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Thuja occidentalis and *Duranta repens* as indicators of urban air pollution in industrialized areas of southwest Nigeria

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Summary

This paper assessed biochemical parameters in leaves of selected ornamental species growing in urban areas of southwest Nigeria to obtain the air pollution tolerance index (APTI) and anticipated performance index (API) for classification into tolerant and sensitive species against air pollution. Four sites and three ornamental species (*Polyalthia longifolia*, *Thuja occidentalis* and *Duranta repens*) common to the sites were used for this study. Results showed significant variations in biochemical variables, hence biochemical parameters cannot be used solely to categorize the species. APTI values obtained suggested that *P. longifolia* is tolerant species to air pollutants while *T. occidentalis* and *D. repens* are sensitive species that can be used as bioindicators of air pollutants. The API value revealed *P. longifolia* and *T. occidentalis* to be poor performer and very poor performer respectively while *D. repens* cannot be recommended for greenbelt development. Therefore, *T. occidentalis* and *D. repens*, can be recommended as bioindicators of poor urban air quality.

Key words

monitoring, APTI, API, air pollution, surrogate indicator

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Introduction

Urban air pollution in developing countries is a contemporary issue (Santos et al., 2015). There has been increasing level of air pollution burden in several urban cities of Nigeria that has become a great challenge to air quality management (Ogunrotimi et al., 2017). The main air pollutants of concern in the urban areas are emissions from traffic that account for 90%-95%, such as nitrogen oxides, sulphur dioxide, particulate matter, and trace elements (Moreira et al., 2016), and industrial emissions. This alluded to the great air pollution in urban cities of Nigeria as 60% of all human activities are carried out via the transport sector (Ogunrotimi et al., 2017). In fact, World Bank data showed that 100% of the Nigerian population is now exposed to atmospheric particulate matter of <2.5 μ m diameter (PM_{2.5}) in levels that exceeded World Health Organization (WHO) guidelines (WHO, 2016).

Plants have been reported to be of significant help in improving the quality of urban environment, not only because of its aesthetic and recreational values, but also have the capability to remove air pollutants (McPherson et al., 1998). In addition, the use of plants as biological indicators has been proved important for the assessment of environmental quality and human health risk (Carreras et al., 1998; Sujetoviene, 2013). Plants play an important role in the maintenance of the ecological balance of the environment by intercepting pollutants (Liu & Ding, 2008a; Kim et al., 2015). Their responses in forms of physiological and biochemical changes can be utilized as a major tool for air pollution monitoring since plants show varied susceptibility to air pollutants; they respond in different ways to similar pollutants (Achakzai et al., 2017). Different air pollutants can directly affect plants via leaves (Steubing et al., 1989) by subjecting plants to various physiological changes before exhibiting visible injuries (Dohmen et al., 1990). Kuddus et al. (2011) reported that plant species existing in polluted environments could be screened for sensitivity and tolerance for pollutants by assessing the biochemical parameters of plants such as total chlorophyll, ascorbic acid, pH of leaf extract and relative water content. Therefore, variation in the levels of sensitivity and response of plants to air pollutants can be utilized. Plant species that are tolerant to air pollutants can be considered as sink whereas the sensitive species can act as indicators of air pollution (Singh et al., 1991; Agbaire & Esiefarienrhe, 2009; Achakzai et al., 2017).

However, using the biochemical variables alone may not provide significant information, so computed index known as air pollution tolerance index (APTI), using the biochemical variables can be adopted and has been used to evaluate tolerance level of plant species (Das and Prasad, 2010; Leghari et al., 2011; Nayak et al., 2015; Ogunkunle et al., 2015). Though, the APTI index has been proved effective in evaluating the effect of air pollutants by biochemical parameters of plants, but in adopting plant species for green belt development, some socioeconomic and biological characteristics must also be considered (Govindaraju et al., 2012). This led to the development of a new index called the anticipated performance index (API) which is an improvement over the APTI and has been used as an indicator to assess the capability of plant species to clean-up of atmospheric pollutants and function in green belt development.

The use of ornamental plants for urban greenery is very important for their role in scavenging atmospheric pollutants aside the aesthetic and recreational purposes. Many ornamental species have been reported to emit volatile organic compounds (VOC) belonging to the isoprenoid family (Loreto and Fares, 2007). These isoprenoids are reported to be strongly reactive and readily react with nitrogen oxides of anthropogenic origin forming tropospheric ozone, which scavenge pollutants from air (Di Carlo et al., 2004; Loreto and Fares, 2007; Vitale et al., 2008). In southwest Nigeria, the practice of landscaping the outdoor environment especially road meridians and house frontages with ornamental plants have gained prominence because of the aesthetic value. However, to the best of the authors' knowledge, studies have not been carried out on ornamental plants in Nigeria using combined APTI and API approach to evaluate species' tolerance to air pollution in order to recommend for urban greenery. Therefore, in this study the biochemical parameters, APTI and API values of some selected ornamental species were considered to assess their potential possibility as bioindicators of urban air quality in some urban areas of the southwest Nigeria.

Materials and Methods

Description of study area and sampling method

The study was carried out in the most industrialized and populated part of the southwest region of Nigeria. The climatic condition of the region is classified as humid tropical climate which is controlled by the Tropical Maritime and Tropical Continental air masses. The climate is characterized by the dry season and the rainy season with prevailing wind direction of southwesterly in the rainy season and northeasterly in the dry season. Level of traffic density, industrialization, urban built-up level and population density were considered in the selection of the five study sites: Ikeja, Ilupeju (Lagos State), Agbara, Sango (Ogun State) and Control site in southwest Nigeria (Fig. 1). Ikeja, Ilupeju, Agbara and Sango fall within the most industrialized region in Nigeria (Owoade et al., 2015). The principal sources of emission in the four sites are vehicular and industrial activities with major gaseous/particulate pollutants like oxides of sulphur, nitrogen, carbon, smoke, dust, fog and mists. Lagos State had very high levels of pollution, especially in traffic areas with both PM₁₀ and PM₂₅ levels higher by several folds than WHO database (Roychowdhury et al., 2016). Ogun State has been reported to be the Nigeria's current industrial hub with Ota as the largest industrial city in the state (Etim, 2012). The Control site was within residential area devoid of industrial activities and traffic density, and located several kilometers away from the four industrial sites.

Pre-sampling survey was carried out in the study locations to ascertain ornamental species common to all study sites. After the survey, *Polyalthia longifolia* Sonn. cv. Pendula, *Thuja occidentalis* L. and *Duranta repens* L. were identified as common to the sites. Sampling of biological materials in this study was carried out in the early part of the dry season (September-November) of 2016. Fresh leaf samples of ornamental plants were collected from the study sites described in Fig. 1. Each study site was divided into six study locations and samples of matured fresh leaves were collected in the morning from the target ornamental species in each study location. Fresh leaf samples of approximately 1 kg



Figure 1. Map showing sampling locations in southwest region of Nigeria (Inset is map of Nigeria)

each (Ogunkunle et al., 2015) were collected from three different stands of the plant species at each location and were immediately transferred into ice cubes to maintain biochemical status before transferring to the laboratory for determination of biochemical parameters. In the laboratory, leaf samples were maintained in refrigerator until biochemical analysis for ascorbic acid content, relative water content, pH of leaf extract and total chlorophyll content.

Chemical analysis

The content of ascorbic acid (AA) in the leaves was determined according to Begum & Harikrishna (2010). Briefly, 4 ml of freshly prepared 0.05 M oxalic acid solution (containing 0.2 mM EDTA), 1 ml orthophosphoric acid, 1 ml of 5% H_2SO_4 , 2 ml ammonium molybdate and 3 ml H_2O were used as extractant for 1 g of fresh leaves of plant sample in a test tube. Absorbance of the solution was read at 760 nm after 15 min and the concentration of ascorbic acid curve. The relative water content (RWC) of the leaf sample was determined based on Equation 1 (Liu & Ding, 2008a).

$$RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$$
 Eq. 1

where FW is the fresh weight (g), TW is the turgid weight (g) after immersion in water overnight and DW is the dry weight (g) of turgid leaves after oven-drying at 115°C for 2 h. The total chlorophyll content of the leaf samples was determined according toHipkins & Baker (1986) as modified by Adenipekun et al. (2009).

The pH of leaf extract was determined after calibration of the pH meter (PHC-3C) with buffers 4 and 9. Briefly, 2.5 g of the fresh leaf samples was homogenized in 10 ml distilled water and pH of extract was read by the pH meter.

In order to evaluate plant species' sensitivity/tolerance ability to atmospheric pollution, the air pollution tolerance index (APTI) proposed by Singh & Rao (1983) was calculated for the ornamental species according to Equation 2.

$$APTI = [A(T + P) + R] / 10$$
 Eq. 2

where A is the ascorbic acid content (mg/g), T is the total chlorophyll (mg/g), P is the pH of the leaf extract, and R is the relative water content of leaf (%). Two classification methods of APTI were adopted in this study. In the first method (Method I), APTI values were used to rank tree species as sensitive (4.0-5.0), intermediate (5.1-6.0), tolerant (6.1-7.0) and highly tolerant

(>7.0) (Singh & Rao, 1983; Okunlola et al., 2016). The second classification (Method II) was according the method of Liu et al. (1983) as described in Zhang et al. (2016). APTI value of each target species was compared with the average APTI value and standard deviation (SD) of all the studied species. Ornamental species were then classified as: tolerant (T) if APTI was higher than the average APTI plus SD; moderately tolerant (MT) if APTI value falled between average APTI and average APTI plus SD; intermediate if APTI value was between average APTI minus SD and average APTI, and sensitive (S) if APTI value was lower than the average APTI minus SD.

Statistical analysis and anticipated performance index (API) assessment

Analysis of Variance (ANOVA) and Duncan Multiple Range Test were employed to separate significant means (P<0.05) using Statistical Package for Social Sciences (SPSS ver 20.0) while presentation of figures was carried out using OriginPro 8 SR0 software v8.0724 (B724), Northampton, USA. The relationship between APTI and the biochemical variables (pH, TChl, RWC and AA) was assessed using the linear regression analysis and principal component analysis (PCA) biplot (correlation matrix) of the Paleontological Statistical Package for Education and Data Analysis (PAST) v 3.15 (Hammer et al., 2001). The air pollution index (API) values of the ornamental plants were determined using the APTI values, relevant biological and socioeconomic parameters listed in Table 1 (Prajapati & Tripathi, 2008; Govindaraju et al., 2012; Ogunkunle et al., 2015). The grading system and classification of plants based on the API are presented in Tables 1 and 2, respectively. According to the grading system, a maximum of 16 positive points can only be allotted to a species and total points secured by a species is always scaled to percentage to categorize the API valuea (Prajapati & Tripathi, 2008; Govindaraju et al., 2012).

Results and discussion

Variation in biochemical parameters of leaves of ornamental species

Ascorbic acid content

Similar pattern of variations in ascorbic acid (AA) was observed among the sites in the ornamental species P. longifolia and D. repens (Fig. 2a). The content of AA in P. longifolia from Ikeja was significantly the highest (P<0.05) and followed by Ilupeju. Likewise, AA content in T. occidentalis and D. repens at Ikeja and Ilupeju were significantly higher (P<0.05) than at Agbara, Sango and Control site. The relatively low amount of AA reported in this study was comparable to results from similar studies (Rai & Panda, 2014; Zhang et al., 2016). AA is an important detoxicant that prevents damaging effect of air pollutants and improves plants' tolerance ability to air pollution (Singh et al., 1991). Large amounts of AA favor defense properties in plants (Lee et al., 1984; Cheng et al., 2007) and indicate level of air pollutants (Bermadinger et al., 1990; Kousar et al., 2014). So, the comparatively higher AA contents observed at Ikeja, Agbara and Sango might be an indication of high level of air pollutants. Ghosh et al. (2014) reported that AA has the potential to protect Abrus

Table 1. Grading method for the studied ornamental plant species
based on the APTI values, biological parameters and socioeconomic
importance

Grading character		Pattern of assessment	Grading allotment
Tolerance	APTI	9.0-12.0	+
		12.1-15.0	++
		15.1-18.0	+++
		18.1-21.0	++++
		21.1-24.0	+++++
Biological and socioeconomic	Plant height	Small	_
		Medium	+
		Large	++
	Canopy structure	Sparse/irregular/ globular	-
		Spreading crown/ open/semi-dense	+
		Spreading dense	++
	Type of plant	Deciduous	-
		Evergreen	+
Laminar structure	Size	Small	_
		Medium	+
		Large	++
	Texture	Smooth	-
		Coriaceous	+
	Hardness	Delineate	-
		Hardy	+
	Economic value	< 3 Uses	-
		3 or more Uses	+
		5 or more Uses	++

Source: Prajapati and Tripathi (2008); Govindaraju et al. (2012); Ogunkunle et al. (2015)

Table 2. Classification of anticipated performance index (API) for plant species

Grade	Score (%) Category			
0	<30	Not recommended		
1	31-40	Very poor		
2	41-50	Poor		
3	51-60	Moderate		
4	61-70	Good		
5	71-80	Very good		
6	81-90	Excellent		
7	91-100	The best		

Source: Prajapati and Tripathi (2008); Govindaraju et al. (2012)

precatorius from damage by activated O_2 during the N_2 fixation in root nodules, thus AA has the importance in the estimation of APTI for plants (Liu & Ding, 2008b). Based on the AA contents, *P. longifolia*, *T. occidentalis* and *D. repens* are considered as potentially sensitive species to urban air pollution due to the relatively low concentrations of foliar ascorbic acid. This observation is in line with the view of Zhang et al. (2016) that species with low AA may be considered as sensitive.

Leaf extract pH

Variations in leaf pH of the species as a result of air pollution levels are presented Fig. 2b. Significant changes in pH (P<0.05) were observed in the three species across the sites. The pH values of leaf extracts of *P. longifolia* ranged from 4.75 to 6.75, and higher pH values (>6.0) of leaf extracts were obtained at Ikeja, Ilupeju and Agbara and significantly lower pH values (P<0.05) were recorded at Sango and Control site. In fact, the pH value of *P. longifolia* at Control site was significantly the lowest (pH=4.75) at P<0.05. Values of leaf pH of *T. occidentalis* at the five sites (Ikeja, Ilupeju, Agbara, Sango and Control site) were \geq 6.0 but varied significantly (P<0.05). Similarly in *D. repens*, leaf pH varied significantly among the sites (P<0.05) but generally lower than 6.0 (pH<6.0).

High pH increases the efficiency of conversion of hexose sugar to ascorbic acid in plants to ameliorate effects of air pollutants (Enete et al., 2013). Hence, the significantly lower (p<0.05) leaf pH observed in control may be adduced to the absence of need to upregulate AA production due to absence of air pollution.

Leaf pH comparison among the species showed that *D. repens* exhibited the lowest pH (pH<6.0) whereas *P. longifolia* and *T. occidentalis* portrayed higher pH values (pH>6.0) at all sites. This clearly shows that *D. repens* has high sensitivity to air pollution; and would normally increase secretion of cellular fluid in leaves in response to pollutants, thereby increasing leaf acidity. This assertion is supported by the postulation of Zhen (2000) that plants that suffer from air pollutants, which gain entry

through stomata and intercellular space from air, so that H_2SO_4 is generated and then leaf pH reduces. It is also noted that the higher the pH values of leaf extract, the stronger the ability of plants to sequester pollutants and tolerate air pollution (Yan-ju & Hui, 2008). The relatively high leaf pH in *P. longifolia* and *T. occidentalis* may be the plants' adaptive strategy against air pollutants. This conforms with the view of Govindaraju et al. (2012) that high leaf pH in plants, especially in polluted conditions, is indicative of the plants to tolerate air pollutants (Govindaraju et al. 2012).

Relative water content (RWC)

P. longifolia and D. repens showed slight variation in RWC among the sites whereas RWC in T. occidentalis, RWC varied significantly (P<0.05) among the sites (Fig. 2c). RWC obtained in P. longifolia for all sites exceeded 55% whereas RWC values for both T. occidentalis and D. repens for all sites were below 50%. This is not unusual as leaf area of P. longifolia is larger than the other two species, hence RWC capacity may also differ. Relative water content has been opined to be important for normal growth and physiological activities of plants (Jiang et al., 2010), and higher RWC in plant has been linked to ability of plants to maintain life processes and tolerate environmental adversity (Zhang et al., 2016). High water content in plants also ensures the maintenance of the physiological balance under stresses such as air pollution (Tsega & Devi-Prasad, 2014). So, the low RWC recorded for T. occidentalis and D. repens may indicate the inability of the species to tolerate the level of air pollutants in the areas. Previous studies have showed the leaf RWC in maize (Liu et al., 2002) decreases when exposed to stress. As an important physiological factor, RWC is affected by the air pollution directly. This has been proved when Elaeocarpus sylvestris suffered from air pollution, their stomatal density increased and led to a decrease in water content of plant tissues (Liao et al., 2011). Thus, higher water content within a plant body can help to maintain physiological balance and enhance tolerance ability under stress conditions (Agarwal & Tiwari, 1997).

Total chlorophyll content (TChl)

Total chlorophyll (TChl) content of plants has been noted as an important indicator of plants' health (Jin et al., 2012). The TChl content obtained in this study differed significantly across the sites for the three species: *P. longifolia*, *T. occidentalis* and *D. repens* (Fig. 2d). TChl contents in *P. longifolia* at Ilupeju, Agbara and Sango were statistically similar but significantly different (P<0.05) from Ikeja and Control site. *Thuja occidentalis* and *D. repens* at Agbara had significantly the highest TChl contents.

In the present study, TChl was comparatively found higher in poluted sites and lower at the Control site contrary to some other studies (Rai et al., 2013). The increased TChl compared to control may be attributed to species' responses to air pollutants in the poluted areas as higher content might favor tolerance to pollutants (Joshi et al., 1993). Furthermore, comparison among the three ornamental species, TChl follows the pattern: *P. longifolia* (5.18-14.49 mg/g)>*D. repens* (7.35-11.76 mg/g)>*T. occidentalis* (3.76-7.51 mg/g). Air pollutants have strong influence on TChl content in plants' leaves, thereby affecting their health (Zhang et al., 2016). So, the generally high TChl contents in *P. longifolia* may be an indication of tolerance to air pollution. High chlorophyll content in leaves has been reported to improve plants' tolerance



to air pollutants (Prajapati & Tripathi, 2008). The relatively low TChl in *T. occidentalis* and *D. repens* in the study showed that those ornamental species cannot be recommended for air pollution abatement but can only serve as bioindicators of air pollution.

Gradation of APTI and API and Tolerance Evaluation

Mean values of APTI for the three species at the four studied sites are presented in Table 3. APTI values for P. longifolia followed the pattern thus: 9.02±0.47 (Agbara) > 8.00±0.75 (Ilupeju) > 7.40±0.86 (Sango) > 7.09±0.51 (Ikeja). The mean APTI for P. longifolia from the four sites (7.87±0.85) was comparable to APTI for the species at Control site (7.80±0.52). Lower APTI values were recorded for T. occidentalis and D. repens for the four sites, however both species shared similar pattern of APTI at the sites. For instance, mean APTI values of T. occidentalis and D. repens at Ikeja were 4.58±0.82 and 4.75±0.45 while the APTI values at Sango for both species were 4.32±0.95 and 4.33±0.46, respectively (Table 3). The highest APTI value for T. occidentalis was in Agbara (6.04±0.98) while D. repens had the highest APTI in Ikeja (4.75±0.45). It is also important to note that mean APTI values of T. occidentlis (4.85±0.80) and D. repens (4.32±0.34) decreased by 27.3% and 34.9% respectively compared to the observed APTI values at the Control site (6.67±0.57 and 6.64±0.49 respectively). Based on the categorization by Method I (Singh & Rao, 1983; Okunlola et al., 2016), P. longifolia can be classified as highly tolerant species to air pollution whereas T. occidentalis and D. repens are sensitive species and can be regarded as potential bioindicator of air pollution. Similarly for Method II (Liu et al., 1983), P. longifolia was classified as tolerant species while T. occidentalis and D. repens were grouped as intermediate species. Sensitive species have been reported to be ideal bioindicators of air pollution (Kousar et al., 2014), and species with high APTI values are tolerant to air pollution and can be used as sinks (Tsega & Devi-Prasad, 2014).

The evaluation and grading of the ornamental species according to APTI values and the relevant socioeconomic and biological parameters are presented in Table 4. Grade allotted to the species were 8, 5 and 4 for P. longifolia, T. occidentalis and D. repens, respectively. The grades when transformed to percentage were all \leq 50%; *P. longifolia* had 50% while *Thuja occidentalis* and D. repens had 31.25% and 25.0%, respectively (Table 4). The API evaluation assessment (Table 4) rated P. longifolia as poor performer in scavenging air pollutants. T. occidentalis was rated as very poor performer whereas D. repens was not recommended due to very low API. Therefore, P. longifolia, T. occidentalis and D. repens are not ideal species for green belt development because of their low capability to scavenge air pollutants. They can only serve as bioindicators for urban air pollution as species for green belt development are plants with high API values (Tsega and Devi-Prasad, 2014; Gupta et al., 2016).

Regression and PCA biplot analyses of variables

The linear regression plots of individual biochemical variables with APTI (Fig. 3) show that a high positive correlation exists between APTI and RWC (R^2 =0.902). APTI was poorly correlated with leaf extract pH (R^2 =0.113), chlorophyll content (R^2 =0.042), and ascorbic acid (R^2 =063). It indicates that RWC is the most significant parameter and determining factors that influenced the APTI. The fact that APTI and RWC had a strong positive correlation (Fig. 4), due to the acute angle between the variables, suggests that the APTI strongly depends on the RWC. It can be

	Mean APTI per site (n=24)				Mean±SD	Cate		
	Ikeja	Ilupeju	Agbara	Sango	APTI value	Method I	Method II	Control
Polyantia longifolia	7.09±0.51	8.00±0.75	9.02±0.47	7.40±0.86	7.89±0.85	Highly tolerant	Tolerant	7.80±0.52
Thuja occidentalis	4.58±0.82	4.47 ± 0.47	6.04±0.98	4.32±0.95	4.85±0.80	Sensitive	Intermediate	6.67±0.57
Duranta repens	4.75±0.45	3.92±0.53	4.29±0.44	4.33±0.46	4.32±0.34	Sensitive	Intermediate	6.64±0.49
	5.68±1.91							

Table 3. Air pollution tolerance index (APTI) of the studied ornamental species

Table 4. Evaluation based on APTI value, some biological and socioeconomic characters and API of the ornamental plants

Species	APTI	Tree habit	Canopy structure	Tree type	Laminar		Economic importance	Hardness	Grade Allotment	Percentage	API value	Assessment
					Texture	Size			Total plus (+)			
Polyantia longifolia	-	+	+	+	+	+	+	++	8	50%	2	Poor
Thuja occidentalis	-	+	+	+	+	-	_	+	5	31.25%	1	Very poor
Duranta repens	_	+	+	+	_	_	_	+	4	25%	0	Not recommended



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Component 1 (44.75%)

Figure 4. PCA biplot of APTI and biochemical variables in the three species (*Polyantia longifolia, Thuja occidentalis* and *Duranta repens*)

assumed that the higher the content of RWC in the ornamental plants, the greater the APTI and the tolerance potential. Leaf extract pH also influenced the APTI values in the species with the presence of an acute angle between the two parameters. Total chlorophyll and AA were both at right angle to APTI, suggesting both variables impose little or no influence on the species' APTI values. From those results, it can be assumed that RWC and pH of leaves in *P. longifolia, T. occidentalis* and *D. repens* may be surrogate indicators of the APTI values in ornamental species.

Conclusion

Component 2 (26.39%)

From this study, it is noticed that biochemical variables cannot be used singly to identify ornamental plants as sensitive or tolerant to air pollution due to the different pattern of variations among the species and across the sites. APTI values obtained from the two methods in the study indicated that T. occidentalis and D. repens are sensitive in nature and can be used as air pollution indicators while P. longifolia is not useful as bioindicator. Regression and PCA biplot analyses indicated RWC to be the most significant factor that the APTI depends on in the ornamental species while the leaf pH may also be an indicator of the APTI. The API values indicated that *P. longifolia* is a poor performer whereas *T. occidentalis* and *D.* repens are very poor performer and not recommended respectively for green belt development. The findings of this study suggest that there is a need to further investigate the possibility of including more ornamental species to study their tolerance against air pollution for further understanding of suitability of ornamental species as air pollution indication and abatement in the southwest Nigeria.

Declaration of Interest

The authors declare no conflict of interest

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