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Article

# Environmental Factors and the Microbial Quality of Urban Drinking Water in a Low-Income Country: The Case of Madagascar

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**Abstract:** Access to piped water is often limited to urban areas in low-income countries, and the microbiological quality of drinking water varies due to technical and environmental constraints. To analyse the parameters that modulate the contamination of these systems, this study examines 16 years of microbial quality data for water supplied in 32 urban areas of Madagascar. A discriminant statistical approach and agglomerative hierarchical clusters were applied to environmental and climatic data. The microbial contamination varied between sites from 3.3 to 17.5%, and 78% of the supply systems showed large variations between years or months. Agglomerative hierarchical clusters (AHCs) revealed four supply system profiles that share a similar bacteriological evolution. Heavy rainfall and dry periods sustained increasing contamination, as reflected in levels of spores of sulphite-reducing clostridia (SSRC) and/or total coliforms (TC). SSRC were dominant in three profiles, with faecal indicator bacteria (FIB) dominant in the other. Principal component analysis demonstrated the main drivers of contamination: type of water source, implemented treatment, location of the site, population growth, lack of protection, agriculture, urbanization/sanitation, and flooding threats. Contamination increased over the 16-year period, reaching alarming levels. The protection of water sources should be a concern for public authorities.

**Keywords:** water supply; spores of sulphite-reducing clostridia; total coliforms; Madagascar; climate change; agriculture

## 1. Introduction

Access to safe and continuous water supply is an essential prerequisite for health, and poor water supply is associated with significant health risks [1]. Nearly 842,000 people are estimated to die each year because of unsafe drinking water, inefficient sanitation, and poor hand hygiene [2]. Most economic activities are concentrated in metropolitan areas. The United Nations predicts that half the population in Africa and Asia will be living in towns by 2030. Towns in low and middle income countries (LMICs) are growing at a rate of five million residents per month, essentially due to natural population increase (50%) and migration from rural areas (25%) [3]. Urbanization presents opportunities and challenges for poverty reduction [4], but rapid urbanization and inadequate urban planning can disturb the functional organization of the city [5]. This results in the development of informal settlements and slums. Around 62% of the urban population of sub-Saharan Africa lives in slums, with limited access to good quality drinking water and sanitation facilities [6]. In this context, surface water is often

contaminated by animal and human faeces, in part due to septic tank and sewage discharge [7,8]. Default in water supply (i.e., ineffective water treatment, frequent shortages, or low pressure) and direct use of raw water for drinking [9] thus result in a high incidence of waterborne diseases.

The Millennium Development Goals focus on access to improved water sources, which does not reliably predict the microbial safety of drinking water [10]. Madagascar has not yet achieved these goals, offering only limited access to improved sources of water and facing many challenges to improving access to an adequate and safe drinking water supply for its population [11]. Nearly half of the Malagasy population lives in severe poverty [12], and the urban population is growing at twice the rate of the rest of the world (annual rate of 4.69%, 2010–2015). Nowadays, the city of Antananarivo has more than one million inhabitants, while five other cities have populations ranging from 300,000 to 100,000 inhabitants (Toamasina, Antsirabe, Fianarantsoa, Mahajunga, and Toliara) [13]. Supply of water and electricity is managed by a state-owned service (JIRAMA: Jiry sy Rano Madagasikara), which only concentrates on urban areas. Aside from private customers, the water systems also provide many urban public kiosks or taps, maintained by non-governmental organizations or community associations. The national water supply system is old and faces a rapidly growing population. However, despite poor management and increasing water demand, 82% of the urban population has access to improved water sources, while only 18% has access to improved sanitation facilities. [11]

Climate change is threatening water quality as water supply is dependent on surface water and rarely groundwater. The quality of water production can thus be impacted by climate conditions, especially if the purification system is overloaded [14]. Intensive rainfall, alternation between dry and wet periods, massive deforestation, and slash-and-burn agricultural methods strongly increase soil erosion [15,16]. These are among the most significant threats to the water sources of Madagascar, where some of the rivers carry red water. In addition, increasing periods of drought and intensity of rain exacerbate flooding and water contamination. In the past 20 years Madagascar has experienced 35 cyclones, 8 floods, and 5 periods of severe drought, with high variation in water quality [17]. Open defecation, lack of sanitation, heavy rainfalls, and hurricanes are also significant threats for the urban water sources [18,19].

In terms of delivery of water, with a rapidly increasing population new areas have to be equipped with treatment stations. It is time to define the best design for the new equipment. The aim of this study is to analyse data and parameters describing each water supply network already working in the country to determine the contamination response to climatic and environmental threats and to propose the best strategy of treatment for the future stations. For this purpose, Madagascar has a national water collection system to survey water quality that provides samples going back decades. These samples are collected monthly or daily depending on the site. For the last 20 years, our laboratory has overseen the microbial testing for all samples collected all over the country. The first part of the study analyses samples collected in towns other than the capital.

Environmental threats can heavily impact water quality as the catchment of surface water is widely used for urban water supply in the eastern highlands and in east littoral area. Groundwater resources are available in the sedimentary area of the extreme south, northwest, and alluvial aquifers of the highlands [20]. The rainfall regime is thus very important in this system for providing both surface water and as a source of contamination of resources.

## 2. Materials and Methods

### 2.1. Sampling Strategy and Microbial Analysis

A national strategy for water sampling was set up based on the JIRAMA organization, which comprises seven divisions throughout the whole country. As shown in Figure 1 ([www.jirama.mg](http://www.jirama.mg)), the headquarters in Toliara manages 10 sites in the south, including Bezaha and Toalagnaro. It borders the Fianarantsoa division, which manages nine sites including Farafangana, as well as the Antsirabe division, which has six sites including Antanifotsy. The Antananarivo division manages 10 sites located

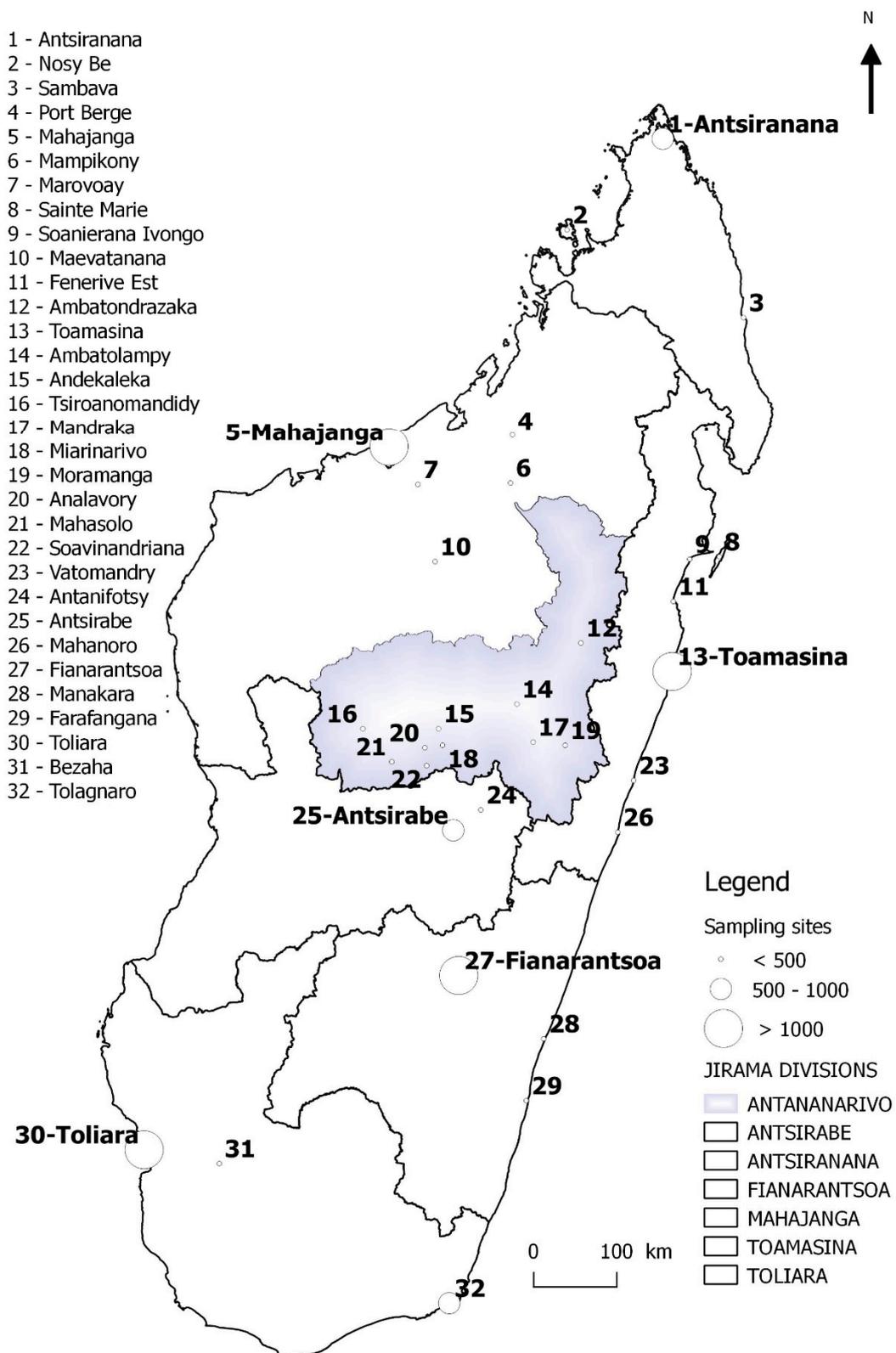
in the highlands from Ambatondrazaka to Tsiroanomandidy, including the capital. The Tamatave division manages six sites on the east side of the island, from Soaniera Ivongo to Mahanoro. The Mahajunga division manages the west coast and part of the western highlands, including the cities of Maevatanana, Marovoay, and Port Berge. The north division (Antsiranana) includes Nosy-be and Sambava.

Large cities and urban municipalities are sampled monthly and quarterly, respectively, whereas the distribution system of the capital Antananarivo is sampled daily. Each sampling leads to 2–4 water samples at the same site, which can be private premises, community taps, or administrative buildings. Each sample is collected in sterile 500-mL containers with 10 mg of sodium thiosulfate, which are stored at 4 to 10 °C and delivered to the laboratory within 18 to 24 h.

Legal microbial limitations are first based on faecal indicator bacteria (FIB), including *Escherichia coli* (EC) and intestinal enterococci (IE) [18]. Non-compliance (positive samples) is registered when FIB, spores of sulphite-reducing clostridia (SSRC), IE, total coliforms (TC), and EC are detected in 100 mL. Detection of spores of sulphite-reducing clostridia (SSRC) and total coliforms (TC) can be linked to the water quality of pipes or to the effectiveness of water treatment procedures [18]. The microbiological examination of samples is done following standardized methods of filtration or inclusion (International Organization for Standardization). Since July 2014, analyses of TC, EC, and IE have been performed using IDEXX US Water Microbiology Colilert<sup>®</sup>-18, Quanti-Tray<sup>®</sup>, Enterolert<sup>®</sup>-DW, and Quanti-Tray<sup>®</sup> kits. Since November 2010, SSRC have been examined after filtration of 100 mL of sample (NF EN 26461-2), instead of the incorporation of 20 mL (NF T90-415).

## 2.2. Samples Selected for the Analysis

From January 1999 to December 2015, water samples were collected in 66 cities and urban municipalities, out of which only the most complete series of data over the 16-year period were retained for analysis. Some sets were removed, such as the data from the year 2009, which was marked by a political crisis. Sites with one year or two consecutive quarters of missing data were also removed. The city of Antananarivo provided more than half of the total samples but was also excluded from the study in order to conduct a separate analysis. Overall, 32 out of 66 public water supplies were selected for the analysis, providing data on 12,495 drinking water samples (described in Table S1). Seven major cities (Toamasina, Mahajanga, Toliara, Fianarantsoa, Antsirabe, Antsiranana, and Tolagnaro) provided 6855 samples (55% of the total). They were investigated twice per month, with a total of eight water samples per month and per city. Seven smaller towns in the western and eastern highlands (Ambatondrazaka, Andekaleka, Moramanga, Mandraka, Ambatolampy, Analavory, and Soavandriana) and one on the east coast (Manakara) were sampled at an average of two samples per month. They provided 2499 samples (20% of the total). The other cities were sampled every two months (Nosy-Be, Farafangana, Fenerive Est, Soaniera Ivongo, Vatomaniry, Maevatanana, and Marovoay) or every three months (Sambava, Mahanoro, Sainte Marie, Antanifotsy, Mahasolo, Port-Bergé, Tsiroanomandidy, and Mampikony), with two samples per survey. These last two groups provided 1769 (14%) and 1372 (11%) samples, respectively (Figure 1).



**Figure 1.** Map of selected water supply systems. The map shows location of the 32 studied water supply systems and the administrative division of the JIRAMA in Madagascar. Three-quarters of these supply systems provided fewer than 500 samples per system over 16 years. A major part of these systems is located from the east littoral area to the western highlands (Toamasina and Antananarivo divisions).

The sources of water varied according to the site. Fifteen water supply systems use groundwater, providing 5040 samples (40%), while the others relied on surface water (7455 samples, 60%) (Table 1).

**Table 1.** Repartition by water sources and treatment processes of 12,495 samples provided by 32 of Madagascar's water supply systems from 1999 to 2015.

|                                 | Conventional Treatment |                          | Unconventional Treatment |                           | N of Stations | Total (%)                  |
|---------------------------------|------------------------|--------------------------|--------------------------|---------------------------|---------------|----------------------------|
|                                 | Classic                | Classic + Neutralization | Chlorination             | Filtration + Chlorination |               |                            |
| <b>Surface Water</b>            |                        |                          |                          |                           |               |                            |
| Lakes                           | 1113                   | 1419                     | 0                        | 0                         | 6             | 2532 (20%)                 |
| Rivers                          | 3487                   | 1436                     | 0                        | 0                         | 11            | 4923 (39%)                 |
| <b>Total</b>                    | 4600                   | 2855                     | 0                        | 0                         |               | 7455 <sup>2</sup> (60%)    |
| <b>Groundwater</b>              |                        |                          |                          |                           |               |                            |
| Boreholes                       | 0                      | 1335                     | 1458                     | 0                         | 5             | 2793 (22%)                 |
| Wells                           | 144                    | 1014                     | 0                        | 0                         | 6             | 1158 (9%)                  |
| Water springs                   | 0                      | 533                      | 248                      | 308                       | 4             | 1089 (9%)                  |
| <b>Total</b>                    | 144                    | 2882                     | 1706                     | 308                       |               | 5040 (40%)                 |
| <b>Total number of stations</b> | 11                     | 16 <sup>3</sup>          | 4                        | 1                         | 32            |                            |
| <b>Total (%)</b>                | 4744 (38%)             | 5737 (46%)               | 1706 (14%)               | 308 (2%)                  |               | 12,495 <sup>1</sup> (100%) |

<sup>1</sup> The 12,495 collected drinking water samples were reported by water sources and treatment processes. <sup>2</sup> 7455 (60%) samples were provided by 17 surface water sources. <sup>3</sup> 16 sites that were treated by a conventional process with final neutralization provided 46% of samples.

Rivers supplied water for cities located in the eastern highlands (Andekaleka), from the forest of Moramanga to the east coast (Toamasina, Soanierana Vongo, Sainte Marie), on the southeastern coast (Manakara, Fianarantsoa), in the southern (Fianarantsoa and Farafangana) and central highlands (Tsiroamomandidy), and in the north (Port Berge and Antsiranana). Lakes and springs are also used for water supply in the central highlands (lakes: Antanifotsy, Mandraka, Ambatolampy, and Antsirabe; springs: Miarinarivo, Soavinandriana, Analavory, and Ambatondrazaka). Wells and boreholes were mainly used in cities in the west (Mampikony, Maevatanana, Mahajunga, and Marovoay) and south (Bezaha and Toliara). Sand sheets were used on the east coast (Vatomandry, Mahanoro, and Sambava) [20]. Conventional or unconventional treatment processes were implemented to purify these water sources. The conventional process consists of the following sequence: (1) raw water intake; (2) coagulation; (3) flocculation; (4) sedimentation; (5) filtration; (6) disinfection by chlorination; and (7) facultative neutralization by the addition of lime. The process was applied to 10,481 samples, of which 5735 were subjected to a final neutralization step. The unconventional processes consisted of the following basic steps: (1) raw water intake; (2) basic filtration; and (3) disinfection by chlorination, or (1) raw water intake; and (2) disinfection by chlorination. These processes were applied to only 2014 samples (Table 1).

### 2.3. Parameters Selected for the Description of the Water Supply Systems

Climate and temperate data were extracted from <https://fr.climate-data.org> [21] based on the Köppen–Geiger classification (Cwa, Cwb, Cfa, Aw, Am, Af, Bwh, and Bsh) [22]. The daily rainfall in each city from 2007 to 2015 (no previous complete data for all concerned cities) was obtained from the International Institute for Research on Climate and Society (IRI). The climate in Madagascar is mainly tropical (type A, Köppen–Geiger) along the coast, arid in the south (type B), and temperate inland (Type C) [21,22]. The average annual rainfall is around 1500 mm, but with large regional variation from 3000 mm in the east littoral area to less than 400 mm in the extreme south. The whole country experiences a rainy season from November to March and a dry season from April to October. Inter-season periods are less frequent or shorter according the climate type.

Settlements were defined as the population supplied by a water system (hamlet, village, town, and city) and were estimated according to the last one population census conducted in 1993 [23]. Technical variables describing the water system were recorded from JIRAMA sources, for which latest data are from 2015 [24], including: (1) the type of treatment process; (2) the monthly production of treated water (only in 2015); (3) the monthly yield or efficiency (percent of produced water effectively sold, only in 2015); (4) the analytical strategy (monthly count of samples collected from each supply system); and (5) the nature of the water source (rivers, lakes, wells, boreholes, or springs).

The environmental threats are grouped into six major categories: (1) lacking protection of the source (which includes the encroachment of riparian areas by settlers, peasants, or livestock); (2) watershed degradation (deforestation, bush fires, riparian erosion phenomena, or disturbance of surface water flow); (3) nuisances related to agriculture (when agricultural activities are found upstream of the catchment); (4) urbanization and sanitation problems (when sanitation or waste management systems are inefficient and when the expansion of the city disturbs the resource); (5) floods or infiltrations in the resources or in the network and illegal connections; and (6) twinning of hydroelectric and drinking water treatment plants. The 2008 and 2012 environmental reports provide a description of all threats that could impact the quality of water sources for each supply system in Madagascar. For each city, the increase of the water demand was estimated based on the population growth between the 1993 census and the 2013 estimation (in percent of increase) [23].

#### 2.4. Statistical Analysis

The frequency of microbial contamination of samples was calculated for the monthly, seasonal, yearly, and 16-year periods as the ratio of the number of positive samples to the total number of samples in the period considered (in percent). Each specific measure of contamination (SSRC, IE, EC, TC, or FIB) was expressed as the ratio of the number of positive samples to the total number of samples. The rainfall data were summarized as the cumulative precipitation per season (quarterly starting in December) for each site and an average for relevant group of sites was calculated.

The statistical analysis was done in XLSTAT (Addinsoft: New York, NY, USA, 2016), with a significance level set at 0.05. A non-parametric Kruskal–Wallis test was used to analyse differences between populations [25]. For each site, identification of the main type of contamination was done by computing over 16 years the Pearson's correlation coefficient between the frequency of positive samples in each indicator (specific contamination) and the frequency of total positive samples. Values of 0.3 to 0.7 suggest a weak correlation, and values of 0.7 to 1 suggest a strong positive correlation [26].

Contamination of the water systems were compared over the sites, by building agglomerative hierarchical clusters (AHC) [27,28] using: (1) annual and monthly cumulative data of sample contamination (Additional Materials, Table S2); (2) overall contamination frequency over 16 years (Table S2); and (3) correlation coefficients between specific contamination and overall contamination (Table S3). Absence of significant difference between sites within a specific cluster of contamination was tested using Kruskal–Wallis. Difference between clusters was assessed using analysis of variance, (ANOVA,  $p < 0.05$ ). For each cluster, temporal trends of contamination were searched by plotting frequencies of total or specific contaminant over time (Figure 6). Statistical significance of trends was tested using a Mann–Kendall trend test for temporal series.

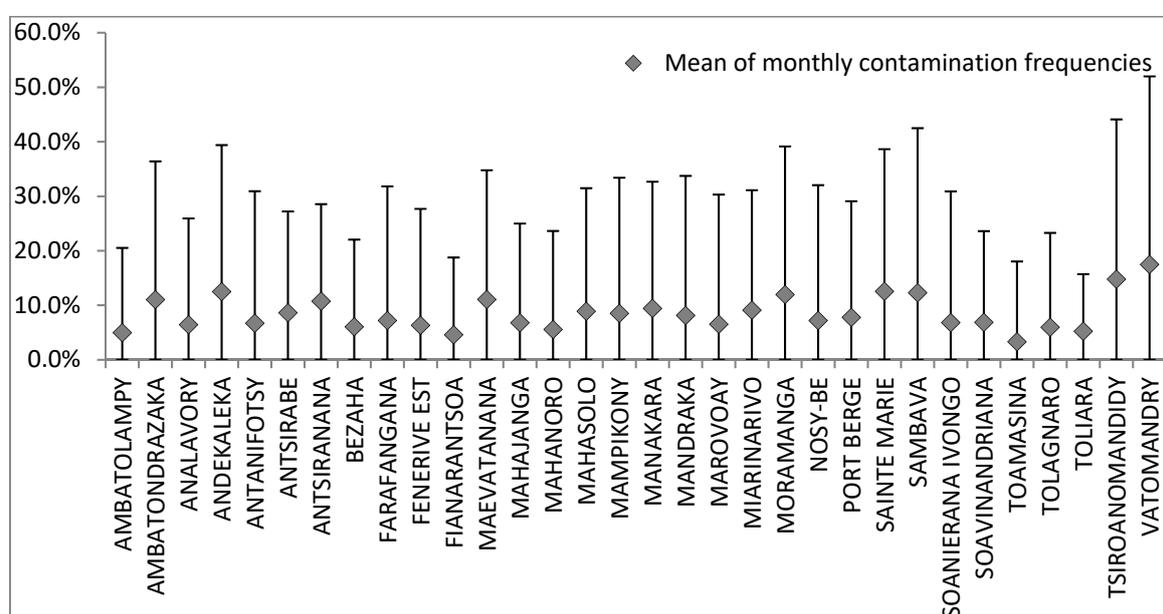
Typology of all the water systems was built using three independent sets of parameters used as active variables: (1) environmental variables (water source, region, type of climate); (2) technical parameters related to the water system (water treatment, monthly water production, water yield, and analytical pressure); and (3) variables describing the environment of the water source (land use and perimeter of protection, watershed degradation, nuisances related to agriculture, expansion of the city impacting sanitation, waste management, and population growth) (Tables S3–S5, respectively) (Pearson's matrix in Table S5). Three principal component analyses (PCAs) were built using these sets. Previous clusters of contamination were used as supplementary variables in the PCA map to detect the major factors characterizing each clusters of contamination. Parameters associated with

the contamination of water were determined using Pearson’s correlation coefficients with the clusters of contamination.

### 3. Results

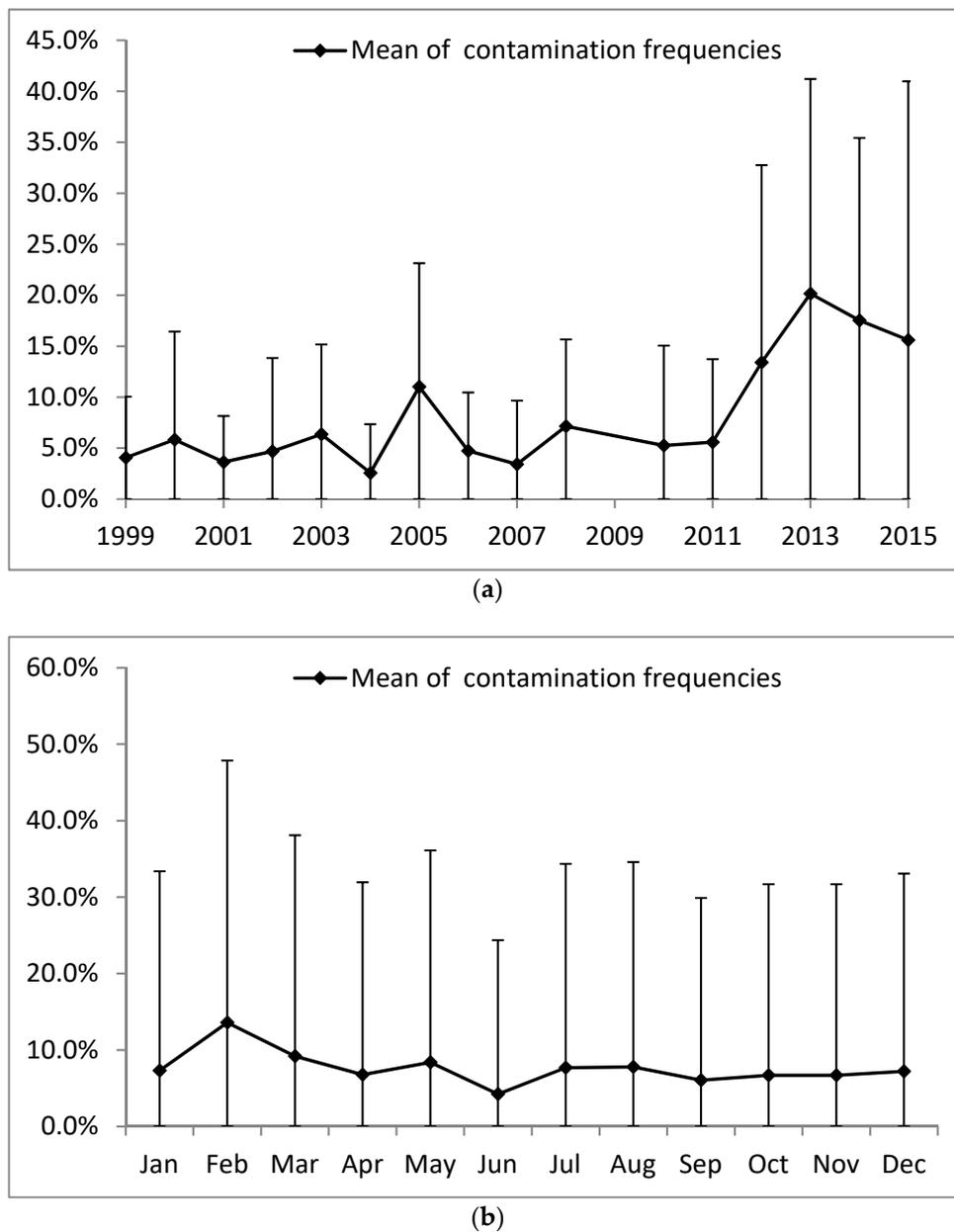
#### 3.1. Spatio-Temporal Variation of Water Contamination

Over the whole period, 944 (7.5%) of the 12,495 samples were contaminated (479, 331, 303, and 126 samples with SSRC, TC, IE, and EC, respectively). The frequency of contamination varied with the site of sampling and ranged from 3.3% (Toamasina) to 17.5% (Vatomandry) (Figure 2). The overall frequency of contamination was greater than 10% for nine sites, while it was less than 6% in eight sites. Differences between sites were significant (Kruskal–Wallis,  $p < 0.001$ ). Overall, and over the 16 years, the occurrence of contaminated samples was punctual (median and third quartile = 0%). Only two sites experienced more frequent contamination events, namely Antsiranana and Vatomandry (third quartile = 14.3% and 28.6%, respectively).



**Figure 2.** Mean of monthly contamination frequencies related to 32 of Madagascar’s drinking water supply systems from 1999 to 2015, except 2009. Means (dots) with error bars show the proportion of contaminated samples over 16 years and its variation within each sampling system.

For half of the sites, monthly or annual variations of contamination were high (variation coefficient greater than 100%). Port Berge was the most impacted by temporal variation (variation coefficient VC 192% and 197%, respectively), while Toliara was the most stable (72% and 76%, respectively). In addition, means of contamination frequencies increased from 2012, with high variation between supply systems during the last years. (Figure 3a). The average contamination frequency values for the rainy months of February were higher ( $13.6 \pm 34.2\%$ ), but also showed the most standard deviation (spatial and annual high variation, Figure 3b).



**Figure 3.** Means of contamination frequencies related to 32 of Madagascar's drinking water supply systems from 1999 to 2015 (except 2009) by specific periods. Means (dots) with error bars show: (a) the average of contaminated samples proportion by year (from 1999 to 2015); (b) the average of contaminated samples proportion by type of months (from January to December).

### 3.2. Definition of Profiles of Water Contamination Based on Temporal Variation and Most Relevant Contamination

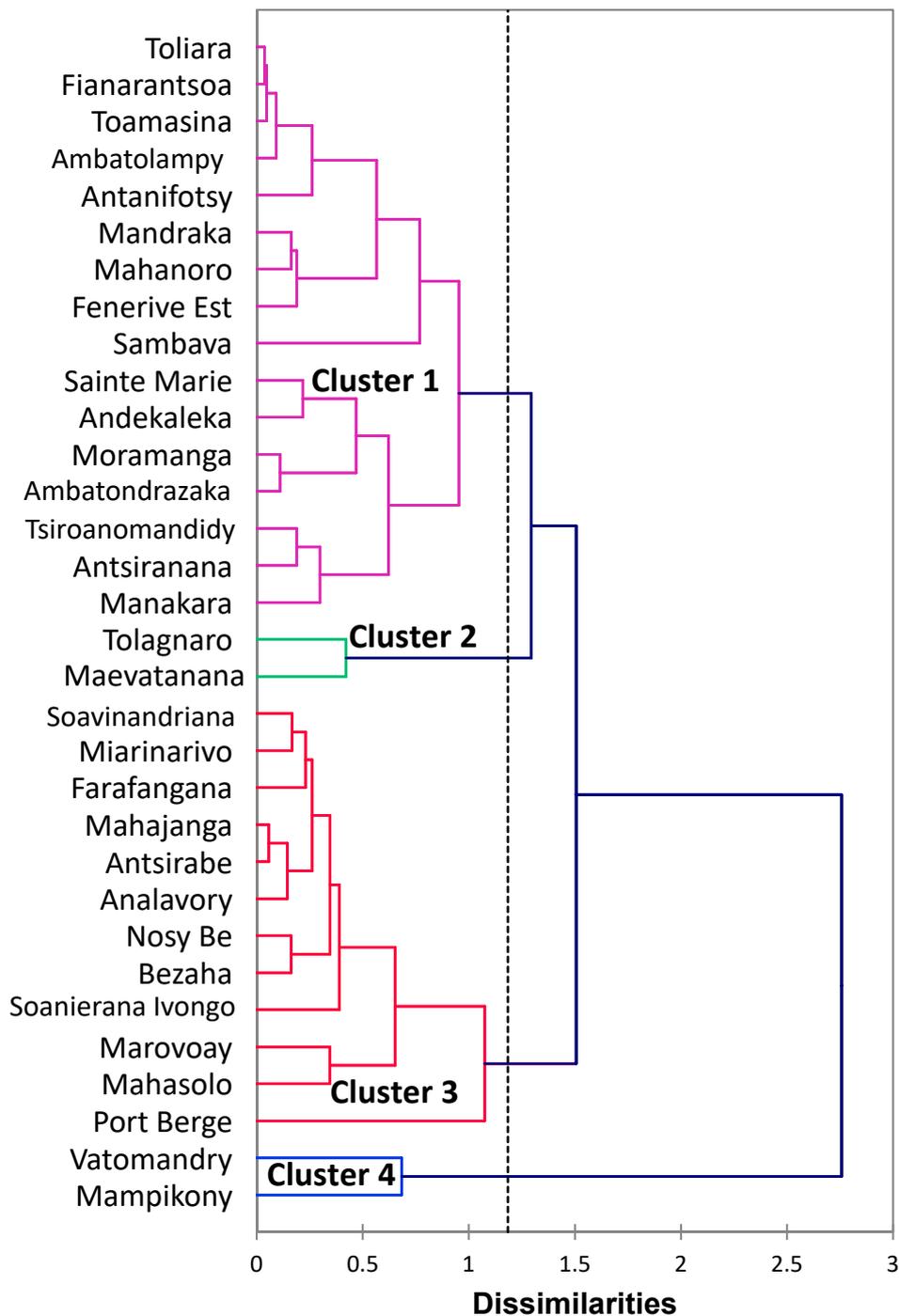
Contamination of samples can be sustained by different types of bacteria, such as EC, IE, TC, and SSRC (Pearson's test over the 16-year period, Table 2). Half of the sites with a low level of contamination (including Toliara, Fianarantsoa, and Toamasina) showed no predominance of any type of contamination and low monthly variation of contamination. For the others (Tolagnaro, Mahanoro, and Bezaha), samples were more frequently contaminated by SSRC and IE and harboured high monthly and annual variation of contamination. The frequency of contamination increases when one type of bacteria predominates, suggesting presence of a main source of contamination releasing bacteria in the system. For some site, a stable predominant contamination was observed throughout the 16 years (correlation between a specific pathogen and total contamination  $>0.7$ ) (Table 2).

**Table 2.** Correlation between specific and overall contamination frequency for 32 Malagasy water supply systems. From 1999 to 2015 (except 2009), Pearson's correlation coefficients were reported.

| Division                | Sites                          | FIB <sup>1</sup> | IE <sup>2</sup> | EC <sup>3</sup> | TC <sup>4</sup> | SSRC <sup>5</sup> |
|-------------------------|--------------------------------|------------------|-----------------|-----------------|-----------------|-------------------|
| Toamasina               | Vatomandry <sup>Bcn</sup>      | 0.290            | 0.290           | 0.144           | 0.357           | 0.876             |
|                         | Sainte Marie <sup>Rc</sup>     | 0.844            | 0.816           | 0.442           | 0.526           | 0.394             |
|                         | Soaniera Ivongo <sup>Rc</sup>  | 0.744            | 0.744           | 0.000           | 0.641           | 0.259             |
|                         | Fenerive Est <sup>Wcn</sup>    | 0.620            | 0.620           | 0.000           | 0.436           | 0.671             |
|                         | Mahanoro <sup>Wc</sup>         | 0.489            | 0.489           | 0.000           | 0.345           | 0.860             |
| Toamasina <sup>Rc</sup> | 0.535                          | 0.535            | 0.308           | 0.578           | 0.656           |                   |
| Antananarivo            | Tsiroanomandidy <sup>Rcn</sup> | 0.411            | 0.411           | 0.316           | 0.489           | 0.785             |
|                         | Andekaleka <sup>Rc</sup>       | 0.723            | 0.575           | 0.475           | 0.387           | 0.552             |
|                         | Moramanga <sup>Rc</sup>        | 0.553            | 0.419           | 0.387           | 0.576           | 0.724             |
|                         | Ambatondrazaka <sup>Su</sup>   | 0.642            | 0.596           | 0.326           | 0.520           | 0.768             |
|                         | Miarinarivo <sup>Scn</sup>     | 0.717            | 0.662           | 0.326           | 0.570           | 0.536             |
|                         | Mahasolo <sup>Wcn</sup>        | 0.734            | 0.541           | 0.541           | 0.817           | 0.483             |
|                         | Mandraka <sup>Lc</sup>         | 0.596            | 0.596           | 0.000           | 0.341           | 0.778             |
|                         | Soavandriana <sup>Scn</sup>    | 0.628            | 0.628           | 0.235           | 0.756           | 0.628             |
| Antsirabe               | Analavory <sup>Su</sup>        | 0.781            | 0.695           | 0.488           | 0.649           | 0.600             |
|                         | Ambatolampy <sup>Lcn</sup>     | 0.588            | 0.454           | 0.370           | 0.588           | 0.698             |
| Antsirabe               | Antsirabe <sup>Lcn</sup>       | 0.677            | 0.629           | 0.359           | 0.619           | 0.609             |
|                         | Antanifotsy <sup>Lc</sup>      | 0.727            | 0.727           | 0.414           | 0.590           | 0.661             |
| Fianarantsoa            | Manakara <sup>Rc</sup>         | 0.674            | 0.448           | 0.492           | 0.674           | 0.607             |
|                         | Faranfangana <sup>Rcn</sup>    | 0.694            | 0.694           | 0.342           | 0.647           | 0.420             |
|                         | Fianarantsoa <sup>Rc</sup>     | 0.660            | 0.537           | 0.378           | 0.574           | 0.706             |
| Toliara                 | Bezaha <sup>Bu</sup>           | 0.859            | 0.754           | 0.398           | 0.398           | 0.634             |
|                         | Tolagnaro <sup>Lc</sup>        | 0.220            | 0.220           | 0.000           | 0.382           | 0.915             |
|                         | Toliara <sup>Bu</sup>          | 0.598            | 0.534           | 0.421           | 0.583           | 0.671             |
| Mahajanga               | Maevatanana <sup>Wcn</sup>     | 0.426            | 0.000           | 0.426           | 0.543           | 0.884             |
|                         | Mampikony <sup>Wcn</sup>       | 0.431            | 0.431           | 0.000           | 0.500           | 0.804             |
|                         | Port Berge <sup>Rcn</sup>      | 0.751            | 0.562           | 0.562           | 0.751           | 0.630             |
|                         | Mahajanga <sup>Bcn</sup>       | 0.772            | 0.764           | 0.336           | 0.587           | 0.598             |
|                         | Marovoay <sup>Bu</sup>         | 0.848            | 0.719           | 0.504           | 0.671           | 0.504             |
| Antsiranana             | Sambava <sup>Bu</sup>          | 0.476            | 0.386           | 0.386           | 0.515           | 0.798             |
|                         | Antsiranana <sup>Rcn</sup>     | 0.427            | 0.338           | 0.275           | 0.492           | 0.832             |
|                         | Nosy Be <sup>Lcn</sup>         | 0.738            | 0.598           | 0.545           | 0.647           | 0.694             |

<sup>1</sup> faecal indicator bacteria; <sup>2</sup> *Escherichia coli*; <sup>3</sup> intestinal enterococci; <sup>4</sup> total coliforms; <sup>5</sup> spores of sulphite-reducing clostridia. <sup>Bcn</sup>: supplied from boreholes and treated with a final neutralization step; <sup>Rcn</sup>: supplied from rivers and treated with a final neutralization step; <sup>Rc</sup>: supplied from rivers and treated by the classic process; <sup>Wcn</sup>: supplied from artesian wells and treated with a final neutralization step; <sup>Su</sup>: supplied from springs and treated by the unconventional process; <sup>Scn</sup>: supplied from springs and treated with a final neutralization step; <sup>Lcn</sup>: supplied from lakes and treated with a final neutralization step; <sup>Lc</sup>: supplied from lakes and treated by the classic process; <sup>Bu</sup>: supplied from boreholes and treated by the unconventional process. <sup>Wc</sup>: supplied from artesian wells and treated by the classic process.

To analyse fluctuations of contamination of the water systems according to time and their most relevant contamination, the systems were first grouped according to their microbiological contamination characteristics using AHC. Four clusters were identified (Figure 4).



**Figure 4.** Clustering of 32 Malagasy water supply systems according to contamination. Dendrogram shows the Euclidean distance between the different supply systems and the four uneven clusters selected.

The relevance of the system clustering was confirmed by verifying the absence of significant differences between sites within each cluster for monthly and annual contamination frequencies (Kruskal–Wallis,  $\alpha = 0.05$ ). No significant difference was found between sites of Clusters 2, 3, and 4 ( $p > 0.05$ ), but significant differences were found between the sites of Cluster 1 ( $p < 0.05$ ). Cluster 1 was then split into two sub-clusters using its first node (Figure 2). Cluster 1/1 includes Toliara, Fianarantsoa, Toamasina, Ambatolampy, Mandraka, Mahanoro, Fenerive Est, and Sambava (Kruskal–Wallis,  $p = 0.502$ ). Cluster 1/2 includes Sainte Marie, Andekaleka, Moramanga, Ambatondrazaka, Tsiroanomandidy, Antsiranana, and Manakara (Kruskal–Wallis,  $p = 0.985$ ). The contamination of samples increased

annually since 2012 for all clusters, but this increase was significant for only Cluster 3 (Mann–Kendall test,  $p < 0.001$ ) and Cluster 1/2 ( $p = 0.053$ ).

### 3.3. Impact of Rainfall Pattern on Contamination

An ANOVA test performed on the different clusters and the explanatory factors demonstrated significant differences ( $p < 0.05$ ) in the means of contamination for the years 2005, 2010 and from 2012 to 2015 (Figure 5a–f). A significant difference between the clusters has also been demonstrated for the wet months of February and March, as well as for the dry months of July. (Figure 5g–i)

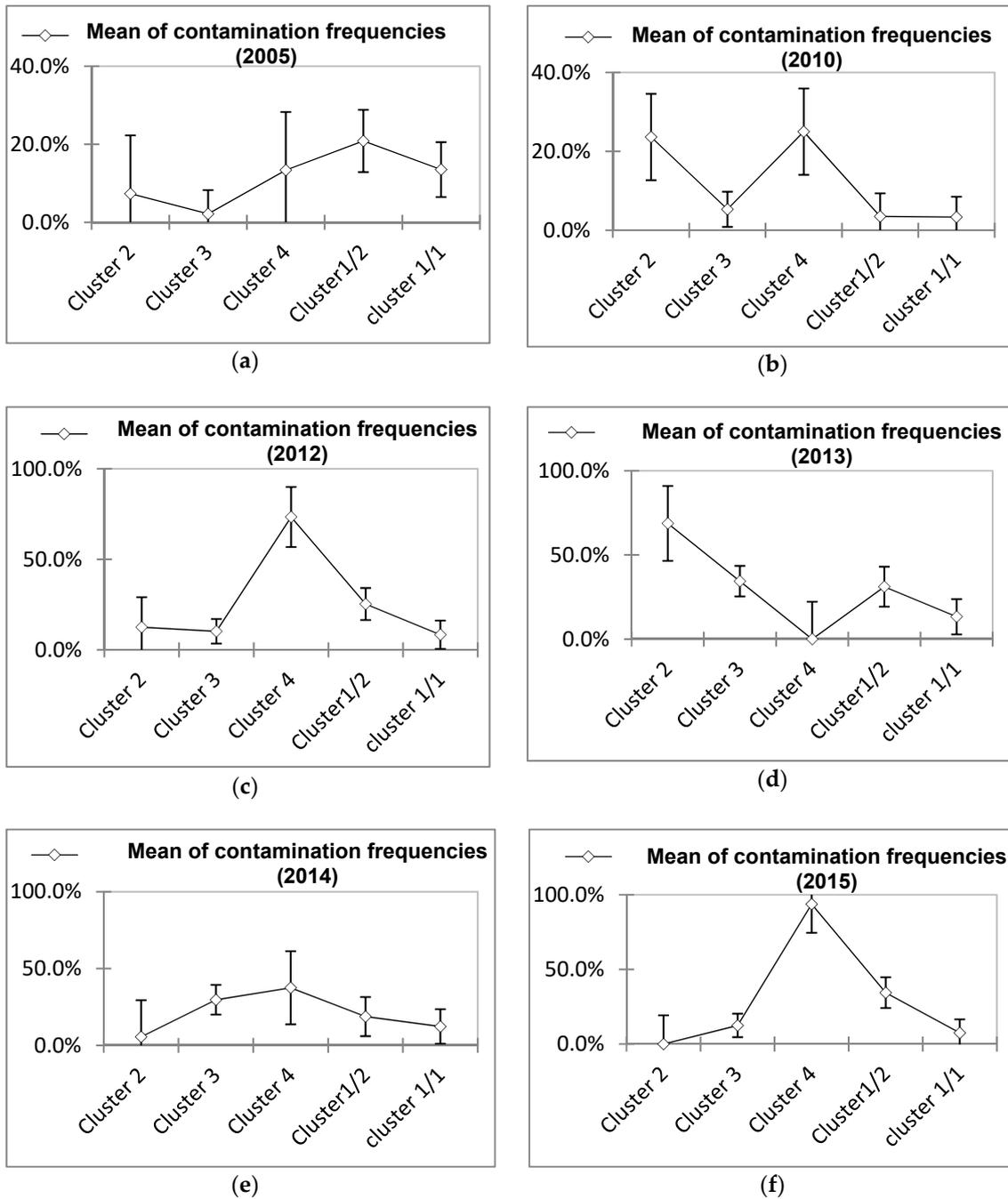
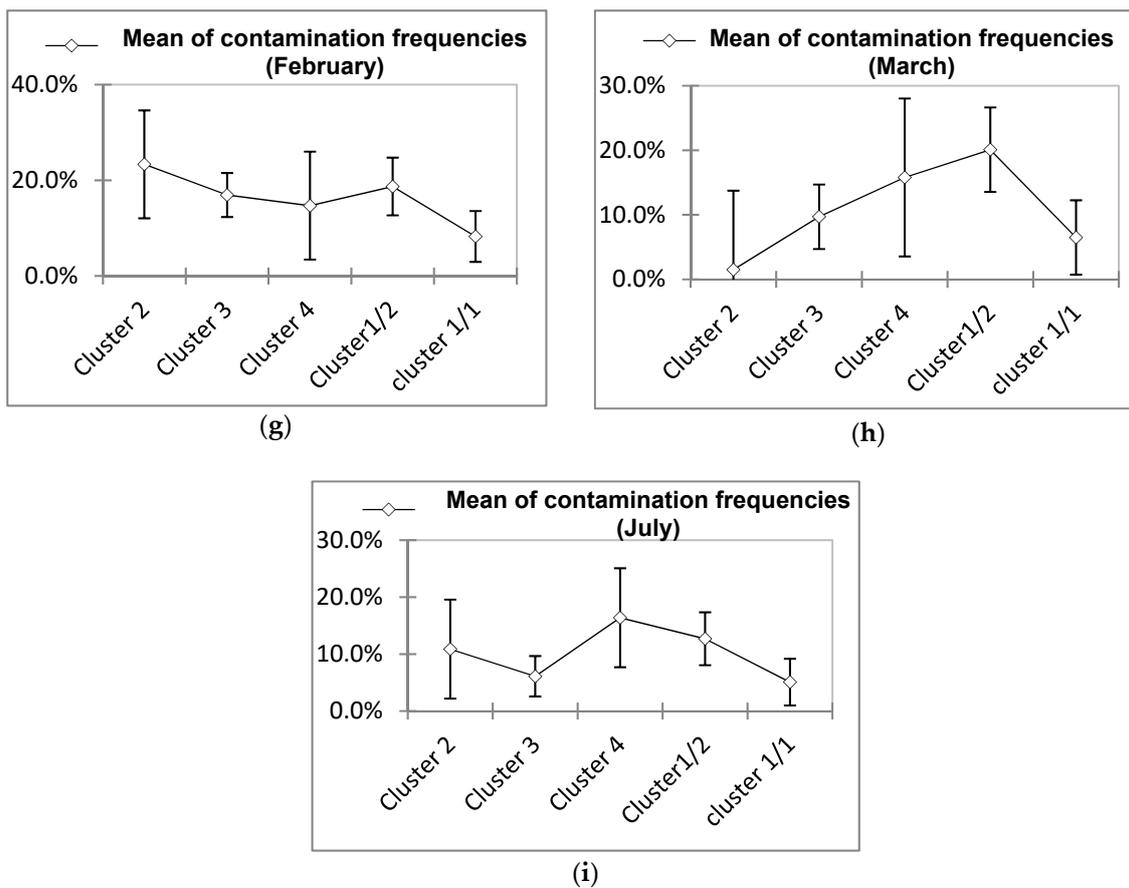


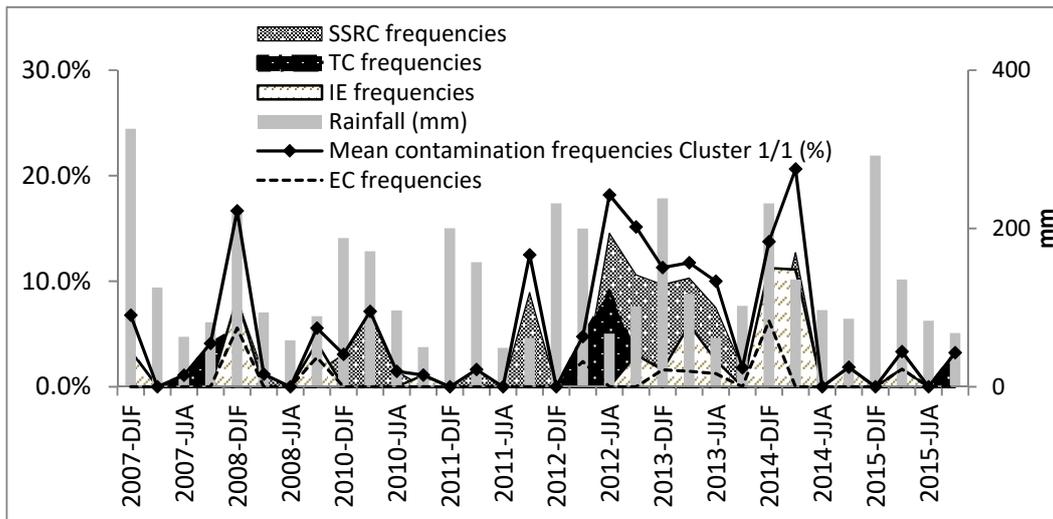
Figure 5. Cont.



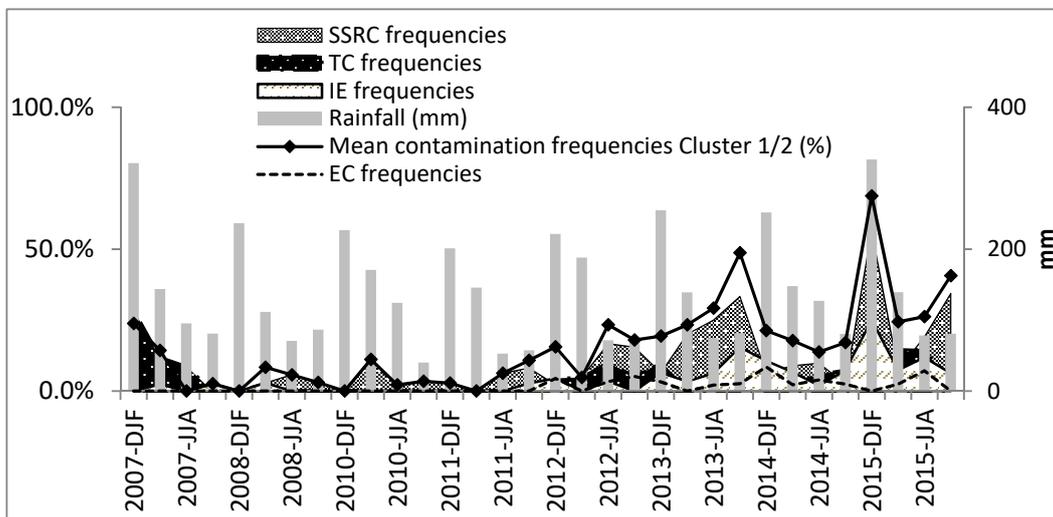
**Figure 5.** Means of contamination frequencies of Madagascar's drinking water systems for periods related to significant differences (ANOVA,  $p < 0.05$ ) between five clusters from hierarchical clustering analysis according to temporal variation and most relevant contamination: (a) for the year 2005; (b) for the year 2010; (c) for the year 2012; (d) for the year 2013; (e) for the year 2014; (f) for the year 2015; (g) for the month of February; (h) for the month of March; (i) for the month of July.

The variation of contamination according to months and years suggested an impact of the weather. To analyse this relation, the fluctuation of contamination of the four described clusters was plotted with the cumulative rainfall of the same period (Figure 6). Strong contamination was statistically associated with heavy precipitation (Figure 6,  $\chi^2$  of trend). In 2013, 2014, and 2015, heavy rainfall from December to February paralleled an increase in sample contamination by IE (Clusters 1, 3, and 4), TC (Clusters 2 and 3), and SSRC (all clusters except 3). Few cases of EC were detected, except during the 2014 wet season for Cluster 1/1. The increase of contamination frequencies was more associated with a conjunction of several bacterial types than a higher density of only one type of bacteria (Clusters 1, 3, and 4).

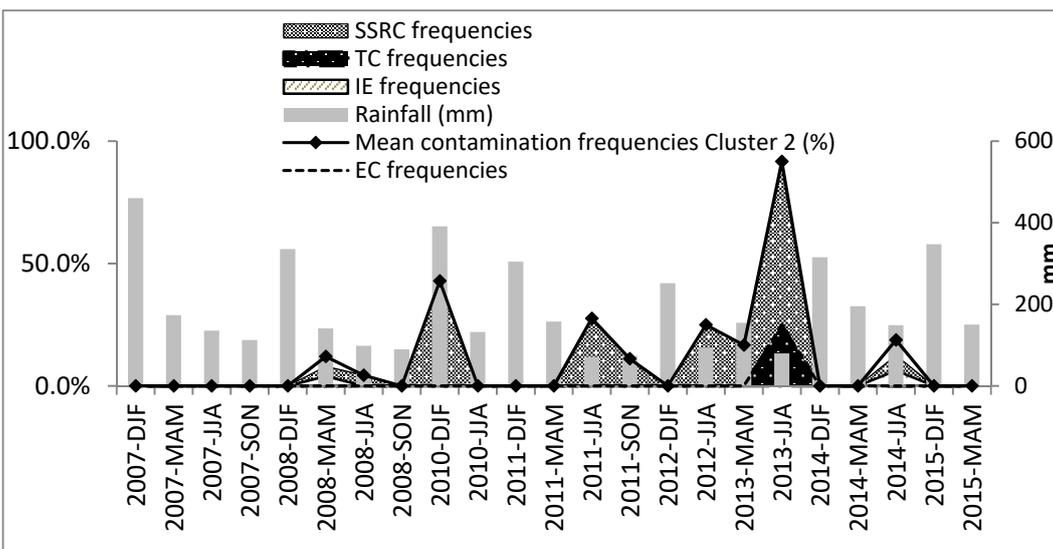
Whatever the season, SSRC contributed to maintaining the contamination of water in all clusters. During the dry period, the reason could have been low flow or low levels of water sources, but in the wet period, it could be linked to erosion phenomena. The frequency of SSRC contamination fluctuates from month to month, which indicates inefficient filtration steps. Similarly, the persistence of TC (clusters 1/2 and 3) in any season can be explained by regular inefficiency of the treatment systems, by survival or multiplication of the bacteria in the water networks themselves, or by problems in network integrity.



(a)

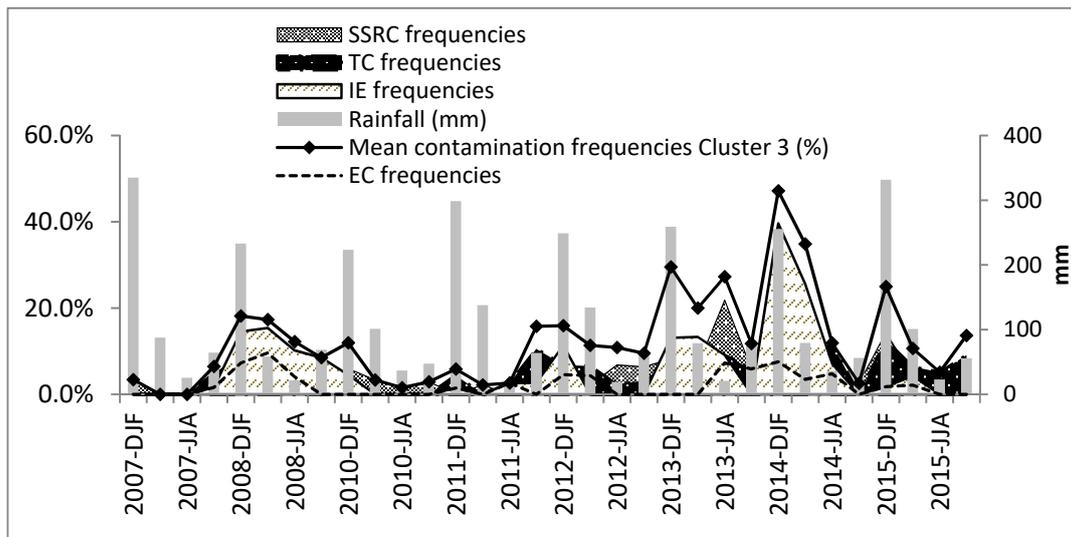


(b)

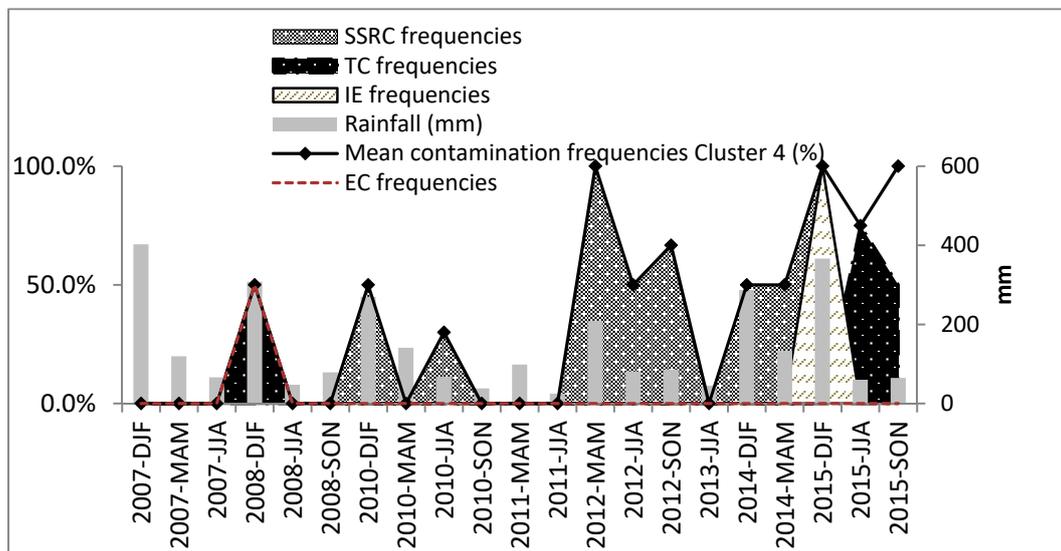


(c)

Figure 6. Cont.



(d)



(e)

**Figure 6.** Relationship between rainfall and contamination of the water samples in 32 Malagasy water supply systems from 2007 to 2015. Graphs show for each quarter (DJF: December to February; MAM: March to May; JJA: June to August; SON: September to November): mean of cumulative rainfall (■); mean of specific contamination frequencies (%) in spores of sulphite-reducing clostridia (SSRC), in intestinal enterococci (IE), in total coliforms (TC), and in *Escherichia coli* (EC); and means of overall contamination frequencies (line —◆—). For the legend of each cluster see Figure 4: (a) Cluster 1/1; (b) Cluster 1/2; (c) Cluster 2; (d) Cluster 3; (e) Cluster 4.

### 3.4. Main Factors Influencing Water Quality (Environmental Clustering)

According to environmental parameters the whole set of water systems, six main component factors were obtained, explaining more than 80% of the total variance of the dataset. The first axis (component) compares the variables “surface water” plus “classic treatment type” to “groundwater” plus “classic + neutralization treatment type” (Figure 7a). This axis also correlates with the environmental threat “population growth”. The second axis had a large positive association with “unconventional treatment type” (45% of the variability of dataset).

Using these main component factors, four groups of systems can be observed on the observations plot (Figure 7b).

Group 1 represents water systems that are supplied by water from conventionally treated surface water with a final neutralization step (Ambatolampy, Antsirabe, Farafangana, Nosy-Be, Port Berge, and Tsiroanomandidy). Agricultural activities were present in the watershed, and more than half of the water systems did not have a protection perimeter. Aside from Ambatolampy, they all had more than 7% contaminated samples in the 16-year study period.

Group 2 is also supplied with surface water treated by conventional treatment without a final neutralization step (Andekaleka, Antanifotsy, Fianarantsoa, Manakara, Mandraka, Moramanga, Sainte Marie, Soanierana Ivongo, Toamasina, and Tolagnaro). With the exception of Sainte Marie, Soanierana Ivongo, and Toamasina, agricultural activities were also recorded in the watershed. Mahanoro is the only system associated with this group that is supplied by groundwater treated with the conventional process with no final neutralization step. In this group, half of the systems had low contamination frequency, whereas the others were highly contaminated.

Group 3 was supplied by groundwater treated by conventional treatment with a final neutralization step (Fenerive Est, Maevatanana, Mahajunga, Mahasolo, Mampikony, Miarinarivo, Sambava, Soavandriana, and Vatomandry). There was no protection perimeter at many sites. One-third of the water sources are subjected to environmental threats resulting from urbanization, poor sanitation, and population growth (more than 9%), favouring faecal contamination (IE). Indeed, SSCr are regularly detected in this group. In contrast, Fenerive Est had the lowest contamination throughout the 16 years, with no environmental threats; boreholes are better protected than wells (both are infrastructures of depth).

Group 4 included sites provided with water springs and boreholes treated by simple filtration or chlorination (i.e., Ambatondrazaka, Analavory, Marovoay, Bezaha, and Toliara). Apart from Ambatondrazaka, these supply systems demonstrated the lowest frequencies of contamination from the 16 years. Ambatondrazaka had a surface water profile similar to Moramanga with agricultural nuisances and was subject to at least two types of environmental threat.

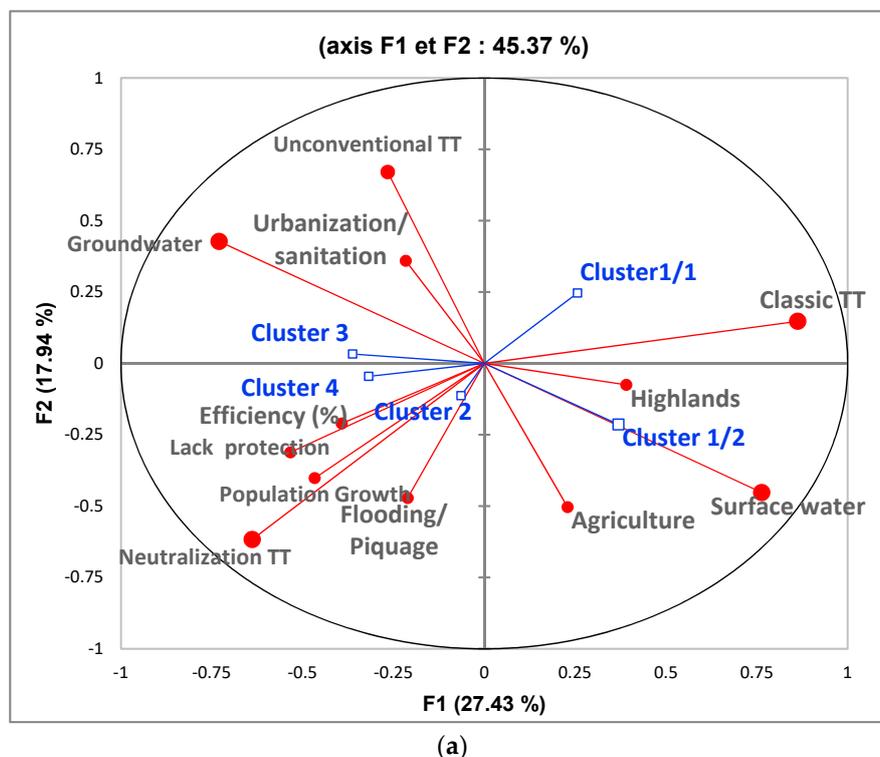
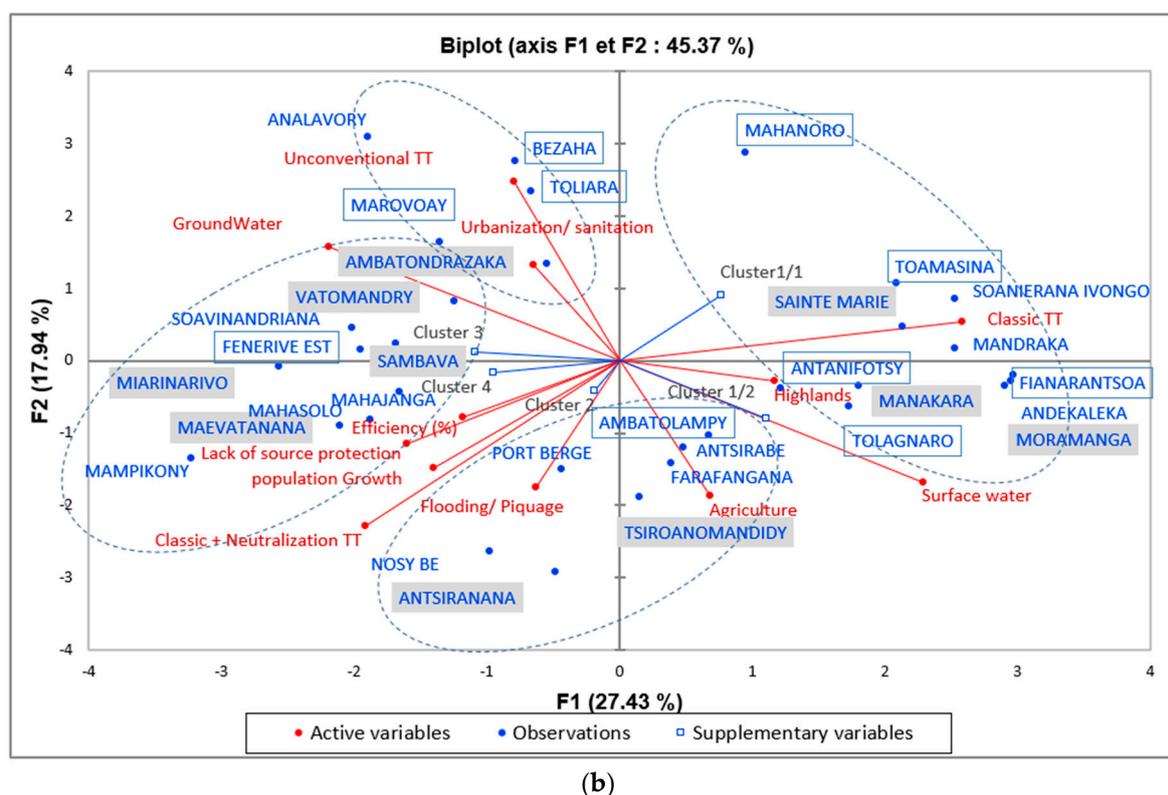


Figure 7. Cont.



**Figure 7.** Principal component analysis map of the 32 water supply systems using environmental and technical variables, and the main environmental threats. (a) Circle of correlation between active (point) and supplementary variables (square); (b) Joined map of contamination data and descriptive variables (• active variable; ◻ supplementary data; • observations). Four groups (circles) can be discriminated according to the type of water source, the treatment implemented, and the type of threats on the water source.

#### 4. Discussion

The systems deployed to provide water in the country are adapted to the local geography. Rivers supplied water for cities located in the rainy parts of the country, i.e., the highlands, the east coast, and in the north. Lakes and springs are also used for water supply in the central highlands. When rainfall decreases, wells and boreholes are preferred (on the west coast and in the south). Sand sheets are also used near the sea of the east coast [20]. Two types of treatments are implemented (conventional or unconventional) to purify these water sources. The conventional process was applied to 85% of the water and for the remainder the unconventional basic process was applied. The use of chlorine-based disinfectants, widespread in low-income countries, is the common denominator of these treatment processes since chlorine is inexpensive and easy to use [29]. A low rate of samples contaminated with *Escherichia coli* (less than 1%) has been observed over the past 16 years, contrary to what can be reported in low-income countries for improved water sources [30]. This is certainly due to an adequate chlorination implementation during treatment process. A higher prevalence of intestinal enterococci (3%) indicates that the water networks may be punctually contaminated by faecal indicator bacteria (FIB) and thus relativize the effectiveness of chlorination alone, even if it is adequate [31,32]. Enterococci are indeed more resistant to chlorination than EC. SSRC are generally more prevalent in the collected samples, hence the need to improve or better control the processes of coagulation, flocculation, and filtration [33,34]. These are the main processes for removing forms of chlorine-resistant microorganisms, such as spores, oocysts or cysts [35]. Even though the SSRC test method changed in November 2010 (media, volume, filtration vs. incorporation), and the increase of the test sample volume from 20 to 100 mL facilitated the detection of positive samples, it remains

obvious that some treatment plants have difficulties in treating consistently raw water. However, it is important to distinguish faecal contamination indicators from indicators to assess the effectiveness of treatment and their health significance.

While treatment plants have shown point or more frequent failures, this study suggests that some environmental factors lead to overloading the treatment systems. Madagascar faces environmental issues [16], such as climatic changes, [36] deforestation, bushfires, erosion, unplanned urbanization, and inefficient sanitation [37]. One-third of the water sources are subjected to environmental threats resulting from urbanization, poor sanitation, and population growth (more than 9%). In this context, FIB, especially IE, are prevalent in contaminated samples [38,39]. With a few exceptions, these waters are mainly provided by boreholes, for which the design (depth) appears to be able to limit the impact of environmental factors. Despite unconventional (incomplete) treatments, the samples collected are among the least contaminated. On the other hand, artesian wells or some less protected sources are under the influence of surface water (predominance of SSRC) and are not able protect themselves from surface pollution (predominance of FIB) [39]. They generally provide a high proportion of contaminated samples, regardless of the type of implemented treatment.

Most supply systems that are among the highest means of contamination have watersheds that are threatened by agricultural activities. Highland rivers are particularly vulnerable, especially since the area is carved by the paddy fields and is strongly eroded [40,41]. The highland landscape usually generates sediments to rivers [42]. This correlates to the prevalence of SSRC in contaminated samples even the most comprehensive treatment systems (coagulation–filtration–neutralization and chlorination) may fail. Similarly, the type of activities could affect the type of contamination, with FIB being more prevalent in livestock areas [43] (Bezaha) or production of vegetable crops (Ansirabe) with use of manure [43,44]. The water supplied from lakes in the same environmental context is of better quality, certainly because sediment deposition alleviates the treatment plants. Surface water seems vulnerable to threats from agricultural activities [45], possibly due to a lack of protective perimeter [18].

Throughout the 16 years, stable predominant contamination was observed in few supply systems. These point sources of contamination would be related to an inadequate water source conception (depth, soil type) or to an inadequate treatment plan design. In this case, rainfall affects the proportion of contaminated samples and not the indicator population. Non-point source pollution, in contrast, is of concern to most supply systems. For half of the sites with a low level of contamination, no predominance of any type of contamination and low monthly variation of contamination was observed. Here, rainfall seems to influence both the proportion of contaminated samples [46,47] and the indicator populations. The increase of contamination frequencies was more associated with a conjunction of several bacterial types than a higher density of only one type of bacteria [48]. In inadequate sanitation conditions (open defecation or use of wastewater open channels), and without management of storm water, urban runoff is an important part of non-point-source pollution. Thus, the capacity and efficiency of the treatment plants determine high and low averages of contamination over the 16-year period. Overall, the frequency of contamination varied with the site ranging from 3.3% to 17.5% (average 7.5%). Contamination events are related to an overloading of the decontamination process, resulting in a high level of contaminants in the water sources during storms, leaching, or flooding [49]. In the opposite way, SSRC contamination can persist during the dry period, certainly due to low flow or low levels of water sources.

Since 2012 the quality of supplied water has deteriorated for sites undergoing demographic growth, with developing environmental threats due to an increase of non-point-source pollution (prevalence of IE during rainy events). The increase in water demand can also affect the treatment time and decreases the performance of already aged systems.

## 5. Conclusions

To improve the public delivery of water in Madagascar, the systems need to be optimized in different towns facing environmental and climatic threats. Although a low level of EC contamination is maintained, a risk assessment approach at the catchment scale would be appropriate. Agricultural runoff associated with erosion phenomena as well as urban runoff are major threats and impact indicator populations in drinking water samples. Heavy rainfall events reinforce this impact and progressively contribute to overloading the treatment plants. The systems can no longer face to climate and urbanization threats. Thus, water source protection and an extensive improvement of treatment plants must take place all over the country. This is a priority for the country.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4441/10/10/1450/s1>: Table S1. Repartition of the number of collected water samples from 32 Malagasy water supply systems per month and per year (1999 to 2015). Part 1: per month; Part 2: per year. Table S2. Monthly and yearly contamination frequency of drinking water samples from 1999 to 2015, except 2009. Part 1: monthly; Part 2: yearly period. Table S3. Description of the 32 water supply systems—environmental and technical descriptive variables. Table S4. Description of the 32 water supply systems—environmental threats, descriptive variables, and contamination profiles. Table S5. Pearson’s correlation matrix used for detection of explanatory variables influencing each cluster in principal component analysis of 32 water supply systems.

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