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Anatomical variability of the trunk wood and root tissues of *Rhizophora racemosa* (G. Mey) and *Avicennia nitida* (Jacq.) and bio-accumulation of heavy metals in mangrove trees

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ABSTRACT

The aim of this study was to investigate the anatomical structure of the trunk wood and the roots of *A. nitida* and *R. racemosa*, two mangrove trees from Gabon. The anatomical differences between the trunks and the roots were used to understand their bio-remediating differences through heavy metals. It was found that the roots of *A. nitida* were less abundant in cells number/mm² than its trunk which exhibited the largest cells diameter. The roots and the trunk of *R. racemosa* didn't exhibit significant difference between their cells number. Nevertheless, the trunk of that mangrove tree displayed the largest cells diameter and somewhat traumatic channels. Any interspecies variability was found between their trunk vessels diameter. However, a significant difference was found regarding their vessels number/mm², the trunk of *A. nitida* was richer in vessels compared to *R. racemosa* one. The roots of the latter were more abundant in vessels and they displayed the largest cells diameter than *A. nitida*. Broad parenchyma bands and sclerous cells lacked within *R. racemosa* while they were richer in *A. nitida* roots and trunk. The occurrence of those anatomical structures which storage substances was thought to act in the highest heavy metals bio-remediation of Avicenniaceae than Rhizophoraceae.

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Keywords: Mangrove, parenchyma bands, sclerous cells, bioremediation, heavy metals.

INTRODUCTION

The wastewater treatment is one of the major environmental issues. Its amount increases with increasing population and industrial activities (Herteman, 2010) near rivers or waterways; which provokes

significant environment pollution (Kamal et al., 2010), insalubrity and medical risks. The wastewaters from these uncontrolled activities are discharged in the sea, releasing like this toxic organic products and serious mineral pollutants including heavy metals.

National strategies for efficient depollution of seas or waterways are a challenge for underdeveloped countries where mangrove is growing interest because it should give response to sea level or climate changes (Blasco et al., 1996). On the other hand, mangrove is a natural stock of active molecules; stem bark from mangrove plants like *Xylocarpus granatus* which contain tannins displaying antiradical and antioxidant activity (Chacha, 2010) and the roots of marine mangrove such as *Ceriops tagal* within chemotherapeutic drugs have been identified (Chacha, 2011) were found in that ecosystem indeed. In addition, mangrove ecosystem was described as potential for carbon stock (Donato et al., 2011; Jones et al., 2014).

However, financial stakes and pollutions risks connected to human and goods transport in the mangrove ecosystem (Ajonina et al., 2013, 2015) as well as the decrease of socio-economic profits for rural communities as consequence of mangrove degradation by drought and hydraulic dam has been pointed out (Tendeng et al., 2016). Nevertheless, the ability of mangrove for metals accumulation was previously discussed (MacFarlane et al., 2003; Ramos e Silva et al., 2006; Almahasheer et al., 2014), and about 400 species including mangrove trees were identified as potential factories for toxic heavy metals storage from wastewaters (Jemal and Ghorbal, 2002). *R. racemosa* and *A. nitida* belonging to the family of Rhizophoraceae and Avicenniaceae respectively, have roots system with high capability for filtering wastewater nutrients and decrease hazardous metals in the costal seas or mangrove soils (Wong et al., 1995). Previous studies have found that *Avicennia* wood concentrated more metals than *Rhizophora* (Marchand, 2003) one. Furthermore, *Avicennia marina* roots concentrated more heavy metals compared to its air parts (MacFarlane and Burchett, 2002). On the other hand, the high capacity of trunk woody tissues to allocate metals was described to be

an alternative for the recovery excess metallic elements from degraded soils (Almeida et al., 2007).

The trend of Rhizophoraceae and Avicenniaceae to concentrate heavy metals was also described (Kamaruzzaman et al., 2009; Varz et al., 2012). But, the anatomical variability between the trunk wood and the roots of *R. racemosa* and *A. nitida* regarding parenchyma as storages for substances (Ndoutoume and Njankouo, 2012) or other characteristics like sclerous cells, vessels number or diameter has received little attention.

The aim of this study was to investigate the anatomical variability between the trunk and the roots of *R. racemosa* and *A. nitida* concerning the vessels diameter and number as well as the sclerous cells width and number in the cross section of the wood, in order to understand the differences observed in the storage of heavy metals within those mangrove wood tissues.

MATERIALS AND METHODS

Chemicals

Blue of methylene, ethanol (90%), and concentrated xylene (98.5%) were purchased from Aldrich and used without any purification. Javel La Croix (8° chl eq.2.4% c.a.) and distilled water were obtained from MEDILAB.

Area of study

The samples were collected in the mangrove of Ayeme Maritime, located approximately at 40 km from Libreville in the province of Estuaire. This zone is characterized by a low tide in the morning; high tide in afternoon and the mangrove is consequently invaded by water.

Wood sampling

Collection of the wood samples

Four trees of each mangrove wood were randomly harvest on the morning in Ayeme Maritime in the month of March 2015 at high tide. The trees were randomly chosen

following their vigorous aspect, good conformity of stem and superior or equal to the minimum 16-18 cm of diameter. *R. racemosa* was in the mud, at the edge of the river; that mangrove tree was sampled between 940' 31.22 " for longitude and 017' 17.41 " for Northern latitude. *Avicennia nitida* was on the same area but in the opposed side, in a totally dry medium located between 940' 26.57 " for longitude and 017' 07.15 " for Northern latitude. For each tree tank, the geographic data were taken with a Garmin map 60 csx GPS mark. Then, small wood discs of 10 cm of thickness were collected at 1.30 m of height from each tree with a chainsaw STILL 066 trade mark.

Samples were placed on shelves and air dried to avoid any deterioration by the light or biological agents. Then, wood discs were polished and three types of sticks were sawn in the radial direction and in North exposition: the first according to the East-West direction, the second following the Nord-South direction and the third in the oblique direction at more or less +45 °C of the first setting stick.

Samples cutting for analysis

The polished wood discs were cut into 128 test-tubes of 10 cm x 10 cm x 10 cm in the radial, tangential and longitudinal (R x T x L) direction according to Normand (1982) procedure.

Anatomical structure analysis by microscopy

The anatomical structure of *R. racemosa* and *A. nitida* was performed by an adaptation of a published procedure (Berrichi, 2009) as follows: the test-tubes were softened in hot distilled water for 24 and 18 hours, respectively. The heating was stopped when the sticks fell down to the vessel bottom. Then, the softened wood sticks were cut with a semi-automatic microtome "TBS 2500" in the transversal direction to obtain samples for which thickness was between 20-30 µm. The slant between the microtome knife and the section surface was about +8 °.

The thin wood test-tubes were soaked in water and scalded in a Javel: water (10:90) mixture until fading the samples. The faded wood stickers were soaked once in distilled water to eliminate any traces of Javel. The rinsed wood stickers were then coloured in blue of methylene and successively dehydrated by soaking in ethanol solution and concentrated xylene. The colorful cuts were then deposited on a blade carrying an object, and a cover-object was carefully set down above the cuts to avoid bubbles of air between the blade and the gill. The blades were dried to eliminate any traces of chemicals used for dehydration.

The anatomical analysis was performed with an optic microscope "Motic 2.0" to a magnification of forty x. The observation was facilitated by a Ken-A-Vision camera connected to a computer. The software Vision 4 allowed to take pictures and to measure the constituent of the woody plan. The following anatomical characters were measured: the number of vessels or sclerous cells by mm² and the lumen vessels diameter in transverse section.

Data analysis

All the data were analysis using the one-way analysis test of variance (ANOVA) followed by the Fischer's LSD (last significant difference) test at $\alpha=0.05$ level of significance with Rr643.0.2 software.

RESULTS

Variability within *R. racemosa* in transverse section

The anatomical structure of *R. racemosa* trunk is depicted in Figures 1 and 2, and the roots can be observed in Figure 3. Some parts of the trunk exhibited larges and hallow pellets looking like traumatic channels located along the broad and dark bands having the appearance of growth ring limits. However, these traumatic channels were not noticeable in the root tissues of *R. racemosa* (Figure 3). The mean vessels number/mm² of the trunk and the roots didn't exhibit

significant difference ($P>0.05$), while significantly difference ($P=0.01$) was found between their mean vessels diameter (Table 1). No evidence of sclerous cells was found within the trunk and the root tissues from *R. racemosa* mangrove tree (Table 1).

Variability within *A. nitida* in transverse section

Figures 4 and 5 show clearly that *A. nitida* trunk and roots contained sclerous cells along their large parenchyma band limits. Furthermore, that mangrove tree has pointed out a within specie variability concerning the anatomical parameters as corroborated by the strong significant difference ($P<0.0001$) obtained from Table 1. The trunk was not only richer in vessels number/mm² than the roots, it exhibited the largest vessels diameter also. Nevertheless, the trunk of *A. nitida* was less abundant in sclerous cells number/mm² compared to the roots, even if that aerial part

of *A. nitida* wood displayed the highest sclerous cells diameter with regard to its roots.

Interspecies variability between *R. racemosa* and *A. nitida* in transverse section

The data collected in Table 1 pointed out that the trunk of *A. nitida* was the most abundant in vessels number/mm² with a high level of significant ($P<0.0001$) regarding *R. racemosa* one. Nevertheless, *R. racemosa* roots were the richest in vessels number/mm² ($P<0.0001$) than *A. nitida*. However, no significant difference ($P=0.43$) was found between the mean vessels diameter of the trunks from the two mangrove trees, while the vessels diameter of *R. racemosa* roots were higher ($P<0.0001$) with regard to those from *A. nitida*. Furthermore, the trunk and the roots of *R. racemosa* did not exhibit any sclerous cells compared to *A. nitida* trunk and roots within they were in a higher extend (Table 1).

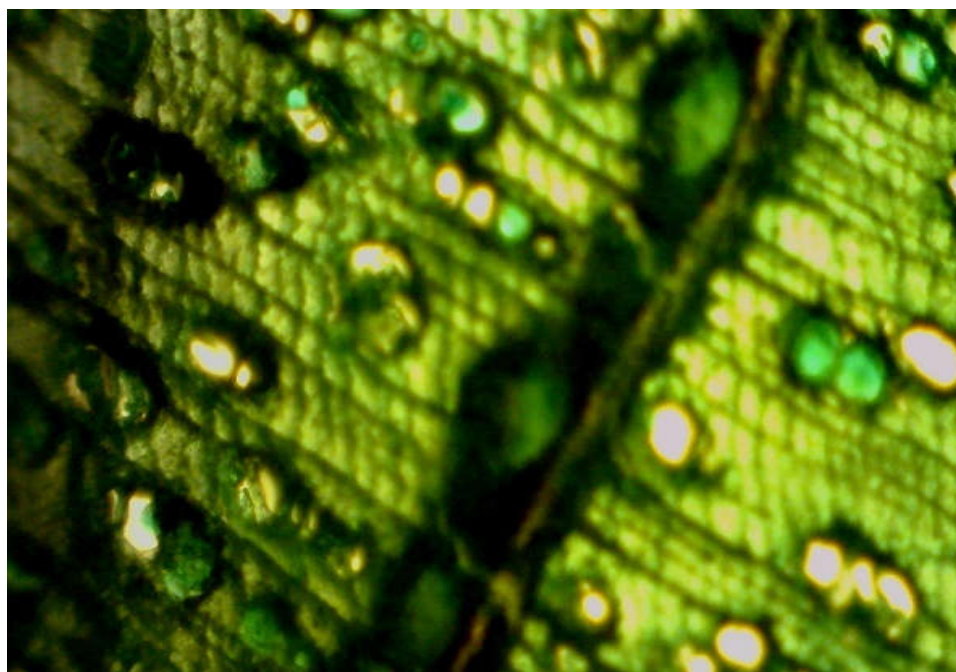


Figure 1: Trunk of *R. racemosa* in transverse section with traumatic channels along the growth rings there was no evidence of parenchyma bands (GX40).

Table 1: Vessels and sclerous cells number/mm², vessels and sclerous cells diameter mean in the transverse section of the trunk and the roots of *R. racemosa* and *A. nitida*.

	Vessels number/mm ²		Vessels diameter (μm)		Sclerous cells number/ mm ²		Sclerous cells diameter (μm)	
	<i>R. racemosa</i>	<i>A. nitida</i>	<i>R. racemosa</i>	<i>A. nitida</i>	<i>R. racemosa</i>	<i>A. nitida</i>	<i>R. racemosa</i>	<i>A. nitida</i>
Trunk	10.89±5.56 ^a	17.22±6.54 ^b	62.12±7.78 ^d	63.72±8.28 ^d	0	1.19±0.38 ^g	-	225.05±51.46 ⁱ
Roots	12.09±4.8 ^a	5.24± 3.05 ^c	57.21±7.73 ^e	47.67±11.09 ^f	0	2.11±0.63 ^h	-	153.89±37.98 ^j

Means with the same letters are not statistically different at α=0.05 level of significant.

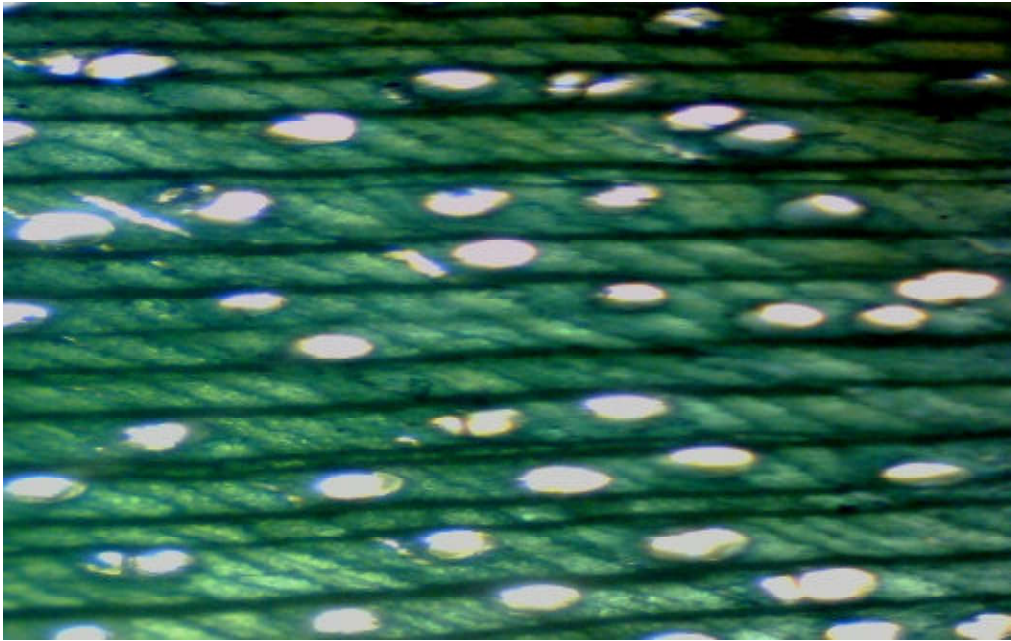


Figure 2: Trunk of *R. racemosa* without traumatic channels, sclerous cells in transverse section or broad parenchyma bands. Evidence of scalariform vessels (G 40x).

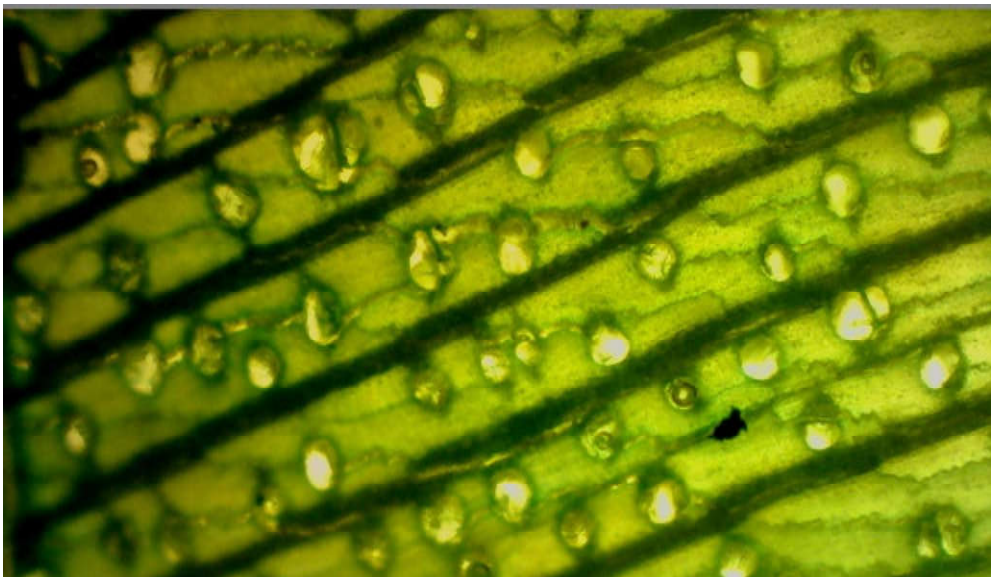


Figure 3: Roots of *R. racemosa* in transverse section without sclerous cells or parenchyma broad bands. Evidence of scalariform vessels (GX40).

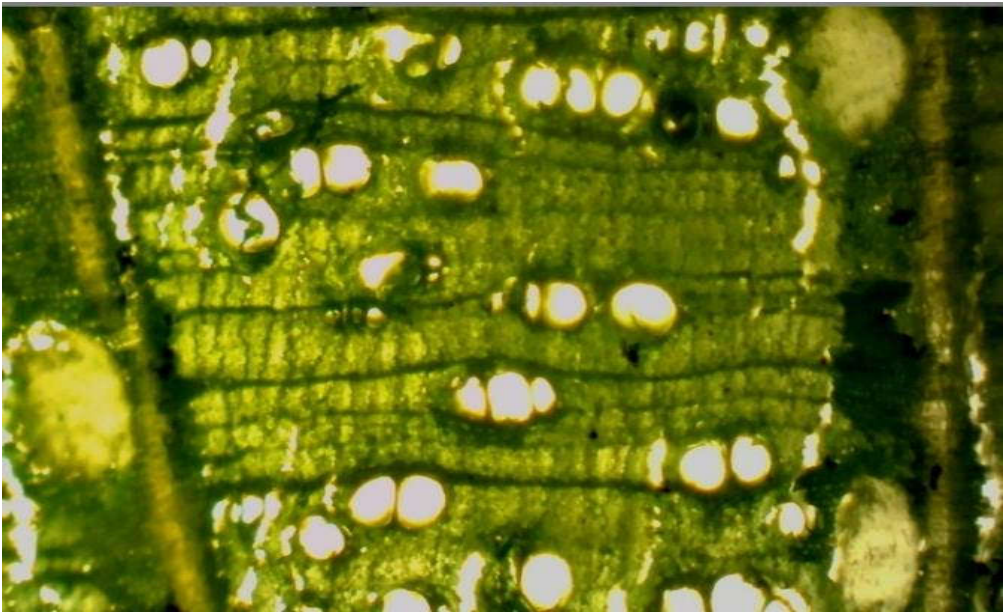


Figure 4: Trunk of *A. nitida* in transverse section with evidence of sclerous cells and parenchyma broad bands and simple perforations (GX40).

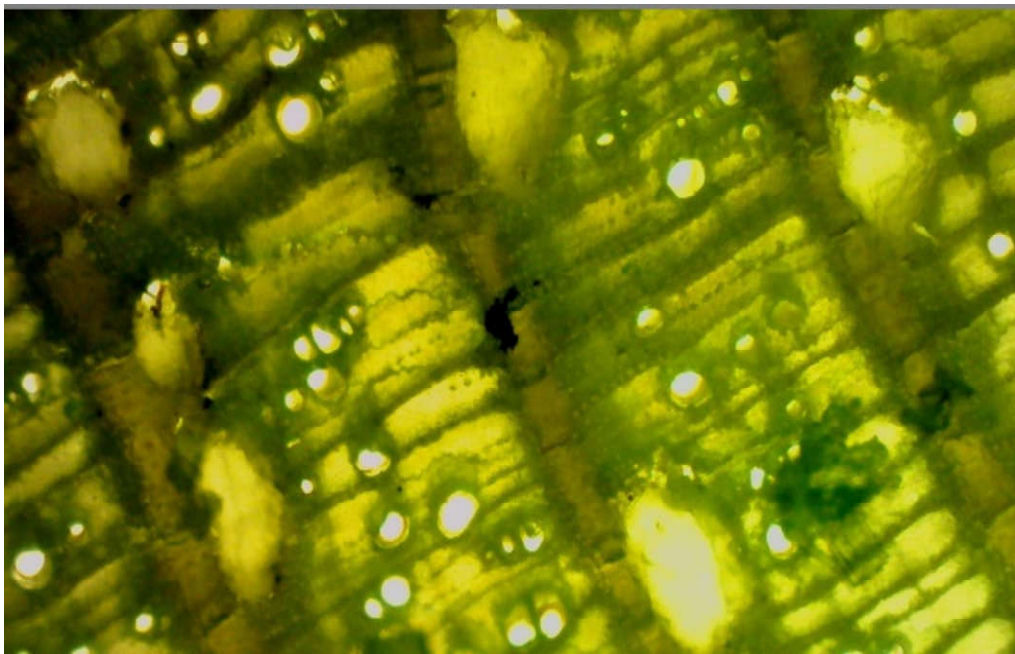


Figure 5: Roots of *A. nitida* in transverse section with evidence of sclerous cells, high content of parenchyma broad bands and vessels with small diameter (GX40).

DISCUSSION

The roots of *R. racemosa* didn't point out traumatic channels neither growth ring limits. But both of those anatomical structures were found within the trunk tissues of that mangrove tree. Their occurrence was in agreement with that previously found by Beeckman et al. (2007) who showed that within mangrove trees such as *Rhizophora mucronata*, clear wood bands were formed during the dry season while a dark wood shape was noticeable in the rainy season. So, assuming that species belonging to the same family possess common properties (Ndoutoume and Njankou, 2012), the alternated bands observed within *R. racemosa* trunk should be assigned to growth rings (Figure 1). Nevertheless, the closed channels located along the growth rings remained controversial and were discussed for a long time. According to Boureau (1956), species attacked by pathologies should produce traumatic channels in order to face the medium pressures; and those traumatic channels should be distinct from the normal channels by their position at the end of growth rings in cross and tangential section. Therefore, these channels of big size and missing constancy in all the trunk of *R. racemosa* should result from pathological origin. Thought that the specific environmental conditions inside with the mangrove tree grow include decay processes which should affect the organic matter (Marchand, 2003; Marchand et al., 2006) of that specific ecosystem. Regarding the vessels numbers/mm², any within specie variability was found for *R. racemosa* (Table 1). The statistical analysis didn't exhibit significant difference ($P > 0.05$) between the trunk and the roots of *R. racemosa* indeed; whereas a significant difference was obtained for their vessels diameter ($P = 0.01$). The trunk of *R. racemosa* displayed the highest vessels diameter than the roots ones (Table 1) and that should explain the better water conduction of the scalariform perforating slabs of

Rhizophora sp. planting woods, as observed by Balde (2010).

However, a strong significant difference ($P < 0.0001$) was found between the vessels number/mm², and the same trend was observed concerning the vessels diameter means of the trunk and the roots of *A. nitida* which exhibited a very significant difference ($P < 0.0001$) too. It was noteworthy that the trunk displayed not only the highest vessels number content, but also the largest vessels diameter than the roots (Table 1). That intra-specie variability within the wood of *A. nitida* was in agreement with that published for a specie like *Picea mariana* (Mill.) whose these anatomical characters varied from the bottom towards the high parts of the wood (Braido Dos Santos, 2014). Furthermore, the data collected in Table 1 corroborated the intra-specie variability between the trunk and the roots of *A. nitida*. The sclerous cells number/mm² displayed a significant difference ($P < 0.0001$) between the two parts of *A. nitida* wood; and the root tissues were richer in sclerous cells number than the trunk ones. On the other hand, the sclerous cells diameter of the trunk and the roots displayed a strong significant difference ($P < 0.0001$), and the sclerous cells diameter were the highest inside the trunk tissues. The occurrence of those sclerous cells located at the limits of the broad parenchyma bands were previously described for *Avicennia marina* (Normand, 1972), which suggests that sclerous cells and parenchyma bands are typical of Avicenniaceae. Those sclerous cells contain sclerenchyma composed of dead cells, and the sclerous cell walls are thickened with lignin conferring hardness and rigidity to *A. nitida* which can easily face to environmental constraints like tide movements (Détienne et al., 1988).

The data depicted in Table 1 have pointed out an inter-species variability for the vessels number/mm² as well as the sclerous cells number/mm² and diameter which varied very significantly ($P < 0.0001$) between the trunks and the roots of *R. racemosa* and *A.*

nitida (Table 1). A strong inter-species variability was found also between the vessels diameter ($P < 0.0001$) of the roots of the two mangrove woods. However, any significant difference was found ($P > 0.05$) between the vessels diameter of the trunk tissues from *R. racemosa* and *A. nitida*.

Although the accumulation and distribution of heavy metals in plants depends on factors such as plant species, element species, chemical and bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Cheng, 2003); the general tendency of the roots for concentrating more heavy metals than the other parts of Rhizophoraceae and Avicenniaceae mangrove trees was fully discussed (Kamaruzzaman et al., 2011; Mejias et al., 2013; Erakhrumen, 2014; Rashidi et al., 2015). The strong ability of *Rhizophora apiculata* roots to concentrate more Cu and Pb than the leaves and the bark was found by Kamaruzzaman et al. (2009). Despite the significant ($P < 0.05$) low vessels diameter of the roots ($57.21 \pm 7.73 \mu\text{m}$) than the trunk ($62.12 \pm 7.78 \mu\text{m}$) tissues we found in our study, the trend of *R. racemosa* roots for having a high level of bio-accumulation of heavy metals such as Fe ($402.43 \pm 0.31 \text{ mg/kg}$), Ni ($40.08 \pm 0.09 \text{ mg/kg}$), Cd ($24.39 \pm 0.22 \text{ mg/kg}$) than the stem wood was described by Erakhrumen (2014). In addition, the results obtained by Zheng et al. (1997) displayed the strongest bio-remediating capability of the roots of *Rhizophora stylosa* to retain more heavy metals such as Ni ($208.4 \mu\text{g m}^{-2} \text{ yr}^{-1}$), Cd ($56 \mu\text{g m}^{-2} \text{ yr}^{-1}$), Pb ($229.8 \mu\text{g m}^{-2} \text{ yr}^{-1}$) compared to the trunk wood which exhibited concentrations as follows: Ni ($96.9 \mu\text{g m}^{-2} \text{ yr}^{-1}$), Cd ($2.6 \mu\text{g m}^{-2} \text{ yr}^{-1}$), Pb ($51.6 \mu\text{g m}^{-2} \text{ yr}^{-1}$). Those results suggest that the vessels diameter should not be a major factor for metal concentration within *Rhizophora*.

On the other hand, the highest bio-concentration of heavy metals in Avicenniaceae through the roots or even via their stems and leaves for their accumulation in the plant tissues which take up elements

selectively was investigated (Defew et al., 2005; Akshayya et al., 2007; Ramos e Silva et al., 2006; Kamaruzzaman et al., 2009; Almasheer et al., 2013). It was noticeable that despite the highest vessels number/ mm^2 and vessels diameter of the trunk tissues we obtained for *A. nitida*; previous works have found that the roots of that mangrove tree concentrated more heavy metals than the trunk (MacFarlane et al., 2003). That was in close agreement with the result displayed above by *R. racemosa*. Thus, the vessels number and the vessels diameter didn't look like major factors for metal concentration within the trunk and the roots of *A. nitida* also.

Furthermore, the microscopic analysis of tissues from the studied mangrove trees showed a high content of the broad parenchyma bands within the roots than the trunk tissues of *A. nitida* (Figure 5). Thought that parenchymas are storages for substances (Ndoutoume and Njankou, 2012), their high content within the roots of Avicenniaceae should lead them to concentrate more organic wastes and heavy metals than the trunk. That should support in some extent, the high heavy metals concentration previous authors have found inside the roots of *A. nitida* (MacFarlane et al., 2003; Kamaruzzaman et al., 2009; Almasheer et al., 2013).

The abundance of sclerous cells and broad parenchyma bands within the wood of *A. nitida* (Table 1) should explain the results obtained by Marchand (2003) who found the following metals concentration for the wood of *Avicennia*: Zn ($12.5 \mu\text{g/dry wt}$), Cu ($13.2 \mu\text{g/dry wt}$) and Cr ($26.5 \mu\text{g/dry wt}$) while the heavy metals content inside *Rhizophora* which was lacking sclerous cells and broad parenchyma bands was as follows: Zn ($00.00 \mu\text{g/dry wt}$), Cu ($00.00 \mu\text{g/dry wt}$) and Cr ($11.40 \mu\text{g/dry wt}$).

Conclusion

This study has pointed out anatomical differences between the trunk and the roots of *R. racemosa* and *A. nitida* from the mangrove trees of Gabon. It was demonstrated that the

trunk vessels number/mm² was the highest for *A. nitida*. However, the roots of *R. racemosa* were the richest in vessels number. But no significant difference was found between the trunk vessels diameter of the two mangrove woods, whereas the root vessels diameter was larger for *R. racemosa* than *A. nitida*. On the other hand, *R. racemosa* trunk exhibited few traumatic channels located along its growth rings; but they did not appear in all the trunk and they were lacking in its roots. Those traumatic channels cannot assume to be characteristic of Rhizophoraceae tissues. It was observed that the roots and the trunk of *A. nitida* displayed sclerous cells located along the broad parenchyma band limits. These anatomical structures which seemed to be characteristics of Avicenniaceae were not found within the trunk and the roots tissues of *R. racemosa* while they were strongly abundant in the trunk wood of *A. nitida* for which the root tissues exhibited the highest content of those anatomical structures.

However, the lack of studies on the anatomical variability between the trunk and the root tissues of those mangrove trees, and the very limited information regarding the correlation between the type of heavy metals and the storage cells render difficult further comparison and discussion. But the general trend for heavy metal concentration within the roots tissues of the studied mangrove species as well as the better capability of *A. nitida* tissues which were found to be richer in sclerous cells and broad parenchyma bands than *R. racemosa* will be further investigated in order to discriminate between the environmental conditions and the anatomical structure control on the bioremediating capability of *Rhizophora racemosa* and *Avicennia nitida* towards heavy metals accumulation.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

RST is the principal investigator of the subject. He acted as group leading of that research program which concerned the anatomical structure and the bio-accumulation of heavy metals within the wood tissues from the mangrove trees of Gabon. SBMI is a PhD student in wood sciences who collected all the wood samples and the GPS data. He made also the anatomical and the statistical analysis of the samples. PS and SI contributed actively to correct the final manuscript. NKM was a Master of Sciences student in wood sciences who participated to the anatomical studies and the microscopic calibration. NC contributed to the discussion dealing with the anatomical characters of the wood.

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