

# Investigations on Implications of Gas Flaring on Some Phytochemicals and Trace Metal Content of Bitter leaf (*Vernonia amygdalina*)

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## Abstract

The effect of gas flaring on some phytochemicals and trace metals in *Vernonia amygdalina* in Obrikom, a gas flaring community (GF) and Rumualogu, a non gas flaring community (NGF) in Rivers state, Nigeria was investigated. There was significant increase ( $P>0.05$ ) in the composition of alkaloid (NGF;  $1.32\pm 0.044$  and GF;  $3.10\pm 0.001$ ) and tannin (NGF;  $0.03\pm 0.001$  and GF;  $0.31\pm 0.007$ ) in the *Vernonia amygdalina* when the leaf samples from Rumualogu(NGF-Community) was compared with the Obrikom(GF-Community) samples. However, there was no significant difference ( $P<0.05$ ) in the flavonoid (NGF;  $0.79\pm 0.012$  and GF;  $0.88\pm 0.009$ ) and saponin (NGF;  $1.20\pm 0.009$  and GF;  $1.27\pm 0.018$ ) compositions. There was significant increase ( $P>0.05$ ) in the levels of Fe ( $2.21\pm 0.01$  to  $2.96\pm 0.01$ ), Zn ( $0.86\pm 0.01$  to  $1.10\pm 0.01$ ) and Pb ( $0.14\pm 0.03$  to  $0.29\pm 0.02$ ) when the leaves grown in a non gas flaring site were compared with the gas flaring site samples. There was no significant difference in Cr concentration; Cr ( $0.01\pm 0.01$  to  $0.02\pm 0.01$ ). Cadmium level was below detection limit (BDL) in the vegetable from both sites. The implication of these findings is a possible change on the nutritional and medicinal values of *Vernonia amygdalina*.

**Keywords:** Gas flaring, Obrikom, Phytochemicals, Rumualogu

## 1. INTRODUCTION

One of the environmental problems associated with crude oil exploration and exploitation in Nigeria is linked to gas flaring (Obioh, 1999; ERA/FOE Nigeria, 2005). 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).

According to Chijoke, (2002), Nigeria has an estimated 180 billion cubic feet of proven natural gas, making it the ninth largest concentration in the world. 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).

*Vernonia amygdalin*, a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. *V. amygdalina* typically grows to a height of 2-5m. The leaves are elliptical and up to 20 cm long. Its bark is rough (Ijeh and Ejike, 2011). *Vernonia amygdalina* is commonly found in West Africa and *Vernonia galamensis* in East Africa. True to its name, this leaf is bitter to taste, but surprisingly delicious in meals (Farombi and Owoeye, 2011). Other names with which this plant is known includes: Orugbo (amongst the Itsekiri and Urobo tribes in Nigeria), Onugbu (Ibos), Ewuro (Yoruba), Mojunso (East Africa-especially Tanzania). The leaf can be eaten fresh like spinach in soup or dried too. These leaves have great nutritional, herbal and medicinal value (Idu and Onyibe, 2007). It contains very high amount of zinc, important in many enzyme function and keeping the skin fresh. However, it has been found, that *Vernonia amygdalina* have an astringent taste, which affects its intake (Bonsi *et al.*, 1995a). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Buttler and Bailey, 1973; Ologunde *et al.*, 1992. At least 13 other new compounds or vital ingredients have been found in these leaves, after a 40 years study and have the following benefits: Anti-malaria, anti-bacteria, anti-parasites, anti-cancer. It is also effective in preventing indigestion, scurvy, sciatica and rheumatism (Cherepy *et al.*, 1997; Bakowska *et al.*, 2003).

A phytochemical is a natural bioactive component of plant that works with nutrients and dietary fibre to protect against diseases. Research has shown that phytochemicals work with nutrients and are found in fruits, vegetables and nuts and may help to slow 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 to macro- and biochemical micro-organisms through direct influence on the 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 Lead) 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, move easily in plants (Garbisu and Alkorta, 2001). The consequence of trace metals in food have been of considerable interest to man (Asaolu, 1995).

The aim of this study is to investigate the implication of gas flaring on phytochemical and some trace metal composition of *Vernonia amygdalina* 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. 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 bitter leaf (*Vernonia amygdalina*). 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 bitter leaf (*Vernonia amygdalina*) were sorted to eliminate any dead matter and other unwanted particles.

### 2.3. Analysis

#### Quantitative determination of phytochemicals:

##### Alkaloid (Harborne (1993) method)

The sample of 5g 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:

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

##### Flavonoid (Borm and Kocipal-Abyazan (1994))

The plant sample of weight 10g 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:

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

##### Saponin (Obadoni and Ochuko (2001))

The samples were ground and 20g of each plant sample was put into a conical flask and 100cm<sup>3</sup> of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 minutes with continuous stirring at about 55°C. 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 90°C. The concentrate was transferred into a 250ml separating 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 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:

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

##### 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 acid standard were prepared in the range of 0-2.5 ml aliquots in 25 ml volumetric flasks then added with 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:

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

#### Determination of metals from plant samples

Concentrated HNO<sub>3</sub> (3ml) and 0.5ml H<sub>2</sub>SO<sub>4</sub> were added to a 50ml flask containing 1g ground oven-dried plant sample and 1ml of 60% HClO<sub>4</sub> and 0.5 ml concentrated H<sub>2</sub>SO<sub>4</sub>. 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 absorbances 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 result of phytochemical composition (mg/100g) of bitter leaf (*Vernonia amygdalina*) grown both in gas flaring and non gas flaring sites are presented in the Table 1.0 below. There was significant increase (P>0.05) in the composition of alkaloid (NGF; 1.32±0.044 and GF; 3.10±0.001) and tannin (NGF; 0.03±0.001 and GF; 0.31±0.007) in the *Vernonia amygdalina* when the leaf samples planted in Rumualogu was compared with the Obrikom samples. However, there was no significant increase in flavonoids (NGF; 0.79±0.012 and GF; 0.8 ±0.009) and saponin (NGF; 1.20±0.009 and GF; 1.27±0.018) compositions. Although alkaloids and tannins act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste which is the major property of *Vernonia amygdalina* as it is commonly called bitter leaf (Rhoades, 1979). An increase in these phytochemicals may be as a result of the gas flaring effect in the area. The flavonoid and saponin contents of the plant are maintained. Thus, keeping the function of flavonoids which act as antioxidants and help to fight heart disease, protect vision, protect against breast cancer, colon, ovarian and prostate cancer, fight inflammation, protects the arteries and fight allergies and bacteria (Putievsky, et al., 1989). Saponins may serve as anti-feedants and to protect the plant against microbes and fungi. Some plant saponins may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste and so can reduce plant palatability, e.g. in livestock feeds or even imbue them with life threatening animal toxicity (Adebayo *et al.*, 2005).

There was significant increase (P>0.05) in the levels of Fe (2.21±0.01 to 2.96±0.01), Zn (0.86±0.01 to 1.10±0.01) and Pb (0.14±0.03 to 0.29±0.02). However, there was no significant difference in Cr concentration; Cr (0.01±0.01 to 0.02±0.01) and Cd level was below detection limit (BDL) of the vegetable from both sites. Trace metal contamination is of great concern due to its effects as being carcinogenic in nature. Investigation of the compositions in the vegetables is important for establishing baseline concentrations from which anthropogenic effects can be measured. 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. Lead being a serious cumulative body poison was found to be high and can enter 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 through gas flaring, irrigation water, farm soil or due to pollution from the highways traffic (Qui *et al.*, 2000). The permissible limit of lead in vegetables for human consumption is 2.0-2.5 mg/kg dry weight (Samara *et al.*, 1992). In general, lead concentration in food crops has increased in recent decades owing to human activities. 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 vegetable might be advantageous for their useful biochemical functions in human nutrition (Asaolu and Asaolu, 2010).

**Table 1: Phytochemical composition (mg/100g) of the dried leaves of the Bitterleaf (*Vernonia amygdalina*)**

Vegetable	Bitter leaf	
	(BL)	
Phytoche. (mg/kg)	NGF	GF
Alkaloids	1.32±0.044 <sup>a</sup>	3.10±0.001 <sup>b</sup>
Flavonoids	0.79±0.012 <sup>a</sup>	0.88±0.009 <sup>a</sup>
Saponins	1.20±0.009 <sup>a</sup>	1.27±0.018 <sup>a</sup>
Tannins	0.03±0.001 <sup>a</sup>	0.31±0.007 <sup>b</sup>

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 Bitter leaf (*Vernonia amygdalina*)**

Vegetable	Bitter leaf	
	(BL)	
Heavy metals (mg/kg)	NGF	GF
Fe	2.21±0.01 <sup>a</sup>	2.96±0.01 <sup>b</sup>
Pb	0.14±0.03 <sup>a</sup>	0.29±0.02 <sup>b</sup>
Cr	0.01±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>
Cd	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
Zn	0.86±0.01 <sup>a</sup>	1.10±0.01 <sup>b</sup>

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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