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Seasonal influence on suitability of masquerade tree as a bioindicator of vehicular pollution along University of Ilorin Road, Nigeria

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Abstract: This study focused on the seasonal impacts on the suitability of masquerade (*Polyalthia longifolia*) as a bioindicator of vehicular pollutants. Some leaves of the tree were plucked along the roadside and from a control site with no vehicular emissions. Biochemical parameters, including pH, ascorbic acid content, relative water content, total chlorophyll and air pollution tolerance index (*APTI*), were determined in both dry and wet seasons using standard methods. Potentially toxic elements (PTEs) commonly associated with automobile pollutants (Pb, Zn, Cr, Mn, Fe and Cu) were analyzed using atomic absorption spectroscopy, after acid digestion. The concentrations of PTEs were slightly higher in leaves from roadside masquerade tree than in those from the control site, except for Cr and Cu at some sampling points during the wet season. The biochemical properties in the roadside samples indicated the presence of pollutants compared to the control site in both seasons. *APTI* of the roadside samples showed higher sensitivity (mean value = 10.30) in the dry season, indicating a gradual loss in tolerance to pollution; however, a slight increase (mean value = 11.23) in tolerance was observed in the wet season. The masquerade tree demonstrated its sensitivity to vehicular pollution in both seasons. It is more sensitive in the dry season but tends to tolerate pollutants in the wet season by increasing *APTI* through improved defense mechanisms.

Keywords: bioindicator; vehicular pollution; masquerade tree; potentially toxic elements.

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INTRODUCTION

High levels of vehicular pollution are a common problem in urban areas, which can harm public health and air quality.[1] This is a consequence of using low--quality fuel, old and poorly maintained cars on the road,[2] and some other processes as friction between tyres and the road surface.[3,4] Air pollution through vehicular activities generates a lot of pollutants such as oxides of carbon (CO and CO_2), nitrogen (NO_x), sulphur (SO_x), polyaromatic hydrocarbons (PAHs) and potentially toxic elements.[5] This menace of air pollution needs immediate and long-term monitoring and mitigation as it has both direct and indirect effects on human health and the environment.

One potential approach to monitoring and mitigating this pollution is the use of bioindicators or living organisms, that can serve as sensitive indicators of environmental conditions or sinks for pollutants. Two major sinks of pollutants are plants and soil.[5,6] Air pollution is among the many environmental challenges that plants, a lovely gift from nature, can help mitigate.[7] Most air pollutants are deposited on the plants' leaves and are removed through stomata on their surfaces.[8,9]

Several trees and plants have reportedly been used as bioindicators in many urban cities worldwide to monitor levels of air pollution caused by vehicular emissions and other activities, such as industrial discharges. Different plant species exhibit varying levels of sensitivity and tolerance to different forms of air pollution.[6] Bello *et al.*[10] reported the utilization of Mexican sunflower (*Tithonia diversifolia*) for monitoring air pollution around the iron smelting industry and the study concluded that there was an impact of the industry on the plant. For vehicular emission, *Polyalthia longifolia* (masquerade tree), *Caesalpinia pulcherrima* (peacock flower or barbados pride tree), *Delonix regia* (flamboyant tree), *Tamarindus indica* (tamarind tree), *Terminalia catappa* (tropical almond tree), *Mangifera indica* (mango tree), *Ficus platyphylla* (broad-leaved fig tree), *Ficus benghalensis* L. (banyan tree), *Azadirachta indica A. Juss* (neem tree), *Ficus religiosa* L. (bodhi tree), *Ficus benjamina* L. (as weeping fig tree) and *Bougainvillea glabra* (paper flower tree) that were exposed to roadside automobile pollution, stress was reported.[2,5,9] The air pollution tolerance index (*APTI*) is typically used to assess how sensitive and tolerant certain plants are to air pollution.[6,11] A measure of pH, ascorbic acid content (*AAC*), relative water content (*RWC*) and total chlorophyll in the leaves of a particular tree is used to determine how plants respond to pollution.[9] Plants that are sensitive to pollutants are used as indicators, while those that are tolerant consume the pollutant and reduce the level of pollution in the environment.[11,12]

Masquerade trees (*P. longifolia*), among others, have been widely studied as bioindicators of vehicular emissions. No wonder it is planted along the main road leading into the University of Ilorin's campus in Nigeria. This tall, evergreen tree native to tropical Asia is also known as the Indian mast tree.[13] Various positions have been taken on its application as a bioindicator. Kirthika and Vishnuprasad[14] reported that it has an intermediate tolerance to vehicular pollution based on its *APTI* value. Its potential as a bioindicator of vehicular pollutants was also related to its ability to accumulate potentially toxic elements.[15] Similarly, Umar *et al.*[16] stated that *P. longifolia* has the highest dust-carrying capacity among the studied plant species, indicating its potential as a bioindicator of vehicular pollution. Also, Azam *et al.*[17] reported that it is less sensitive to vehicular pollution than the other trees used in their study, based on *APTI* values.

According to the existing research, the masquerade tree is a viable bioindicator for tracking air pollution and vehicle emissions in urban environments. However, there is little or no information on the effect of seasons on its efficiency in response to pollution. Therefore, this present study investigated the effect of seasonal conditions on its ability to serve as a bioindicator of vehicular pollutants.

EXPERIMENTAL

Description of study area

The University of Ilorin (Better by Far), also known as Unilorin, is a federal government-owned University located in Ilorin, Kwara State, Nigeria. The institution has a large landmass of approximately 15,000 ha and is situated in the southern part of the city. The University, with approximately 3,040 staff and 34,999 students, experiences a high number of vehicles plying the road from the gate to the campus, especially the University's main car park. The dry and wet seasons are the two main seasons in the region. The dry season covers November and April of the following year. The wet season lasts between April and October.

Sample collection

Leaf samples of masquerade tree (*P. longifolia*) were collected in January (dry season) and May (wet season), 2023. These sites were the University roadsides from the Fountain roundabout towards the gate and walkways (control site). Four samples were collected from the roadside at the following coordinates: 8.47650°N 4.67212°E (RS1), 8.47617°N 4.67182°E (RS2), 8.47556°N 4.67165°E (RS3) and 8.47499°N 4.67100°E (RS4). The control sample was taken at 8.48105°N, 4.67400°E. The samples were collected in a sealed, air-tight bags.

Determination of potentially toxic elements (PTEs) concentration

A 20 mL of an aqua regia solution (15 mL 37 % HCl and 5 mL 65 % HNO_3) was added to 1 g of the finely ground leaf samples in the digestion beaker. This solution was then heated using a hot plate until it became transparent. The heating process then continued for an additional 20 min, after which deionized water (50 mL) was added. The mixture was filtered into a 100 mL standard flask using a funnel and Whatman filter paper. The content was brought up to the 100 mL mark and stored in a sample bottle for PTEs analysis.[18] Concentrations of selected PTEs (Pb, Zn, Cr, Mn, Fe and Cu) were analyzed in the samples using a Buck Scientific Accusys 230 atomic absorption spectrophotometer.

pH measurement

The pH of the leaves was determined using a slightly modified method reported by Pandey *et al.*[19] Here, 5 g of the leaves were crushed and homogenized with a pestle and mortar. It was mixed thoroughly for 5 min with 10 mL of distilled water. Then, the mixture was separated by centrifugation and the supernatant obtained was subjected to pH estimation using a digital table-top pH meter (Watson pH-2602).

*Relative water content (*RWC*)*

1 g of fresh masquerade tree leaves was accurately weighed and immersed in water for 12 h. The fully hydrated weights of the leaves were measured after they were saturated with water. The leaves were further dried in an oven (Gallenkamp OV-160) at 105 °C overnight, and their dry weight was subsequently measured. Then the *RWC* (%) was calculated as:

$$RWC(\%) = \frac{F - D}{T - D} \times 100 \tag{1}$$

where *F* is the weight (g) of the fresh leaves; *T* is the hydrated weight (g) and *D* is the dry weight (g) at 105 °C.[11,20]

*Ascorbic acid content (*AAC*)*

The *AAC* in each sample was quantified and expressed as mg per 100 g using a slightly modified method of Vahid.[21] 10 g of the leaves were blended with a 4 % oxalic acid solution, and the volume was made up to 100 mL using the same 4 % oxalic acid solution. The solution was then filtered as an extract using a 110 mm grade 1 Whatman filter paper. A 5 mL aliquot of the sample extract was titrated with a standardized solution of 2,6-dichlorophenolindophenol (dye). The *AAC* was then calculated as:

$$AAC(\text{mg (100 g}^{-1})) = 100\frac{\text{Dye factor} \times 100V_2}{V_1W} \tag{2}$$

where *W* is the weight (g) of a sample taken for extraction with oxalic acid; V_1 is the volume (mL) of sample extract taken for titration and V_2 is the volume (mL) of dye required (titer value). The dye factor was calculated from the standardization of the dye solution.

*Total chlorophyll (*TCh*)*

50 mg of the fresh leaves were crushed and added to a vial containing 7 mL of dimethyl sulfoxide (DMSO), and then incubated at 65 °C for 30 min. The mixture was centrifuged, separated into 10 mL standard and then made up to 10 mL with DMSO. The sample was analyzed using a spectrophotometer (VWR UV-6300PC) at wavelengths of 648 and 665 nm, with a blank as a reference. Then the *TCh* was calculated as:

$$TCh(\text{mg g}^{-1}) = \frac{20.34A_{648} + 7.49A_{665}}{1000W}V \tag{3}$$

where *V* is the initial volume of extract; *W* is the weight of the sample taken; A_{648} and A_{665} are the optical density (*OD*) values measured at 648 and 665 nm, respectively. 20.34 and 7.49 are the absorption coefficients. [22]

*Air pollution tolerance index (*APTI*)*

APTI of the tree was calculated using the expression: [23]

$$APTI = \frac{AAC(TCh + \text{pH}) + RWC}{10} \tag{4}$$

Statistical analysis

For each sampling point and season, the mean values of each biochemical property, along with their standard deviations, are presented. Using Microsoft Excel 2016 Tool Packs for Data analysis, the relationship between biochemical properties and the *APTI* was analyzed using correlation analysis.

RESULTS AND DISCUSSION

Concentration of potentially toxic elements (PTEs)

The concentration of PTEs in the leaves of the masquerade tree from the four sampling points (RS1–RS4) and that of the control site (CS) are presented in Table I for dry and wet seasons.

TABLE I. Concentration of potentially toxic elements (mg L^{-1}) in the masquerade leaves during dry and wet season; ND means not detected

Site	Element					
	Pb	Zn	Cr	Mn	Fe	Cu
			Dry season			
RS1	0.086	0.278	0.054	0.469	1.533	0.116
RS2	0.036	0.371	0.052	0.505	1.615	0.121
RS3	0.055	0.341	0.052	0.512	1.445	0.105
RS4	0.099	0.210	0.048	0.287	1.747	0.107
Mean±*SD*	0.069±0.02	0.30±0.06	0.052±0.002	0.443±0.09	1.585±0.11	0.112±0.01
CS	0.023	0.108	0.047	0.163	0.866	0.062
			Wet season			
RS1	0.047	0.623	0.032	0.558	2.511	0.051
RS2	0.087	0.520	0.038	0.492	2.578	0.089
RS3	0.048	1.221	0.040	0.409	1.604	0.058
RS4	0.046	1.832	0.084	0.297	1.836	0.012
Mean±*SD*	0.057±0.02	1.049±0.53	0.0489±0.02	0.439±0.10	2.132±0.42	0.053±0.03
CS	ND	0.301	0.044	0.272	0.384	0.109

It is observed that all selected PTEs, which are usually associated with vehicular emissions, are present in roadside samples. Their presence in the control sample is lower, except for chromium, which showed a slightly higher concentration in the CS than in the three RS (RS1, RS2 and RS3) and copper, which had a higher concentration in the CS than in all the RS samples during the wet season. This exception may be due to the high mobility of the metal ions during the wet season. Similarly, stormwater containing PTEs from various sources can be readily deposited and may affect the control sample.[24,25] Interestingly, a major PTE (Pb) attributed to vehicular emissions was not detected in the CS during the wet season. Additionally, the leaves of the plant from RS1 to RS4 recorded significantly higher values of potentially toxic elements than those of the CS in the dry season. The observation is justified by the plant's proximity to the road and the long accumulation of dust containing these potentially toxic elements on its leaves, which prevents rainwater from washing it away.

The mean concentrations of the potentially toxic elements from the roadside sampling points RS1–RS4 are more than the control site (CS) except copper in the wet season. The presence of potentially toxic elements in such amounts (higher than those at the control site) is likely due to contamination of the ambient environment by vehicular emissions. The presence of PTEs in uncontaminated environmental media (air, soil and water) is not unlikely, but vehicular emissions are a significant source in urban environments. These metallic elements originate from various car parts and operations, including fuel combustion, tyre friction, brake wear and road surface abrasion.[3,4]

*Biochemical properties and air pollution tolerance index (*APTI*)*

This section presents the results on biochemical properties and the computed *APTI*. These biochemical parameters include ascorbic acid content, pH, relative water content and total chlorophyll content of the tree's leaves. The results for each roadside sample point RS1–RS4 are compared with those of the control site (CS).

pH. The pH value of the leaves of the masquerade tree from the four roadside sampling points RS1–RS4 along the road with the University of Ilorin, and that of the control site (CS) are presented in Table II.

TABLE II. pH of the leaves from the masquerade tree at the roadside (RS) and control site (CS) samples

Season	Sampling point					
	RS1	RS2	RS3	RS4	Mean±*SD*	CS
Dry	6.47	6.44	6.45	6.49	6.46±0.02	6.53
Wet	7.05	6.75	6.78	6.97	6.89±0.13	7.14

It is observed that all the samples, including the control, are slightly acidic in the dry season. Although the control site (CS) is less acidic (pH 6.53) compared to the individual roadside samples and their mean value (pH 6.46). The tree recorded higher pH levels during the wet season than in the dry season, with the highest value of 7.14 at the control site and the lowest value of 6.75 at RS2, which can be approximated to 7.0. An acidic pH indicates that NO_x and SO_x are present in the air, and their presence can be attributed to vehicular emissions from the burning of fossil fuels.[26,27] Plant health and soil composition are greatly impacted by acid rain, which is brought on by SO_x and NO_x emissions in the atmosphere.[28] It lowers the pH of rainwater below 5.6 and consequently affects the photosynthetic processes, influencing plant development, productivity, and yield.[28,29] It induces oxidative stress by generating reactive nitrogen species (RNS) when it reacts with reactive oxygen species (ROS) and damages lipids, proteins and nucleic acids.[30] Plants with a pH of around 7.0 are tolerant.[27] In this study, the pH level of the masquerade tree's leaves from a less polluted control site (CS) is 7.14, indicating that the tree is naturally (without pollution) air pollution-tolerant plant.

*Relative water content (*RWC*)*

The results of the *RWC* are presented in Table III. The highest value was observed in the control sample, at 97.88 and 98.21 % in the dry and wet seasons, respectively.

TABLE III. Relative water content (%) of the leaves from the masquerade tree at the roadside (RS) and control site (CS) samples

Season	Sampling point					
	RS1	RS2	RS3	RS4	Mean±*SD*	CS
Dry	94.39	94.89	96.13	91.24	94.16±1.80	97.88
Wet	96.26	96.48	97.84	94.31	96.22±1.26	98.21

However, all selected locations showed a significant *RWC* value. *RWC* of a leaf represents the water it retains compared to its fully turgid state and serves as a crucial indicator of plant response to pollution. Although the wet season affects the plant, the *RWC* of the roadside sample is still lower than that of the control site in the dry season. The result indicates roadside pollution, as plant *RWC* is reduced in contaminated environments. In stressful situations, such as exposure to air pollution, when transpiration rates are often high, a plant's high water content aids in maintaining its physiological equilibrium. [31] Plants with high relative water content under polluted conditions may be tolerant to pollutants.

*Ascorbic acid content (*AAC*)*

The *AAC* of tree leaves is a major biochemical property that is strongly affected by vehicular pollution, along with other factors such as chlorophyll content. The results of the ascorbic acid content in the leaves of the masquerade tree, in this study, are presented in Table IV.

TABLE IV. Ascorbic acid content (mg g^{-1}) of the leaves from the masquerade tree at the roadside (RS) and control site (CS) samples

Season	Sampling point					
	RS1	RS2	RS3	RS4	Mean±*SD*	CS
Dry	1.056	1.036	1.023	1.234	1.087±0.08	0.99
Wet	1.538	1.386	1.564	1.736	1.556±1.02	1.367

The results of the study revealed that the masquerade tree recorded its lowest ascorbic acid content at the control site compared to the individual roadside sampling points RS1–RS4 and their mean values in both the dry and wet seasons. The higher *AAC* observed at the roadside sampling point is likely due to their biochemical response to stress caused by pollution.[32,33] The tree maintains higher ascorbic acid levels under polluted conditions, indicating its tolerance to air pollutants.

However, the *AAC* was higher at all sampling points, including the control site, during the wet season compared to the dry season.

*Total chlorophyll content (*TCh*)*

The highest *TCh* was observed at one of the roadside sampling sites, RS1, at 2.839 and 3.448 mg g^{-1} for the dry and wet seasons, respectively (Table V). However, the mean values in both seasons are lower than those at the control site. The lower total chlorophyll value in the roadside samples (mean value) compared with the control site indicates the impact of automobile pollutants on the plant.

Iqbal *et al.*[32] reported that elevated levels of automobile pollution reduce chlorophyll content in plants growing near roadways. However, chlorophyll content in plants varies based on species, leaf age, pollution levels and various biotic and abiotic factors.[35] Vehicular pollutants like SO_2, NO_x and O_3 react with the leaf's chloroplast and consequently decrease the chlorophyll content.[36]

TABLE V. Chlorophyll content (mg g^{-1}) of the leaves from the masquerade tree at the roadside (RS) and control site (CS) samples

Season	Sampling point					
	RS1	RS2	RS3	RS4	Mean±*SD*	CS
Dry	2.839	1.387	1.024	1.643	1.723±0.68	2.396
Wet	3.448	1.749	2.153	2.538	2.472±0.62	3.232

*Air pollution tolerance index (*APTI*)*

The *APTI* of the masquerade tree from the roadside (RS) and control site (CS) are presented in Table VI. The results obtained from all the roadside sampling points RS1–RS4 and control site (CS) compared with the *APTI* categories (Table VII) reported by Lakshmi *et al.*[12] suggested that the plant is sensitive to air pollutants. It is observed that the masquerade tree is sensitive to air pollution levels along the University's roadside.

TABLE VI. The *APTI* of the masquerade tree from the roadside sampling points (RS) and the control site (CS)

Sampling point	Season	
	Dry	Wet
RS1	10.42	11.24
RS2	10.29	10.83
RS3	10.38	11.18
RS4	10.13	11.08
CS	10.67	11.24

The seasonal effect showed that its sensitivity is marginally higher during the dry season compared to the wet season. A plant's ability to mitigate air pollution is indicated by its *APTI*, where higher index values signify greater tolerance.[37] It is an index for determining how plants react biochemically and physiologically to environmental conditions. Pollution-sensitive plants aid in detecting pollution, whereas tolerant plants serve as sinks in polluted areas to help reduce pollution.[11,12]

TABLE VII. *APTI* categories

Category	*APTI* value range
Tolerant species	30–100
Intermediate-tolerant species	17–20
Sensitive species	1–16
Very sensitive species	< 1

The correlations between biochemical properties and *APTI* for the dry and wet seasons are presented in Table VIII. A significant positive correlation ($r = 0.8450$) was observed between the plant's *RWC* and *APTI* during the dry season. During the wet season, the relationship is weak, although positive ($r = 0.1322$). A positive correlation between *APTI* and *RWC* was reported by Punit and Rai.[36] Other factors, such as leaf thickness and soil moisture, also affect the relative water content, even in the presence of pollutants. The total chlorophyll of the leaves also showed a weak but positive correlation ($r = 0.3132$) in the dry season, but a strong positive correlation ($r = 0.7776$) in the wet season. The amount of chlorophyll in plants' leaves varies depending on the species, age, season, drought stress and pollution level.[8,38] Other parameters, pH ($r = –0.5556$) and *AAC* ($r = –0.88722$), indicated a negative relationship with *APTI* in the dry season. This result implies that the lower pH and *AAC* observed in the tree leaves correlated with higher sensitivity (lower *APTI*). The result is likely due to higher levels of vehicular pollutant dust during the season. Reports have shown that *APTI* values generally decrease (with higher sensitivity) in polluted areas with lower pH and *AAC* compared to control sites.[7,39] However, moderate positive correlations were found between the pH and *APTI*

TABLE VIII. Correlation between biochemical properties and APTI during dry and wet season

Parameter	pH	*RWC* / %	*AAC* / mg g^{-1}	*TCh* / mg g^{-1}	*APTI*
Dry season					
pH	1				
RWC / %	–0.86411	1			
AAC / mg g^{-1}	0.876296	–0.97247	1		
TCh / mg g^{-1}	0.429714	–0.23069	0.06662	1	
APTI	–0.5556	0.845035	–0.88722	0.313231	1
Wet season					
pH	1				
RWC / %	–0.56025	1			
AAC / mg g^{-1}	0.549513	–0.60149	1		
TCh / mg g^{-1}	0.938304	–0.24072	0.367135	1	
APTI	0.631617	0.132173	0.522431	0.776754	1

($r = 0.6316$) and between *AAC* and *APTI* ($r = 0.5224$) in the wet season. Consequently, the increase in pH and *AAC* with increasing *APTI* value tends to reduce sensitivity and improve tolerance, suggesting a defence mechanism against air pollutants.

CONCLUSION

This study focused on the seasonal impact on the suitability of the masquerade (*P. longifolia*) as a bioindicator of vehicular pollutants along the University of Ilorin Road. The results of potentially toxic elements and biochemical properties at the roadside sampling points (RS1–RS4) compared with the control site (CS) indicated the presence of air pollution during both dry and wet seasons. The seasonal effect on the plant is not significant since the values are much closer to 7 in both seasons. The roadside masquerade trees can be considered pollution-tolerant due to their high ascorbic acid content, especially during the wet season. The relative water content was high in both seasons, but higher at the control site and in the wet season than at the roadsides and in the dry season. The plant's leaves showed the highest total chlorophyll content during the wet season. The *APTI* values in both seasons suggest that the plant is sensitive to air pollutants. Consequently, the overall conclusion is that the masquerade tree is sensitive to vehicular pollutants in both seasons, making it a suitable bioindicator. However, there was a reduction in sensitivity, resulting in improved tolerance during the wet season.

ИЗВОД

СЕЗОНСКИ УТИЦАЈ НА УПОТРЕБЉИВОСТ ДРВЕТА *Polyalthia longifolia* КАО БИОИНДИКАТОРА САОБРАЋАЈНОГ ЗАГАЂЕЊА НА ПОДРУЧЈУ УНИВЕРЗИТЕТА У ИЛОРИНУ, НИГЕРИЈА

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Ова студија имала је за циљ испитивање сезонског утицаја на погодност дрвета *Polyalthia longifolia* као биоиндикатора загађења ваздуха, пореклом од саобраћаја. Листови су прикупљени дуж саобраћајнице изложене интензивним саобраћајним емисијама, као и са контролне локације без присуства саобраћаја. Током сушне и кишне сезоне анализирани су биохемијски параметри, укључујући pH вредност листа, садржај аскорбинске киселине, релативни садржај воде, укупни хлорофил и индекс толеранције на загађење ваздуха (*APTI*), применом стандардних аналитичких метода. Концентрације потенцијално токсичних елемената повезаних са аутомобилским загађивачима (Pb, Zn, Cr, Mn, Fe и Cu) одређене су методом атомске апсорпционе спектроскопије, након киселе дигестије узорака. Добијени резултати указују на то да су концентрације потенцијално токсичних елемената у већини случајева биле више у листовима прикупљеним дуж саобраћајнице у поређењу са контролном локацијом, изузев Cr и Cu на појединим местима узорковања, током кишне сезоне. Разлике у биохемијским параметрима између узорака са саобраћајнице и контролне локације потврђују утицај саобраћајног загађења у обе испитиване сезоне. Вредности *APTI* указале су на већу осетљивост биљке током сушне сезоне (средња вредност = 10,30), што сугерише смањену толеранцију на загађење, док је током кишне сезоне забележено благо повећање толеранције (средња вредност = 11,23). Резултати потврђују да *P. longifolia* показује изражену осетљивост на саобраћајно загађење током обе сезоне, која је виша у сушном периоду, док је способност толеранције повећана у кишној сезони, вероватно услед активирања ефикаснијих физиолошких одбрамбених механизама.

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