***Original Research Article***

**Influence of tree leaf surface characteristics and wettability in heavy metals accumulation in tropical urban environment**

**ABSTRACT**

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| --- |
| The urban air pollution is a major environmental concern, particularly in the developing countries and in their major cities. In these cities, plants can play a key role in removing pollutants. Therefore, the differences in leaf heavy metals accumulation, surface wettability and leaf surface characteristics in urban ornemental species, including *Jatropha interrigima* and *Ficus benjamina* , were studied at roadsides and Parks. The two species showed distinct trends in leaf heavy metal accumulation. During the study, the leaf heavy metals content of *J*. *interrigima*, a species with adaxial hairy leaf surfaces, was arithmetically higher than that of *F*. *benjamina*, a species with waxy leaf surfaces. The contact angles of water droplets (DCA) on leaves increased with leaf on the abaxial and abaxial surface for both species which, remained highly-wettable (40° < DCA < 90°). However, the DCA on *F*. *benjamina* was higher when compared with those of *J*. *interrigima* at roadsides. A significant and positive relationship existed between leaf heavy metals content and DCA surface of leaves of both species. |

*Keywords: Leaf wettability; heavy metals; leaf hairy; leaf wax; tropical urban environment.*

**1. INTRODUCTION**

Urbanized and industrialized areas are known to be subjected to high concentrations of air pollutants (Kardel et al., 2011). Atmospheric pollution is the main result of different components such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), particulate matter (PM0.1, PM2.5 and PM10) and organic components, which can originate from various sources. The main sources of these pollutants in the urban environment are road traffic and industrial activity (Petroff et al., 2008). Air pollution is a serious threat to human health (Gratani et al., 2008).

Metals such as lead, cadmium, mercury and copper are cumulative poisons. They have been reported to be exceptionally toxic (Ellen et al., 1990). Lead has been associated with intoxications leading to problems in the kidney and liver, the central nervous system, reproductive organs and anaemia. Although copper is an essential trace element in the functions of the human body, chronic and excessive intake has been linked with digestive tract problems and cirrhosis of the liver (Dzierżanowski et al., 2011).

Plants play an important role in filtering ambient air by absorbing and accumulating heavy metals such as lead, cadmium and copper (Buszewski et al., 2000; Olivares, 2003).

Lead and copper monitoring of leaves can, therefore, provide an easy and inexpensive way for monitoring the spatial and temporal distribution of atmospheric pollutants in urban environments (Jouraeva et al., 2002), and thus offer an ideal tool for spatial lead and copper assessment in low income countries.

Some species-specific features of leaves may strengthen this air filtration process, e.g., trichomes (Smith and Staskawicz, 1977) and the chemical composition and structures of epicuticular waxes (Jouraeva et al., 2002). Leaf surface wettability or hydrophobicity, which is measured by calculating the contact angle between a water droplet and a leaf surface plays an important role in a plant’s ability to capture particulate pollution.

The present study aims to (1) evaluate heavy metals (lead and copper) concentrations in plant species exposed in different habitats and leaf wettability (2) determine the quantitative interrelationship between heavy metals content accumulated by leaves and leaf wettability.

**2. MATERIAL AND METHODS**

**2.1 Description of Study Area and Sampling Sites**

Abidjan is located in south west of Côte d’Ivoire (5˚00'N - 5˚30'N, 3˚50'W - 4˚10'W). The climate has 4 seasons with a first rainy season (May-July) and a second rainy season (September-November). The drought seasons occur between the wet seasons. Abidjan is characterized by a high level of industrialization and urbanization. The city has a significant growing old automobile park, of which 70% are secondhand vehicles. Many parks and green spaces were preserved in the city, but these parks disappear quickly due to urbanization. Industies are specialized in various domains of which the oil products and its derivatives, textile and the agroalimentary. Traffic density and industrial smokestacks could be potential sources of pollution.

Two contrasting urban habitat types were selected, i.e., the main road and Parks. Main roads were composed of two busy roads: Lagoon Boulevard and North Highway, where traffic intensity was larger than 6000 vehicles per hour in rush-hour traffic (Barima et al., 2014). Also two parks were selected: municipal plant nursery and botanical garden. In these green areas, human influence is relatively weak and pollution activities were most controlled relatively to the road traffic.

**2.2 Species Selection**

The study was performed on two ornamental species: *Ficus benjamina* L. (Moraceae) and *Jatropha interrigima* Jacq. (Euphorbiaceae). *F*. *benjamina* is a tropical evergreen tree reaching a height rarely exceeds 3 m when used as an ornamental plant. Leaves are glossy, oval of 6-13 cm long, with an acuminate tip. Moreover, the leaves of *F. benjamina* have abaxial wax and smooth cuticle. *J*. *interrigima* is an evergreen shrub or small tree with glossy leaves and densely hairy leaves when young. The plant has a rounded or narrow domed form and gets up to 4 m tall with a spread of about 3 m, although in cultivation it is usually smaller. Leaves are extremely variable; they may be entire and elliptic or oval, or they may be fiddle shaped, or they may have three sharp pointed lobes. These two species differ in leaf shape, size and surface characteristics (Table 1).

**Table 1. Leaf shape, mean hair density (hair.cm2), mean vein density (vein.cm2) and mean leaf area (cm2) (± standard deviation, n = 30)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Leaf shape | Adaxial hair density | Abaxial hair  Density  | Vein density | Mean leaf area |
| *F*. *benjamina* | Oval with an acuminate tip  | 0 | 0 | 9 ± 2  | 13.73 ± 3.30 b |
| *J*. *interrigima* | Entire and elliptic or oval | 120 ± 65 | 0 | 5 ± 1  | 34.29 ± 7.24 a |

Different lowercase letters indicated significant differences in leaf area from different species according to an ANOVA procedure (Tukey-HSD; *P* < 0.05).

**2.3 Experimental Design**

The experimental pots were placed near Main roads (4 sites) of Abidjan highways and in Parks (2 sites) during three months. Within Main roads, the pots were arranged in three rows separated about two meters between the rows. Each row was consisted of height pots (four pots in two replicates). The distance between two pots was 1 m and between two replicates in the same rows was 1 m.

**2.4 Heavy Metals Monitoring**

**2.4.1 Leaves Sampling**

At each sampling location, and for each species, six mature undamaged leaves were collected on the same plant each month and carefully placed in paper envelopes and directly dried at ambient temperature. No discrimination between leaf surface-accumulated and leaf-encapsulated heavy metals were done in order to quantify total lead and copper present on and in leaves. Leaf area was quantified with ImageJ software after scanning the leaves in the laboratory soon after sampling.

**2.4.2 Heavy Metals Determination**

Chemical determination of trace elements (Pb, Cu) was carried out by means of ICP-MS, Perkin-Elmer Elan 6000 (Serveis Científico-Tècnics, University of Barcelona). The ICP-MS were equipped with a Meinhard concentric nebuliser, cyclonic spray chamber, Pt cones and quadruple mass analyser, measuring time 50 ms, integration time 1 s and 3 replicates were used for this study. Typical instrument operating conditions for the ICP-MS were: RF power 1.150 W, plasma Ar flow rate 15 L.min-1, nebuliser Ar flow rate 0.8 L.min-1. Leaf samples (100 mg) were digested in Teflon TM containers using HNO3 (1–2 ml) and H2O2 (0.5–1 ml) for 14 h at 90°C. All concentrations are expressed in mg.g–1 on a dry weight basis.

The calibration was done with 4 standard solutions Cu (0, 4, 8, 20, 40 ppb) and Pb (0, 2, 4,10, 20 ppb), prepared by dilution of standard solutions 1.000 ppm certified traceable to National Institute of Standards and Technology (NIST). All standards were prepared daily after subsequent appropriate dilution with high-purity deionised water (Millipore, USA). The isotopes used for the measurement were 63Cu and 208Pb, and Rhodium (103Rh) was used as internal standard corrector. Rhodium allows us to correct for matrice-induced variation and instrumental drift (Angaman et al., 2017).

**2.5. Leaf Surface Wettability**

Leaf surface wettability was estimated from measurements of the drop contact angle (DCA), i.e., the angle between the perimeter of a droplet on a leaf surface, and the leaf surface. In practice, following criteria picked up by Aryal and Neuner (2010), leaves were termed; “super-hydrophilic” if DCA < 40°; “highly-wettable” if 40° < DCA < 90°; “wettable” if 90° < DCA < 110° (Crisp, 1963); “non-wettable” if 110° < DCA < 130°; “highly non-wettable” if 130° < DCA < 150° (Smith and McClean, 1989); and super-hydrophobic if DCA > 150° (Yoshimitsu, 2002). Eight mature and undamaged leaves of *F*. *benjamina* and *J*. *interrigima* were collected at Main roads and in Parks. After cutting, leaf samples were immediately transported to the laboratory. During transport to the laboratory, and until the moment of analysis, samples were kept in a cool box. Images were taken in laboratory conditions with a Canon EOS 550D digital camera and macro objective (EF-S 55-250 mm f/4-5.6 IS) after placing a drop of 7.5 μl distilled water with a micropipette on leaf surface. This operation was repeated on the right and left sides and on the adaxial and abaxial surfaces of leaves. Drop contact angles were measured using a manual method described by Kardel et al. (2012) with ImageJ software as the average of the contact angles on the left and right side of the drop from the adaxial (DCAad) and abaxial (DCAab) leaf surface.

**2.6. Variability of Drop Asymmetry**

Leaf surface heterogeneity, which may be related to heterogeneity in leaf surface topology, wax deterioration or erosion (Kardel et al., 2012), was estimated by the drop asymmetry (DA) (dimensionless) as a proxy for intra-leaf variability in DCA :

$$DA=2 x \left|\frac{R-L}{R+L}\right|$$

where R and L are the angles (◦) measured at the right and left side of the drops, respectively.

**2.7 Statistical Analysis**

All data were analyzed using Statistica software, version 7.1 (StatSoft Inc., 1984-2005). Analyses of variance (ANOVA) with Tukey’s honestly significant difference (Tukey-HSD) were applied to determine significant differences as well as in heavy metals (Cu and Pb) in leaves and in DCA and DA on adaxial and abaxial surface of two study species between Main roads and Parks. Linear regression analysis was performed to identify the relationship between heavy metals, DCA and DA. A given effect was assumed significant at *P <* 0.05.

**3. RESULTS**

**3.1 Leaf Heavy Metals Accumulation**

Figures 1-2 show the leaf Cu and Pb amounts from *F. benjamina* and *J. interrigima* at Main roads and in Parks. Leaf Cu and Pb amounts were significantly higher on Main roads than in Parks in all species (*P <* 0.05). Thus, the leaf Cu content ranged from 6.6 mg.g-1 and 7.97 mg.g-1 at roadsides respectively for *F. benjamina* and *J. interrigima*, against 2.74 mg.g-1and 2.36 mg.g-1 in Parks (Fig. 1), while the leaf Pb content ranged from 3.03 mg.g-1and 4.45 mg.g-1 at roadsides respectively for *F. benjamina* and *J. interrigima*, against 0.52 mg.g-1and 0.86 mg.g-1 in Parks (Fig. 2). A high concentrations of heavy metals were observed for *J. interrigima*; however, no significant differences (*P* > 0.05) were found between leaf Cu and Pb amounts accumulated by the two species in each habitat.

A ; a

B ; a

A ; a

B ; a

**Fig. 1. Copper concentrations of leaves from two plant species (*F*. *benjamina* and *J*. *interrigima*) in the habitats considered (Main roads and Parks)**

*Different capital letters indicate significant differences between habitats for each considered species Different small letters indicate significant differences between the species within each land use class Error bars are standard deviation Significant if P < 0.05*

**Fig. 2. Lead concentrations of leaves from two plant species (*F*. *benjamina* and *J*. *interrigima*) in the habitats considered (Main roads and Parks)**

*Different capital letters indicate significant differences between habitats for each considered species*

*Different small letters indicate significant differences between the species within each land use class*

 *Error bars are standard deviation*

 *Significant if P < 0.05*

**3.2. Drop Contact Angle**

Higher values of drop contact angle (DCA) were found on *F*. *benjamina* adaxial (84.79°) and abaxial (86.25°) leaf surface at roadsides. The lowest mean of DCA for adaxial (65.15 °) and abaxial (77.5°) leaf surface were observed with *F*. *benjamina* in Parks (ANOVA, Tukey test) (Table 2). On adaxial surface, these angles vary from 79.5°- 84.79° in Main roads and 65.15° - 80.3° in Parks. On abaxial surface, DCA ranged from 81.5°- 86.25° in Main Roads and 77.5° - 77.73° in Parks. significant differences (*P* > 0.05) were observed between DCA mean of these two species from adaxial and abaxial leaf surface considered (Table 2). At the intraspecific level, DCA at roadsides were generally higher than those obtained in Parks (*P* < 0.05). significant change was found in adaxial and abaxial surface DCA at Main roads (*P* > 0.05).

**Table 2. Mean drop contact angle (° ± standard deviation) on the adaxial and abaxial leaf surfaces from plant species in Main roads and Parks**

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Leaf surface | Main roads | Parks |
| *F*. *benjamina* | Adaxial | 84.79 ± 14,11a,B | 65.15 ± 8.59 a,A |
| Abaxial | 86.25 ± 15.43 a,B | 77.5 ± 15.51a,A |
| *J*. *interrigima* | Adaxial | 79.5 ± 10,10 a,B | 80.3 ± 11.09 a,B |
| Abaxial | 81.5 ± 15.05 a,B | 77.73 ± 13.54 a,B |

Different capital letters above indicate significant differences between both habitats.

Mean values on each column between the different leaf surfaces followed by the same small letters above are not significantly different.

Significant differences if *P* < 0.05.

**3.3 Variability of Drop Asymmetry**

Lower drop asymmetry (DA) values were obtained on both waxy surface of *F*. *benjamina* vary between 0.08 - 0.10 at Main roads, while in the Parks, the lowest DA values were obtained on leaf surfaces of *J*. *interrigima* (Table 3): 0.11 and 0.12 respectively from adaxial and abaxial surfaces. The highest values of DA were observed on leaf surfaces: 0.15 and 0.16 respectively from adaxial hairy and abaxial non-hairy of *J*. *interrigima* on Main roads and betwen 0.17 and 0.21 for *F*. *benjamina* in Parks (Table 3).

**Table 3. Mean drop asymmetry (dimensionless) (DA ± standard deviation) on the adaxial and abaxial leaf surfaces from plant species in Main roads and Parks**

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Leaf surface | Main roads | Parks |
| *F*. *benjamina* | Adaxial | 0,10 ± 0,10 a,A | 0,17 ± 0,13 b,B |
| Abaxial | 0,08 ± 0,11 a,A | 0,21 ± 0,11 b,B |
| *J*. *interrigima* | Adaxial | 0,15 ± 0,13 b,B | 0,11 ± 0,09 a,A |
| Abaxial | 0,16 ± 0,10 b,B | 0,12 ± 0,08 a,A |

Different capital letters above indicate significant differences between both habitats.

Mean values on each column between the different leaf surfaces followed by the same small letters above are not significantly different.

Significant differences if *P* < 0.05.

**3.4 Relationship Between Heavy Metals Content, DCA and DA**

For the two investigated species, we observed a positive correlation between drop contact (DCA) angles on adaxial and abaxial surfaces and leaf heavy metals content accumulated, but the strong and higher correlation (R2 ˃ 0.50, *p* < 0.05) were found on *F*. *benjamina* adaxial and abaxial leaf surface in Main roads (Fig. 3, (a) and (b)), while for *J*. *interrigima* the same trend was observed on abaxial leaf surface in both investigated habitats (Fig. 4, (b)). Contrary to DCA, the linear regression between drop asymmetry (DA) on adaxial and abaxial surfaces and leaf heavy metals content showed, generally, a negative correlation (Fig. 3-4, (c) and (d)).

 **(a)** (**b)**

 **(c)**  (**d)**

**Fig. 3. Correlations between heavy metals content and mean drop contact angle (DCA) (a and b) / Drop Asymmetry (DA) (c and d) on adaxial and abaxial leaf surfaces of *F*. *benjamina*.**

 **(a)**   **(b)**

 **(c)**  **(d)**

**Fig. 4. Correlations between leaf heavy metals and mean drop contact angle (DCA) (a and b) / Drop Asymmetry (DA) (c and d) on adaxial and abaxial leaf surfaces of *J*. *interrigima*.**

**4. DISCUSSION**

**4.1 Leaf Heavy Metals Accumulation**

In this study, mean heavy metals concentrations measured in Main roads and parks for *F*. *benjamina* and *J*. *interrigima* revealed high levels of copper (Cu) and lead (Pb) along roads. These results also suggest that the major source of pollution were car exhaust and confirm those obtained by Bukowiecki et al. (2010), Kardel et al. (2012) in temperate regions also in West Africa by Fatoki (1987). The high heavy metal contents in plant samples collected from roadsides are due mostly to the density of traffic. The sources of Pb on roads are automobile emissions of gasoline combustion; Cu from overhead wires and brake pads usury (Kakareka et al., 2004). The habitat quality in Main roads might, therefore, be considered to be low compared to Parks as already demonstrated in several studies like Cavanagh et al. (2009), Serbula et al. (2010).

 *J*. *interrigima* leaf area (34.29 cm2) and hair density (120 hairs.cm2) were the highest among species tested (Table 1); mean heavy metals concentrations was arithmetically higher than *F*. *benjamina* (Fig. 1-2), even if these differences were not significant. The complexity of *J*. *interrigima* leaf surface would make this species most likely to intercept air pollutants than *F*. *benjamina*. Indeed, studies have shown that leaves with complex shapes, fine hairs or emitting sticky substances may accumulate particles efficiently (Freer-Smith et al., 2004; Wang et al., 2013). However, *F*. *benjamina* leaves, although having no roughness, had heavy metals comparable to those obtained with *J*. *interrigima* probably because of its wax layer cuticle. Indeed, studies have shown that some waxy species, during the growth, accumulates particle in wax formation (Dzierżanowski et al., 2011).

**4.2 Leaf Surface Wettability**

In this study, leaf wettability varied between habitat and both leaf sides. DCA ranged from 65° to 87°, indicating that (based on criteria of Crisp, 1963) both species were highly-wettable. Previous studies showed that waxy cuticles and outgrowths, such as trichomes, increase the wettability (Holder, 2012). The highest DCA were found on *F*. *benjamina* (wax layer cuticle) adaxial and abaxial leaf than those of *J*. *interrigima* (most complex surface in term of hair and vein densities) surface at roadsides. The difference in leaf wettability can be related to the chemical composition of the surface wax layer (Burkhardt, 2010), surface or the ultrastructure of the epicuticular wax (Barthlott et al., 1998). The higher leaf DCA on Main roads areas might be due to erosion of the epicuticular wax, which is related to pollution stress. These results confirm Kardel et al. (2011) observations on scanning electron microscope images of *Alnus glutinosa*, *Acer pseudoplatanus*, *Betula pendula*, *Quercus robur* and *Sambucus nigra*.

**4.3 Leaf Surface Heterogeneity**

Higher values of drop asymmetry (DA) were observed at the adaxial and abaxial leaf surfaces of *J*. *interrigima* compared to *F*. *benjamina* in Main roads. This result confirmed the high surface heterogeneity of *J*. *interrigima* (hairy and more vein density) than the waxy *F*. *benjamina*. Habitat significantly influenced the adaxial and the abaxial DA of *J*. *interrigima* (*P* < 0.05). Previous studies showed that for many species, an increased exposure to pollution leads to an increased damage of the leaf surface (Khavaninzadeh et al., 2003; Kardel et al., 2012). Furthermore, particles deposition on the leaf surface can be heterogenic (Lindberg and Harriss, 1981), since many of them are composed of materials that are readily wetted or dissolve in water (Neinhuis and Barthlott, 1998). As a result, water adheres more easily to the surface, and damaging substances might be concentrated during the drying process and resulted in leaf damage. This is what we probably observed in this study for the adaxial and abaxial leaf surface of *J*. *interrigima*.

**4.4 Relationship Between Leaf Heavy Metals Content, DCA and DA**

For the two investigated species, a positive correlation was found between DCA on adaxial and abaxial surfaces and leaf heavy metals amounts. This result could be due to the high-wettability of study leaves. Indeed, Wang et al. (2013) revealed a significantly negative correlation between leaf contact angles of water droplets on adaxial surfaces and particulate matter (PM) retention amounts for *Sophora japonica* and *Cedrus deodara* because of their high non-wettability. Besides, in our study, the strong and higher correlation (R2 ˃ 0.50, *P* < 0.05) were found on *F*. *benjamina* adaxial and abaxial leaf surface in Main roads. According to Bradley et al. (2003) and Sase et al. (2008), stored water on leaf surfaces increases the potential for leaf damage from pollutants. This leaf property induced a higher capacity of investigation leaf species to encapsulate more heavy metals as the DCA increases. Contrary to DCA; the linear regression between DA on adaxial and abaxial surfaces and leaf heavy metals content showed generally a negative correlation. This trend, could suggested that leaf heavy metals accumulation may be not one of the factors affecting leaf surface. Leghari and Zaidi (2013), attested that it is often difficult to estimate the effects of air pollutants because the organisms are exposed to a wide range of uncontrolled variables (parasites, weather conditions, complex mixture of pollutants).

**5. CONCLUSION**

Plant leaves samples, showed differences in heavy metals (Cu and Pb) accumulation at roadside and in parks. Main roads were significantly more polluted than Parks confirming that the main sources of pollution determined with heavy metals were car exhaust for the two investigated species. All tested species were highly-wettable because of drop contact angle was greater than 40° and less than 90°. However, *F*. *benjamina* with its leaf wax were more wettable than *J*. *interrigima* with its leaf trichomes. A significantly positive correlation was found between leaf surface wettability and leaf heavy metals content. Accordingly, leaf surface properties, and leaf wettability in particular, may be one of the regulatory factors influencing deposition and accumulation of heavy metals at the leaf level.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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