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Comparative Phytochemical and Proximate Analysis of the Response of Some Roadside Plants to Highway Exhausts on East-West Road, Obio/Akpor L.GA, Rivers State

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Abstract

In a rapidly developing city like Port Harcourt, high vehicular emissions are to be expected. These emissions pose a chronic environmental stress on plants, effectively altering their structural and biochemical frameworks. This study investigate how highway exhaust along the East-West Road in Rivers State, Nigeria, changes chemical traits of two roadside plants that are abundant on the East-West Road. The plants are *Amaranthus spinosus* L. (Amanranthaceae) and *Bidens pilosa* L. (Asteraceae). Fresh, mature plant samples were collected from two high-traffic points; Eleme Junction and Obiri Ikwerre Flyover. The samples were compared against control samples taken at low traffic points away from the polluted site for each site; 3.6km away for Eleme Junction, 3.1km for Obiri Ikwerre Flyover, all in Obio/Akpor L.G.A. Analysis was done on the leaf samples as the leaves serve as the major source of exhaust intake into the plant. The proximate composition of the leaf samples was determined using the methods of the Association of Official Analytical Chemists (A.O.A.C). Phytochemical profiling of the leaf samples was carried out using Gas Chromatography–Mass Spectrometry (GC-MS), different compounds were identified at different retention times. The phytochemical analysis revealed twenty-nine compounds detected in *A. spinosus*, with each of them having different abundance across four sites. Phytol ranged from 12.89±0.71 at Obiri Ikwerre to 8.63±0.52 with the control sites having 12.46±0.68 and 12.10±0.65 while Phytic acid ranged from 10.48±0.63 at Obiri Ikwerre to 7.89±0.51 at Eleme Junction while the control sites had 3.94±0.32 and 3.80±0.89. For *B. pilosa*, Phytol ranged from 15.78±0.84 at Obiri Ikwerre to 10.78±0.61 at Eleme junction while the control sites had 13.51±0.72 and 5.90±0.42 while Phytic acid ranged from 8.42±0.55at Obiri Ikwerre to 5.72±0.43 at Eleme junction while control sites had 2.45±0.22 and 3.30±0.26. For proximate composition, the present study found that in *A. spinosus*, protein was highest at Eleme junction control at 27.46±1.15, and lowest at Obiri Ikwerre with 19.22±0.93, while carbohydrate showed the opposite pattern, highest at Obiri Ikwerre 46.91±1.13 and lowest at control 1 33.63±0.95. In *B. pilosa*, protein was highest at control 1 22.74±1.02 and carbohydrate ranged from 39.98±1.11 to 44.64±1.23 across the four sites, with fiber highest at Obiri Ikwerre. It can be concluded that according to the proximate analysis of *A. spinosus*, plants found at the control site has higher nutritional value than those found at the polluted siteswhile *B. pilosa* has the same range along the four sites. This study recommends that *A. spinosus* and *B. pilosa* can be explored for their nutritional values provided other factors like soil quality are considered and can also be used as bio indicators of pollution as polluted sites seems to thrive more than the unpolluted sites.

Keywords: *Amaranthus spinosus*, *Bidens pilosa*, Phytochemical composition, proximate analysis, Air pollution

INTRODUCTION

For every developing city, the highway is an integral part as major developments are built around it but the environmental implications are often overlooked. The automobile exhausts that are constantly released from vehicles using this highway are a major cause of environmental pollution. These exhausts are not just simple gases but a mixture of gases and particulate matter. Roadside plants are the major recipients of these gases and particulate matter (Uka *et al*., 2019; Uka & Belford, 2020; Muthu *et al*., 2021; Guo *et al*., 2023). This exposure varies with traffic volume, fuel quality, and weather, the stress it places on vegetation is at once persistent and unpredictable. The leaf bears the brunt of it: it is where gas exchange, photosynthesis, and transpiration all occur, and it is consequently where the visible and invisible signatures of pollution first appear (Singh *et al*., 2020; Uka & Belford, 2020; Singh, 2021; Shakeel *et al*., 2023).

Roadside plants cannot move away from direct impact of this exhaust as animals can and are therefore continuously exposed to these pollutants throughout their life cycles, forcing them to absorb, accumulate and integrate pollutants on their surfaces. Therefore they serve as vital biological sinks and indicators of environmental health. This comes at a high cost as constant exposure to automobile exhaust brings about various morphological, biochemical, anatomical and physiological deformities (Hasnain, *et al.,* 2024).

Rivers State, situated in the South-South geopolitical zone of Nigeria, serves as the economic hub of the Niger Delta region. The state experiences unique environmental pressures due to the convergence of intensive oil and gas operations, industrial estates, and an overstretched municipal transport network (Gam &Amadi, 2025). Major highways like the East-West road suffer from frequent traffic congestion, resulting in sustained, high-concentration exposure of roadside ecosystems to automobile exhausts (Gam &Amadi, 2025).

Understanding the boundaries between natural variation and pollution driven plasticity is highly vital especially for highly resilient ethnobotanically important species like *Amaranthus spinosus* and *Bidens pilosa* that were found to be abundant in the highway used for this study.

Amaranthus spinosus belongs to the family Amaranthaceae, a large and, by most taxonomic accounts, rather unruly group in which species frequently display considerable morphological plasticity and ecological adaptability distinguished from other Amaranths by this spiny stems and petioles(Kumar *et al*., 2014; Assad *et al*., 2017; Ayed& Ahmed, 2026).

Bidens pilosa belongs to the family Asteraceae and the genus *Bidens*, itself a large and widely distributed group comprising somewhere between 230 and 240 species globally (Silva *et al*., 2011; Bartolome *et al*., 2013; Kuo *et al*., 2020). It is generally described as an annual or short-lived perennial erect herb, often ruderal in habit and capable of considerable vigour under favourable conditions (Connelly, 2009; Silva *et al*., 2011; Kuo *et al*., 2020).

For this study, we investigated the phytochemical and proximate responses of *A. spinosus and B. pilosa* to air pollutants from automobile exhausts.

MATERIALS AND METHODS

Study Site

The study was carried on the East-West Road, a major highway corridor in Obio/Akpor Local Government Area of Rivers State, Nigeria. Table 1 and Figure 1 identify the different sampling points used in the study.

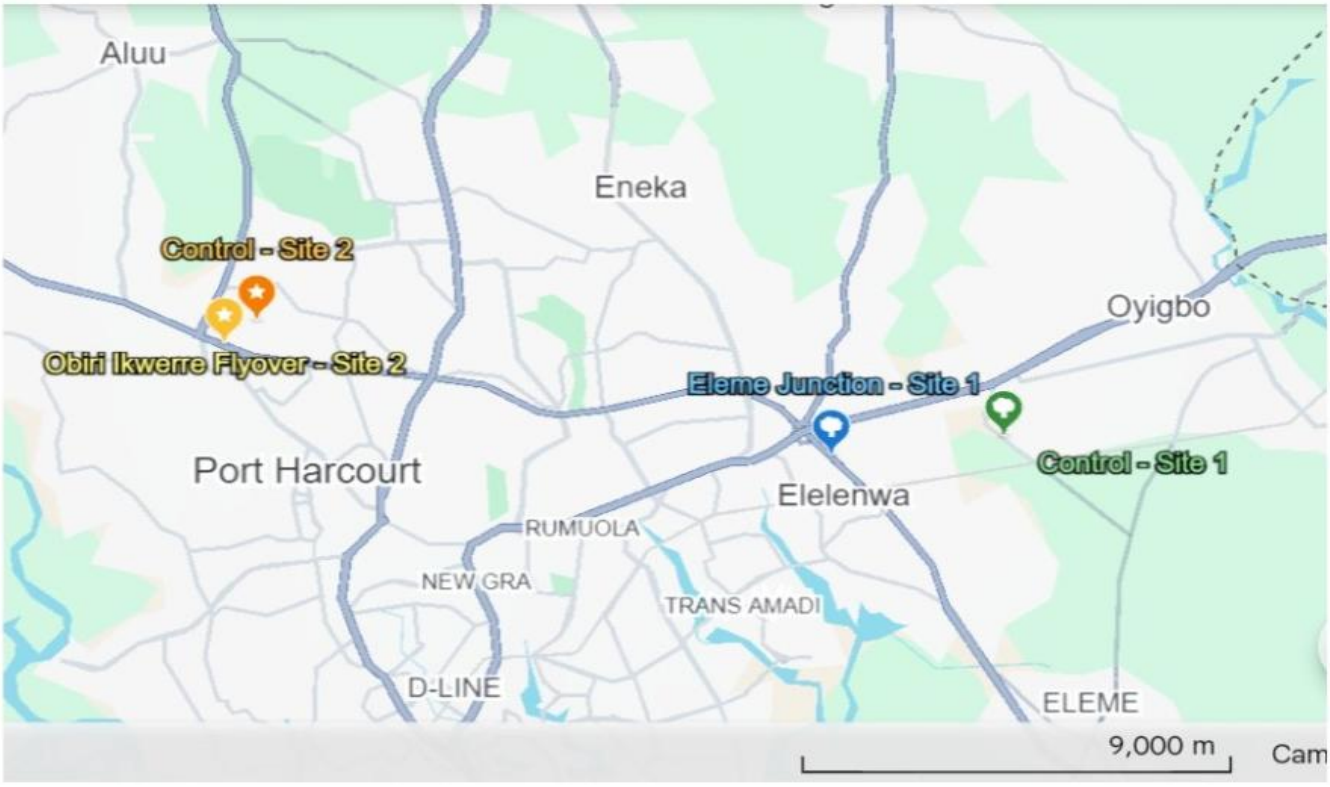

Fig 1: Location of the Sampling sites

Table 1: Point of Collection of Materials Used for the Study

SITE	LONGITUDE (E)	LATITUDE (N)	ELEVATION (m)
Eleme junction (site 1)	7° 04' 19.36"	4° 51' 01.18"	20.02
Control (3.6km from site 1)	7° 06' 14.54"	4° 51' 15.28"	17.33
Obiri Ikwerre (site 2)	6° 57' 34.14"	4° 52' 24.22"	17.25
Control (3.1km from site 2)	6° 58' 58.38"	4° 53' 17.44"	11.38

Plant sample collection

Fresh, mature plant samples were collected from two high-traffic points; Eleme Junction and Obiri Ikwerre Flyover. The samples were compared against control samples taken at low traffic points away from the polluted site for each site; 3.6km away for Eleme Junction, 3.1km for Obiri Ikwerre Flyover, all in Obio/Akpor L.G.A. Identification and authentication were done at the Herbarium of Rivers State University. Ten mature healthy leaves were harvested per plant species, with visibly damaged or diseased leaves excluded from collection. Leaves were placed in clean, labeled polythene bags and transported to the laboratory.

Chemical Analysis

Proximate Study

The proximate composition of the leaf samples was determined using the methods of the Association of Official Analytical Chemists (A.O.A.C). The parameters analyzed were moisture content, ash content, crude protein, crude lipid, crude fiber, and carbohydrate content.

Moisture Content Determination

Five grams of each leaf sample was weighed into a dried, pre-weighed crucible. The sample was placed in a moisture extraction oven at 105°C and heated for three hours. The dried sample was transferred to a desiccator, allowed to cool, and reweighed. This process was repeated until a constant weight was obtained. The difference in weight was calculated as a percentage of the original sample weight, as shown below:

$$\text{Percentage moisture} = \frac{(W2-W1)}{(W2-W3)}\ x\ 100$$

Where W1 is the initial weight of the empty dish, W2 is the weight of the dish plus the undried sample, and W3 is the weight of the dish plus the dried sample.

Ash Content Determination

Two grams of each leaf sample was weighed into a crucible, heated in a moisture extraction oven at 100°C for three hours, and then transferred into a muffle furnace at 550°C until the residue turned white and was free of carbon. The sample was removed from the furnace, cooled in a desiccator to room temperature, and reweighed immediately. The weight of the residual ash was then calculated as a percentage of the original sample weight, as shown below:

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{original weight of sample}}\ X\ 100$$

Crude Protein Determination

The micro-Kjeldahl method described by A.O.A.C. (1990) was used. Two grams of each leaf sample was mixed with 10 ml of concentrated H_2SO_4 in a heating tube, and one tablet of selenium catalyst was added before the mixture was heated inside a fume cupboard. The digest was transferred into distilled water, and a 10 ml portion of the digest was mixed with an equal volume of 45% NaOH solution and poured into a Kjeldahl distillation apparatus. The mixture was distilled, and the distillate was collected into 4% boric acid solution containing three drops of methyl red indicator. A total of 50 ml of distillate was collected and titrated. Each sample was analyzed in duplicate, and the average value was taken. The nitrogen content obtained was multiplied by 6.25 to derive the crude protein content, expressed as percentage nitrogen, as shown below:

$$\text{\% Nitrogen} = \frac{(100\ \text{x N X 14 X VF X T})}{(100\ \text{X Va})}$$

Where N is the normality of the titrant (0.1N), VF is the total volume of the digest (100 ml), T is the titre value, and Va is the aliquot volume distilled.

Crude Lipid (Fat) Determination

Two grams of each leaf sample was loosely wrapped in filter paper and placed in a thimble fitted to a clean, dried, and pre-weighed round-bottom flask containing 120 ml of petroleum ether. The sample was heated using a heating mantle and allowed to reflux for five hours. Heating was then stopped, and the thimble containing the spent sample was retained and later weighed. The difference in weight represented the mass of fat extracted, expressed as a percentage of the sample weight, as shown below:

% Crude lipid = $\frac{(W2-W1)}{W3}$ X 100
Where W1 is the weight of the empty extraction flask, W2 is the weight of the flask plus extracted oil, and W3 is the weight of the sample.

Crude Fiber Determination
Two grams of each leaf sample and one gram of asbestos were added to 200 ml of 1.25% H_2SO_4 and boiled for 30 minutes. The solution and its contents were poured into a Buchner funnel fitted with muslin cloth and secured with an elastic band, and the mixture was filtered. The residue was then transferred into 200 ml of boiling NaOH, and boiling was continued for a further 30 minutes before the mixture was transferred to the Buchner funnel and filtered again. The residue was washed twice with alcohol and thrice with petroleum ether, then placed in a clean, dry crucible and dried in the moisture extraction oven to constant weight. The crucible was removed, cooled, and weighed, and the difference in weight (loss on ignition) was recorded as crude fibre and expressed as a percentage, as shown below:
% Crude fibre = $\frac{(W1-W2)}{Wt}$ X 100
Where W1 is the weight of the sample before incineration, W2 is the weight of the sample after incineration, and Wt is the weight of the original sample.

Carbohydrate Content Determination
The nitrogen-free extract method described by A.O.A.C. (1990) was used. Carbohydrate content was calculated by difference, as 100 minus the summation of the other proximate parameters, as shown below:
NFE (% carbohydrate) = 100 − (m + p + F1 + A + F2)
Where m is moisture, p is protein, F1 is fat, A is ash, and F2 is crude fiber.

Phytochemical Study
Phytochemical profiling of the leaf samples was carried out using Gas Chromatography–Mass Spectrometry (GC-MS). Analysis was performed using an Agilent 6890N Gas Chromatograph coupled with an Agilent 5975 Mass Selective Detector (Agilent Technologies, 2005). Equipment used included auto-sampler vials, 150 µL vial inserts and crimp seals, a vial crimper and decrimper, a 2.5 mL airtight syringe or 3 mL disposable hypodermic syringe, a 10-microliter autosampler syringe, a 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5) of 1-µm film thickness, and a Soxhlet extractor. Reagents used included Air–Zero grade, Helium gas of UHP grade, n-hexane, anhydrous sodium sulfate, and standard plant-chemical internal standards.

A 20-g aliquot of leaf sample was homogenized, and a 10-g aliquot was spiked with the labelled compounds. The sample was mixed with anhydrous sodium sulfate, allowed to dry for a minimum of 30 minutes, and extracted for 18 hours using methylene chloride in a Soxhlet extractor. The extract was evaporated to dryness, and the lipid content was determined.

Extracts for phytochemical analysis were subjected to a sequential methylene chloride–n-hexane (1:1) cleanup specific to these analytes, and 1 µL of the sample was injected into the Agilent 6890 gas chromatograph fitted with the 5975 MS detector and the 0.32-mm ID fused-silica capillary column described above. The following temperature ramp was used: injector at 250°C, oven initially at 200°C held for one minute and then heated to 230°C at 1.5°C per minute, held for a further ten minutes. Characterization and identification of phytochemicals from the leaf extract was completed in SCAN mode with an m/z range of 35 to 450. The flow

rate of the helium carrier gas was 1 mL per minute, with manual injection of a 1 µL injection volume. Interpretation of the GC-MS mass spectra was carried out using the database of the National Institute of Standards and Technology (NIST). The mass spectrum of each unknown compound was compared with the spectra of known compounds stored in the NIST library (National Institute of Standards and Technology, 2020), and major components were identified using authentic standards alongside records from computerized spectral libraries.

Statistical Analysis

Data collected were subjected to a two way analysis of variance (ANOVA) using Excel Data Analysis Toolpak. Where significant interaction were detected ($p \leq 0.05$), mean separation was performed using Fisher's Least Significant Difference (LSD) post hoc test.

RESULTS

Proximate Composition

The proximate composition for the two plant species as shown in Table 2 showed that for *A. spinosus*, the moisture content was more at the Eleme junction (11.48%) as compared to the control site (9.13%). Obiri Ikwerre also showed the same pattern with moisture content at the polluted site (10.74%), higher than its control site (9.25%). Ash content followed a different pattern with the control sites considerably higher than the two polluted sites. Protein, lipids and crude fibers followed the pattern of Ash with control sites higher than polluted sites while Carbohydrate followed the pattern of moisture with polluted sites higher than control sites.

For *B. pilosa*, the polluted sites had higher moisture content (11.24% and 10.78%) than the control sites (10.20% and 9.85%), lipid content followed a different pattern with control sites having a higher value (4.08% and 4.15%) than the polluted sites (8.89% and 10.57%). Ash, protein followed the pattern of lipid while carbohydrate and fiber followed the pattern of moisture content.

Table 2: Proximate Composition of *Amaranthus spinosus* and *Bidens pilosa*

Species	Parameter (%)	Control	Eleme junction	Obiri Ikwerre	Control 2
Amaranthus spinosus	**Moisture**	9.13 ± 0.19^{a}	11.48 ± 0.27^{b}	10.74 ± 0.33^{b}	9.25 ± 0.22^{a}
	Ash	13.05 ± 0.53^{a}	9.36 ± 0.36^{b}	8.52 ± 0.43^{b}	12.40 ± 0.48^{a}
	Protein	27.46 ± 1.15^{a}	21.90 ± 0.99^{b}	19.22 ± 0.93^{c}	25.80 ± 1.05^{a}
	Lipid	4.98 ± 0.29^{a}	4.41 ± 0.23^{a}	4.76 ± 0.25^{a}	4.91 ± 0.27^{a}
	Fiber	11.75 ± 0.64^{a}	10.24 ± 0.49^{a}	9.85 ± 0.56^{a}	11.42 ± 0.60^{a}
	Carbohydrate	33.63 ± 0.95^{a}	42.61 ± 1.13^{b}	46.91 ± 1.23^{c}	36.22 ± 1.02^{a}
Bidens pilosa	**Moisture**	10.20 ± 0.25^{a}	11.24 ± 0.29^{b}	10.78 ± 0.33^{ab}	9.85 ± 0.24^{a}
	Ash	10.24 ± 0.46^{a}	8.89 ± 0.35^{b}	10.57 ± 0.52^{a}	10.95 ± 0.49^{a}
	Protein	22.74 ± 1.02^{a}	19.26 ± 0.87^{b}	20.85 ± 0.94^{ab}	23.10 ± 0.98^{a}
	Lipid	4.08 ± 0.23^{a}	3.78 ± 0.18^{a}	3.92 ± 0.21^{a}	4.15 ± 0.22^{a}
	Fiber	11.35 ± 0.53^{a}	12.19 ± 0.57^{a}	13.90 ± 0.71^{a}	11.80 ± 0.58^{a}
	Carbohydrate	41.39 ± 1.03^{a}	$44.64 \pm 1,23^{b}$	39.98 ± 1.11^{a}	40.20 ± 1.08^{a}

Values are presented as Mean ± Standard Deviation (SD), n = 3 replicates
Superscripts with different letters within rows are significantly different according to the LSD ($p \leq 0.05$)

Phytochemical Composition

Across most of the twenty-nine compounds detected in *Amaranthus spinosus*,the control sites carries the highest relative abundance while Eleme junction carries the lowest, with Obiri Ikwerre falling in between.This pattern holds for hexadecanoic acid methyl ester (5.89% at control 1 against 1.48% at Eleme junction, 5.54% at control 2 against 2.63 at Obiri Ikwerre). Also true for ergostrol, squalane, ethyl butyrate and terpinolene.Phytic acid broke from this pattern, recording 10.48% at Obiri Ikwerre, 7.89% at Eleme, and 3.94% at the control site 1 and 3.80% at control site 2.Campesterol showed the widest spread of any compound, at 2.72% at Obiri Ikwerre, 11.62% at Eleme, and 18.74% at control site 1 and 18.5% at control site 2.Alpha-Amyrin (second peak) also rose steadily across sites, at 2.24% at Obiri Ikwerre, 5.73% at Eleme, 8.42% at the control 1, and 8.20% at control site 2.Table 3 describes the pattern for all compounds detected.

Table 3: Phytochemical Profile -*Amaranthus spinosus* (% Peak Area)

Compound	% Control	% Eleme junction	% Obiri Ikwerre	% Control 2
Hexadecanoic acid methyl ester	5.89 ± 0.42^{a}	1.48 ± 0.15^{b}	2.63 ± 0.24^{c}	5.45 ± 0.38^{a}
Phytol	12.46 ± 0.68^{a}	8.63 ± 0.52^{b}	12.89 ± 0.71^{a}	12.10 ± 0.65^{a}
2,3-Butanedione	5.13 ± 0.39^{a}	3.74 ± 0.31^{b}	2.36 ± 0.21^{c}	4.85 ± 0.35^{a}
Ergostrol	6.28 ± 0.45^{a}	3.08 ± 0.26^{b}	5.18 ± 0.39^{c}	6.10 ± 0.42^{a}
Phytic acid	3.94 ± 0.32^{a}	7.89 ± 0.51^{b}	10.48 ± 0.63^{c}	3.80 ± 0.29^{a}
n-Hexadecanoic acid	8.15 ± 0.53^{a}	6.42 ± 0.44^{b}	9.62 ± 0.58^{a}	8.28 ± 0.50^{a}
Carpaine	3.78 ± 0.29^{a}	2.86 ± 0.24^{b}	3.79 ± 0.30^{a}	3.65 ± 0.27^{a}
Squalane	2.64 ± 0.23^{a}	1.43 ± 0.14^{b}	1.52 ± 0.16^{b}	2.58 ± 0.21^{a}
Alpha-Pinene	5.60 ± 0.41^{a}	4.77 ± 0.36^{b}	6.89 ± 0.48^{b}	5.35 ± 0.38^{a}
Stigmasterol	4.76 ± 0.36^{a}	3.83 ± 0.30^{a}	4.44 ± 0.34^{a}	4.68 ± 0.32^{a}
15-Hydroxypentadecanoic acid	8.14 ± 0.52^{a}	6.57 ± 0.45^{b}	8.74 ± 0.55^{a}	8.05 ± 0.48^{a}
Alpha-Amyrin	5.63 ± 0.41^{a}	4.89 ± 0.37^{a}	4.60 ± 0.35^{a}	5.50 ± 0.39^{a}
Beta-Pinene	6.86 ± 0.47^{a}	6.42 ± 0.44^{a}	9.85 ± 0.61^{b}	6.70 ± 0.45^{a}
3-Carene	3.17 ± 0.26^{a}	2.13 ± 0.19^{b}	3.90 ± 0.31^{a}	3.05 ± 0.24^{a}
Ethyl butyrate	3.54 ± 0.28^{a}	1.46 ± 0.14^{b}	1.51 ± 0.15^{b}	3.40 ± 0.25^{a}
Alpha-Amyrin (2)	8.42 ± 0.56^{a}	5.73 ± 0.42^{b}	2.24 ± 0.20^{c}	8.20 ± 0.52^{a}
Eicosane	5.22 ± 0.39^{a}	2.86 ± 0.24^{b}	4.38 ± 0.34^{a}	5.10 ± 0.36^{a}
Terpinolene	3.67 ± 0.29^{a}	2.11 ± 0.19^{b}	1.94 ± 0.18^{b}	3.55 ± 0.27^{a}
Alpha-Farnesene	4.83 ± 0.37^{a}	3.52 ± 0.28^{b}	4.68 ± 0.36^{a}	4.72 ± 0.35^{a}
Lupeol	4.39 ± 0.34^{a}	2.74 ± 0.23^{b}	3.26 ± 0.27^{b}	4.25 ± 0.31^{a}
Methyl linoleate	3.13 ± 0.26^{a}	2.69 ± 0.22^{a}	2.90 ± 0.24^{a}	3.08 ± 0.23^{a}
Beta-Sitosterol	8.46 ± 0.53^{a}	7.30 ± 0.48^{a}	8.55 ± 0.54^{a}	8.35 ± 0.50^{a}
Campesterol	18.74 ± 0.95^{a}	11.62 ± 0.70^{b}	2.72 ± 0.23^{c}	18.15 ± 0.91^{a}
Methyl stearate	5.20 ± 0.39^{a}	3.49 ± 0.28^{b}	4.49 ± 0.35^{c}	5.08 ± 0.36^{a}
Camphene	3.69 ± 0.29^{a}	2.52 ± 0.22^{b}	2.36 ± 0.21^{b}	3.60 ± 0.26^{a}
Pyrrole,2-methyl-5-phenyl	2.40 ± 0.21^{a}	1.84 ± 0.17^{b}	3.24 ± 0.26^{b}	2.30 ± 0.19^{a}
2-Furancarboxaldehyde	6.78 ± 0.47^{a}	4.53 ± 0.35^{b}	5.18 ± 0.39^{b}	6.60 ± 0.44^{a}
Beta-Myrcene	3.53 ± 0.28^{a}	2.68 ± 0.23^{b}	2.36 ± 0.21^{b}	3.45 ± 0.26^{a}
Neophytadiene	5.76 ± 0.42^{a}	3.08 ± 0.25^{b}	4.69 ± 0.36^{c}	5.60 ± 0.39^{a}

Values are % peak area ± SD, Superscripts with different letters within rows are significantly different according to the LSD ($p \leq 0.05$)

Across most of the twenty-nine compounds detected in *Bidens pilosa* , Obiri Ikwerre carries the highest relative abundance among the four roadside stations while Eleme carries the lowest, with the two control sites falling in between.This pattern holds for phytol (15.78% at Obiri Ikwerre against 10.78% at Eleme), squalane (9.36% against 6.51%), beta-sitosterol (14.10% against 9.36%), campesterol (9.24% against 6.13%), alpha-pinene (5.77% against 3.68%), and alpha-farnesene (5.89% against 3.75%).Control values for these same compounds fell between the two, at 13.51% and 5.90% for phytol, 7.37% and 1.30% for squalane, 11.18% and 10.90% for beta-sitosterol, 6.47% and 6.25% for campesterol, 6.41% and 5.10% for alpha-pinene, and 7.85% and 4.65% for alpha-farnesene. Lidocaine broke from this pattern, recording 4.71% at control 1, 4.55% at control 2, 1.65% at Eleme junction, and 1.89% at Obiri Ikwerre. Phytic acid also departed from the general pattern, peaking at Obiri Ikwerre (8.42%), with Eleme at 5.72%, control 2 at 3.30% and control 1 lowest at 2.45%.Table 4 describes the pattern for all compounds detected.

Table 4: Phytochemical Profile -*Bidens pilosa* (% Peak area)

Compound	% Control	% Eleme	% Obiri Ikwerre	% Control 2
8-Methyloctahydrocoumarin	5.83 ± 0.41^{a}	2.54 ± 0.28^{b}	4.46 ± 0.35^{c}	4.10 ± 0.32^{a}
Phytol	13.51 ± 0.72^{a}	10.78 ± 0.61^{b}	15.78 ± 0.84^{c}	5.90 ± 0.42^{a}
2,3-Butanedione	2.68 ± 0.25^{a}	1.86 ± 0.19^{b}	3.51 ± 0.31^{c}	4.75 ± 0.36^{a}
Benzphetamine	5.18 ± 0.39^{a}	3.43 ± 0.29^{b}	4.89 ± 0.36^{a}	7.50 ± 0.50^{a}
Phytic acid	2.45 ± 0.22^{a}	5.72 ± 0.43^{b}	8.42 ± 0.55^{c}	3.30 ± 0.26^{a}
*Pyridine,3-propyl**	3.42 ± 0.27^{a}	1.89 ± 0.17^{b}	1.45 ± 0.15^{b}	2.50 ± 0.21^{a}
Carpaine	2.98 ± 0.24^{a}	2.44 ± 0.21^{a}	4.93 ± 0.38^{b}	3.40 ± 0.27^{a}
Squalane	7.37 ± 0.51^{a}	6.51 ± 0.47^{a}	9.36 ± 0.62^{b}	1.30 ± 0.13^{a}
Alpha-Pinene	6.41 ± 0.45^{a}	3.68 ± 0.31^{b}	5.77 ± 0.42^{a}	5.10 ± 0.38^{a}
n-Hexadecanoic acid	4.26 ± 0.34^{a}	3.74 ± 0.30^{a}	7.14 ± 0.49^{b}	7.60 ± 0.51^{a}
15-Hydroxypentadecanoic acid	6.08 ± 0.44^{a}	4.69 ± 0.36^{b}	6.42 ± 0.47^{a}	3.40 ± 0.27^{a}
2-Butanone	4.93 ± 0.38^{a}	3.52 ± 0.29^{a}	8.78 ± 0.59^{b}	4.10 ± 0.31^{a}
Beta-Pinene	7.75 ± 0.53^{a}	5.07 ± 0.39^{b}	9.94 ± 0.67^{c}	10.90 ± 0.65^{a}
3-Carene	3.44 ± 0.28^{a}	3.31 ± 0.27^{a}	5.46 ± 0.41^{b}	6.25 ± 0.43^{a}
Ethyl butyrate	2.63 ± 0.23^{a}	1.89 ± 0.18^{b}	3.89 ± 0.32^{c}	4.55 ± 0.33^{a}
Propionaldehyde	3.54 ± 0.29^{a}	1.89 ± 0.17^{b}	2.45 ± 0.22^{b}	6.70 ± 0.46^{a}
Beta-Phellandrene	1.37 ± 0.14^{a}	0.46 ± 0.08^{b}	1.87 ± 0.18^{c}	3.40 ± 0.26^{a}
Terpinolene	5.26 ± 0.40^{a}	2.13 ± 0.20^{b}	5.53 ± 0.42^{a}	2.48 ± 0.20^{a}
Alpha-Farnesene	7.85 ± 0.54^{a}	3.75 ± 0.31^{b}	5.89 ± 0.44^{c}	4.65 ± 0.35^{a}
D-Limonene	3.54 ± 0.29^{a}	4.20 ± 0.34^{a}	8.36 ± 0.57^{b}	2.50 ± 0.21^{a}
Sparteine	4.24 ± 0.33^{a}	3.48 ± 0.28^{ab}	4.76 ± 0.36^{b}	4.10 ± 0.31^{a}
Beta-Sitosterol	11.18 ± 0.69^{a}	9.36 ± 0.58	14.10 ± 0.81^{c}	10.90 ± 0.65^{a}
Campesterol	6.47 ± 0.46^{a}	6.13 ± 0.44^{a}	9.24 ± 0.61^{b}	6.25 ± 0.43^{a}
*Lidocaine**	4.71 ± 0.36^{a}	1.65 ± 0.16^{b}	1.89 ± 0.18^{c}	4.55 ± 0.33^{a}

Camphene	6.95 ± 0.49^{a}	3.87 ± 0.32^{b}	4.63 ± 0.35^{c}	6.70 ± 0.46^{a}
*Pyrrole,2-methyl-5-phenyl**	3.51 ± 0.28	2.42 ± 0.22	3.42 ± 0.27	3.40 ± 0.26^{a}
2-Furancarboxaldehyde	2.53 ± 0.22^{a}	3.13 ± 0.26^{ab}	2.59 ± 0.23^{b}	2.48 ± 0.20^{a}
Beta-Myrcene	4.79 ± 0.37^{a}	4.90 ± 0.38^{a}	3.74 ± 0.30^{b}	4.65 ± 0.35^{a}
4-Hexen-2-one	2.63 ± 0.23^{a}	3.46 ± 0.28^{a}	5.84 ± 0.43^{b}	2.50 ± 0.21^{a}

(Values are % peak area ± SD, Superscripts with different letters within rows are significantly different according to the LSD ($p \leq 0.05$)

DISCUSSION

Proximate comparisons

For proximate composition, the present study found that in *Amaranthus spinosus*, protein was highest at control sites and lowest at polluted sites, while carbohydrate showed the opposite pattern, highest at polluted sites and lowest at control sites. In *Bidens pilosa*, protein was highest at control sites with fiber highest at Obiri Ikwerre.

These protein figures stand out as notably high when set against similar studies. Rjeibi *et al*. (2017) reported *Amaranthus spinosus* protein of only 1.94% to 3.54% across their Tunisian sites, far below the present study's range, even though their work also showed strong site-to-site variation in moisture, fat, and ash, matching the present study's general pattern of location-driven differences. Isoje *et al*. (2025), working in Nigeria, recorded protein of 17.52% in the dry season and 7.55% in the rainy season, again lower than the present study's values but consistent with the idea that timing and environment change nutritional content. Oluwole *et al*. (2023), sampling *Amaranthus spinosus* from dumpsites in Lagos, found much lower protein and lipid than the present study, while still showing the same underlying pattern that different collection points give different results. Ogundare and Tanimola (2022) and Singh and Punia (2020), both working with processed amaranth flour rather than fresh leaves, reported moisture close to the present study's figures but lower protein, fiber, and ash, a difference likely tied to the fact that flour is a processed product rather than a whole leaf. Boyiako *et al*. (2026) reported much higher moisture, but this was because their results were expressed on a fresh-weight rather than dry-weight basis, a reminder that the units and basis a study uses can make two pieces of work look more different than they really are.

For *Bidens pilosa* , Alikwe *et al*. (2014), working with leaf meal in Nigeria, reported protein of 15.86%, lower than the present study's range, though their ash and fiber values were broadly similar. Sengupta *et al*. (2023), working in India, reported much lower protein and fat for the whole plant, a clear contrast with the present study. Rahelivololoniaina (2023), studying leafy vegetables in Madagascar, found *Bidens pilosa* carbohydrate of 20.85%, well below the 39.98% to 44.64% recorded in the present study, even though the plant was still described there as one of the more lipid-rich of the leafy vegetables sampled. Adedapo *et al*. (2011), comparing this plant in a South African and Nigerian context, found the same broad mix of moisture, ash, protein, lipid, fiber, and carbohydrate present, without giving exact figures to compare directly. Hsu *et al*. (2021) reported an unusually high carbohydrate value of 78.8 g per 100 g in *Bidens pilosa* pollen from Taiwan, a figure that contrasts sharply with the present study simply because pollen and leaves are different plant parts with very different compositions.

Pulling all of this together, the closest match in overall pattern is not any single paper but the repeated finding across the literature that proximate composition in Amaranthus and related leafy vegetables shifts with collection site, as shown both for *Amaranthus spinosus*

specifically (Rjeibi *et al*., 2017) and for leafy vegetables generally in the Niger Delta (Orisa *et al*., 2020). The present study's *Amaranthus spinosus* protein values are unusually high compared with most of the literature reviewed, which mostly falls between about 2% and 17.5% depending on plant part and preparation, and its *Bidens pilosa* fiber and ash values sit comfortably within published ranges even though its protein is again on the higher side. Overall, there is agreement in the general trend, that composition depends on where and how the plant is collected, but frequent disagreement in the actual magnitude, especially for protein and carbohydrate, which supports reading the Rivers State differences recorded in this study as a response to local environmental conditions rather than as fixed values for either species.

Phytochemical comparisons

In the present study, the results showed that among the twenty-nine compounds detected in *Bidens pilosa*, Obiri Ikwerre generally recorded the highest amounts, Eleme recorded the lowest, and control sites fell in between for most of the compounds, including phytol, squalane, beta-sitosterol, campesterol, alpha-pinene, and alpha-farnesene. A few compounds moved differently: lidocaine was highest at the control sites, phytic acid peaked at Obiri Ikwerre, and 2-butanone also spiked at Obiri Ikwerre. This pattern, where one location consistently carries more of most compounds while another carries less, agrees with what researchers like Xuan and Khanh (2016) reported about this same plant in other parts of the world, even though the exact compounds involved are usually not the same.

Xuan and Khanh (2016), in a broad review of the plant's chemistry, reported that its phytochemical content changes under different environmental conditions and that the amount of secondary metabolites it produces is strongly shaped by the surrounding environment. This lines up with what the present study found, since the four roadside stations gave clearly different compound levels even though the plant species was the same. The same review showed that oils from Argentina contained three major compounds at 11.2%, 39.5%, and 3.3% that were completely missing in the Japanese samples, while the Japanese samples were instead dominated by other compounds present at only 2.09% and 3.71%. This is the same result reported in the present study: the idea that location changes plant chemistry holds true everywhere, but the specific compounds that go up or down are rarely identical from one place to another.

This idea is repeated in several other countries. In China, Jiang *et al*. (2026) found that the amount of volatile oil produced by the plant ranged from 0.93% to 2.99% depending on which of the eleven regions the sample came from, and the strength of its antioxidant activity also changed with origin, with Bozhou oil giving a DPPH value of 38.06 mg/mL and Guiyang oil giving different values for two other antioxidant tests. Ye *et al*. (2026), also working in China, found that samples from Guangxi had higher and more balanced levels of the measured compounds than samples from other regions, and that harvesting in August gave the best results, showing that both where and when the plant is collected can raise its chemical content. Both of these findings support the present study's conclusion that station location on its own can explain much of the difference recorded between the different sampling sites.

Elsewhere, the differences are much sharper. In Japan, Deba*et al*. (2008) found that fresh leaf and flower oils were led by β-caryophyllene, at 10.9% in the leaves and 5.1% in the flowers, and by τ-cadinene, at 7.82% and 6.13%; none of these compounds were among the leading compounds recorded in the present study. In India, Singh *et al*. (2017) reported that one single compound made up 57.82% of the volatile peak area in a methanolic leaf extract, a

level far above anything recorded at any of the four Rivers State stations, showing how much extraction method and plant part can shift the outcome. In Colombia, Quintero-Rincón*et al*. (2026) found the plant to be rich instead in phenolic acids, and in Ecuador, Ruiz-Reyes *et al*. (2022) recorded high phenolic and flavonoid content together with strong antioxidant activity, again pointing to a chemical profile shaped more by phenolic compounds than by the terpenoids and sterols that stood out in the present study.

Closer to home, direct comparisons from Rivers State, or even from Nigeria as a whole, were not available in the literature reviewed, so the closest evidence comes from related West African work. Ajanaku *et al*. (2018), working with *Bidens pilosa* collected in West Africa, found that the amount of alkaloids, tannins, and steroids in leaf extracts changed depending on the solvent used, reaching as high as 1.12% for alkaloids and 1.31% for steroids, and that antimicrobial activity also varied, again pointing to local growing conditions and extraction method as drivers of chemical differences. Edu *et al*. (2025), working with a different Nigerian plant, *Jatropha*, found that the number of compounds detected by GC–MS ranged from 12 to 33 depending on which state the samples came from, reinforcing that this kind of location-based chemical variation is common in Nigerian plants generally, not unique to *Bidens pilosa* . Taken together, the Obiri Ikwerre-high, Eleme-low, control sites-intermediate pattern recorded in the present study fits comfortably within a well-established global picture: this plant's chemistry is strongly controlled by its growing environment, but the present study is among the first to document this specific pattern for roadside stations within Rivers State, since no directly matching local study was found in the literature reviewed.

In *Amaranthus spinosus*, the present study found a different pattern: across most of the twenty-nine compounds detected, the control sites carried the highest levels, Eleme, the roadside station, carried the lowest, and Obiri Ikwerre fell in between. This held for hexadecanoic acid methyl ester, ergostrol, squalane, ethyl butyrate, and terpinolene, though phytic acid, campesterol, and alpha-amyrin moved differently, with campesterol in particular showing the widest spread of any compound, from 2.72% at Obiri Ikwerre up to 18.74% at control 1.

This same broad idea, that one location can be chemically richer than another for this plant, is well supported elsewhere. Rjeibi *et al*. (2017), working in Tunisia, found that gallic acid content ranged from 27.22% at one site to 64.93% at another, and that caffeic acid and total antioxidant capacity followed the same site-dependent pattern; this matches the present study's finding that station location alone can account for large swings in individual compound levels. In France, Azri *et al*. (2025) found that amaranth seed oil content ranged from 4.3% to 6.4%, with the highest squalene yield reaching 7.7% in one particular growing location, again pointing to both location and variety as drivers of chemical differences, and Gupta *et al*. (2023) reported squalene levels varying more than fourfold across different plant materials, further supporting this picture of strong quantitative variability within the same species.

Where the present study diverges more sharply from other work is in which compounds actually dominate the profile. In Palestine, Auda *et al*. (2026) found that a hexane leaf extract of the plant was led by isocitronellol at 30.78% and several other terpene alcohols and esters, none of which featured among the leading compounds recorded in Rivers State. Closer to home, in Nigeria, Yusuff *et al*. (2025) reported that an ethanolic leaf extract was dominated by 9-hexadecenoic acid at 26.21% and n-hexadecanoic acid at 17.21%, with phytol and squalene also present at meaningful levels; this gives a partial match with the present study, since squalane and related compounds also feature prominently in the Rivers State results,

even though the overall ranking of compounds differs. Other Nigerian studies, by Mamuru *et al*. (2019) and by Mbazurike *et al*. (2026), reported still different leading compounds again, underlining how much extraction method and even the specific batch of leaves used can change the outcome from one Nigerian study to the next.

The wider Nigerian evidence still supports the underlying principle behind the present study's findings, that environment and season shape the plant's chemistry, even without an exact site-matched comparison. Ogie-Odia *et al*. (2022) and Isoje *et al*. (2025) both reported that phenolic content, antioxidant capacity, and related compounds in *Amaranthus spinosus* shifted considerably between dry and rainy seasons and between different collection sites in Nigeria. Overall, the control-high, Eleme-low pattern recorded in the present study is consistent with the broader literature on environmentally driven variation in this plant's chemistry, but as with *Bidens pilosa*, the exact compounds that dominate differ sharply from country to country, and no directly matching Rivers State study exists to compare against, which makes the present findings a useful addition to that gap in the literature.

CONCLUSION

Based on the findings from this study, traffic pollution took a heavy toll on the metabolic and nutritional makeup of both species. GC-MS profiling showed that the plants reorganized their secondary metabolites to better defend against oxidative stress. Furthermore, proximate analysis revealed a clear drop in the nutritional value of roadside leaves, with crude protein, lipids, and carbohydrates falling significantly compared to plants grown in unpolluted areas. It is worth flagging that, since the control sites in this study recorded the highest levels for most compounds, closeness to a highway appears to be the reason a plant has less of these compounds.

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