of *Telfairia occidentalis* in the study areas. The results obtained from the study would also provide information on the nutritive and medicinal values of the vegetable in the study area.

2.0 Materials and Methods

2.1. Study Area and period

This research was conducted in the tropical area of Obrikom and Rumuaologu of Rivers State, Nigeria between August and October, 2012.

2.2. Study design

The vegetable used for the study was fluted pumpkin leaf *(Telfairia occidentalis*). The vegetable was planted in the farmlands located in Obrikom and Rumuaologu communities in Rivers State. The plants were randomly harvested after twelve (12) weeks from the two farms within each location. Fresh leaves of fluted pumpkin (*Telferia occidentalis)* were sorted to eliminate any dead matter and other unwanted particles.

2.3. Analysis

Quantitative determination of phytochemicals:

Alkaloid (Harborne (1993) method)

5g of the sample was weighed into beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was completed. The whole solution was allowed to settle and the precipitate was collected and washed with ammonium hydroxide and then filtered. The residual was dried and weighed.

Calculation:

$$\frac{\text{weight of filter paper and sample residue-weight of empty filter paper}}{\text{weight of sample used}} \times 100$$

Flavonoid (Borm and Kocipal-Abyazan (1994))

10g weight of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Calculation:

$$\frac{\text{weight of filter paper and sample residue-weight of empty filter paper}}{\text{weight of sample used}} \times 100$$

Saponin (Obadoni and Ochuko (2001))

20g of each of the plant sample was ground and put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 minutes with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of N-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Calculation:

$$\frac{\text{weight of filter paper and sample residue-weight of empty filter paper}}{\text{weight of sample used}} \times 100$$

Tannin (AOAC methods (1980))

The percentage composition of tannin in the plants was determined using the AOAC methods (1980) with some modifications. Folin-Denis reagent and saturated sodium carbonate were prepared in accordance with the procedure to analyze the tannin content. Standard solution of tannic acid was freshly prepared by dissolving 10 mg of tannic acid in 100 ml water. A series of tannic; E3 J. Biotechnol. Pharm. Res. 44 acid standards were prepared in the range of 0-2.5 ml aliquots in 25 ml volumetric flasks, then added to 1.25 ml Folin-Denis reagent and 2.5 ml sodium carbonate solution. The mixture was made up to the volume and the color was measured after 30 min at 760 nm using a spectrophotometer (Perkin Elmer). The samples were prepared by boiling 1 g of their dried powder in 80 ml of water for 30 min. The samples were cooled, transferred into a 100 ml volumetric flask and diluted to mark. The solution was filtered to get a clear filtrate and analyzed as in the standard. Tannin content was determined by a tannic acid standard curve and expressed as milligrams of tannic acid equivalence (TAE) per 100 g of dried sample.

Calculation:

$$\% \text{ soluble tannins} = \frac{C(mg)\ \text{extract vol.}(ml)}{10x\ \text{aliq}(ml) x\ \text{sample wt}(g)}$$

Determination of metals from plant samples

Concentrated HNO_3 (3ml) and 0.5ml H_2SO_4 were added to a 50ml flask containing 1g ground oven-dried plant sample and 1ml of 60% $HCLO_4$ and 0.5 ml concentrated H_2SO_4. The flask was swirled gently and the contents digested slowly on an electrothermal heater to 250°C for 15 minutes. The increase in temperature was gradual until it reached 250°. The digest was then cooled and filtered through 541 Whatman filter paper into a volumetric flask and diluted to 50 ml with distilled water. The residual acid concentration of the digested sample was brought to 1% v/v after digestion. The digested samples were analysed for trace metals, using the Atomic Absorption Spectrophotometer, Model 451 (American Standard Testing on Spectrophotometer (AMST) 1982.). The instrument was calibrated using standard solutions of lead, iron, copper and zinc. The absorbance obtained were used in calculating the concentrations of the metals in the different samples.

Data Analysis: The data obtained were compared for statistical significant differences using Student's t-test, with the aid of SPSS 17 package (SPSS Inc. Chicago III). In all, $P<0.05$ was significant. Data are presented as Mean±S.E.M (standard error of the mean).

3.0. Results/Discussion

The results obtained from the study are shown below: The phytochemical values for plants in gas flaring community are significantly ($p<0.05$) less than those for plants in non gas flaring communities. The quantitative analysis of fluted pumpkin leaf (*Telfairia occidentalis*) was carried out in dry samples. The phytochemical composition (mg/100g) of fluted pumpkin (*Telferia occidentalis*) leaves grown both in gas flaring and non gas flaring sites are presented in Table 1. There was significant decrease ($P<0.05$) in the alkaloid (NGF; 3.34±0.006 and GF; 2.18±0.004), flavonoid (NGF; 6.67±0.009 and GF; 0.83±0.001) and saponins (NGF; 8.21±0.020 and GF; 2.22±0.009) contents of the *Telferia occidentalis* when the leaves grown in a non gas flaring site was compared with the gas flaring site sample except the tannin content (NGF; 0.009±0.001 and GF; 0.460±0.012) that showed a significant increase ($P>0.05$). This significant decrease in alkaloid, flavonoid and saponin may imply that gas flaring tends to pose a negative effect on the nutritional and medicinal values of *Telferia occidentalis* by reducing these phytochemical contents. In addition, a significant increase in the tannin content may reduce digestibility of nutrients following their ingestion by herbivores (Keay *et al.,* 1989) and improve the astringent nature of tannin which increases its ability to either bind and precipitate or shrink proteins (Gill *et al.,* 1992).

There was significant increase ($P>0.05$) in the levels of Fe (2.78±0.01 to 3.51±0.02), Zn (0.90±0.06 to 1.30±0.02), Pb (0.21±0.01 to 0.54±0.01) and Cd (0.00±0.00 to 0.07±0.01) when the leaves grown in a non gas flaring site were compared with the gas flaring site samples. However, there was no significant difference in Cr concentration of the vegetable from both sites; Cr (0.2±0.01 to 0.06±0.01). Trace metal contamination is of great concern due to its effects as being carcinogenic in nature. Investigating the compositions in the vegetables is important for establishing baseline concentrations from which anthropogenic effects can be measured. All trace metals analysed were found present in the soil samples in traceable amount. National and international regulations on food quality set the maximum permissible levels of toxic metals in human food; hence an increasingly important aspect of food quality should be to control the concentrations of heavy metals in food (Radwan and Salama, 2006; Sobukola *et al.,* 2008). This study showed that Fe abundance, characteristic of green vegetables is maintained as the iron concentrations of the vegetable tend to increase in the leaves from gas flaring locations. Highest content of lead (Pb) was observed from fluted pumpkin leaf. Pb being a serious cumulative body poison enters into the body system through air, water and food and cannot be removed by washing fruits and vegetables (Divrikli *et al.,* 2003). The high levels of Pb in some of these plants may probably be attributed to pollutants in irrigation water, farm soil or due to pollution from the highways traffic (Qui *et al.,* 2000). The level of Pb reported in this study is comparable to those reported for apple (0.19 and 0.76 mg/kg); watermelon (0.30 mg/kg); orange (0.15 mg/kg) and banana (0.02 mg/kg) by Parvean *et al.* (2003) and Radwan and Salama (2006). The permissible limit of lead in vegetables for human consumption is 2.0-2.5 mg/kg dry weight (Samara *et al.,* 1992). Thus, this study showed that the Pb concentration is below the permissible limits. One of the most important metals for normal growth and development in human is Zinc (Divrikli et al., 2006). Its deficiency may be due to inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism (Colak *et al.,* 2005; Narin *et al.,* 2005). The high storage of iron and zinc in the leaves of the leaves of fluted pumpkin vegetable might be advantageous for its useful biochemical functions in human nutrition (Asaolu and Asaolu, 2010). In general, lead concentration in food crops has increased in recent decades owing to human activities.

Table 1: Phytochemical composition (mg/100g) of the dried leaves of the Fluted Pumpkin (*Telferia occidentalis*)

Vegetable	Fluted Pumpkin Leaf (FP)	
Phytoche. (mg/kg)	**NGF**	**GF**
Alkaloids	3.34±0.006[a]	2.18±0.004[b]
Flavonoids	6.67±0.009[a]	0.83±0.001[b]
Saponins	8.21±0.020[a]	2.22±0.009[b]
Tannins	0.01±0.001[a]	0.46±0.012[b]

Values are presented as Mean ± S.E.M. (standard error of mean) of the three determinations. Values for each phytochemical with the same subscripts on the same row for the dry sample of each vegetable are not significantly different (P<0.05) using ONE-WAY ANOVA.

Table 2: Trace metal composition (mg/kg) of the dried leaves of the Fluted Pumpkin (*Telferia occidentalis*)

Vegetable	Fluted Pumpkin (FP)	
Heavy metals (mg/kg)	**NGF**	**GF**
Fe	2.78±0.01[a]	3.51±0.02[b]
Pb	0.21±0.01[a]	0.54±0.01[b]
Cr	0.2±0.01[a]	0.06±0.01[a]
Cd	0.00±0.00[a]	0.07±0.01[b]
Zn	0.90±0.06[a]	1.30±0.02[b]

Values are presented as Mean± S.E.M. (standard error of mean) of the three determinations. Values for each metal with the same subscripts on the same row for the dry sample of each vegetable are not significantly different (P<0.05) using ONE-WAY ANOVA.

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