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Full Length Research Paper

Diversity of culturable endophytic fungi of *Hevea guianensis*: A latex producer native tree from the Brazilian Amazon

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Hevea guianensis **is a species of rubber tree native to the Amazon rainforest. This tree is highly exploited for latex extraction but is not cultivated. Therefore, few studies have investigated its microbiota. The aim of this study was to analyze the diversity of endophytic fungi in the leaves, stems and roots of *H. guianensis* trees from the Brazilian Amazon. A total of 92 fungi were isolated from different tissues of this plant species. These isolates were grouped into 28 operational taxonomic units (OTUs). The dominant phylum was Ascomycota (96.73%). The stem cortex showed the greatest fungal richness and diversity, although the frequency of isolates was highest in the leaves. The fungal isolates of leaves were highly heterogeneous than those of stem and roots. *Colletotrichum* was the most well-represented and abundant genera in the leaves; *Diaporthe* was the second most abundant genus in the leaves; *Penicillium* was the main genus obtained from the roots; the genera *Lasiodiplodia*, *Purpureocillium*, *Phyllosticta*, *Daldinia* and *Pseudofusicoccum* were recovered only from the leaves; whereas the genera *Trichoderma* and *Fusarium* were isolated from the stems and roots of *H. guianensis*. Thus, we describe the endophytic fungi of *H. guianensis* of great biotechnological interest, such as *Trichoderma*.**

Key words: Rubber tree, biodiversity, endophytic fungi.

INTRODUCTION

The Amazon rainforest is the largest biodiversity region on the planet, and the first description of genus *Hevea* and its species *Hevea guianensis* in 1775 by Fusee Aublet (Sethuraj and Mathew, 2012) was made in this

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forest. Later, other species were described, such as *Hevea brasiliensis* [(Willd. ex Adr. De Jess) Muell.-Arg] in 1824, *Hevea pauciflora* (Spruce ex Benth), *Hevea spruceana* (Benth) and *Hevea rigidifolia* [(Spruce ex Benth) Muell.-Arg] in 1854, *Hevea nitida* var. *toxicodendroides* (Mart. ex Muell.-Arg), *Hevea microphylla* (Ule), *Hevea camporum* (Ducke), *Hevea benthamiana* (Muell.-Arg) in 1962 and *Hevea camargocina* (Pires) in 1981 (Muller, 1865, 1874; Murca, 1981; Schultes, 1977, 1987; Sethuraj and Mathew, 2012).

Commercially acceptable latex and rubber is obtained from *H. brasiliensis*, *H. benthamiana* and *H. guianensis*. However, *H. guianensis* is exploited for latex extractivism but is not cultivated, unlike *H. brasiliensis*, a closely related species, which is exploited, and extensively cultivated (Sethuraj and Mathew, 2012). Thus, few studies have attempted to obtain *H. guianensis* cultivars resistant to diseases for the production of better quality latex (Cardoso et al., 2014) or to describe its endophytic community, which is capable of producing metabolites of biotechnological interest and with potential applications for the biological control of phytopathogens (Gazis and Chaverri, 2010; Rocha et al., 2011).

Hevea species seem to have evolved in the Amazon rainforest under high and constant humidity, which favours the colonization of pathogens; thus, the development of some degree of resistance is considered essential for plant survival (Gasparotto and Rezende, 2012). Natural rubber production in Brazil has been affected for decades by the high incidence of pathogens, including *Pseudocercospora ulei* (South American leaf blight - SALB) (Hora-Júnior et al., 2014), *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* (anthracnose), *Oidium hevea* (powdery mildew), and *Phytophthora* species (striated canker or panel canker) (Gasparoto and Rezende, 2012). Therefore, countries in Southeast Asia, such as Thailand, Indonesia, Vietnam, India and Malaysia, are the largest producers of rubber worldwide (FAO, 2017).

The symbiotic interaction between microorganisms and plants is an alternative to ensure the preservation of native species because it can increase plant resistance to biotic and abiotic stresses (Zheng et al., 2017; Koide et al., 2017; Saunders et al., 2010; Arnold, 2007), increase plant production (Babu et al., 2015; Murali and Amruthesh, 2015; Khan et al., 2008) and control phytopathogens (Ben Amira et al., 2017; Contina et al., 2017; Larran et al., 2016; Valenzuela et al., 2015; Mbarga et al., 2014; Rocha et al., 2011). However, little is known about the interaction between endophytic fungi and plants from the Amazon. Some studies have examined the communities of microorganisms associated with *H. brasiliensis* and *H. guianensis* distributed in native habitats and rubber trees plantations in Peru, Cameroon (Africa) and Mexico (Gazis, 2012; Gazis et al., 2012, 2011; Chaverri et al., 2011; Rocha et al., 2011; Gazis and Chaverri, 2010). These studies demonstrated the occurrence of a high diversity of endophytic fungi mainly inside the stem despite the high colonization rate of endophytes inside the leaves (Gazis, 2012; Gazis et al., 2012, 2011; Chaverri et al., 2011; Gazis and Chaverri, 2010) and enabled the discovery of a new species of endophytic fungus identified as *Trichoderma amazonicum* (Chaverri et al., 2011), a new class of Xylonomycetes (Gazis et al., 2012) and a wide diversity of basidiomycetes in Peruvian rubber trees (Martin et al., 2015). In addition, it is evidenced in these studies that molecular techniques are efficient tools for the identification of cultivable fungi and for the analysis of their diversity in their habitat.

However, no study has described the diversity of endophytic fungi in *H. guianensis* in the Brazilian Amazon or the differences in the profiles of these microorganisms in the communities present in the different niches of these rubber trees. In addition, few studies have promoted knowledge about the diversity, conservation and biotechnological exploitation of endophytic microorganisms in different Brazilian biomes, although several state and federal programmes have encouraged research on natural resources and biodiversity (Sette et al., 2013; Valencia and Chambergo, 2013).

Thus, this study describes the diversity of endophytic fungi in *H. guianensis* trees in the Brazilian Amazon and the community profiles of these microorganisms in the leaves, stems and roots of this latex producer.

MATERIALS AND METHODS

Isolation of endophytic fungi from different tissues of *H. guianensis*

Leaf, stem and root samples were obtained from six healthy *H. guianensis* trees of similar size, located in the Amazon rainforest, Acre, and distributed at different sampling points between the coordinates 07°44'05.3" S / 72°49'46.8" W and 10°02'16.7" S / 67°40'45.4" W. The collections were carried out in native fragments of forest located near the cities of Xapuri, Boca do Acre and Rio Branco. The samples were collected in the month of July and the trees were randomly selected.

The methods proposed by Wirsel et al. (2001), Evans et al. (2003) and Leite et al. (2013) with modifications were used to isolate the endophytic fungi. The leaves were collected at the interface of the center to the periphery of the tree. The leaves that showed good sanity were packed into paper bags, which in turn were placed into plastic bags and stored at 4°C (Stone et al., 2004). Fragments of the cortex of the lateral roots near the primary root of the tree were collected and the root cortex fragments transported to the laboratory immediately after collection in silica gel tubes. Also, fragments of the stem cortex were collected at breast height and on the same side of the tree. The 3-to-5-cm fragments of the stem cortex were obtained after removal of the outer bark with the aid of a properly sterilized scalpel and immediately inoculated into YMC culture medium (10 g of malt extract, 2 g of yeast extract and 15 g of agar dissolved in 1 L of heated distilled water and then autoclaved) (Evans et al., 2003).

The leaves were washed in running water for 10 min, cut into fragments of approximately 0.25 cm^2 and subsequently subjected to disinfestation treatments. During the disinfestation process, the leaf

fragments were immersed in 70% ethanol solution containing Tween 80 (0.02%) for 1 min, transferred to sodium hypochlorite solution (2.5% active chlorine) for 8 min and then washed twice in sterile distilled water for 2 min. To test the efficiency of the surface disinfestation method, the adaxial portion of some leaf fragments was pressed onto the culture medium used for the isolation (Schulz et al., 1998).

For disinfestation, the roots were washed in sterilized water, cut into fragments of approximately 5 cm, immersed in 70% ethanol and Tween 80 (0.02%) for 1 min, immersed in hydrogen peroxide (3%) for 3.5 min and washed twice in sterilized distilled water for 2 min per wash. Five fragments of leaves and roots were transferred into each Petri dish containing YMC medium plus the antibiotics streptomycin (50 µg/ml) and tetracycline (50 µg/ml). The plates were incubated for 10 days at 25°C ± 2°C in the dark.

The concentrations and exposure times of the leaf and root fragments in sodium hypochlorite and hydrogen peroxide, respectively, were previously tested to obtain and adjust the optimal conditions for endophyte isolation and the proper elimination of epiphytic and saprophytic microorganisms. The effectiveness of the disinfestation process was verified by the inoculation of aliquots of the last washing solution from the leaf and root fragments into liquid YMC medium.

After growth of colonies, the fungi were subjected to monosporic purification and cultured in YMC medium at 25°C ± 2°C for a photoperiod with 12 h of white light and 12 h in the dark for seven days. Then, the isolates were preserved in 10% glycerol, distilled and sterilized water (Castellani, 1939) and stored at 4°C in the Mycology Collection of the Laboratory of Molecular Genetics of Fungi (BIOAGRO - UFV Campus - Viçosa/MG, Brazil).

DNA extraction, amplification and sequencing of the rDNA ITS region

The fungi were grown in YMC medium for seven days, and their mycelia were transferred to Eppendorf tubes with 0.2 ml of glass beads (425 to 600 µm). The DNA from these mycelia was extracted using the Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer instructions with modifcations proposed by Pinho et al. (2012). The extracted DNA was quantified and evaluated for purity by spectrophotometry (A_{260}/A_{280} ratio) (Nanodrop 2000, Thermo Scientific).

The internal transcribed spacer (ITS) region (ITS1-5.8s-ITS2) of the rDNA was amplified by PCR using the primers ITS 1F (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990). Each amplification reaction used 50 ng of DNA, 25 mM $MgCl_2$, 10 mM dNTPs, 5 µM ITS 1F, 5 µM ITS 4, 1 unit of GoTaq® Green MasterMix 2X (Promega, WI, USA) and ultrapure water to 25 µl. The Eppendorf Mastercycler thermocycler (Eppendorf, Germany) was programmed to perform an initial denaturation step at 95°C for 3 min, followed by 36 cycles at 95°C for 1 min, 50°C for 1 min and 72°C for 1 min. After the cycles, there was a final extension at 72°C for 7 min. Next, the PCR products were separated by 1.2% agarose gel electrophoresis and sent to the commercial company Macrogen (Korea) for DNA purification and sequencing.

The forward and reverse sequences of each DNA strand were analysed using the Geneious 8.0.4 program and grouped into contigs. Next, using the BLAST program, the sequences were compared to the sequences deposited in the GenBank database of the National Center for Biotechnological Information (NCBI) and UNITE (Unified system for the DNA-based fungal species) using a nucleotide sequence alignment algorithm (BLASTN). In this process, the sequences from this study with lower e-values, greater query coverage and greater identity in correspondence to the sequences present in the database were considered to belong to the species or genus referring to the isolate with greater sequence identity. Sequences from the ITS regions of the isolates from this study were deposited in GenBank under accession numbers MK026979 to MK027005 and MK027293. Subsequently, the ITS region sequences were grouped into operational taxonomic units (OTUs), with sequences having ≥ 98% similarity considered to belong to the same OTU. Sequences with < 98% similarity were considered to belong to different OTUs even though they were of the same genus, based on Nilsson et al. (2009) results.

Phylogenetic analysis

The nucleotide sequences of the rDNA ITS region of each OTU and the reference or type sequences (Table 1) obtained from the database were aligned with the program MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0 (Tamura et al., 2013). The cluster was performed using Bayesian inference (BI) (Yang and Rannala, 1997) in the program MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001) with the GTR+I+G evolutionary model according to the Akaike Information Criterion (AIC) parameter chosen in the program MrModeltest v2.3 (Nylander, 2004). The phylogenetic trees were inferred in the program MrBayes, in which two independent runs with four Monte-Carlo Markovian chains (MCMC) were run for 10,000,000 generations, with the trees sampled and retained every 1000^{th} generation. During the burn-in phase, the first 1,000,000 tree samples were discarded, and the remaining trees were summarized to generate a consensus tree. *A posteriori* probability BI values above 95% were added in the tree branches and indicated high data reliability with strong statistical support (Harada et al., 1995).

Endophytic fungal diversity

The diversity of endophytic fungal species was measured using the prediction (extrapolation) and rarefaction (interpolation) models of the initial sample to compare species richness and biodiversity among the different fungi isolated from the tissues (leaf, stem and root) of *H. guianensis*.

Extrapolation and rarefaction models based on Hill numbers are empirical estimates that tend to be an increasing function of sampling effort. *q* determines the measure of relative frequency, and the models determine a unified approach for individual-based (abundance) and sample-based (incidence) data for species richness (^{q}D, where $q = 0$). To measure taxon diversity by incorporating the relative abundance, we assume ^{q}D, where $q > 0$ and q = 1 for the exponential of Shannon's index and q = 2 for the inverse of Simpson's concentration index (Chao and Colwell, 2014).

The diversity index analysis and calculation of the standard error within a 95% confidence interval with 1000 bootstrapping replicates were performed in R version 3.1.2.

Analysis of similarity among fungi in different plant tissues

Non-metric multidimensional scaling (nMDS) analysis was used to evaluate the similarity among the fungal communities isolated from the different tissues (leaf, stem and root) of the rubber trees. In this analysis, the distances were measured using the Bray-Curtis index within the R vegan package (Oksanen, 2015).

RESULTS

A total of 92 endophytic fungi were isolated from the tissues of *H. guianensis* trees (leaf: 66 isolates, stem: 8 isolates and root: 18 isolates) located at different

Table 1. Number and frequency of endophytic fungi isolated from the leaves, stems and roots of *Hevea guianensis* from the Amazon forest in the state of Acre per operational taxonomic unit (OTU).

Operational taxonomic units (OTUs)	Total of isolates of *H. guianensis*			Frequency of colonization of isolates (%)		
	Leaf	Stem	Roots	Leaf	Stem	Roots
OTU001 - *Colletotrichum* spp.	27	1	0	40.9	12.5	0
OTU002 - *Fusarium oxysporum*	0	1	2	0	12.5	11
OTU003 - Diaporthaceae	6	0	0	9.0	0	0
OTU004 - *Penicillium* spp.	0	0	9	0	0	50
OTU005 - Diaporthaceae	3	0	0	4.5	0	0
OTU006 - *Trichoderma* spp.	0	1	3	0	12.5	16.6
OTU007 - *Purpureocillium lilacinum*	2	0	0	3.0	0	0
OTU008 - *Lasiodiplodia* spp.	2	0	0	3.0	0	0
OTU009 - *Phomopsis* spp.	1	0	0	1.5	0	0
OTU010 - *Pestalotiopsis mangiferae*	4	0	1	6.0	0	5.5
OTU011 - *Penicillium* spp.	0	1	0	0	12.5	0
OTU012 - *Mucor* spp.	1	0	1	1.5	0	5.5
OTU013 - *Colletotrichum* spp.	1	0	0	1.5	0	0
OTU014 - *Colletotrichum* spp.	5	0	0	7.5	0	0
OTU015 - *Daldinia eschscholtzii*	3	0	0	4.5	0	0
OTU016 - Diaporthaceae	1	0	0	1.5	0	0
OTU017 - *Colletotrichum* spp.	1	0	0	1.5	0	0
OTU018 - *Phyllosticta capitalensis*	2	0	0	3.0	0	0
OTU019 - *Curvularia* spp.	0	0	1	0	0	5.5
OTU020 - *Pseudofusicoccum stromaticum*	3	0	0	4.5	0	0
OTU021 - *Chaetomium globosum*	1	0	0	1.5	0	0
OTU022 - *Penicillium* spp.	0	1	0	0	12.5	0
OTU023 - *Phlebiopsis flavidoalba*	0	1	0	0	12.5	0
OTU024 - *Phyllosticta citriasiana*	2	0	0	3.0	0	0
OTU025 - *Pilidiella wangiensis*	1	0	0	1.5	0	0
OTU026 - Hypocreales	0	0	1	0	0	5.5
OTU027 - *Letendraea helminthicola*	0	1	0	0	12.5	0
OTU028 - *Chaunopycnis* spp.	0	1	0	0	12.5	0
Total	66	8	18	-	-	-

collection points throughout the state of Acre (Table 1). Of the total, 96.73% (89 isolates), 1.08% (one isolate) and 2.17% (two isolates) belonged to the phyla Ascomycota, Basidiomycota and Zygomycota, respectively.

Within the Ascomycota phylum group, 16 OTUs (63 isolates) belonged to class Sordariomycetes, 6 OTUs (11 isolates) to class Dothideomycetes and 4 OTUs (15 isolates) to class Eurotiomycetes. Only 1 OTU (two isolates) belonged to class Mucoromycotina of phylum Zygomycota, and 1 OTU (one isolate) belonged to class Agaricomycetes of phylum Basidiomycota (Supplementary Tables 1 and 2).

The sequences of the isolates used as representatives of each OTU and subjected to phylogenetic analysis via Bayesian inference were grouped with the sequences of the type and reference isolates deposited in GenBank and Unite. Phyla Zygomycota, Ascomycota and Basidiomycota formed clusters, and the genera within these phyla formed clades within their respective families with well-supported branches (greater than 95% bootstrap support (BS) and 0.95 posterior probabilities (PP) (Figure 1).

When comparing the richness and diversity of the fungi recovered from the different plant tissues, greater richness ($q = 0$) and Shannon ($q = 1$) and Simpson ($q = 2$) diversity were observed in isolates obtained from the stem cortex of *H. guianensis.* The richness and diversity values of the fungi isolated from the leaves and roots of these rubber trees did not differ significantly (Figure 2 and Table 2).

The nMDS analysis based on the Bray-Curtis distances between OTUs showed a trend towards cluster formation and a heterogeneous distribution of fungi recovered from leaf tissue compared to isolates from the stem and root cortex ($R = 0.302$, $p < 0.001$) (Figure 3).

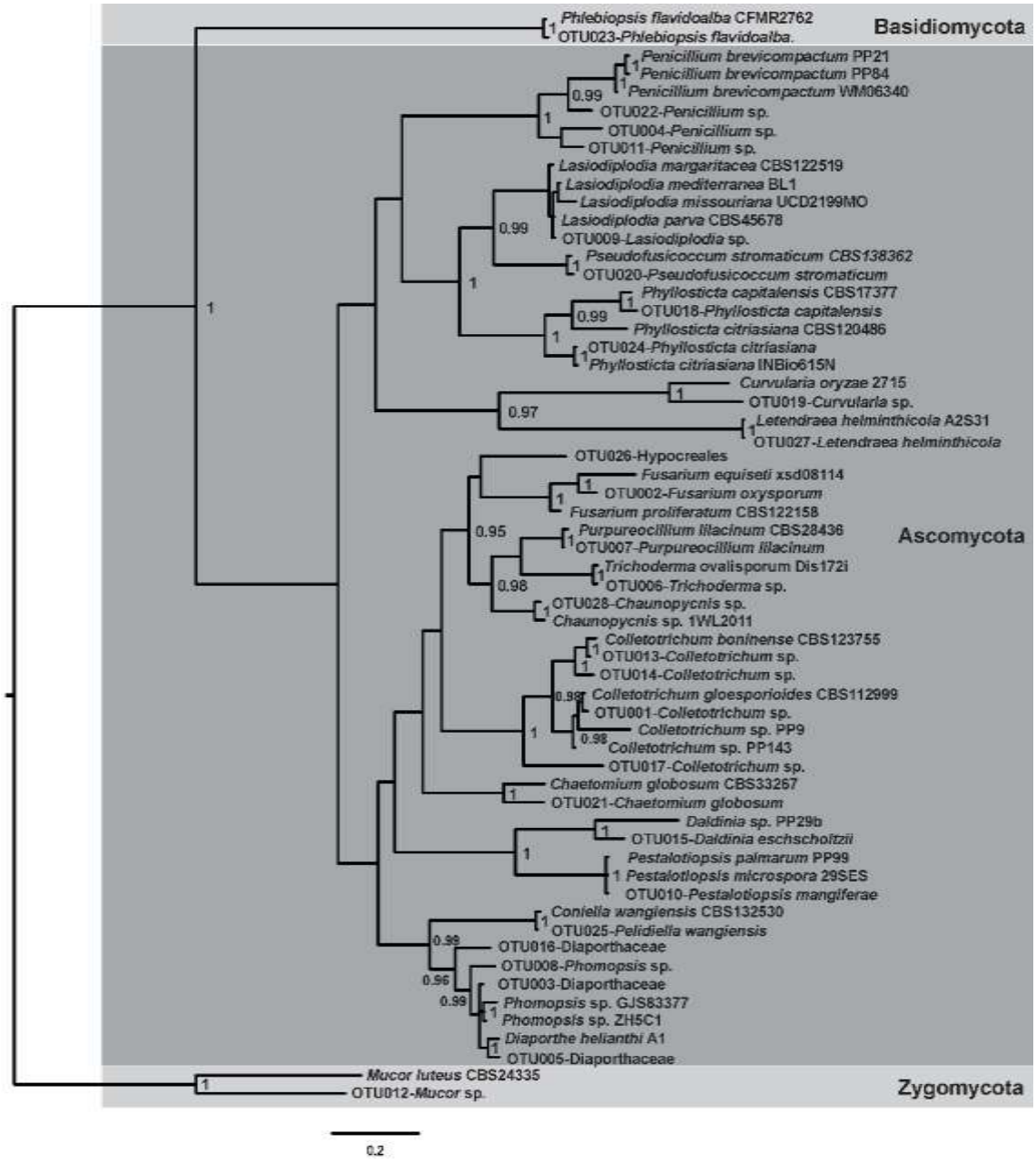

Figure 1. Phylogenetic tree obtained by Bayesian inference (BI) using sequences from the rDNA ITS region of the 28 operational taxonomic units (OTUs) that clustered all 92 endophytic fungi belonging to the phyla Ascomycota, Basidiomycota and Zygomycota. Posterior probability values below 95% were omitted.

Of the 92 isolates cultured from *H*. *guianensis* tissues, 38% of the fungi (35 isolates) belonged to the genus *Colletotrichum* (family Glomerellaceae), of which 97.14% (34 isolates) were obtained from the leaf fragments of the rubber tree; only 2.85% (one isolate) of the isolates from this genus were isolated directly from the stem cortex of the plant (Figure 4, Table 1, Supplementary Tables 1 and 2).

The *Penicillium* (family Trichocomaceae) genus had the second highest number of fungi isolated from *H. guianensis*, representing 11.95% of the total fungi recovered (Figure 4). Representatives of this genus were isolated mainly from the roots, totalling 50% (nine isolates) of the fungi obtained from the roots of *H. guianensis* (Table 1, Supplementary Tables 1 and 2 Material). Also, *Diaporthe* (family Diaporthaceae) is the second genus most abundant within of the tissue of *H. guianensis*, mainly within of the leaf tissue, corresponding

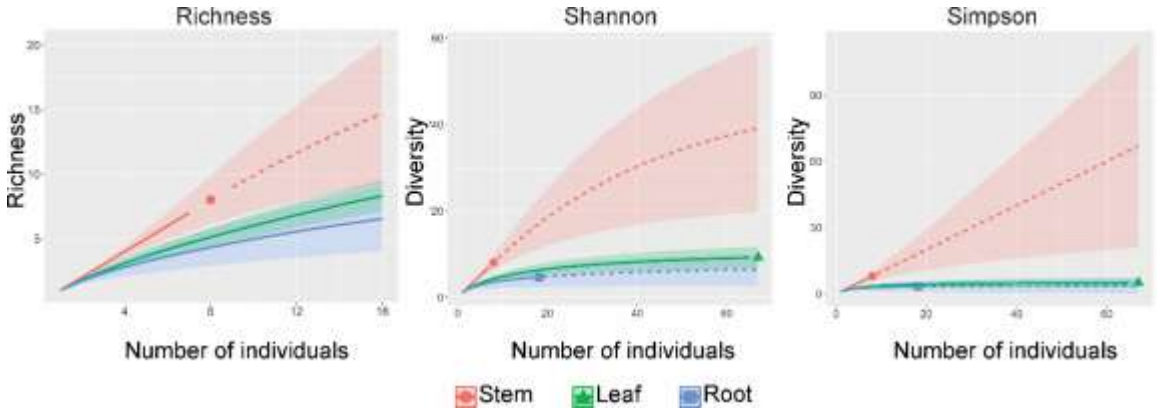

Figure 2. Rarefaction (solid line) and extrapolation (dashed line) curves for twice the size of the reference sample. The rarefaction (solid line) and extrapolation (dashed line) curves compare the species richness (q = 0), exponential of Shannon's entropy index (q =1) and inverse of Simpson's concentration index (q = 2) according to the Hill numbers of endophytic fungi in the different *Hevea guianensis* niches: stem (red line), leaf (green line) and root (blue line), with 95% confidence intervals obtained by the bootstrap method with 200 replications.

Table 2. Comparison of the asymptotic richness estimator (q = 0), exponential of Shannon's entropy index (q = 1) and inverse of Simpson's concentration index (q = 2) of endophytic fungi among the different *Hevea guianensis* niches with their 95% confidence intervals (*).

Sample	Tissues	Richness (q0)	Shannon (q1)	Simpsom (q2)
Acre	Leaf	8.311 ± 0.658[a]	9.193 ± 0.897[a]	4.928 ± 0.897[a]
	Stem	14.710 ± 0.302[b]	39.122 ± 0.909[b]	20.852 ± 0.909[b]
	Root	6.549 ± 0.771[a]	2.659 ± 0.948[a]	3.670 ± 0.948[a]

Means in a column followed by the same letter do not differ at 5% probability according to Tukey procedure using R software version 3.1.2 with 1000 bootstrap replicates.

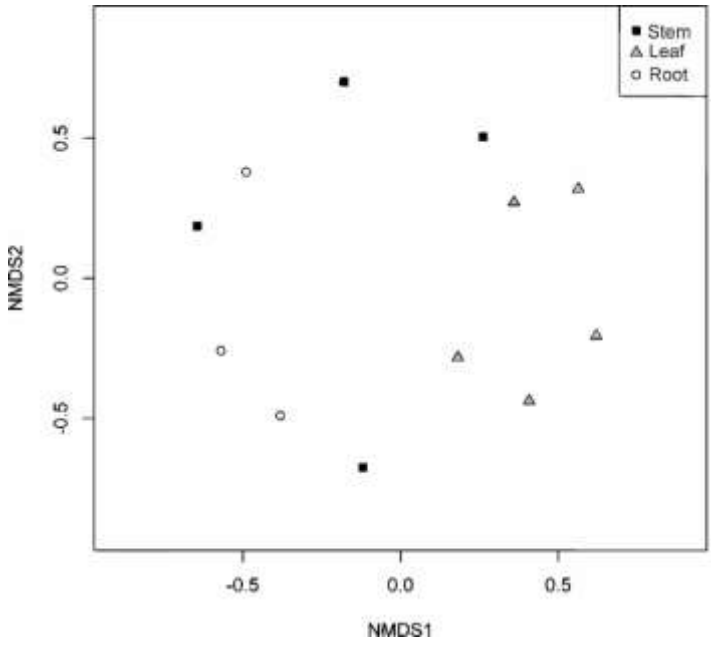

Figure 3. Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance between fungal samples obtained from different *Hevea guianensis* tissues.

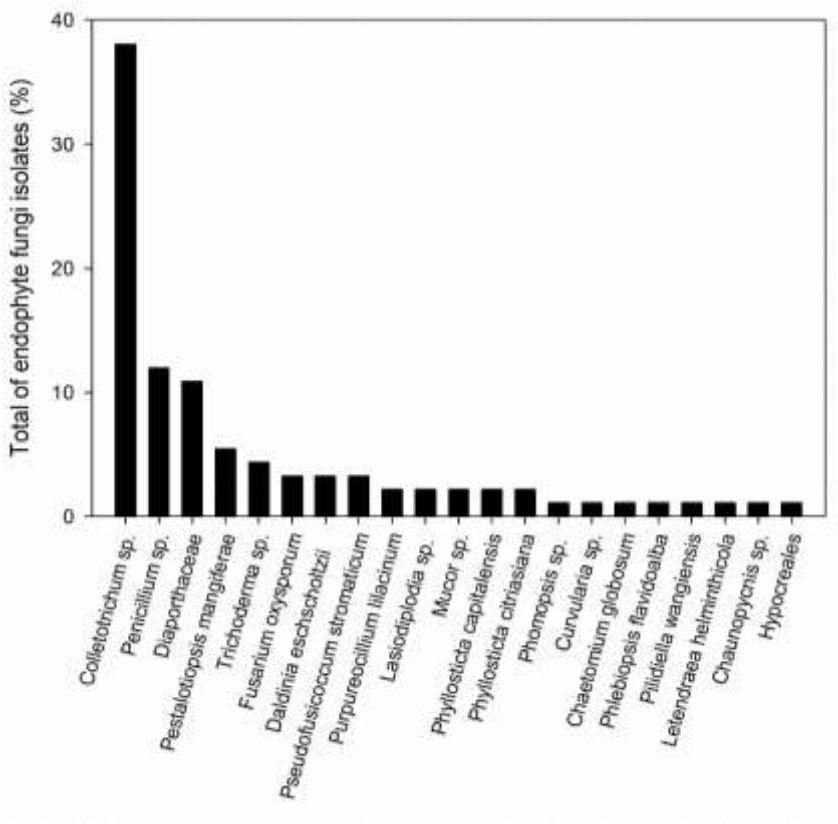

Figure 4. Percentage of endophytic fungi isolated from *Hevea guianensis* in the Brazilian Amazon (State Acre).

to 11.95% (11 isolates) of the isolates distributed into the four OTUs (Table 1, Supplementary Tables 1 and 2).

Fungi of the genus *Pestalotiopsis* were isolated from the leaves and roots, whereas isolates of the genera *Trichoderma* and *Fusarium* were obtained from the stems and roots and the genera *Phyllosticta*, *Daldinia* and *Pseudofusicoccum* were recovered only from the leaves of the rubber trees, with representation of 5.43, 4.34, 3.26, 4.34, 3.26 and 3.26%, respectively (Table 1, Supplementary Tables 1 and 2).

Among the genera that presented lower abundances, representatives of the genus *Mucor* were isolated from the leaves and roots of *H. guianensis*. Fungi belonging to the genera *Lasiodiplodia* and *Purpureocillium* were only isolated from the leaves of the rubber trees, corresponding to 2.17% of the total fungi recovered (Table 1, Supplementary Tables 1 and 2).

DISCUSSION

The fungi isolated from different tissues of *H. guianensis* were more diverse and showed greatest richness in stem cortex than in the roots or leaves, although the frequency of isolates was highest in the leaves. In addition, the endophytic fungi of the leaves showed heterogeneous distribution in relation to the stem and root isolates.

This study is the first to describe the diversity of endophytic fungi in leaves, stems and roots of *H. guianensis* in the Brazilian Amazon. In comparison to the species *H. brasiliensis*, which is extensively cultivated, few studies have investigated *H. guianensis*, exploited in its natural habitat but is not cultivated in terms of the production of varieties that have been genetically improved for disease resistance or production of better quality latex (Cardoso et al., 2014) and descriptions of microorganisms in the tissues with potential biotechnological applications (Rocha et al., 2011; Gazis and Chaverri, 2010).

The morphological similarity between *H. guianensis* and *H. brasiliensis* makes identification of these species difficult in their natural environment. However, we located and identified six *H. guianensis* trees among the *H. brasiliensis* trees in the Amazon forest in the state of Acre.

The 92 fungi isolated were identified when the ITS region sequence was used as a barcode. However, most of the microorganisms that inhabit the interior of plants and other niches are unculturable. Although there a limitation in determining the true richness of fungi that colonize plants, the use of ITS region facilitated the identification of different genera and their clustering into OTUs. Many studies have estimated the diversity and

distributions of species in a microbial community by counting OTUs (Koide et al., 2017; Martins et al., 2016; Angelini et al., 2012; Gazis et al., 2011; Gazis and Chaverri, 2010), and ITS region sequences have been used as an efficient universal barcode to discriminate fungal genera (Schoch et al., 2012) and to cluster these sequences into OTUs with intraspecific variations of 0 to 3% (Nilsson et al., 2009).

As observed in several other studies (Ferreira et al., 2017; Martins et al., 2016; Fernandes et al., 2015; Leite et al., 2013; Gazis and Chaverri, 2010; Hanada et al., 2010), phylum Ascomycota was most abundant in the endophytic fungal community of *H. guianensis* (96.73%), particularly class Sordariomycetes (68.47%). In addition, the estimated richness, Shannon diversity and Simpson diversity were significantly higher in the stems, despite the high proportion of fungi isolated from the leaves (71.73%). Fungi isolated from leaves were distributed into 18 OTUs when compared to the fungi recovered from the stems (8.69% of the total isolates clustered into 8 different OTUs) and from the roots (19.56% of the recovered fungi were present in 7 OTUs) of these rubber trees. The high diversity of endophytic fungi in the stem is due to the high equitability in the distribution of fungi identified and isolated in this plant tissue.

Regarding the estimation of richness and diversity of the endophytic fungi, the results obtained in this study corroborate those from the study of Gazis and Chaverri (2010), who found a high diversity of fungi in the stem cortex despite obtaining a higher frequency of isolate colonization in the leaves of Peruvian rubber trees.

In this study, a trend was revealed towards clustering of the isolates present in the leaves and the separation of these isolates from the fungi recovered from the stem cortex and roots. Several factors may affect the distribution and abundance of the microbial community, such as the environment, chemical composition of tissues and interspecific competition among microorganisms (Zheng et al., 2017; Martins et al., 2016; Gazis and Chaverri, 2010; Suryanarayanan and Vijaykrishna, 2001).

Colletotrichum, *Penicillium* and *Diaporthe* were the predominant genera, while *Trichoderma*, *Pestalotiopsis*, *Fusarium*, *Phyllosticta*, *Daldinia*, *Pseudofusicoccum*, *Mucor*, *Lasiodiplodia* and *Purpureocillium* were obtained in lower frequencies from the tissues of *H. guianensis*.

However, Gazis and Chaverri (2010) studied the diversity of endophytic fungi in Peruvian rubber trees and found that *Penicillium*, *Pestalotiopsis* and *Trichoderma* were the most frequent genera. Thus, there are diffferences among the endophytic fungal communities in rubber trees from different study areas.

In this study, *Colletotrichum* was isolated from the leaf fragments (38%) and from the stem cortex (2,85%) of the *H. guianensis*. *Diaporthe* is the second most important genus isolate inside the leaves. This result was also observed in several studies of the diversity of endophytic fungi in tropical plants (Ferreira et al., 2017; Fernandes et al., 2015; Leite et al., 2013). Nevertheless, Gazis and Chaverri (2010) observed a low frequency of *Colletotrichum* with the tissue of *H. brasiliensis*, a closely related species from Peruvian Amazon forest.

Fifty percent of the endophytic fungi isolated from the roots belonged to the genera *Penicillium*. *Trichoderma*, *Fusarium*, *Pestalotiopsis*, *Curvularia* and *Mucor* were also isolated from the roots of *H. guianensis.* Also, the fungal genera recovered from the rubber tree stems in this study showed greater equitability and were identified as *Colletotrichum* species, *Fusarium oxysporum*, *Trichoderma* species, *Penicillium* species, *Phlebiopsis flavidoalba*, *Letendraea helminthicola* and *Chaunopycnis* species.

Among the factors affecting the microbial community, climate and dispersion are processes that have been reported to significantly influence the endophytic fungal communities in plants (Koide et al., 2017; Zheng et al., 2017). This knowledge has great relevance because climate change can affect the natural environment and plantations of crops of commercial interest. Additionally, the environment can modify the dispersion of endophytic fungi and their effects on plants regarding tolerance to extreme temperature and humidity, as could be the case with rubber trees in the Amazon.

Some species close to fungi genera obtained in the present study are reported in the literature as potentially mutualistic species, which may be tested in the future as biological control agents of plant diseases, may confer resistance to abiotic stresses and/or promote plant growth. For example, in relation to studies on rubber trees, Rocha et al. (2011) isolated a total of 435 endophytic fungi from the leaves of three cultivars of *H. brasiliensis* that were resistant to diseases and found a higher abundance of fungi belonging to the genera *Colletotrichum*, *Diaporthe*, *Fusarium, Pestalotiopsis*, *Microspheropsis* and *Myrothecium*. These latter isolates were able to inhibit the germination of *Pseudocercospora ulei* conidia by 80%. The genera *Colletotrichum*, *Diaporthe*, *Pestalotiopsis*, and *Fusarium* were also obtained in the present study and could be tested as biological control agents in future studies.

Other fungi genera with potential for biological control of diseases, inductors of plant resistance to abiotic stress and/or growth promoters in plants include *Penicillium* (Guijarro et al., 2017; Babu et al., 2015; Murali and Amruthesh, 2015), *Lasiodiplodia* (Xiang et al., 2016), *Fusarium* (Zhang et al., 2014; Rocha et al., 2011), *Purpureocillium* (Lopez et al., 2014) and *Trichoderma* (Ben Amira et al., 2017; Contina et al., 2017; Larran et al., 2016; Mbarga et al., 2014).

In addition, the Amazon region has the greatest biodiversity on the planet as well as different endemism centres, and little is known about the communities of endophytic fungi present (Gibertoni et al., 2016; Gazis and Chaverri, 2010). Analysis of microbial culture collections showed the existence of 46 Brazilian culture

collections registered in the Genetic Heritage Management Council (CGEN) database belonging to the World Federation for Culture Collections, the majority of which were located in south eastern Brazil (Sette et al., 2013). The authors believe that there is still a lack of up-to-date information and studies aimed at obtaining and analysing microbial culture collections (Sette et al., 2013). This information and analysis can promote knowledge about the diversity, conservation and biotechnological exploitation of fungi.

The endophytic fungi isolated and identified from *H. guianensis* in the present study will be used in future studies focusing on the identification of new species, using different locus to phylogenetic analysis, and also their potential use in the promotion of plant growth, the biological control of diseases and in the production of bioactive metabolites of interest.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Table 1. Identification of endophytic fungi grouped in each OTU.

OTUs	Phylum	Class	Order	Family	Genus	Species
OTU001	Ascomycota	Sordariomycetes	Sordariomycetidae	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU002	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium oxysporum*
OTU003	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	-	----------
OTU004	Ascomycota	EurotiomyTTcetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp
OTU005	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	-	----------
OTU006	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma sp*
OTU007	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Purpureocillium*	*Purpureocillium lilacinum*
OTU008	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Phomopsis*	*Phomopsis* sp.
OTU009	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Lasiodiplodia*	*Lasiodiplodia* sp
OTU010	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriaceae	*Pestalotiopsis*	*Pestalotiopsis mangiferae*
OTU011	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU012	Zygomycota	Mucoromycotina	Mucorales	Mucoraceae	*Mucor*	*Mucor* sp.
OTU013	Ascomycota	Sordariomycetes	Sordariomycetidae	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU014	Ascomycota	Sordariomycetes	Sordariomycetidae	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU015	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Daldinia*	*Daldinia eschscholtzii*
OTU016	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	-	----------
OTU017	Ascomycota	Sordariomycetes	Sordariomycetidae	Glomerellaceae	*Colletotrichum*	*Colletotrichum* sp.
OTU018	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Phyllosticta*	*Phyllosticta capitalensis*
OTU019	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Curvularia*	*Curvularia* sp.
OTU020	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Pseudofusicoccum*	*Pseudofusicoccum stromaticum*
OTU021	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium globosum*
OTU022	Ascomycota	*Eurotiomycetes*	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium* sp.
OTU023	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phlebiopsis*	*Phlebiopsis flavidoalba*
OTU024	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Phyllosticta*	*Phyllosticta citriasiana*
OTU025	Ascomycota	Sordariomycetes	Diaporthales	Schizoparmaceae	*Pilidiella*	*Pilidiella wangiensis*
OTU026	Ascomycota	Sordariomycets	Hypocreales	-	-	----------
OTU027	Ascomycota	Dothideomycetes	Pleosporales	Tubeufiaceae	*Letendraea*	*Letendraea helminthicola*
OTU028	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Chaunopycnis*	*Chaunopycnis*sp.

OTU, operational taxonomic unit.

Supplementary Table 2. Identification codes of the isolates composing each OTU.

OTUs	Isolates
OTU001	800F8F-AC; 368F3F-AC; 598F9F-AC; 362F6F-AC; 12F6F-AC; 219F3F-AC; 222F3F-AC; 347F3F-AC; 61F3F-AC; 408F3F-AC; 402F3F-AC; 372F3F-AC; 358F3F-AC; 344F3F-AC; 235F6F-AC; 233F3F-AC; 223F10F-AC; 218F3C-AC; 168F3F-AC; 143F3F-AC; 11F10F-AC; 67F10F-AC; 62F3F-AC; 571F10F-AC; 241F10F-AC; 220F10F-AC; 805F3F-AC; 66F10F-AC
OTU002	664F10C-AC; 329F6R-AC; 215F6R-AC
OTU003	327F3F-AC; 716F3F-AC; 141F6F-AC; 326F6F-AC; 178F6F-AC; 514F6F-AC
OTU004	202F8R-AC; 171F9R-AC; 689F9R-AC; 752F8R-AC; 753F8R-AC; 756F8R-AC; 694F8R-AC; 755F8R-AC; 798F8R-AC
OTU005	512F9F-AC; 617F9F-AC; 333F8F-AC
OTU006	508F9R-AC; 26F9R-AC; 3F13C-AC; 4F9R-AC
OTU007	64F3F-AC; 364F10F-AC
OTU008	470F9F-AC; 626F9F-AC
OTU009	77F3F-AC
OTU010	172F10F-AC; 594F10F-AC; 422F10F-AC; 359F3F-AC; 165F8R-AC
OTU011	197F3C-AC
OTU012	555F10F-AC; 649F9R-AC
OTU013	129F6F-AC
OTU014	343F3F-AC; 406F6F-AC; 572F6F-AC; 401F6F-AC; 367F3F-AC
OTU015	240F3F-AC; 324F8F-AC; 365F3F-AC
OTU016	558F6F-AC
OTU017	234F6F-AC
OTU018	302F10F-AC; 307F10F-AC
OTU019	325F8R-AC
OTU020	396F3F-AC; 551F10F-AC; 601F10F-AC
OTU021	413F10F-AC
OTU022	620F10C-AC
OTU023	179F10C-AC
OTU024	135F3F-AC; 388F3F-AC
OTU025	363F6F-AC
OTU026	216F6R-AC
OTU027	674F10C-AC
OTU028	176F9C-AC

OTU, operational taxonomic unit.

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Impact of faecal bacteria contamination on drinking water supply in Aghien Lagoon, Abidjan, Ivory Coast

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In developing countries, urban surface waters are particularly affected by faecal pollution from domestic wastewaters due to the lack of sanitation and wastewater treatment plants. The presence of pathogenic microorganisms limits the uses of these waters for recreation and economic activities. In Ivory Coast, due to the important gap between water demand and water supply in urban areas, use of surface waters for the production of drinking waters becomes a serious alternative. Actually, there is no monitoring program to control pollution discharges into these surface waters. In this study, a monitoring study was planned from September 2015 to February 2017 in order to evaluate the level of faecal pollution of the Aghien lagoon, a potential drinking water supply located in Abidjan. Based on the enumeration of faecal indicator bacteria (*Escherichia coli* and Intestinal enterococci), microbiological water quality from Aghien Lagoon and its tributaries were evaluated. Abundance of faecal indicators ranged between 1.72×10^1 CFU.100 ml^{-1} and 1.48×10^2 CFU.100 mL^{-1} for *E. coli* and between 2.26×10^3 CFU.100 mL^{-1} and 7.72×10^3 CFU.100 mL^{-1} for IE in lagoon waters. The abundances of FIB observed in tributaries were higher than those observed in lagoon water. The tributaries comparison indicates that, the Djibi River is the most contaminated with an average value of 1.73×10^6 CFU.100 mL^{-1} for IE and 6.92×10^5 CFU.100 mL^{-1} *E. coli* mL. The contributions of tributaries in terms of faecal bacteria discharged into the Aghien lagoon are not negligible and these contributions are significantly different between the dry and rainy season. Therefore, lagoon water may be a potential drinking water supply if wastewater treatment plants are implemented in the Djibi and Bété basins.

Key words: Water quality, faecal bacteria, drinking water supply, tropical lagoon.

INTRODUCTION

In urban area, population growth combined with urbanization poses a serious problem in relation to drinking water supply. This situation is particularly pronounced for urban area in developing countries. Jacobsen et al. (2013), in a report of the World Bank Organization entitled "*The Future of Water in African Cities: Why Waste Water?",* indicates that there is an important gap between water demand and water supply.

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Water demand is increasing at a higher rate than the population growth. Whereas, water availability is shrinking due to the competing demands from agriculture, mining activities, industry deterioration of water quality and climate change. For a long time, ground waters were a major source of drinking water production in developing countries due to their relatively low cost of treatment and their high quantities. The increase of population in urban area is accompanied by a reduction of the quantity of these reservoirs due to pressure. Today, freshwaters are emerging as alternatives for the production of drinking water in developing countries.

To guarantee an access to drinking waters for the population of Abidjan (422 Km², 6 million inhabitants) in ten or twenty next years, the authorities are exploring the potential of lagoon water to serve as reservoir for drinking water production: the Aghien Lagoon. However, the most important problems which limit the use of this lagoon water are its quantity for long term uses and its quality due to industrial, domestic and agriculture pollutions (Traoré et al., 2012; Koffi et al., 2014). It is well known that surface waters from urban area are exposed to different types of pollutions including physicochemical and microbiological parameters (viruses, bacteria, protozoa and helminths) (Ouattara et al., 2011; Passerat et al., 2011; Anyona et al., 2014; Pandey 2014; Marcheggiani et al., 2015). These pollutions may result from industrialization and poor wastewater management strategies (Gigliola et al., 2012; Páll et al., 2013; García-Armisen et al., 2014). The main consequence is that, waterborne diseases that cause mortality of population are difficult to prevent or to control. An example of the absence of surface water management program is the presence of faecal pathogenic microorganisms (bacteria, viruses and protozoa) in these tropical waters (Lu et al., 2016; Nshimyimana et al., 2013; Vincy et al., 2017). The detection and enumeration of all these pathogenic microorganisms potentially present is impossible due to the large diversity of the pathogens, low abundance of each species and absence of standardized and low-cost methods for the detection of each of them. Thus, for routine monitoring, Faecal Indicator Bacteria (FIB) is usually enumerated to evaluate the level of microbial water contamination (Ouattara et al., 2011, Boehm et al., 2014). *Escherichia coli* and intestinal enteroccoci are considered as the best Faecal Indicator Bacteria to predict the sanitary risk associated with freshwaters (Edberg et al., 2000; Passerat et al., 2011).

A short review of literature showed that these indicators are used around the world to evaluate the microbiological water quality of surface waters (European Union Directive, 2006; Griffin et al., 2000). Even if the *E. coli* and intestinal enteroccoci are adopted in temperate area, there is a reasonable doubt concerning their use in tropical waters. In a short review focused on fecal indicators in tropical ecosystem, Rochelle-Newall et al. (2015) highlighted the fact that the fecal indicator bacteria chosen are sometimes applied to tropical systems without taking into account the potential specificities of the tropics such as higher temperature and humidity, differences in nutrient and organic matter availability and higher solar irradiation levels.

The objectives of our research are to evaluate the impact of wastewaters discharged in the Aghien Lagoon and its tributaries using *E. coli* and intestinal enterococci as faecal indicator bacteria and to determine the contribution of each tributary by mean of the quantification of microbial fluxes.

MATERIALS AND METHODS

Study area

The study area is Aghien Basin located in South east of Ivory Coast (Figure 1). The Aghien watershed is composed of two basins: The Bété Basin (68%), Djibi Basin (26%) and an area covered by lagoon water (6%). The Djibi Basin and the Bété Basin are characterized by urbanization and agriculture (more than 60% of these basins) (Table 1). The water capacity of Aghien Lagoon is estimated to be 25 Km^3 with a maximum depth of 13 m (Effebi et al. 2017; Koffi et al*.,* 2014). The lagoon receives water from Bété and Djibi rivers before joining the Mé River in the downstream part of the lagoon. Some other small tributaries located in the Aghien watershed are diverted in the Bété and Djibi rivers so that their waters reach Aghien Lagoon. The Bété River and Djibi River receive domestic sewage from Anyama, Abobo, Brofodoume municipalities and villages located in the watershed without any treatment.

Environmental water sampling and processing

During the monitoring survey conducted in the scope of this study, water samples were collected in the Aghien Lagoon and its tributaries. Twelve sites were investigated from September 2014 to February 2017 (Figure 1); a total of ten sampling campaigns were thus performed. During these campaigns, water samples were collected in the lagoon (6 stations) and its tributaries (6 stations) with a sterile plastic bucket from bridges, halfway between the banks. Samples were stored in 1 L sterile bottles. All the bottles were labeled with the source name, date and time of collection of the samples. The bottles were transported to the laboratory, kept at 4°C and processed within a maximum of 2 h after collection for microbiological analysis.

Physical and chemical parameters

Nutrients, temperature, pH and dissolved oxygen are some of the important factors that play a vital role in the growth of microorganisms in the water body (Qureshimatva et al., 2015). Their importance on the evaluation of water quality affected by sewage waters is well described by Errich et al. (2016). The flow rate is used to calculate the fluxes of faecal bacteria discharged by different tributaries in order to estimate their contribution in terms of faecal pollution. In the scope of this study, three parameters were measured *in situ* during the whole study period. Temperature, dissolved oxygen and pH were measured using multi-parameter HACH HQ40D, according to standardized protocols of Rodier et al. (2009).

Water height and water flow of tributaries (Djibi, bété and Mé)

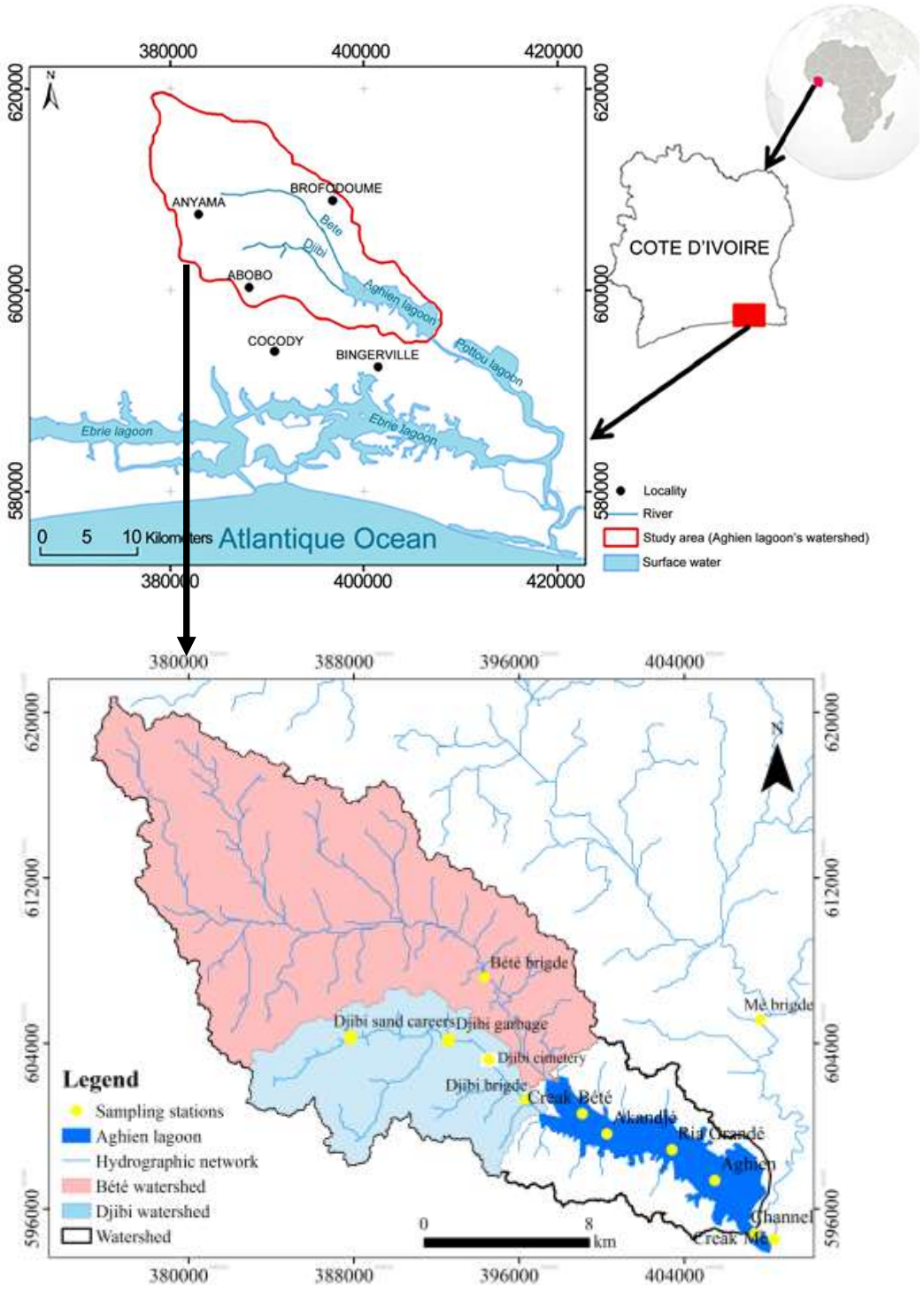

Figure 1. Location of the lagoon Aghien and its main tributaries (Bété and Djibi).

Table 1. Characteristics of Aghien lagoon and its tributaries.

Aghien and tributaries	Length (Km)	Depth (m)	Superficies (Km^2)
Bété	30	4	206
Djibi	16	2	78
Aghien lagoon	11	13	20

Table 2. Physical and chemical parameters measured in the lagoon Aghien and its tributaries.

Parameter	pH	Temperature (°C)	Dissolved oxygen (%)	Flow rate ($m^3.h^{-1}$)
Lagoon Aghien	8.6 (7.7 - 9.6)	26 (25 -28)	80 (70 - 90)	-
Djibi River	6.6 (6.8 - 7.0)	28 (25 -33)	8 (10 - 30)	1.00 (1.63 – 0.60)
Bété River	6.8 (6.5 – 7.1)	26 (25 -28)	65 (60 - 90)	1.80 (4.95 – 0.79)
Mé River	6.9 (6.4 – 7.2)	26 (25 -28)	70 (60 - 80)	31.70 (111.3 – 7.00)

*Concentrations are expressed as average, minimum and maximum values observed during the whole sampling campaigns.

were measured monthly by the Hydrology Department of Nangui Abrogoua University (Data collected from 2014 to 2017) in order to establish a standard curve (relationship water height-flow rate) for these tributaries. During the study period, water heights of tributaries were measured. The flow rate values of tributaries were calculated using this calibration curve. Results of physic and chemical parameters were expressed as maximum, average and minimum (Table 2).

E. coli and IE enumeration by plate count technique

E. coli and IE were enumerated in water samples by standard plate counts on TBX (E. coli) and Slanetz and Bartley agar (Bio-Rad Laboratories, Inc.). These two chromogenic growth media were shown to be highly specific to their corresponding indicator bacteria (ISO 7899-2 (08/2000) and ISO 16649-2:2001). These high levels of specificity were confirmed on samples from Aghien Lagoon and the three River samples at the beginning of the present study. Slanetz and Bartley supplemented with TTC (0. 2%) plates were incubated at 36°C for 24 h then at 44°C for 2 h before enumeration. TBX plates were incubated at 44°C for 24 h. Plate counts were expressed as colony forming units (CFU) per 100 mL of sample. The protocols used to detect E. coli (TBX agar) and IE (Slanetz and Bartley agar) are well described by Vergine et al. (2016) and Tiwari et al. (2018), respectively.

Contribution of tributaries in the microbial pollution of the lagoon

As presented in Figure 1, Aghien Lagoon received water from two main tributaries. In order to determine the contribution of the main tributary in the microbial pollution of the lagoon waters, the values of flow rate (l.h-1) was multiplied by the values of the abundance of faecal bacteria (CFU.l-1) observed in these tributaries during dry season. Then, a comparison was performed between the values of fluxes (CFU h-1) injected by each tributaries. Then, a statistical test (student's t- test) was performed to determine the significance degree of these differences.

Statistical analysis

In this study, all data were subjected to descriptive statistical analysis (95% confidence limit). Statistical tool R was used to determine the variance, average, standard errors and ranges. Student test (t-test) was used to test differences among the sampling sites.

RESULTS

Physical and chemical characteristics of Aghien basin

A summary of the physical and chemical characteristics of the lagoon Aghien and its tributaries is presented in Table 1. Table 2 indicates that pH values vary between 6.6 and 7.2 in the tributaries and between 7.7 and 9.6 in the lagoon. Temperature value varies between 25 and 28°C for Aghien Lagoon, Bété and Mé rivers. High values of temperature were observed in the Djibi River (33°C). Dissolved oxygen values were around 65-90% for Aghien Lagoon, Bété and Mé Rivers. The values of dissolved oxygen observed in the Djibi River were particularly low (less than 10% along the river). The water flow rates expressed as $m^3.h^{-1}$ in the Table 2 were lower in the main tributaries (from 0.60 to 4.95 $m^3.h^{-1}$). The relatively low values were observed in the Djibi River. The most important values of water flow rates were observed in the Mé River located in the outlet of the Aghien Lagoon (Figure 1). For each of them, the high values were observed during the high rainy seasons and the low values were observed in the high dry season. A significant difference was observed between the high rainy seasons compared to the high dry seasons.

E. coli and IE in Aghien Lagoon and its tributaries

Abundance of E. coli and EI in water of Aghien Lagoon

All sampling sites (Channel, Akandje, Ria Grande and Aghien) were located in the proximity of small villages around the lagoon. Abundance of faecal bacteria in the lagoon varied between 1.72×10^1 CFU.100 mL^{-1} and 1.48×10^2 CFU.100 mL^{-1} for *E. coli* and between 2.26×10^3 CFU.100 mL^{-1} and 7.72×10^3 CFU.100 mL^{-1} for Intestinal enterococci (Figure 2). Higher abundances of faecal indicators were observed in the Channel site for *E. coli* and Akandjé site for Intestinal enterococci. Particularly, abundances of Intestinal enterococci were higher than those of *E. coli* at the entire sampling sites.

Level of E. coli and EI in the main tributaries of Aghien Lagoon

Three main tributaries received water from one sub-basin. Average abundance of faecal bacteria in the main tributaries varied between 3.18×10^2 and 8.22×10^3 for *E. coli* and between 8.30×10^3 and 1.41×10^4 for Intestinal enterococci (Figure 3). At the entire sampling

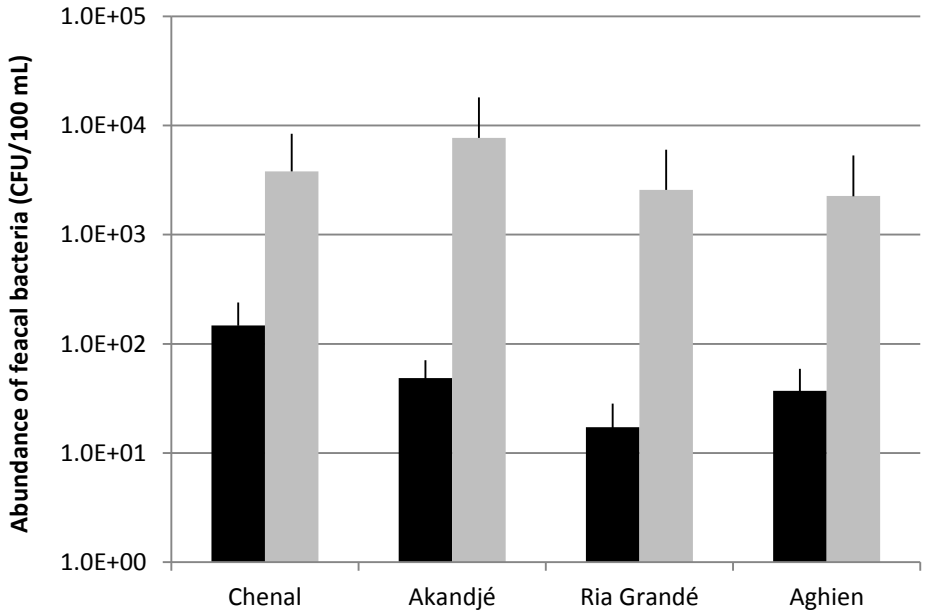

Figure 2. Abundance of faecal indicator bacteria in the Aghien lagoon. *E. coli and* intestinal enterococci abundances are in dark color and in grey color respectively.

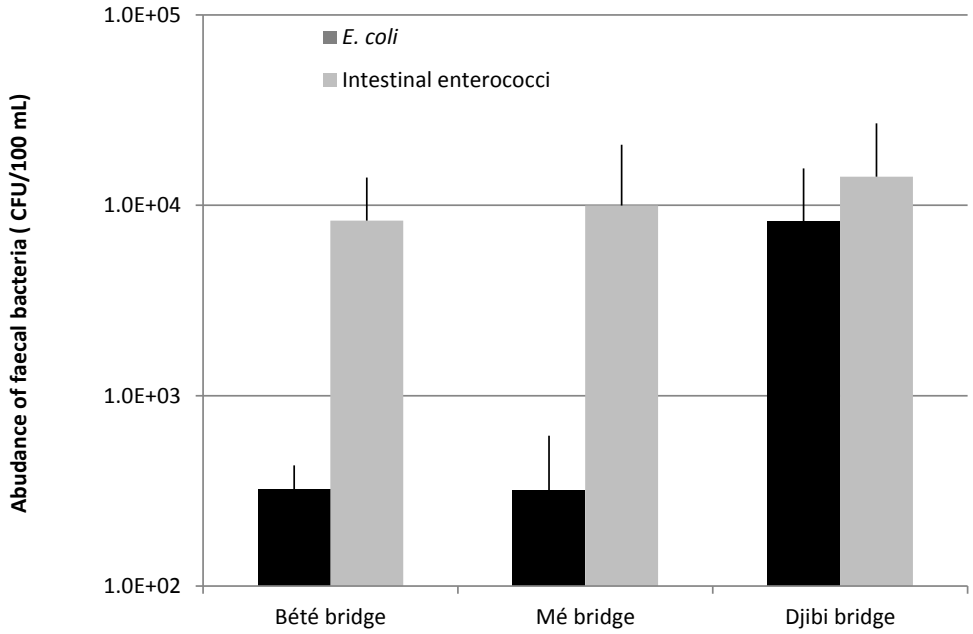

Figure 3. Abundance of faecal indicator bacteria in the main tributaries of Aghien lagoon. *E. coli and* intestinal enterococci abundances are in dark color and in grey color respectively.

sites, there were more abundances of Intestinal enterococci than *E. coli*. For both indicators, their abundances were significantly higher in Djibi River compared to that of Bété River and Mé River (p value <0.01).

Level of E. coli and EI in the Djibi River

The results showed that there were more abundances of faecal indicator observed in the Djibi River than in the other tributaries (Figure 4). To better appreciate why

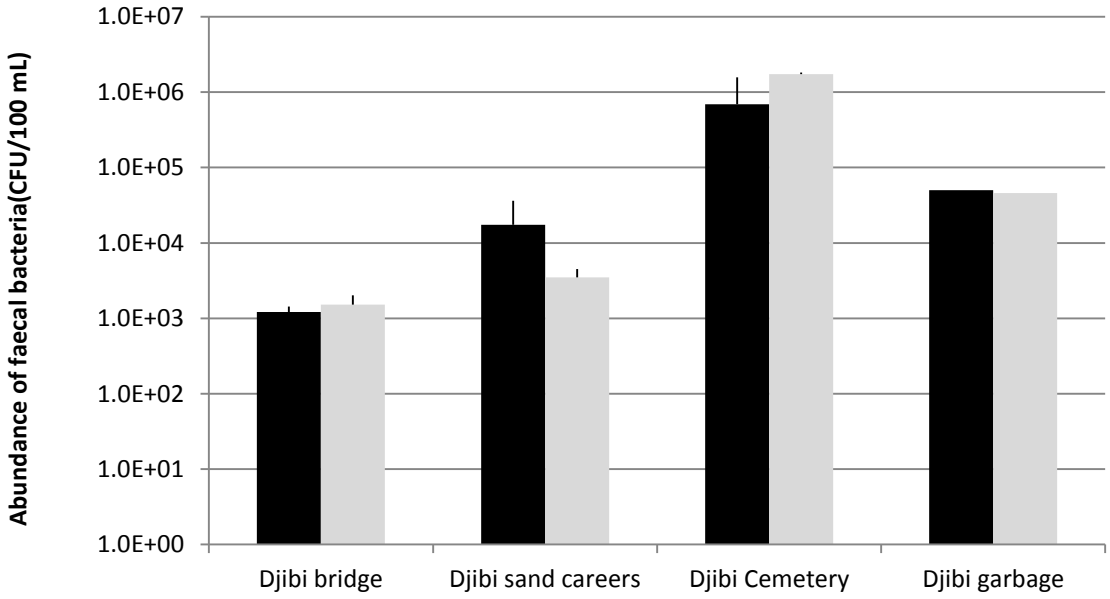

Figure 4. Abundance of faecal indicator bacteria in the main tributaries of Djibi River. *E. coli and* intestinal enterococci abundances are in dark color and in grey color respectively.

there was abundance of FIB determined in Djibi Basin than those in Bété and Mé basins, specific monitoring program was performed in the Djibi Basin. Results of abundances of *E. coli* and intestinal enterococci are presented in Figure 4. There were abundances of FIB at "Djibi cemetery" site than in the others sites of Djibi River. With an average value of 1.73×10^6 CFU.100 mL^{-1} for IE and 6.92×10^5 CFU.100 mL^{-1} *E. coli*, faecal bacteria was significantly abundant than that of the three other sites (p value <0.01).

DISCUSSION

The presence and abundance of faecal bacteria in the lagoon water clearly showed that sewage waters are drained into the Aghien Lagoon. The abundance of *E. coli* in the lagoon is lower than that recommended for recreational activities (US EPA, 1986; Havelaar et al., 2001; EU, 2006). The abundance of intestinal enterococci observed in lagoon is higher than that recommended by international guideline for microbiological water quality. International guideline recommended use of *E. coli* and Enterococci as indicators of faecal contamination of recreational waters even if the quality standards can vary from one country to another one terms of abundances. However, these guidelines do not indicate if it is enough to consider acceptable water quality when one of two criteria is not followed.

Levels of faecal contamination observed in the Aghien Basin are quite similar to those of surface waters encountered in several cities in Africa. For example, Musyoki et al. (2013) who carried out a study in Nairobi River, which crosses Kenyan capital city, Nairobi and its tributary (Athi River) showed that the abundances of faecal indicator bacteria in the waters of the rivers were 1.0×10^4 CFU.100 mL^{-1} for *E. coli* and 3.6×10^3 CFU.100 mL^{-1} for *Enterococcus faecalis)*. Sibanda et al. (2013) also assessed the distribution of faecal-indicator bacteria in Tyume River in the Eastern Cape Province, South Africa. Faecal coliform (including *E. coli*) counts ranged from 1.0×10^2 to 1.6×10^4 CFU.100 mL^{-1} while enterococci counts were in the range of 3.3×10^1 CFU.100 mL^{-1} to 5.1×10^3 CFU.100 mL^{-1}. High levels of faecal indicator bacteria were also observed in the Buffalo River (Chigor et al., 2013) and in the Apies River (Ekwanzala et al., 2017*)* where the abundance of Enterococci reached the concentration of 10^5 CFU.100 mL^{-1}.

Based on the data analysis of *E. coli* and intestinal enterococci abundances observed in surface waters from many urban areas, it appears that their abundances are relatively low compared to the values observed in the outlet of wastewater treatment plants in Europe (Ouattara et al., 2014). This is very surprising when we consider the lack of sanitation systems in most cities in developing countries. We also observed that in most of the sampling sites, abundances of IE were higher than those of *E. coli* particularly in Aghien Lagoon and its tributaries. From literature, *E. coli* abundance measured in the waters (wastewaters and surface waters) is most of the time higher than that of intestinal enterococci. This result is reported by Ouattara et al. (2011, 2014) in wastewaters in Belgium. In surface water, several authors also

showed that the abundance of *E. coli* is higher than that of IE. For example, Passerat et al. (2011) observed in Seine River that the abundance of *E. coli* (1.5E+06 (100 mL)$^{-1}$) was three fold higher than that of IE (4.0E+05 (100 mL)$^{-1}$). Indeed, good correlation between both indicators (*E. coli* and intestinal enterococci) has been demonstrated (Farnleitner et al., 2010; Ouattara et al., 2014). But in this study, due to the fact that sometimes IE abundance was higher than that of *E. coli* , the correlation found is not very good (R^2 = 0.6, n = 40). A possible response to the poor correlation observed in the Aghien Basin Lagoon and its tributaries is that they received wastewaters from septic tanks overfilling. After a long period of transition in these septic tanks, sewage water spilled from septic tank. And then, these waters are drained into lagoon and its tributaries. This fact combined to the high resistance of IE compare to *E. coli* in surface waters may explain this poor correlation between *E. coli* and IE.

In the main tributaries, abundances of faecal indicator bacteria are higher than those observed in the Aghien Lagoon. These tributaries are more impacted by faecal pollution than the Aghien Lagoon. Among these tributaries, Djibi River is much more affected by faecal pollution than the others tributaries. The lower levels of dissolved oxygen and higher abundance of faecal bacteria indicated that, Djibi River is strongly impacted by sewage water. Faecal pollution is particularly pronounced in Djibi River because of its low flow rate. In Bété River and Mé River, their relatively high flow rates contribute to reduce the impact of the faecal pollution (dilution effect). When comparing the contribution of tributaries which impacted the Aghien Lagoon, we observed that during the dry season, the fluxes of faecal bacteria injected by Djibi River in the lagoon are smaller than those of Bété River. During the rainy season, the fluxes of faecal bacteria discharged by Djibi River are in the same order of magnitude with those of the Bété River. At the same time, the student's test performed to evaluate the contribution of the rivers indicated that there is no significant difference between Djibi River and Bété River (p value > 0.5).

Conclusion

Globally, the abundances of intestinal enterococci were higher than the acceptable level for bathing, recreational activities or drinking water, indicating that the water of Aghien Lagoon is impacted by domestic sewage waters. Among the tributaries, Djibi River presented the higher levels of faecal bacteria and low levels of dissolved oxygen, indicating that this tributary is much more affected by wastewater pollution compared to Mé River and the Bété River. The contributions of tributaries in terms of faecal bacteria discharged into the Aghien Lagoon are not negligible and these contributions are significantly different between the dry and the rainy season. In order to preserve the lagoon water quality and to promote its potential uses for bathing, irrigation or drinking water production, the implementation of wastewaters treatment plants in Djibi Basin and Bété Basin is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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