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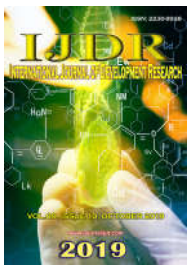

RESEARCH ARTICLE OPEN ACCESS

# QUANTITATIVE EVALUATION OF SURFACE WATER POLLUTION BY ANTIBIOTIC RESIDUES IN YAOUNDE OF CAMEROON

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**ABSTRACT**

Many studies have been undertaken in order to find pollutants of anthropic origin found in surface waters. Recently discovered we reported the emergence of water pollution by drugs. Although present at little concentrations, the water pollution by antibiotics can be at the origin of health and environmental risks which must not be neglected. In order to quantitate their presence in domestic and hospital waste, two molecules of antibiotics: Ciprofloxacin (CIPX) and Ceftriaxone (CFX) have been the subject of this study. The three STEP sampling being: STEP of the CNPS hospital, STEP of "camp Sic Messa", STEP of "camp Sic Cite Verte". We obtained from chromatographic analyses quantify CPX contents of 7.329 µg/L at the entry of the "camp SIC Messa". The CIPX was observed in all the sites of sampling with contents of 46.103 µg/L at the entry of the STEP of the CNPS hospital, of 9.835 µg/L downstream from the Ebogo River, of 15.490 µg/L downstream of the Abiergué River and of 2.459µg/L at the entry of the STEP of the "camp Sic Cite Verte". These results show a persistence of antibiotic molecules in the environment.



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## INTRODUCTION

For several centuries, water has become the dump of all anthropogenic pollution. Today, it is the prey of a so-called emergent pollution: that of the water pollution by the residues of antibiotics. Topical subject still unknown in Cameroon, the use of antibiotics has greatly increased in recent decades, because of their effectiveness in the fight against pathogenic bacteria. However, once ingested by humans and animals, antibiotics are excreted from 50% to 80% in their active form or metabolized in the environment (Tamtam, 2007). Many studies have shown that pharmaceutical residues are present in raw and purified wastewater (Golet et *al*., 2001) and that their presence is of concern because of the direct toxicity of its molecules due to the cumulative or synergistic effect that There may be between different pharmaceutical molecules and other pollutants (Pomati et *al*., 2006). The presence of these antibiotic residues may be of the order of one microgram and even one nanogram, moreover, it has a pseudo-persistent character following continuous releases of drugs into the environment. The overall objective of this work is to quantitatively assess the pollution of surface water by antibiotic residues in at least one sampling site.

To achieve this objective, the sampling of domestic and hospital effluents was done in the SOPREC wastewater treatment plants of the city of Yaounde. The three sites defined during this study are the STEP of the CNPS hospital, the STEP of the "camp SIC Messa" and STEP of the "camp Sic Cite Verte".

## MATERIALS AND METHODS

### Materials

**Reagents:** The standards of Ceftriaxone and Ciprofloxacin purity higher than 95% and the extra-pure water were provided by LANACOME (National Laboratory of quality control and expertise of drugs) table 1. Methanol of 99.9% purity was provided by LASPEE - IRAD. These standards were used for chromatographic analyzes.

**Gray water samples:** The sampling took place between 6.30am and 9.30am, which are the hours thatcorrespond to the strong activities of households and hospital services. Sampling was done in sterile amber glass bottles of 250ml and 200ml, stored in a cooler filled with ice cubes at a third. 100ml of

effluents were used for the microbiological analyzes and 100ml of effluents were filtered on fiberglass before being conserved at -18°C for extraction and to be analyzed by HPLC. 250ml of effluents were used for the physicochemical analyzes. During the analyzes, repetitions were made twice. The map sampling sites it shows by the Figure 1.

In order not to confuse the samples to be analyzed with LASPEE, a coding of the samples was made:

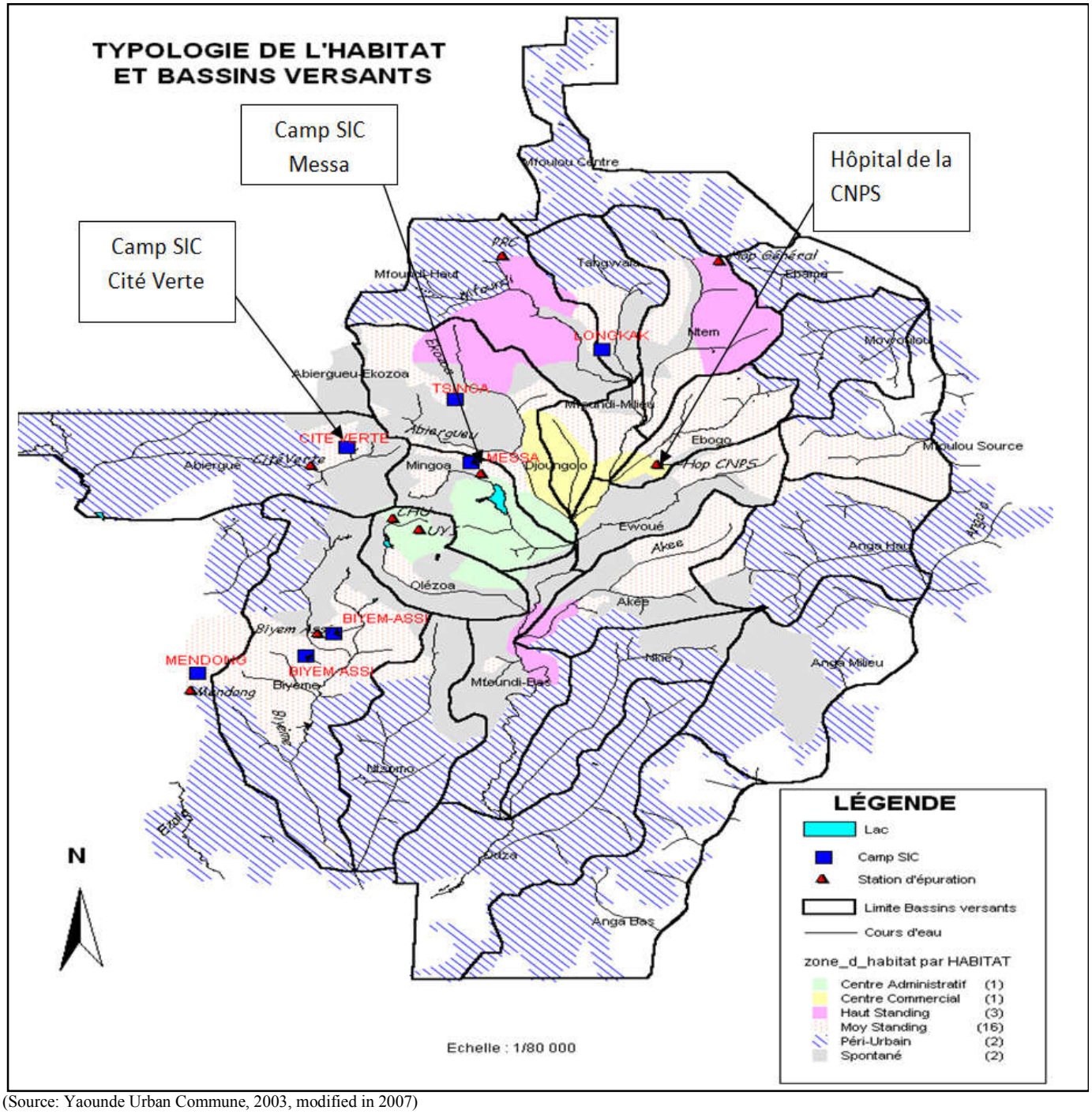
- 242: Entrance STEP hospital of the CNPS;
- 243: STEP hospital output of the CNPS;
- 244: Upstream Ebogo River;
- 245: Aval river Ebogo;
- 246: entrance STEP camp sic Messa;
- 247: first class wells STEP camp sic Messa;
- 248: exit camp sic Messa;
- 249: Municipal lake;
- 250: Entrance STEP Green City;
- 251: STEP Green City exit;
- 252: Abiegué river upstream;
- 253: Downstream Abiergué.

## Methods

### Analysis of antibiotic residues of Ciprofloxacin and Ceftriaxone

### Treatment of water samples

**Method:** The method that was carried out for the preparation of the water samples is the concentration of the water samples at the rota-vapor.




(Source: Yaounde Urban Commune, 2003, modified in 2007)

**Figure 1. Map of sampling sites**

This is an important operation that allows the concentration of trace elements by removing effluents from excess water. It has been developed in the laboratory.

**Operating mode:** The concentration of the effluents was made in order to concentrate the antibiotic residues, eliminating the excess water contained in the samples. 200 ml of water samples were taken for concentration. The extraction conditions that were applied are T=35°C, P°=200mbar, N=100tr/min. This method has been used as an alternative to Solid Phase Extraction (SPE) which uses HLB oasis cartridges.

**Identification and quantification of antibiotic residues**

**Method:** The analytical method that has been used for the identification and quantification of antibiotic residues is high performance liquid chromatography (HPLC).

Pure standards have been named:

- Pc: pure CFX control;
- Pci: pure CIPX control.

During the chromatographic analyzes, four repetitions were made.

**Operating mode:** 20µL of concentrate were introduced at the top of the column via the injection system. The various compounds of the sample were eluted at a flow rate of 0.006 ml/min by the mobile phase (methanol and ultra-pure water) along the column and are partitioned between the mobile phase and the stationary phase. The gradient mode is used during elution, i.e., the composition of the MeOH + $H_2O$ elution mixture varies from 50-75-50%. So MeOH, the most eluent solvent will see its fraction increase according to the program defined at the beginning. At the column outlet, the compounds were identified by a UV detector, which sends an electronic signal to the recorder which retrieves the data.

**Identification of antibiotic residues:** The identification of antibiotics using HPLC was performed by superimposing and comparing retention times of chromatograms of pure standards, and those obtained from chromatograms of water samples.

**Quantification of antibiotic residues:** Quantitative analysis of antibiotic residues was determined by measuring the height of the peaks corresponding to the desired antibiotics. The preparation of standards standard solutions allowed us to determine the response factor of each antibiotic.

**The characteristics of the high performance liquid chromatography used**

- The column which has been used is brand ALLTIMA, of the C18 type, with 5µmicron porosity and a length of 250 mm;
- The elution solvents were methanol and ultra-pure water;
- The elution time was 40 min for water samples and 20 min for white;
- The elution temperature was 25°C;
- The HPLC is a donation from the University of DORTMUND series 99 000 3638;
- The UV detector has been used.

## RESULTS AND DISCUSSION

**Analysis of antibioticresidues of amoxicillin, sulfamethoxazole, ciprofloxacin and ceftriaxone**

**Identification of antibiotic residues.**

i. **CFX identification:** The results of the identification of the CFX are presented in the chromatograms of Figures 2 and 3. The observation of these chromatograms gave the retention time of the pure standard $t_R$=10.739 min and that of the effluent at the STEP inlet. SIC Messa camp $t_R$=11.468min.

ii. **Identification of CIPX:** The results of the identification of the CIPX are presented in the chromatograms of Figures 4, 5, 6, 7 and 8. The observation of the peaks of Figure 4 has shown that, for the pure standard of CIPX, the $t_R$ = 17.462 min. The second peak that appears on the chromatogram of the pure standard is probably due to the presence of impurities, since the repeatability of the tests indicates the retention time of ciprofloxacin in the range of 17.00 min to 18.00 min. Figures 5, 6, 7 and 8 allowed the identification of the CIPX in the following respective sites:

   - STEP entry from the CNPS hospital at $t_R$ = 17.009 min;
   - Aval river Ebogo STEP hospital of the CNPS at $t_R$ = 17,709;
   - Exit of the SIC Camp Messa $t_R$ = 17,178 min;
   - Entrance to the STEP of the SIC camp of the Cité Verte at $t_R$ = 17,760min.

The presence of CIPX in the hospital sites of the CNPS and Cité Verte is due to the fact that it is a broad-spectrum antibiotic widely used in hospitals. But it can also be prescribed for home consumption, hence its presence at the exit of the SIC Messa camp.

**Quantification of antibiotic residues**

The results of the quantification of antibiotic residues are presented in Table 2. It was obtained for CFX contents given 7,113 µg/L at the entrance to the SIC Messa camp. For the CIPX levels, 43.894 µg/L were obtained at the inlet of the CNPS hospital STEP, 9.364 µg/L downstream of the Ebogo River, 14.748 µg/L at the outlet of the STEP. SIC Messa camp and 2,341 µg/L at the entrance to the Green City STEP. These discrepancies between the inlet and the outlet contents of the STEPs are due to the residence time put by the effluents in the STEPs, which is one week. The presence of CIPX in all sites is due to the fact that it is the most widely prescribed broad-spectrum antibiotic in Cameroon. The results of antibiotic residue analyzes using HPLC allowed for the identification and quantification of CFX and CIPX antibiotics at levels of µg/L which is similar to what is observed in the studies from Kümmerer, (2009). Fluoroquinolones and ciprofloxacin were also detected from studies conducted by DINH, (2012). Antibiotics such as beta-lactam antibiotics are rarely encountered in water because of their high solubility, yet they are present in the effluents of the STEP of the CNPS hospital and in the STEP of the Green City, to say that their presence in these effleunts is due to their strong use in hospitals.

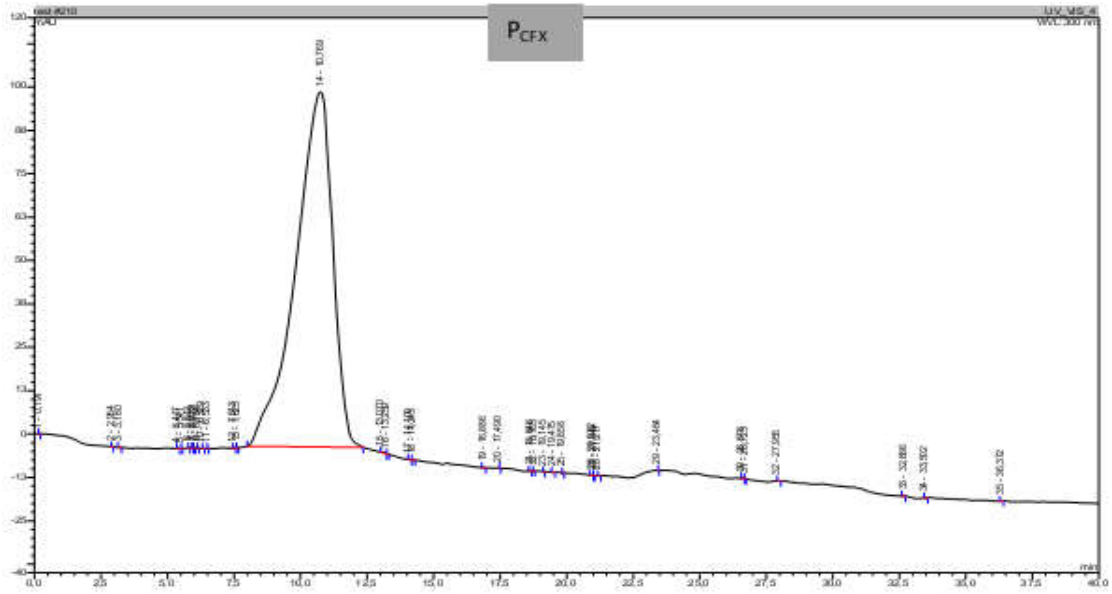



**Figure 2. Chromatogram of the pure standard of Ceftriaxone**

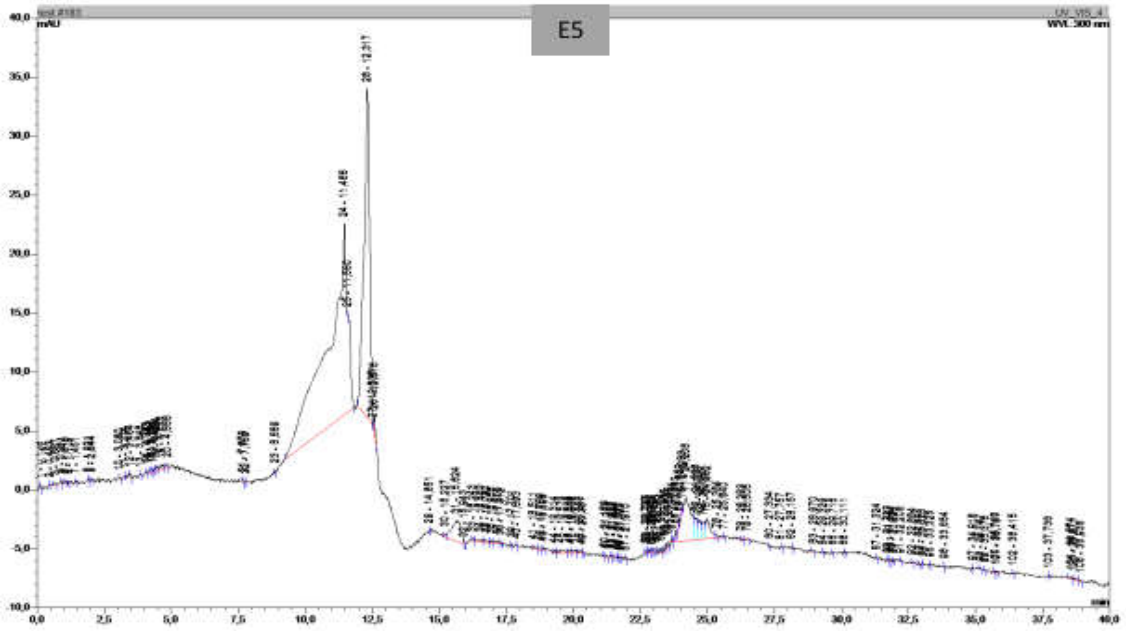



**Figure 3. Chromatogram of sample 256 (STEP camp SIC Messa input)**

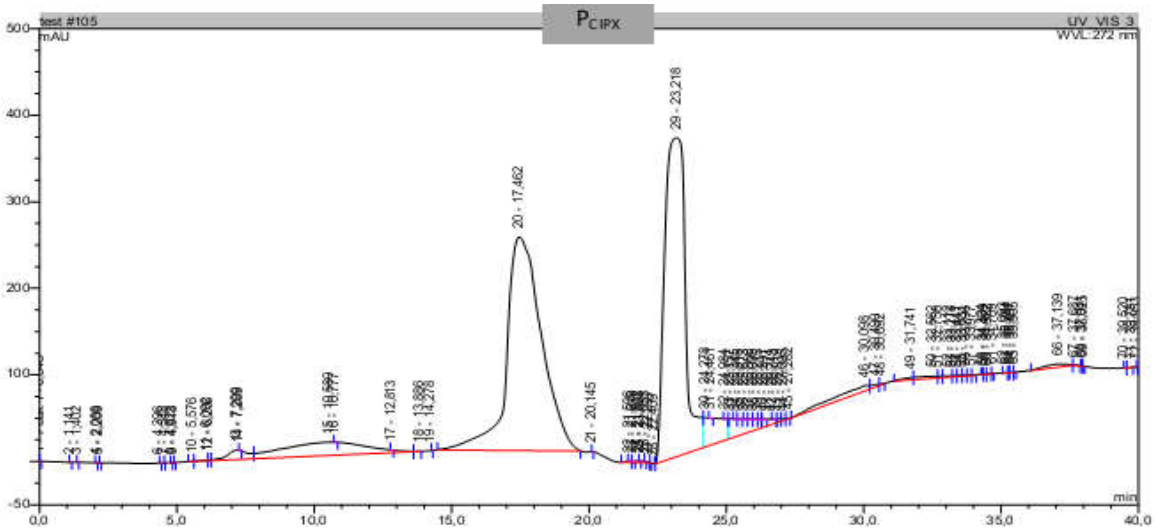



**Figure 4. Chromatogram of the pure standard of Ciprofloxacin**

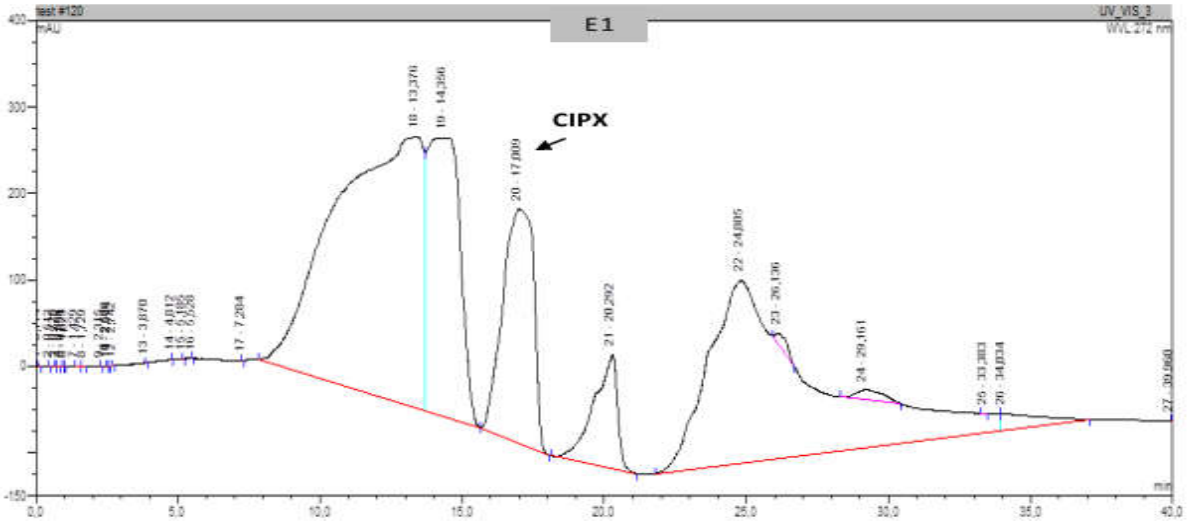



**Figure 5. Chromatogram of sample 242 (entry STEP hospital of the CNPS)**

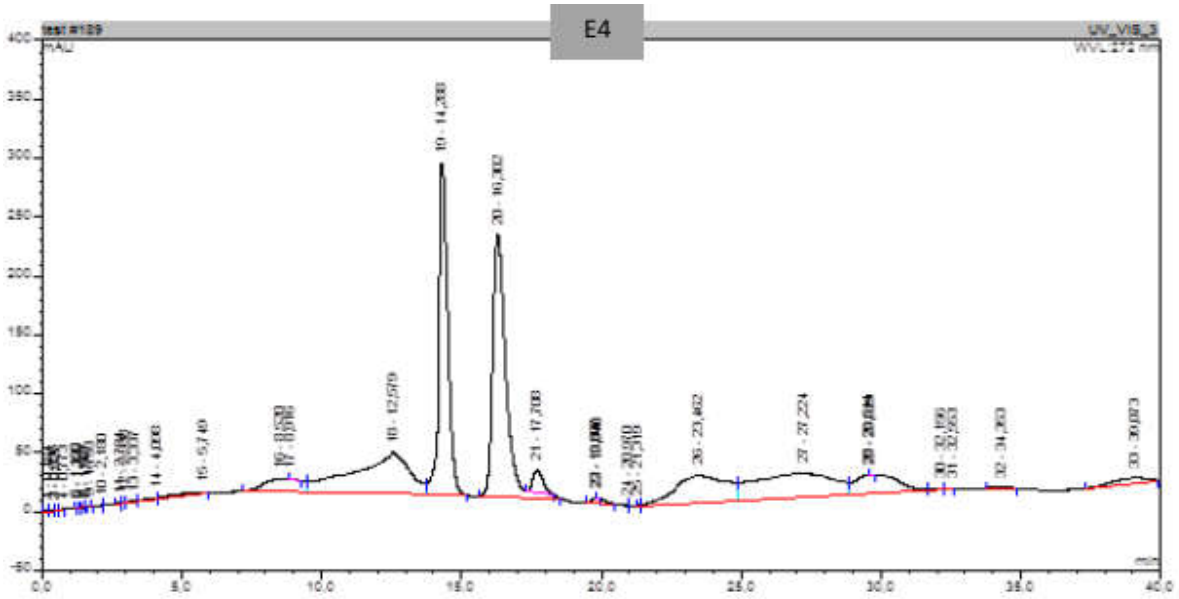



**Figure 6. Sample chromatogram 245 (downstream Ebogo STEP hospital of the CNPS)**

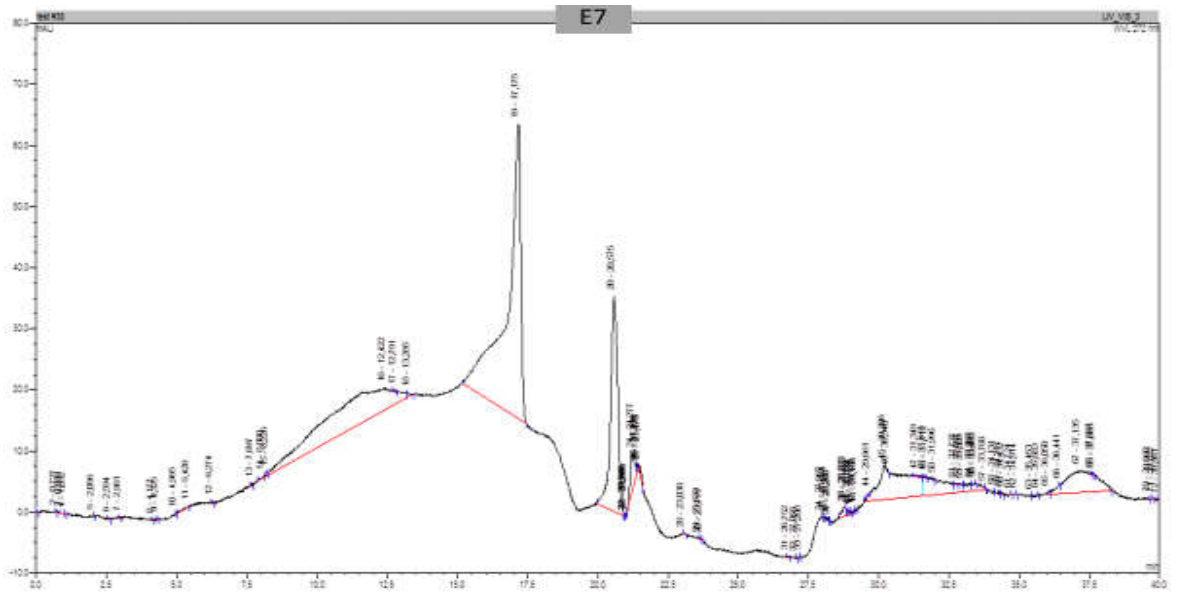



**Figure 7. Chromatogram of sample 248 (STEP camp SIC Messa output).**

**Table 1. Preparation of pure standards of antibiotics**

| Amoxicillin standard name | Concentration (µg/L) | Retention time (min) | Height of peaks (USI) | UV detector wavelength (nm) |
|---|---|---|---|---|
| CFX | 15 | 10,001 | 97 | 272 |
| | 30 | 10,769 | | |
| | 45 | 11,603 | | |
| CIPX | 20 | 17,001 | 253 | 272 |
| | 60 | 17,462 | | |
| | 100 | 17,709 | | |

**Table 2. Determination of the approximate antibiotic residue content at each sampling site**

| Sampling site | n° Sample | CFX (µg/L) | CIPX (µg/L) |
|---|---|---|---|
| CNPS Hospital | 242 | - | 43,894 |
| | 243 | - | - |
| | 244 | - | - |
| | 245 | - | 9,364 |
| SIC Messa Camp | 246 | 7,113 | - |
| | 247 | - | - |
| | 248 | - | 14,748 |
| | 249 | - | - |
| SIC Green City Camp | 250 | - | 2,341 |
| | 251 | - | - |
| | 252 | - | - |
| | 253 | - | - |

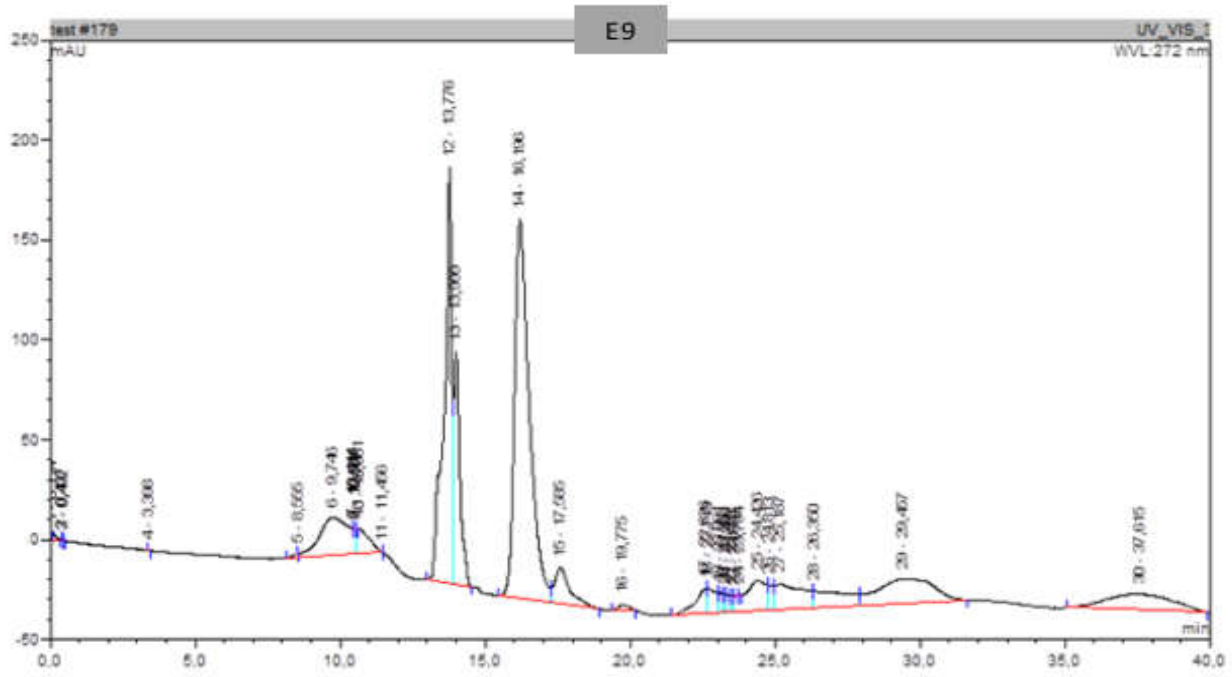



**Figure 8. Chromatogram of sample 250 (STEP camp entry SIC Green City)**

## Conclusion

The objective of this work, which was to evaluate and quantify antibiotic residues in the surface waters of the city of Yaoundé, was achieved. The search for antibiotic residues in the effluents allowed us to identify and quantify the two antibiotics Ciprofloxacin and Ceftriaxone. Their presence in the receiving environment is a proof of the pollution of the surface waters by these antibiotics and also the inefficiency of the treatment plants to eliminate antibiotic residues in greywater. During this analysis, we observed the presence of certain peaks that could not be identified because we did not have standards. These different results testify to the importance of paying particular attention to this new form of pollution. Moreover the fact that the treatment plants are not suitable for the removal of drug residues, we will see this pollution increase if nothing is done.

As prospects it would be interesting to:

- Conduct regular analyzes to determine the amount of drug residues released throughout a year;
- Perform other tests on an HPLC/MS to confirm the presence of these antibiotics;
- Extend antibiogram tests to other pathogenic microorganisms in humans;
- Search for an analytical protocol using HPLC, suitable for the quantification and identification of several pharmaceutical residues in effluents.

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