

## Postdoc Fellowships for non-EU researchers

### Final Report

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<b>Selection</b>	
<b>Host institution</b>	University of Antwerp
<b>Supervisor</b>	Prof. Dr Roeland SAMSON
<b>Period covered by this report</b>	From 10/02/2014 to 09/5/2014 and from 20/6/2014 to 19/9/2014
<b>Title</b>	Potentials of leaf Saturation Isothermal Remanent Magnetisation and physiological leaf parameters as indicators of local air quality in tropical urban environments: a case study for Abidjan (Ivory Coast).

#### 1. Objectives of the Fellowship (1/2 page)

The main objective of this fellowship is to enhance my capacity in research on the interactions between the plant and its environment. This was to allow me to come to University of Antwerp with samples of plants derived from a southern city for analysis in a laboratory of the North.

Another objective of the fellowship is to enable the writing of scientific papers resulting of laboratory research.

Finally, the award was intended to strengthen collaboration with partners of University of Antwerp and also in order to focus our research on new issues.

#### 2. Methodology in a nutshell (1/2/ page)

Study was performed in Abidjan, the economic capital city of Ivory Coast. This city was subdivided into 4 land use classes i.e.: industrial zones (IZ), residential zones (RZ), <sup>SEP</sup>parks and main roads (MR). The geographical distribution of sites can allow to extrapolate results to the whole city of Abidjan.

A botanical inventory was performed at the different land use classes, considering the presence of species. After screening, species well distributed over all land use classes, easy to harvest and whose leaves are wide enough to be easily scanned were selected, i.e.: *Amaranthus spinosus*, *Barleria prionitis*, *Eleusine indica*, *Ficus benjamina*, *Jatropha interrigima* and *Panicum maximum*.

Passive biomonitoring and active biomonitoring were performed in 2012, 2013 and 2014. At each sampling location, and for each species, 6 mature and undamaged leaves were collected at the road-facing side of the plant and carefully placed in paper envelopes. Leaf area was quantified with Image J software after scanning the leaves in the laboratory soon after sampling. Leaves were dried at ambient temperature. Magnetic measurements were carried out at the Department of Bioscience Engineering of the University of Antwerp (Belgium) using a magnetiser and a magnetometer. More details of the method were described in Barima et al. (2014).

Data analysis: For each considered land use class, mean leaf SIRM was calculated and compared using ANOVA procedure. The relations between leaf SIRM and sampling distance from road and height classes were computed through simple and multiple regressions using coefficient of determination. A regression was

performed, by Pearson's method, between SIRM and monthly averages of air temperature, air humidity and wind speed, and total monthly rainfall to determine the main seasonal parameters influencing leaf SIRM. Contact angles of standardized water droplets with a leaf surface were used as a proxy for leaf wettability. Images were taken in laboratory conditions with a Canon EOS 550D digital camera and macro objective after placing a drop of 7.5 µl distilled water with a micropipette on leaf surface. Drop contact angles were measured using a manual method described by Kardel et al. (2012). A significance level of 0.05 was used for all tests.

### 3. Results (6-8 pages)

#### Leaf SIRM per species and land use class

For *Amaranthus spinosus*, *Ficus benjamina* and *Panicum maximum*, the highest leaf SIRM ( $20.1-45.1 \times 10^{-6}$  A or  $20.1-45.1 \mu\text{A}$ ) was measured at MR (Fig. 1). The lowest leaf SIRM was observed at RZ and parks, with values generally below  $10 \times 10^{-6}$  A. In MR, leaf SIRM was lowest for *Panicum maximum*, having about half of the values as measured for *Amaranthus spinosus* and *Ficus benjamina*. In IZ, the highest and lowest SIRM-values were observed for *A. spinosus* and *P. maximum* ( $15.7 \times 10^{-6}$  A), while *E. indica* and *F. benjamina* had intermediate values. For *F. benjamina*, leaf SIRM in parks is slightly higher, although not significantly, than in RZ.

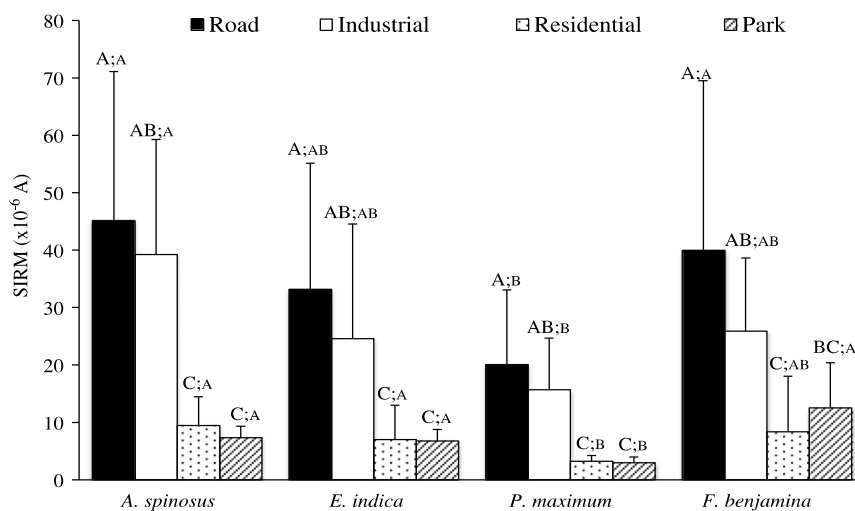


Fig 1. Leaf SIRM for *A. spinosus*, *E. indica*, *P. maximum* and *F. benjamina* as observed in the considered land use classes. Different capital letters indicate significant differences between land use classes for each considered species. Small letters indicate significant differences between the species within each land use class. Error bars are standard deviation. Significant if  $p \leq 0.05$ .

#### SIRM variation across land use classes

The results showed a great variability of leaf SIRM in the considered land use classes in Abidjan. The most polluted land use classes were major roads and industrial areas opposed to parks and residential areas. These results are in agreement with those obtained by Kardel et al. (2012b) who found high levels of SIRM in urban (tram lines, highways, main roads and intersections) and harbour-industrial areas compared to green and sub-urban areas in the city of Gent (Belgium). These results also suggest that the main sources of pollution determined with SIRM were car exhaust (Kardel et al., 2012b; Bukowiecki et al., 2010) and industrial emissions (Hansard et al., 2011). The habitat quality in these areas might, therefore, be considered to be low compared to parks and residential areas as already demonstrated in several studies like Mitchell and Maher (2009) and Diaz et al. (2012). High, or at least better, air quality was observed in parks and residential areas as opposed to roads and industrial areas which is in agreement with results of other studies, including those of e.g. Weijers et al. (2004) and Serbula et al. (2010). These authors showed that air quality was better within parks and worsened when approaching roads.

#### Inter-species differences in leaf SIRM

Of the considered species the highest leaf SIRM was observed for *A. spinosus* and *F. benjamina*. For *A. spinosus* the high leaf SIRM, and thus PM deposition, might be explained by its leaf characteristics,

especially when young these leaves are slightly hairy on both sides. Indeed, the presence of hairs is known as a facilitator for capturing PM (Mitchell et al., 2010; Speak et al., 2012). Leaves of *F. benjamina*, although having no hairs and being smooth, showed high SIRM values, comparable to those obtained for *A. spinosus* having hairy leaves, and superior to those of the considered Poaceae species, which have a low hair density. In general, the deposition of atmospheric particulate matter on trees, and thus leaf SIRM, is 2 to 16 times greater than on low vegetation (Fowler et al., 1989; Hiemstra et al., 2008). This is often explained by their greater total leaf area. In addition, according to Reyes et al. (2012), the secretion of latex of *F. benjamina*, make it an excellent adsorbent of particles. Kardel et al. (2012b) observed high SIRM values, and thus a high deposition of particles on *Tilia* leaves which were sticky due to honeydew produced by aphids. Another explanation for the high leaf SIRM of *F. benjamina* is the presence of wax on the leaves, which seems to be one of the factors favouring PM accumulation (Sæbø et al., 2012). Kardel et al. (2011) suggested that deposited particles could be built in the wax during regeneration, resulting in an increase of leaf SIRM during the growing season, and this process might be important for species with waxy leaves like *F. benjamina*. Moreover, this species is an evergreen species, keeping its leaves during several years, resulting in a longer time increase in leaf SIRM, as was observed by Lehndorff et al. (2006) for *Pinus nigra*.

### Effect of distance to road and height on leaf SIRM

For all herbaceous plants, leaf SIRM normalized by traffic density ( $SIRM_{norm}$ ) decreased (exponentially or logarithmically) with increasing distance from the roadside (Fig. 2). Indeed, the best fits obtained were statistically significant ( $p < 0.05$ ). The highest was obtained with *E. indica* (69%, exponential fit) and the lowest with *A. spinosus* (33%, logarithmic fit).

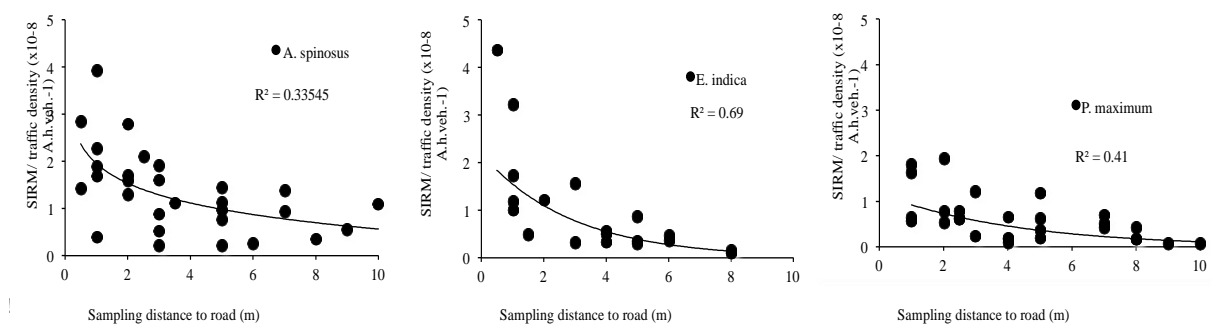


Fig. 2. Leaf SIRM normalised for traffic density in function of distance from the major road for the considered species. Regressions are statistically significant ( $p < 0.05$ ).

### Leaf SIRM and distance from roadside

These results are consistent with most of the work on this topic (Kardel et al., 2012b; Rooney et al., 2012), indicating that vehicle exhaust is the main source of pollution in the vicinity of MR. Kozawa et al. (2012) and Baldauf et al. (2013) showed that in general, the concentration of the main primary gases resulting from motorised traffic (CO, NO, NO<sub>2</sub>) is high in the immediate vicinity of the road and then decreases with distance, while further from the road they dilute in the atmosphere. Hagler et al. (2009) showed that traffic impact is very little beyond 300 m from both sides of the road, and is particularly noticeable in the first 80-100 m (Beckerman et al., 2007; Hagler et al., 2009). Moreno et al. (2003) showed that PM content significantly reduced within 3 m of the road, where the coarser particles are deposited, while only the finer particles are routed at greater distances.

Leaf SIRM of *F. benjamina* varied with height, and this variation differed between the considered land use classes (Fig. 3). In MR, SIRM decreased with height. A decrease of 62% was observed for the upper canopy layer compared to the lower layer. In IZ and parks the opposite trend was observed. The highest SIRM-values were observed for the highest sampled canopy layer, and values decreased by 84% and 50% for the lowest canopy layers in the IZ and parks, respectively. In RZ no clear height effect was observed.

This height trend in MR confirms that the main pollution source in this land use class is from fuel driven traffic (diesel engines mainly) as already reported by many authors (Sagnotti and Winkler, 2012; Speak et al., 2012). Unlike in industrial zones, particles are ejected into the atmosphere from smokestacks. Particles fall down by gravity or rain and are first intercepted by the upper canopy level, before reaching the lower ones. One explanation for height canopy trend of PM in parks can be related to convection effect between

urban areas (high temperature) and parks (lower temperature compared to urban) and movement of air flow from urban area (or other urban features around parks area) to top of the canopy layer in the parks and thus deposition of PM at the top of the canopy.

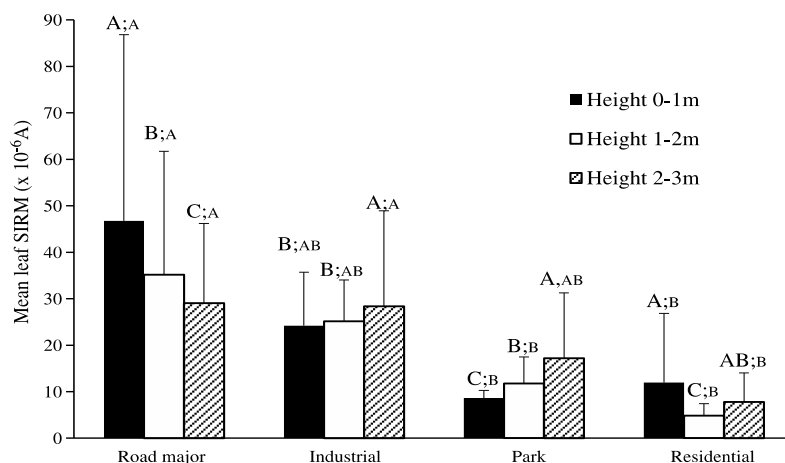


Fig. 3. Variation in leaf SIRM in function of canopy height for *Ficus benjamina*. Different capital letters indicate significant differences between land use classes. Small letters indicate significant differences between leaf SIRM at a canopy height within each land use class. Error bars are standard deviation. Significant if  $p \leq 0.05$ .

### Seasonal variation in *Ficus benjamina* leaf SIRM

*F. benjamina* leaf SIRM varies significantly between and within seasons (Fig. 4). This in-season variation differs among the considered land use classes. Except for July (last rainy month), leaf SIRM values obtained during the dry season are higher than those obtained during the rainy season. For MR and IZ, respectively, leaf SIRM decreased from 152.10  $\mu\text{A}$  to 47.90  $\mu\text{A}$  and from 133.0  $\mu\text{A}$  to 62.70  $\times 10^{-5}$  A from February to June ( $p < 0.05$ ). However, at the end of rainy season (July), leaf SIRM rose to tend towards the value obtained during the end of the dry season. The same trends were observed for the active biomonitoring approach (Figure 4). In sum, whatever the month and season, leaf SIRM were higher during the dry season than the rainy season ( $p < 0.05$ ).

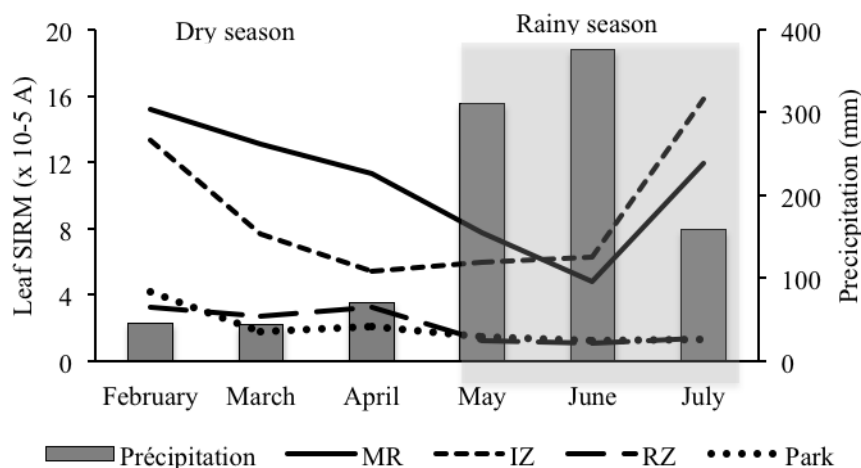


Fig. 4. Leaf SIRM of *Ficus benjamina* and monthly rainfall from February to July 2012 for the passive biomonitoring. February to April is the dry season and May to July is the rainy season.

In general, leaf SIRM during the dry season was higher than during the rainy season. In temperate climatic regions and for deciduous species, several authors like Mitchell et al. (2010), Kardel et al. (2011) and Hofman et al. (2014a) also found a seasonal effect of leaf SIRM, but they observed an increase of leaf SIRM with increasing exposure time. Using PM sensors, other authors found similar trends. Instead authors like Kassomenos et al. (2014) obtained in Greece and Spain, PM concentrations higher during the warm period higher PM concentrations and suggesting the larger relative contribution of secondary and natural particles during hot and dry days. These variations of leaf SIRM or PM content in the atmosphere showed that the

concentration of these pollutants is influenced by weather or meteorological conditions (Kassomenos et al., 2014).

In our study area, and in West African tropical zone in general, PM prevalence in the atmosphere during the dry season could be explained by the harmattan dust. Indeed, in the dry season, from December to March, the West African region is subject to a dry dust-laden wind, the harmattan, blowing from the Sahara Desert. The harmattan wind carries the Sahara mineral dust to long distances (Gonçalves et al., 2014). Transport of particles by these winds have already been demonstrated in Mediterranean countries including Greece (Kassomenos et al., 2014), Portugal (Wagner et al., 2009) and Spain (Rodríguez-Germade et al., 2014). In sum, in dryer atmospheric conditions, PM concentrations are higher, and thus also deposition. Moreover, due to the lack of rain, there is little wash-off, which is observed during the rainy season and which is in contrast to the findings of Kardel et al. (2011) and Hofman et al. (2014a).

### Effects of meteorological variables on leaf SIRM in a passive biomonitoring approach

Among the meteorological studied, precipitation seems to be best correlated to changes in leaf SIRM. This negative correlation is observed in all land use classes (Table 1). The highest correlations were obtained in MR ( $r = -0.94$ ;  $p = 0.004$ ) and RZ ( $r = -0.89$ ;  $p = 0.010$ ). Although the correlation coefficients for the other variables are generally larger than 30% they are not statistically significant.

Table 1. Results of simple regressions performed with *F. benjamina* leaf SIRM in a land use class and meteorological variables (temperature, humidity, monthly rainfall and wind speed.  $r$ : Pearson coefficient of correlation. Negative  $r$  expresses a negative linear link between the variables. Number of samples per month = 109.  $p$  is p-value. Significant correlations ( $p < 0.05$ ) are given in bold.

		Temperature (°C)	Humidity (%)	Monthly Rainfall (mm)	Wind speed (km.h <sup>-1</sup> )
Main roads	$r$	0.29	-0.69	-0.94	0.38
	$p$	0.570	0.128	<b>0.004</b>	0.450
Industrial zones	$r$	-0.57	0.07	-0.34	0.13
	$p$	0.234	0.896	<b>0.040</b>	0.804
Residential zones	$r$	0.60	-0.79	-0.89	0.57
	$p$	0.202	0.062	<b>0.010</b>	0.232
Parks	$r$	0.27	-0.71	-0.59	-0.68
	$p$	0.604	0.116	0.221	0.134

It is well known that atmospheric PM and dust concentrations vary due to variation in meteorological conditions, even with constant emissions to the environment, as meteorological conditions play a vital role in governing the fate of air pollutants (Deshmukh et al., 2013). Among the considered meteorological variables, rainfall is statistically negatively correlated to leaf SIRM (Table 1, Fig. 4 and 4). It seems that the heavy rain events washes off the intercepted dust from the leaves. This wash off effect was also found by other authors (e.g. Matzka and Maher, 1999; Sant'Ovaia et al., 2012; Li et al., 2014), while others did not observe such an effect (Kardel et al., 2011; Hofman et al., 2014b).

Studies showed that leaf surface characteristics have a strong influence on the retention of particles by the leaf surfaces, and therefore on the intensity of wash off effect. Thus, leaves with complex shapes, waxy cuticles, ridged surface fine hairs or emitting sticky substances may accumulate particles efficiently (Freer-Smith et al., 1997; Kardel et al., 2011; Wang et al., 2013). However, within these characteristics, atmospheric PM amount a species with ridged leaf surfaces, was significantly higher than species with waxy leaf surfaces (Wang et al., 2013). Furthermore, plants accumulated atmospheric PM both on foliage surfaces and in waxes. With regards to quantity, atmospheric PM on leaf surface generally exceeded wax PM, with variable rates depending on the species and land use classes (Przybysz et al., 2014). In nature, atmospheric PM on leaf surface can easily be washed off from foliage by rain, as also pointed out by other authors (Beckett et al., 2000; Van Heerden et al., 2007). Moreover, increased direct exposure to rain event conditions might cause a more intensive mechanical and chemical abrasion of the wax layer (Wang et al., 2013) like *F. benjamina* (simple and waxy leaves) in our study case.

### Effect of *Ficus benjamina* exposure duration to air on leaf SIRM – Seasonal effect

The comparison of leaf SIRM of *F. benjamina* derived from active biomonitoring in the dry (April to May) and rainy season showed a statistical difference near the MR ( $p < 0.05$ ) and in IZ ( $p < 0.05$ ). Mean leaf

SIRM is higher in dry season (148.3  $\mu\text{A}$  for MR and 80.07  $\mu\text{A}$  for IZ) than in rainy season (71.75  $\mu\text{A}$  for MR and 98.40  $\mu\text{A}$  for IZ). During the dry season (February to April), SIRM of leaves subjected to one month exposure (“young leaves”; 180.50  $\mu\text{A}$ ) is important than those subjected to three months exposure (“old leaves”; 116.10  $\mu\text{A}$ ) ( $p < 0.05$ ). A reversed trend was observed, during the raining season (May to July), SIRM of “old leaves” (97.10  $\mu\text{A}$ ) was higher than that of the “younger” leaves (46.40  $\mu\text{A}$ ) ( $p < 0.05$ ) (Table 2). No clear statistical difference was found in the intra seasonal variations of the other land use classes (Table 2).

Table 2. Seasonal evolution of mean leaf SIRM ( $\mu\text{A} \pm$  standard deviation) derived from active in the dry (April to May) and rainy season (May-July).  $t$  : Student t-test.  $p$ : P-value. Significant effects are shown in bold ( $p < 0.050$ ). Letters indicate mean leaf SIRM ranking obtained in different months. Values assigned by the same letter are not statistically different (ANOVA, Turkey test). ns = not significant from ANOVA test.

	Main roads	Industrial zones	Residential zones	Parks
February	180.50 $\pm$ 136.20 a	69.76 $\pm$ 9.90 ab	35.80 $\pm$ 20.40 ns	38.60 $\pm$ 2.90 a
April	116.10 $\pm$ 77.90 a	11.31 $\pm$ 22.50 a	17.90 $\pm$ 0.80 ns	14.10 $\pm$ 1.90 b
$t$	2.98	-4.87	1.29	7.52
$p$	<b>0.013</b>	0.128	0.418	0.084
May	46.40 $\pm$ 25.40 b	36.80 $\pm$ 5.40 a	11.50 $\pm$ 1.80 ns	11.20 $\pm$ 2.10 b
July	97.10 $\pm$ 71.00 b	61.60 $\pm$ 13.30 a	21.50 $\pm$ 5.90 ns	15.20 $\pm$ 4.60 b
$t$	-3.29	-1.87	-1.83	-0.84
$p$	<b>0.007</b>	0.312	0.318	0.554
Seasonal mean				
$t$	3.038	2.546	1.214	1.123
$p$	<b>0.003</b>	<b>0.043</b>	0.270	0.123

Some authors have proposed that part of the particulate material responsible for the magnetic signal is not found on the leaves' surface but rather is incorporated into their structure through the stomata cavities or their cuticle waxy protective layer (Sagnotti and Winkler, 2012; Burkhardt and Pariyar, 2014; Przybysz et al., 2014) as explained in the section below.

*F. benjamina*, an evergreen tree, showed an increase in leaf SIRM with time during the rainy season. This result suggests that the encapsulation of magnetic particles into the leaf tissue mainly occurs during the rainy season (growth phase) compared to the dry season probably due a higher wax regeneration or formation activity during this growth season (Mitchell et al., 2010; Kardel et al., 2011). Mitchell et al. (2010) showed the evergreen trees may incorporate a larger proportion of particles within the leaf structure. However, lower leaf SIRM after three months of exposure to air during the dry season could be explained, in tropical humid area, during this season, by the severity of drought and harmattan is more rigorous (Weinstein et al., 2010). Plant adaptation to drought stress (high temperature and low rainfall) and harmattan lead to stomata closure (Koffi et al., 2014) and a reduction of epicuticular wax production and reducing the encapsulation of particles by leaf and thus a subsequent SIRM decline (Hofman et al., 2014a). In any event, rain-induced wash off of magnetic particles from tree leaves is likely to vary according to species and leaf characteristics, in addition to rain intensity, canopy position and degree of shelter (Mitchell et al., 2010; Hofman et al., 2014a).

#### Leaf SIRM for young and mature leaves

Leaf SIRM is higher on mature leaves than on young leaves on Main roads. Leaf SIRM was thus 1.80, 2.21 and 1.31 times higher in mature leaves than in young leaves for respectively *B. prionitis*, *F. benjamina* and *J. interrigima* at roadside (Fig. 5). However, there are no significant differences ( $p > 0.05$ ) between SIRM of young and mature leaves in Parks for leaves that have undergone the same treatment (washed or unwashed) (Fig. 5; Table 2).

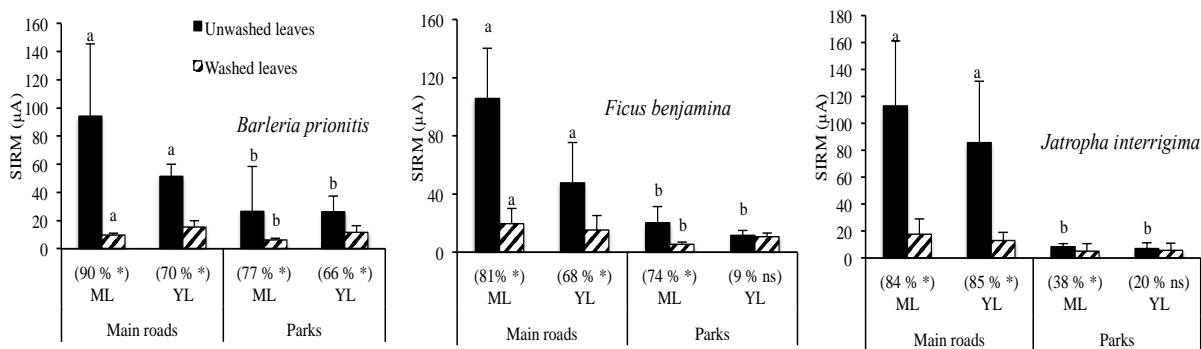


Fig. 5. Washed and unwashed leaf SIRM for mature and young leaves for three species. Letters above the histograms represent significantly different between mean leaf SIRM (washed and unwashed) between Main roads and parks. Letters below histograms indicate the percentage of leaf SIRM loss of washed leaved compared to unwashed leaves. \* show significant proportions. ns = non significant, ML= mature leaves, YL= young leaves. Significant differences if  $p \leq 0.05$ .

Table 2. Leaf SIRM ( $\mu\text{A} \pm$  standard deviation) for *B. prionitis*, *F. benjamina* and *J. interrigima* sampled at Main roads and Park. Different letters indicate significant differences between species for each considered habitat according to an ANOVA (Tukey-HSD test) procedure. Significant if  $p \leq 0.05$ .

		Unwashed leaves		Washed leaves	
		Main roads	Parks	Main roads	Parks
Mature leaves	<i>B. prionitis</i>	94 ± 51	12 ± 3	10 ± 1	5 ± 1 <sup>a</sup>
	<i>F. benjamina</i>	106 ± 34	20 ± 11	20 ± 10	5 ± 2 <sup>a</sup>
	<i>J. interrigima</i>	113 ± 48	8 ± 3	18 ± 11	2 ± 0.3 <sup>b</sup>
Young leaves	<i>B. prionitis</i>	52 ± 9 <sup>b</sup>	18 ± 6	16 ± 4	12 ± 5
	<i>F. benjamina</i>	48 ± 28 <sup>ab</sup>	12 ± 3	15 ± 10	11 ± 2
	<i>J. interrigima</i>	86 ± 45 <sup>a</sup>	7 ± 4	13 ± 6	6 ± 5

Leaf SIRM is higher on mature leaves than on young leaves in Main roads. This result may suggest a particle accumulation in leaves over time as already observed by Mitchel et al. (2010), Kardel et al. (2011), Rodriguez-Germade et al. (2014) and Hofman (2014b). Indeed, pollutants particles (determined from the SIRM) appear gradually settle on the leaf surface until dynamic equilibrium between particle deposition and particle loss is reached; this equilibrium depends on the species (Mitchell et al., 2010). *J. interrigima* leaf area (34.29 cm<sup>2</sup>) and hair density (120 hairs.cm<sup>2</sup>) were highest among species tested (Table 1) ; leaf SIRM was (mathematically) high than the other species studied (Table 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 *B. prionitis* and *F. benjamina*. Indeed, studies have shown that leaves with complex shapes, ridged surface fine hairs or emitting sticky substances may accumulate particles efficiently (Freer-Smith et al., 1997; Kardel et al., 2011; Freer-Smith et al., 2004; Wang et al., 2013). However, within these characteristics, atmospheric PM amount a species with ridged leaf surfaces, was significantly higher than species with waxy leaf surfaces (Wang et al., 2013). However, *F. benjamina* leaf, although having no roughness, had leaf SIRM comparable to those obtained with *J. interrigima* probably because of the wax layer present on his leaves. Indeed, studies have shown that some waxy species, during the growth, accumulates particle during in wax formation (Lehndorff et al., 2006; Dzierzanowski et al., 2011; Terzaghi et al., 2013). In parks, given the low presence of pollutants unlike roads, the difference between young and mature leaves was not clear.

#### Washed effect on leaf SIRM of *Barleria prionitis*, *Ficus benjamina* and *Jatropa interrigima*

Leaf SIRM erosion by water varied strongly between species and between leaf age and habitat (Fig. 5). In MR, leaf SIRM is statistically lower after washing ( $p < 0.05$ ); erosion rates were generally over 70 %. With 19 % of leaf SIRM encapsulated in mature leaves and 32% in young leaves, *F. benjamina* was the species that encapsulate most leaf SIRM among study species at roadsides. In Parks, differences between washed and unwashed leaf SIRM was statistically different for mature leaf SIRM. The largest loss in leaf SIRM was observed with *B. prionitis* (77 %,  $p < 0.05$ ) and lowest with *J. interrigima* (38 %,  $P < 0.05$ ) for mature leaves. However, for young leaves in Parks, there was no significant difference between washed and unwashed leaf SIRM of *F. benjamina* and *J. interrigima*.

### **Washed effect on leaf SIRM**

Results showed leaf SIRM erosion varied strongly between species and between leaf age and habitat (Fig. 5). The fact that the largest losses were obtained at roadsides makes sense, because this habitat is the most polluted compared to Parks, as we explained in the section above. At Main roads, washed leaf SIRM was statistically lower than unwashed leaves; erosion rates were generally over 70%. The SIRM detected after washing leaves is derived from cuticular encapsulation of surface-deposited particles as already demonstrated by Kardel et al. (2011), Dzierżanowski et al. (2011), Lehndorff et al. (2006), Terzaghi et al. (2013) and Hofman et al. (2014b). Indeed, the particulate material responsible for the magnetic signal is not found only on the leaves' surface but rather is incorporated into their structure through the stomata cavities or their cuticle waxy protective layer (Sagnotti and Winkler, 2012; Burkhardt and Pariyar, 2014; Przybysz et al., 2014). Dzierżanowski et al. (2011) and Terzaghi et al. (2013) showed that cuticular encapsulation occurs mainly for small (<10 µm) particles and is negligible for particles larger than 10.6 µm.

With 20% leaf SIRM encapsulated in mature leaves and 32% in young leaves, *F. benjamina* (waxy) seems to be most particle encapsulated than other two species tested, though hairy. The presence of hair on leaves could constitute a sort of particle barrier preventing particles to penetrate leaves cuticle. On such surfaces, the contact area between a particle and the underlying leaf surface is reduced (Wang et al., 2013). Since the particles are trapped in hair network were therefore easily washed off. The high encapsulation in *F. benjamina* leaves may suggest an accumulation of PM during wax formation as already demonstrated by Dzierżanowski et al. (2011) on *Acer campestre*, *Physocarpus opulifolius* and *Tilia cordata*. In our study, SIRM encapsulated by the young leaves of *F. benjamina* was 32 % higher than which encapsulated by mature leaves (Fig. 2) confirming that the encapsulation of magnetic particles into the leaf tissue mainly occurs during the growth phase (compared to mature) probably due a higher wax regeneration or formation activity during this phase (Mitchell et al., 2010; Kardel et al., 2011). Nevertheless, as we did not quantify wax layer amounts, we are not able to attribute the observed SIRM variation of the washed leaves to the thickness of the epicuticular wax layer. Also, not only the amount of waxes is important for trapping particles, but chemical composition and structures of wax layer which are a species- specific traits are also essential (Terzaghi et al., 2013).

### **Drop contact angle and drop asymmetry**

The lowest mean DCA for adaxial (71.0°) and abaxial (65.9°) leaf surface were observed with *B. prionitis* at roadsides and Parks (ANOVA Tukey test). The highest DCA were found on *F. benjamina* adaxial and abaxial leaf surface in both investigated habitat. On adaxial surface, these angles vary from 80.9° - 83.4° in Main roads and 63.2° - 65.7° in Parks. On abaxial surface, DCA were ranged from 83.2° - 84.6° in Main Roads and 65.6° - 76.5° in Parks. Any significant differences (t test;  $p > 0.05$ ) were obtained between mean DCA of these two species for adaxial and abaxial leaf surface considered. At the intraspecific level, DCA at roadsides were always higher than those obtained in Parks. Any significant change was founded in adaxial and abaxial surface DCA at Main roads. It is the same trend in Parks for *B. prionitis* and *J. interrigima*. However, in this habitat, DCA on the abaxial surface is greater than on adaxial surface. The highest values of Drop Asymmetry (DA) were observed at the adaxial and abaxial leaf surface of *B. prionitis*: 17.2 and 26.0 respectively for adaxial and abaxial surface on Main roads and 7.6 and 18.5 in Parks. Lower DA values were obtained on both waxy surface of *F. benjamina* with value vary between 7.6-11.2 at Main roads and 10.8-16.6 in Parks. Habitat significantly influenced the adaxial DA of *B. prionitis* and the abaxial DA of *J. interrigima* (test t;  $p < 0.05$ ). Any habitat influence was observed on the both surface of *F. benjamina* leaves and any significant differences (t test;  $p > 0.05$ ) were also obtained between mean adaxial and abaxial DA for all species in habitats.

### **Relationship between leaf SIRM, SIRM encapsulated and DCA and DA**

For the three investigated species, a significantly positive correlation was found between Drop contact angles on adaxial and abaxial surfaces and leaf SIRM (Fig. 6, A and B) and leaf SIRM encapsulated (Fig. 6A and B). Pearson's correlation coefficients were  $r = 0.60$  and  $r = 0.43$  respectively for adaxial (Fig. 6A,  $p = 0.002$ ) and abaxial (Fig. 6B;  $p = 0.036$ ) leaf SIRM. For leaf SIRM encapsulated,  $r = 0.53$  and  $r = 0.37$  respectively on adaxial (Fig. 6A,  $p = 0.007$ ) and abaxial (Fig. 6B;  $p = 0.070$ ) surfaces. No significant correlations were founded between leaf SIRM or leaf SIRM encapsulated and the drop asymmetry (Fig. 6).



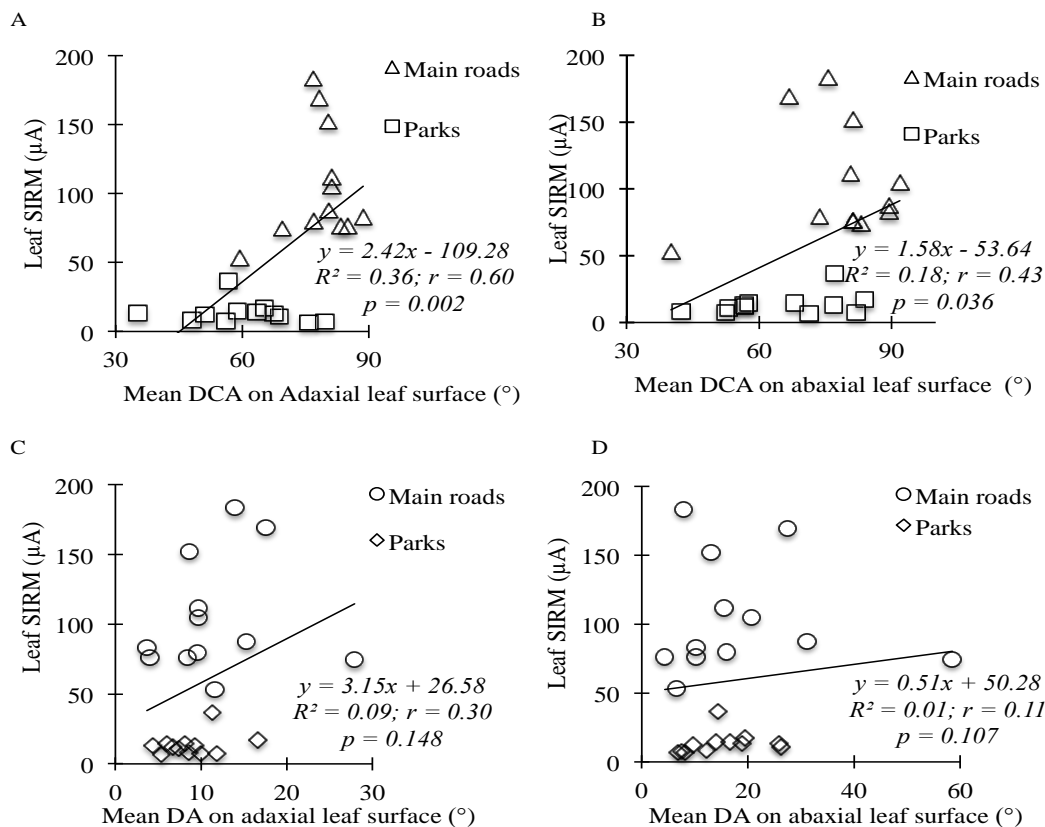


Fig. 6. Correlation between total leaf SIRM and mean Drop Contact Angle (DCA) (A and B) and Drop Asymmetry (DA  $\times 10^2$ ) (C and D) on adaxial and abaxial leaf surfaces.

## Leaf wettability

### Drop contact angle between species

In this study, drop contact angle varied between species, habitat and between the adaxial and abaxial surface of a leaf (Table 3). DCA were higher for *F. benjamina* (waxy) and *J. interrigima* (most complex surface in term of hair and vein densities) than on *B. prionitis*. According to Kardel et al. (2011), a species with a waxy, hydrophobic leaf surface, expressed by a large drop contact angle or small leaf wettability, is unable to accumulate as many magnetic particles on its surface as species with a hydrophilic leaf surface (large leaf wettability). However, no significant differences were found between the species leaf SIRM even if arithmetically, *F. benjamina* and *J. interrigima* leaf SIRM were generally higher than *B. prionitis* leaf SIRM on roadsides. The absence of significant difference is due to the inhomogeneity of studied road characteristics resulting in a large standard deviation (Table 3). For example, traffic density is higher on the Lagoon Boulevard than on North Highway during rush hour. Traffic jams, and thus the high production of combustion residues from engine vehicle, on Lagoon Boulevard is more important than one North Highway. In addition, North Highway is wider than Lagoon Boulevard. According to the criteria synthesized by Aryal and Neuner (2010) all study species were highly-wettable because DCA were greater than  $40^{\circ}$  and less than  $90^{\circ}$  (Table 3) confirming that most leaves from non-freezing tropical and subtropical origins were highly wettable while temperate leaves were non-wettable and subalpine and alpine leaves were highly non-wettable (Aryal and Neuner, 2010). However, trichome leaf of *J. interrigima* and waxy leaf of *F. benjamina* were more wettable than *B. prionitis* leaf. Previous studies showed that waxy cuticles and outgrowths on leaf surface, such as trichomes, increase the wettability or hydrophobicity of leaf surface and facilitate the removal of polluted particles from leaf surface (Mundo et al., 1995; Wagner et al., 2003; Holder, 2012).

### Effect of habitat leaf wettability

Drop contact angle and thus leaf wettability was higher in Main roads than in Parks for all species and for adaxial and abaxial surfaces (Table 4). The higher leaf wettability 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. (2012a) observations on scanning electron microscope images of *Alnus glutinosa*, *Acer pseudoplatanus*, *Betula pendula*, *Quercus robur* and *Sambucus nigra*. The potential for cuticular perturbations in unsuitable habitats is high, due to different interactions occurring at the leaf surface, such as gas exchange and invasion

by pathogens and insects. Many studies indeed revealed that the epicuticular wax is affected by gaseous pollutants, through dry and wet deposition (Baker and Hunt, 1986; Cape et al., 1995; Barnes and Brown, 1990; Holder, 2012). Nevertheless, the degree to which habitat type affected leaf wettability depended on tree species and time of sampling.

Table 3. Mean Drop Contact Angle ( $^{\circ} \pm$  standard deviation) at the adaxial and abaxial leaf surface of the considered tree species and habitat (LUC). Number of samples per species = 32. A and B in parenthesis above DCA indicate significant differences between species for adaxial and abaxial DCA for considered habitat (Student t test). ns = non significant difference between the adaxial and abaxial surfaces for species and habitat considered. Lowercase letters above the averages show differences between DCA of three investigated species for considered leaf surface (ANONA, Tukey-HSD test). pValue represent results of DCA comparison between Main roads and Parks. Significant differences if  $p \leq 0.05$ .

Species		Main roads	Parks	pValue
<i>Barleria prionitis</i>	Adaxial	71.0 $\pm$ 12.9 (ns; b)	52.7 $\pm$ 14.4 (ns; b)	0.001
	Abaxial	65.9 $\pm$ 24.2 (ns; b)	52.4 $\pm$ 13.6 (ns; b)	0.001
<i>Ficus benjamina</i>	Adaxial	83.4 $\pm$ 13.1 (ns; a)	63.2 $\pm$ 10.6 (B; a)	0.001
	Abaxial	83.2 $\pm$ 10.8 (ns; a)	76.5 $\pm$ 19.4 (A; a)	0.001
<i>Jatropha interrigima</i>	Adaxial	80.9 $\pm$ 13.7 (ns; a)	65.9 $\pm$ 15.1 (ns; a)	0.001
	Abaxial	84.6 $\pm$ 15.9 (ns; a)	65.6 $\pm$ 16.1 (ns; a)	0.001

Table 4. Mean drop asymmetry (DA  $\pm$  standard deviation) on the adaxial and abaxial leaf surface of the considered species in Main roads and Parks. Letters in parenthesis above DA are ranking from ANOVA of DA means comparison with Tukey-HSD test. DA with the same letter above was not statistically different. DA between Main roads and Parks was compared using a student t-test and significant differences are shown in bold. Significant differences if  $p \leq 0.05$ .

Species		MR	Parks	p
<i>Barleria prionitis</i>	Adaxial	17.2 $\pm$ 12.1 (a)	7.6 $\pm$ 4.7 (a)	<b>0.006</b>
	Abaxial	26.0 $\pm$ 21.3 (a)	18.5 $\pm$ 13.8 (a)	0.246
<i>Ficus benjamina</i>	Adaxial	7.6 $\pm$ 4.9 (b)	10.8 $\pm$ 8.3 (b)	0.180
	Abaxial	11.2 $\pm$ 9.4 (b)	16.6 $\pm$ 10.2 (b)	0.099
<i>Jatropha interrigima</i>	Adaxial	10.7 $\pm$ 10.1 (ab)	8.9 $\pm$ 14.0 (ab)	0.440
	Abaxial	17.5 $\pm$ 14.0 (ab)	8.0 $\pm$ 5.6 (ab)	<b>0.018</b>

#### Relationship between leaf SIRM, leaf SIRM encapsulated and leaf wettability

For *B.a prionitis*, *F. benjamina* and *J. interrigima*, a significantly positive correlation was found between Drop contact angles on adaxial and abaxial surfaces and leaf SIRM and leaf SIRM encapsulated. This result could be due to the high-wettability of study leaves. Indeed, stored water on leaf surfaces increases the potential for plant pathogens and the potential for leaf damage from pollutant particles (Bradley et al., 2003; Sase et al., 2008). This leaf property induces a higher capacity of investigation leaf species to encapsulate more pollutants as the DCA increases, as was the case in this study. If the leaves were not wettable, encapsulation particles would be difficult or impossible (Koch et al., 2009) because the contact area between a particle and the underlying leaf surface is considerably reduced. As a consequence, the physical adhesion forces between the particle and the surface will be reduced owing to leaf surface free energy characteristics (Wang et al., 2013). If water rolls over such a hydrophobic surface, contaminating particles are picked up by water droplets, or they adhere to the surfaces of water droplets, and are then removed with the droplets as they roll off the leaves (Wang et al., 2013). For wettable or high-wettable leaf surfaces with low contact angles ( $< 90^{\circ}$ ), the much larger contact area may lead to much stronger force between particles and leaf surfaces. Accordingly, mature leaves of *B. prionitis*, *F. benjamina* and *J. interrigima*, highly-wettable surfaces, which promote the accumulation and deposition of particles on leaf surfaces, making them appropriate species for air quality biomonitoring in humid environment such as African tropics.

#### 4. Perspectives for future collaboration between units (1 page)

The work carried out during the postdoctoral position helped to highlight the capacity of tropical plants to be used as bio-indicators of air pollution. This study also showed the importance of meteorological parameters on the deposition and encapsulation of particulate matter in leaves. Further work should be undertaken to

determine the particle deposition processes in or onto leaves. We will identify the different proteins involved in PM sequestration process. In this perspective, collaboration will be established and strengthened with the Research Group on Environmental Ecology & Microbiology and specifically in the Laboratory of Environmental and Urban Ecology.

In partnership with the Laboratory of Environmental and Urban Ecology, we plan to measure the spatial distribution of particulate matter in several West African capitals using common plants.

The Laboratory of Environmental and Urban Ecology will also be involved in the training of two PhD students of my home university who work on the biomonitoring of urban air quality from anatomical and spectral characteristics of ornamental species in the city of Abidjan. As part of this work, leaf samples will be analyzed in the Laboratory of Environmental and Urban Ecology of the University of Antwerp. Prof. Roeland Samson will participate to both thesis committees.

## **5. Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...**

During this postdoctoral position, the following articles were published:

- Assessing atmospheric particulate matter distribution based on Saturation Isothermal Remanent Magnetization of herbaceous and tree leaves in a tropical urban environment. *Science of the Total Environment*, 470–471: 975–982.
- Les caractéristiques des stomates des feuilles de *Ficus benjamina* L. comme bioindicateurs potentiels de la qualité de l'air dans la ville d'Abidjan (Côte d'Ivoire). *Journal of Applied Biosciences*, 78: 6675 - 6684.
- Detoxifying hydrogen peroxide enzymes activity in two plant species exposed to air pollution in Abidjan city (Côte d'Ivoire). *International Journal of Plant, Animal and Environmental Sciences* 5(1).
- Distribution spatiale intra-urbaine des particules fines : monitoring par l'Aimantation Rémanente Isotherme à Saturation des feuilles (SIRM) en milieu tropical urbain (Côte d'Ivoire). *Journal of Applied Biosciences*, 81: 7186 - 7197.
- Biomonitoring of urban pollution by leaves spectrals and anatomicals characteristics of *Ficus polita* Vahl. *International Journal of Innovation and Applied Studies*, 8: 861-870.

Two articles being submitted:

- Active and passive air quality biomonitoring in the tropics: intra-urban and seasonal variation in atmospheric particels estimated by leaf saturated isothermal remanent magnetisation of *Ficus benjamina* L.
- Leaf age, wettability and wash-off effect on leaf SIRM of tropical species.

## **6. Skills/Added value transferred to home institution abroad (1/2 page)**

Through this postdoctoral position, we strengthened our expertise in plant responses to environmental stress. Specifically, techniques and analysis of plant reactions to air pollution have been learned and developed. This knowledge has been incorporated into the training we provide in Master in my home university.

We have also created new contacts with researchers from the University of Antwerp and strengthen existing collaborations with our former partners. These collaborations allow the expedition to plant samples from Ivory Coast to the University of Antwerp for analysis by technician of the Laboratory of Environmental and Urban Ecology.

Through this training, a series of scientific papers were published; these publications will participate in a better visibility of my home university and my research team at national and international level.