Occurrence and Seasonal Variation of Antibiotics in Fez-Morocco Surface Water

1,5 Oualid Chaib, 1,6 Btissam Arhoune, 2 Sanae Achour, 3 Elodie Moreau-Guigon, 3 Fabrice Alliot, 3 Marc Chevreuil, 5 Samira El Fakir, 5 Ilham El Arabi and 1 Bouchra Oumokhtar

1 Laboratory of Microbiology and Molecular Biology, Faculty of Medicine and Pharmacy, University Sidi Mohammed Ben Abdallah USMBA, Fez, Morocco
2 Laboratory Central of Medical Analysis, CHU Hassan II, Faculty of Medicine and Pharmacy, University Sidi Mohammed Ben Abdallah USMBA, Fez, Morocco
3 EPHE, PSL, Sorbonne Université, CNRS, Milieux Environnementaux, Transferts et Interactions dans les Hydrosystèmes et les Sols, METIS, 75005 Paris France
4 Laboratory of Epidemiology and Clinical Research, Faculty of Medicine and Pharmacy, University Sidi Mohammed Ben Abdallah USMBA, Fez, Morocco
5 Laboratory of Physiology, Pharmacology and Environmental Health, Faculty of Sciences Dhar El Mehraz, University Sidi Mohammed Ben Abdallah USMBA, Fez, Morocco
6 Laboratory of Biotechnology, Faculty of Sciences Dhar El Mehraz, University Sidi Mohammed Ben Abdallah USMBA, Fez, Morocco

Abstract: The presence and accumulation of antibiotics in the water environment has become emerging contaminants of concern causing disruption of ecosystems worldwide. We describe here the seasonal variation and the occurrence of antibiotic residues in Fez city surface water (Morocco). During one year between February 2014 and January 2015, 8 surface water samples were collected monthly. Quantification of the 7 antibiotics was performed by on-line Solid Phase Extraction (SPE) liquid chromatography–tandem mass spectrometry (LC–MS/MS). A total of 96 surface water samples were investigated and the results revealed that 100% of the sites were contaminated by at least one antibiotic. Amoxicillin had the highest concentration with maximum concentration (4107 ng L⁻¹), followed by ciprofloxacin (1058 ng L⁻¹) and sulfamethoxazole was the most widely detected (93%). Seasonal variation showed that the concentration of antibiotics was higher in winter for trimethoprim (96 ng L⁻¹), ciprofloxacin (438 ng L⁻¹) and in summer for amoxicillin (1113 ng L⁻¹), sulfamethoxazole (162 ng L⁻¹) and erythromycin (47 ng L⁻¹). The results from this research show that antibiotics are frequent contaminants in Fez city surface water. This is the first attempt to assess the occurrence of these 7 pharmaceutical residues in water samples in Fez Morocco.

Keywords: Contamination, Antibiotics, Surface Water, LC/MS/MS, Seasonal Variation, Fez

Introduction

Water, the essential substance to humans and the source of life, can become a serious hazard to our health, as well as to the life of the flora and fauna. Water bodies are generally sources of drinking water for many people and are used in agriculture for irrigation processes. Water quality can be affected by the increasing discharge of organic contaminant coming from different applications.

The presence of antibiotic in water bodies have been drawing extensive attention because of their high water solubility, resistance to degradation and potential risks to ecosystem and human health (Tang et al., 2015). These emerging contaminants may originate from different sources and many studies have shown that antibiotics were introduced to the environment from Wastewater Treatment Plants (WWTPs) (Leung et al., 2012; Proia et al., 2016; Zhang et al., 2017), hospitals (Szekeres et al., 2017), land application of animal manure (Karcı and Balcıoğlu, 2009), Aquaculture (Cabello, 2006), and industrial garbage (Collado et al., 2014; Shishir et al., 2011).
Several studies from different countries reported contamination by antibiotics in tap water (Cai et al., 2015), surface water (Batt and Aga, 2005), groundwater (Ruixue et al., 2015), sediments (Kim and Carlson, 2007), wastewater treatment plant effluents (Khan et al., 2013). In Morocco, the antibiotics consumption has increased from 9.68 in 2003 to 13.85 Defined Daily Dose/1000 Inhabitant/Day (DDD) in 2012 (Inouss et al., 2015). The public has easy accessibility to antibiotics in low and middle-income countries, from a variety of sources, including drugstores, over-the-counter chemical shops, hospitals, and roadside stalls (Lerbech et al., 2014). Antibiotics can sometimes be bought at pharmacies and drugstores without prescription, despite prohibiting legislation’s to stop selling (Bekoe et al., 2014; Lerbech et al., 2014). Extensive accessibility to antibiotics in developing countries could lead to continuous exposure to antibiotics via food and water (Belalche, 2014). Therefore, the existing treatment facilities of wastewater treatment plants, which are designed for removing biodegradable organics and nutrients, cannot effectively eliminate these recalcitrant chemicals, leading to considerable discharge of pharmaceuticals into aquatic environments (Jin-Lin and Wong, 2013; Tamtam et al., 2008). The use of wastewater for irrigation is common in low and middle-income countries (Drechsel and Keraita, 2014). Usually, untreated and/or partially treated wastewater from urban areas is discharged into drains, smaller streams and other tributaries of larger water bodies, where it is mixed with storm and freshwater (diluted wastewater) before it is used by farmers. This surface water is referred to as low quality water (Raschid-Sally and Jayakody, 2008).

Thus, the monitoring program of the present study is being implemented for determining the occurrence and seasonal variation of seven antibiotics in Fez city surface water (Morocco).

Material and Methods

Target Chemicals

In this study, 7 molecules of antibiotics belonging to several families were selected as target chemicals regarding their rates of consumption in either human or veterinary practices in Fez city (Belalche, 2014), their detections and persistence in the water environment. Nevertheless, these molecules have been chosen in accord to the availability of analytical standards in the market. Detailed information of the target chemicals is summarized in Table 1.

Description of Study Area

Oued Fez is the main water body crossing the city of Fez in Morocco (Derwich et al., 2008). It is a tributary of the Sebou River, the biggest river system in Morocco with its 40,000 km² catchment (Fig. 1). The river flows in an easterly direction from the springs of “Ras el Ma” (elevation = 420 m a.s.l.) through the Fez medina and into the Sebou, 4 km downstream from the city of Fez (elevation = 210 m a.s.l.).
Table 1: Uses of selected antibiotic targets

| Family          | Compound          | Uses                                      |
|-----------------|-------------------|-------------------------------------------|
| β-lactams       | Amoxicillin       | Veterinary, Human                          |
| Tetracycline    | Tetracycline      | Veterinary, Human and Pisciculture        |
| Macrolide       | Erythromycin      | Human                                     |
| Sulfamides      | Sulfamethoxazole  | Veterinary, Human                          |
| Fluoroquinolone | Ciprofloxacin     | Human                                     |
| Quinolone       | Oxolinic Acid     | Veterinary                                 |
| Diaminopyrimidines | Trimethoprim    | Veterinary, Human and Pisciculture        |

Table 2: Geophysical coordinates, land use and location of the sampling points

| Sampling point | Geophysical Coordinates | Land use                   | Sections of the main stream |
|----------------|-------------------------|----------------------------|-----------------------------|
| S1             | 34°2’25.03’’-5°3’40.21’’ | Rural with farming activities | Upstream                    |
| S2             | 34°3’19.14’’-5°0’14.62’’ | Urban                      |                             |
| S3             | 34°2’31.35’’-4°59’38.63’’ | Urban                      | Midstream                   |
| S4             | 34°3’19.66’’-4°58’29.85’’ | Urban with farming activities |                             |
| S5             | 34°3’13.49’’-4°58’59.95’’ | Urban                      |                             |
| S6             | 34°4’28.71’’-4°57’36.14’’ | Urban                      |                             |
| S7             | 34°4’29.77’’-4°56’20.36’’ | Rural with farming activities |                             |
| S8             | 34°4’48.61’’-4°54’58.31’’ | Rural with farming activities | Downstream                  |

Its main course is 33 km long and its catchment area is 615 km$^2$ (Lombard-Latune et al., 2010). The climate is characterized by hot and dry summers and cold winters. Diurnal temperature swings are large; the mean range is 17-34°C in July and 4-15°C in January. The temperature can, however, rise above 40°C in the summer, especially when the desert wind, is blowing from the Sahara, and fall below 0°C in winter. The rainfall maximum is in the winter period whereas the summer period is almost completely dry and daily hours of bright sunshine varies between about 6h in December and 11 h in July (Perrin et al., 2014). All of Fez’s sewage estimated 38 million m$^3$ (or 110.000 m$^3$/j) by Autonomous distribution control of water and electricity Fez) is flushed directly into nearby watercourses. This includes industrial effluents generated by many industries including tanneries, oil mills, metal works, potteries, resulting in serious degradation of water quality. Tanneries, where the different processes are still carried out traditionally, are probably the most polluting and flush, day after day, considerable amounts of chemicals including chromium and ammonium in addition to organic matter into the river (Nazer et al., 2006; Prabhavathy, 2010).

Experimental

Reagents and Chemicals

The following antibiotics were purchased from A2S via CIL-Cluzeau (Sainte-Foy-la-Grande, France): amoxicillin (AMO), erythromycin (ERY), sulfamethoxazole (SMX), tetracycline (TET), ciprofloxacin (CIP), oxolinic acid (OXO), Trimethoprim (TRI) was meanwhile purchased from LGC Standards (Molsheim, France).

$^{13}$C- or deuterium-labelled compounds were used as internal standards (ISs): ethyl-d5-oxolinic acid (OXO-d$_5$) was purchased from Sigma-Aldrich (Oxo-d$_5$) was purchased from Sigma-Aldrich which was sold in MeOH or in acetonitrile except for OXO-d$_5$ and $^{13}$C-AMO who was sold in powder. OXO-d$_5$ was prepared in MeOH with 0.1% NaOH 50% to increase her solubility. For the same reason, $^{13}$C-AMO was

Sampling Schedule

During one year between February 2014 and January 2015, we collected 96 surface water samples from various urban environments (S1-S8) to cover major land use types in Fez city (Fig. 1), which includes rural areas with farming activities (n=36), urban (n=48) and urban area with farming activities (n=12) (Table 2).

Water samples were collected from the surface of water bodies with 1L glass bottles, which has been conditioned before sampling to avoid contamination by organic compounds. Sample bottles were stored in a cooler with ice until transportation to the laboratory. The samples were filtered using 0.7 µm Whatman glass fiber filters immediately after collection. Filtrates were stored at -18°C until analyses.
prepared in UP-water. These stock solutions were stored in amber glass vials at −18°C.

Working mix solutions of ISs and native compounds were prepared in MeOH (0.1 ng µL⁻¹).

Sample Collection and Preparation
Fifty milliliter of water samples were filtered through 25 mm glass fiber GF/F filters, nominal cut-off size 0.7 µm (Whatman, Fontenay Sous Bois, France). pH was adjusted at 7 with 5% ortho-phosphoric acid or 5% NaOH. Then 2 mL of the so-obtained filtrate was passed through 0.2 µmnylon membrane filters (Millipore, France) and 0.1% Na2-EDTA 1 M and ISs (at 0.1 ng µL⁻¹) was added just before analyze. EDTA was used as a chelating agent to reduce antibiotic binding to major cations, thereby promoting analyte retention on the SPE cartridge.

On-line SPE–LC–MS/MS Analysis of Antibiotics
We used the method On-line SPE-LC-MS/MS as described previously with a few modifications (Dinh et al., 2011). Separation in the LC column was achieved using an Agilent Zorbax Eclipse XDB-C 18 column (4.6 mm I.D. × 50 mm, 1.8 m particle size) with a 0.2 µm prefilter upstream to protect the analytical column, the total run time in separation was 15 min and the IS used for quantification were 13C-AMO was used for AMO, 13C-SMX was used for SMX, TRI and TET, 13C-ERY was used for ERY and 13C-CIP was used for CIP and OXO-d5 for OXO.

All steps of the LC-MS/MS analytical methods, the validation of the method and the limits of quantification (LOQs) are detailed in the Supplementary Information.

Results
Spatial Distribution of Antibiotic Residues in Surface Water
Results of our study revealed that 100% of the study sites were contaminated by at least one antibiotic (positive detection). The highest positive samples percentage was recorded in downstream respectively with 63% and 59% respectively in S7 and S8, followed by midstream 48% in S3, 46% in S4, 31% in S5and 57%in S6. Finally, the contamination rate in upstream was lower with 28% and 32% respectively in S1 and S2.

Concerning antibiotic detection, sulfamethoxazole was the most frequent antibiotics detected in all sites (93%) followed by ciprofloxacin (75%). Trimethoprim came in third range with (69%), amoxicillin and erythromycin were detected at (60%). However, the least detected antibiotics were tetracycline and oxolinic acid with 3% (Fig. 2).

Table 3 summarized the variation of antibiotic concentration of the 8 sample sites analyzed during one year from February 2014 to January 2015. The results showed that the maximum concentration was recorded to amoxicillin with maximum of 4107 ng L⁻¹ recorded in site S8 on July and to ciprofloxacin with maximum of 1058 ng L⁻¹ recorded in site S7 on December. Besides, SMX comes in second range with respectively 553ng L⁻¹ recorded in site S8 on August, followed by ERY with a maximum of 114 ng L⁻¹ recorded in site S6 on December for and TRI with 264ng L⁻¹ recorded in site S8 on Julyng L⁻¹.

Fig. 2: Detection percentages of antibiotics in 96 surface water samples
Table 3: Minimum/maximum, median and extended antibiotic concentrations in ng L\(^{-1}\) of the 96 samples analyzed

| Site | AMO Ranges<158.3 | CIP <7.2-20 | SXM <111.4 | TET <1.9-5 | TRI <7.8 | ERY <12.2 |
|------|------------------|-------------|------------|------------|---------|----------|
| S1   | Mean <158.3      | 7           | 4          | 0          | 1       | 25       |
|      | Median <158.3    | 8           | 4          | <111.4     | <1.9    | <7.8     |
|      | S2 Ranges <158.3-205 | <7.2-20 | 4-47   | <111.4     | <1.9-5  | <7.8-65  |
| Mean | 23               | 62          | 12         | 0          | 1.1     | 46       |
| Median | <158.3   | 8           | 6          | <111.4     | <1.9    | 53       |
| S3   | Ranges <158.3-2323 | <7.2-357 | <1.9-327  | <111.4     | <1.9-44 | <7.8-65  |
| Mean | 914              | 133         | 49         | 0          | 13.4    | 38       |
| Median | 554  | 70           | 17         | <111.4     | 1       | 55       |
| S4   | Ranges <158.3-1350 | <7.2-277 | <1.9-52   | <111.4-286 | <1.9-22 | <7.8-70  |
| Mean | 362              | 34          | 15         | 24         | 6       | 25       |
| Median | 211  | 9            | 9          | <111.4     | 7       | <7.8     |
| S5   | Ranges <158.3-286 | <7.2-30   | 4-35      | <111.4     | <1.9-7  | <7.8-64  |
| Mean | 37               | 6           | 10         | 0          | 2       | 24       |
| Median | <158.3 | 6           | 8          | <111.4     | <1.9    | <7.8     |
| S6   | Ranges 325-4005  | 11-1023     | 12-385     | <111.4-134 | 10-264  | <7.8-114 |
| Mean | 1165             | 207         | 80         | 11         | 50      | 31       |
| Median | 708  | 58           | 43         | <111.4     | 19      | 6        |
| S7   | Ranges 389-3204  | 26-1058     | 23-410     | <111.4-142 | 12-242  | <7.8-84  |
| Mean | 1715             | 381         | 182        | 12         | 95      | 45       |
| Median | 1687 | 109          | 126        | <111.4     | 70      | 38       |
| S8   | Ranges <158.3-4107 | <7.2-951 | 15-553     | <111.4     | 3-120   | <7.8-81  |
| Mean | 2247             | 188         | 178        | 0          | 56      | 30       |
| Median | 2247 | 109          | 154        | <111.4     | 47      | 16       |

Fig. 3: Annual variations of antibiotic concentrations in midstream

However, the lowest value was recorded to tetracycline with a maximum of 286 ng L\(^{-1}\) recorded in site S4 on December, followed by oxolinic acid 64 ng L\(^{-1}\) recorded in site S4 on November (Table 3).

**Seasonal Variation of Antibiotic Concentrations**

Seasonal variation of antibiotic concentration has shown that during periods with high flow, the concentration was 6 times lower than during low flow periods (Fig. 3). Depending on the season, the river flow ranged from 0.058 to 0.827 m\(^3\) s\(^{-1}\) which corresponded to a theoretical dilution factor from 6 to 15.

Annual variations of the antibiotic flux in Fez city midstream ranged between from 0.004 to 5.28 g d\(^{-1}\), with the presence of two substantial peaks recorded during the winter period (February 2014 and January 2015) respectively, corresponding to high-flow events (Fig. 4).
Discussion

Because compound detection and concentration are dependent on source strength, hydrologic condition, timing of sampling and other factors (Focazio et al., 2008) interpretations of these reconnaissance data are limited. Surface water samples recorded higher contamination levels by most of the pharmaceuticals than reported worldwide (Locatelli et al., 2011; Kasprzyk-Hordern et al., 2007; Boix et al., 2015). Once entering into water, these antibiotics may have direct toxicity to aquatic organisms, even at low concentrations (ng L$^{-1}$ or µg L$^{-1}$ level) (Chen and Zhou, 2015). For example, they could cause phytoplankton toxicity, inhibition of microbial activity and change the microbial community structure (Liao et al., 2017). Additionally, antibiotics could promote the development of bacterial resistant (Chaib et al., 2017), which might be potentially harmful to the ecosystem and human health (Zhang et al., 2011).

The level of antibiotics in upstream (sites S1 and S2) were approximately two folds lower than midstream (from sites S3 to S6). This large difference may be directly related to the contamination sources in midstream (urban area), where was little disturbance by human activities and the presence of several private hospitals, clinics, and medical analysis laboratories. In addition, the antibiotic detection percentage was much higher in site S6 (57%) than the other urban sites. In fact, site S6 is a well-known as a hot-spot and has a denser population around 612489 of inhabitants, this finding highlight the contribution of excessive antibiotics consumption in water environment contamination. Besides, the level of antibiotics in downstream were much higher than up and midstream. Sites S7 and S8 (rural with farming activities) are impacted by the agricultural activities and the continuously WWTP’s discharges into surface water.

We showed that AMO was the most concentrated and SMX was the most frequent in surface water samples. β-lactam antibiotics are among the most frequently used and consumed in large quantities in Morocco and worldwide (Belâiche, 2014), their detection is difficult and the studies which investigated the presence of β-lactam and antibiotics are scarce, because of the poor stability of the β-lactam ring under pH conditions (Moreno-Bond et al., 2009). Some studies were not successful in detecting β-lactam and cephalosporin antibiotics in water matrices (Gros et al., 2013; Zhou and Chen, 2013). Also, SMX is among the most widely used prescribed antibiotics, the large scale of livestock and poultry farming and the high population density result in high usage of SMX (Binh et al., 2018). Levels of CIP found was higher, this result was not surprising because CIP is typically used in human and veterinary medicine to treat and prevent diarrhoea and other intestinal infections (Chen et al., 2012; Liang et al., 2013). Additionally, ERY is widely used in concentrated animal feeding operations to treat bacterial infection diseases of animal and human, such as respiratory diseases, intestinal infections (Lien et al., 2016). More even, TET constitute one of the most extensively used antibiotic classes due to their low cost, ease of use and relatively minor side effects (Li et al., 2016; Ahmed et al., 2017). Tetracycline are also the most widely used veterinary drugs and feed additives in aquaculture and livestock.
industries. TET is also added at the sub-therapeutic level to animal feed to prevent infection and act as growth promoters (Sarmah et al., 2006). Besides, OXO is widely used in aquaculture to cure and prevent skin infections in fish. The pisciculture production is usually made in the winter because of the low temperature; the non-negligible fish farming activity present around the city could be involved in the release of the measured OXO (Tamtam et al., 2008).

The seasonal variation of antibiotic concentration has shown that during periods with high flow, the antibiotics concentration was 6 times lower than during low flow periods. This difference in sampling periods can be explained by the variation in antibiotics consumption, physicochemical behavior such as photo-degradation and river flow conditions between summer and winter.

The period of high flows in Oued Fez from December to February (with an average precipitation of 32.6 mm) could lead to dilution on the antibiotics concentration in surface water. Studies have reported that the Seasonal variation in antibiotics concentration is related to production, consumption, excretion or environmental factors such as solar irradiation, precipitation, and temperature (Conley et al., 2008). Our results have been confirmed by other studies reporting higher concentrations in summer (Jiang et al., 2011; Gracia-Lor et al., 2012). This variation can partially be explained by the fact that more antibiotics are used and discharged into the aqueous environment during summer because they are utilized to treat higher rates of gastrointestinal infection and diarrhea among both humans and domestic animals (Gracia-Lor et al., 2012). In contrast, levels of antibiotics in waterways during the dry season because there is much lower flow runoff in rivers, although the consumption of antibiotics is reduced to some extent due to fewer outbreaks of gastrointestinal infection (Jiang et al., 2011). However, the differences observed in Antibiotic flux in midstream might be related to sediment resuspension by the flood under high river flow events. Moreover, antibiotic persistence in sediment (Hektoen et al., 1995) and their possible desorption towards the aqueous phase have been reported (Smith and Samuelsen, 1996; Simon, 2005).

The worldwide comparison of the 7 pharmaceuticals in the river surface water is presented in Table 4. The highest concentration of each of the pharmaceuticals obtained in the streams surface water was at moderate to high levels of contamination compared to the concentrations in other regions of the world. In the case of β-lactams for example, AMO (4107 ng L⁻¹) was detected at much higher levels than that of Brazil (8.9 ng L⁻¹, Locatelli et al., 2011), United Kingdom (245 ng L⁻¹, Kasprzyk-Hordern et al., 2007). For the macrolide, ERY (114 ng L⁻¹) was detected at a higher concentration than that of Spain (5 ng L⁻¹, Boix et al., 2015), Serbia (9.1 ng L⁻¹, Petrović et al., 2014) but was much lower than that of the China (810 ng L⁻¹, Gulkowska et al., 2006). In addition, OXO (64ng L⁻¹) was detected at low level than that of France (140 ng L⁻¹, Tamtam et al., 2008) and higher than that of Denmark (5ng L⁻¹, Sørensen et al., 2004).

### Conclusion

The results of this study confirm that several antibiotics residues originating from domestic, hospital waste and farming contaminate Fez surface water. This finding is very interesting and may constitute the basis for future work on other Moroccan cities surface water and other environmental matrices. There is a need to continuously predict the concentrations of these antibiotic compounds and to design strategies to minimize exposure to these compounds.

---

**Table 4: Global comparison of antibiotic concentrations in surface water**

| Antibiotics       | Our results         | Other results          | Reference                                  |
|-------------------|---------------------|------------------------|--------------------------------------------|
| Surface water     |                     |                        |                                            |
| Amoxicillin       | 159 to 4107ng L⁻¹   | 8.9 ng L⁻¹             | Kasprzyk-Hordern et al. (2007)             |
|                   | 39 to 245ng L⁻¹     | 39 to 245ng L⁻¹        | Locatelli et al. (2011)                    |
|                   | 6.9ng L⁻¹           | 6.9ng L⁻¹              | Petrović et al. (2014)                     |
|                   | Not detected        | Not detected           | Locatelli et al. (2011)                    |
|                   | 470 to 810ng L⁻¹    | 470 to 810ng L⁻¹       | Gulkowska et al. (2006)                    |
| Erythromycin      | 78 to 114ng L⁻¹     | 9.1ng L⁻¹              | Petrović et al. (2014)                     |
|                   | 5ng L⁻¹             | 5ng L⁻¹                | Boix et al. (2015)                         |
| Sulfamethoxazole  | 1.9 to 553ng L⁻¹    | 160ng L⁻¹              | Locatelli et al. (2011)                    |
|                   | 0.3 to 56.8ng L⁻¹   | 0.3 to 56.8ng L⁻¹      | Yan et al. (2013)                          |
| Tetracyclin       | 111.4 to 286ng L⁻¹  | 39.3 to 142ng L⁻¹      | Tambam et al. (2008)                       |
|                   | 11ng L⁻¹            | 11ng L⁻¹               | Locatelli et al. (2011)                    |
| Oxolinic acid     | 12.2 to 64ng L⁻¹    | 2 to 5ng L⁻¹           | Sørensen et al. (2004)                     |
|                   | 25 to 140ng L⁻¹     | 25 to 140ng L⁻¹        | Tambam et al. (2008)                       |
| Ciprofloxacin     | 8 to 1058ng L⁻¹     | 0 to 28.2ng L⁻¹        | Petrović et al. (2014)                     |
|                   | 0 to 1250ng L⁻¹     | 0 to 1250ng L⁻¹        | Vertlicchi et al. (2014)                   |
Adoption of effective proceeds in WWTP's must be applied to eliminate antibiotics. A risk assessment of antibiotic residues study will be very helpful to target the environmental impacts, to sensitize population and medical professional about the severity of the antibiotic presence in environment and to seek urgent actions.

**Study Limitation**

To perceive the variations of the antibiotics in water body, and significant variations among the sampling sites, a risk assessment of antibiotic concentration will be dealt in our next article. Also, there is a need to demonstrate the contribution of hospitals effluent, WWTP’s and agricultural activities in the hydrological system.

**Acknowledgements**

This work was supported by Faculty of Medicine, USMBA, Fez.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

**References**

Ahmed, M.B., J.L. Zhou, H.H. Ngo, W. Guo and M.A.H. Johir *et al*., 2017. Competitive sorption affinity of sulfonamides and chloramphenicol antibiotics toward functionalized biochar for water and wastewater treatment. Bioresource Technol., 238: 306-312.

Batt, A.L. and D.S. Aga. 2005. “Simultaneous analysis of multiple classes of antibiotics by ion trap LC/MS/MS for assessing surface water and groundwater contamination. Analytical Chemistry, 77: 2940-47.

Bekoe, S.O., S.A. Bak, E. Björklund, K.A. Krogh and N.N. Okine *et al*., 2014. Determination of thirteen antibiotics in drug products—A new LC-MS/MS tool for screening drug product quality. Analytical Methods, 6: 5847-5855.

Belalche, A., 2014. Le profil de la consommation des antibiotiques au Maroc. Infosanté. La revue des professionnels de la santé et du médicament n°2: 26-31.

Binh, V. N., N. Dang, N.T.K. Anh and P.K. Thai, 2018. Antibiotics in the aquatic environment of Vietnam: Sources, concentrations, risk and control strategy. Chemosphere, 197: 438-450.

Boix, C., M. Ibáñez, J.V. Sancho, J. Rambla and J.L. Aranda *et al*., 2015. Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry. Talanta, 131: 719-27.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environ. Microbiol., 8: 1137-1144.

Cai, M.Q., R. Wang, L. Feng and L.Q. Zhang, 2015. Determination of selected pharmaceuticals in tap water and drinking water treatment plant by high-performance liquid chromatography-triple quadrupole mass spectrometer in Beijing, China. Environ. Sci. Pollution Res., 22: 1854-1867.

Chaib, O., S. Achour, S. El Fakir, I. El Arabi and B. Oumokhtar, 2017. Occurrence of antibiotics resistant bacteria in fez Surface water (Morocco). Asian Jr. Microbiol. Biotech. Environ. Sc., 19: 2017: 65-71.

Chen, K. and J. Zhou, 2015. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere, 95: 604-612.

Chen, Y., H. Zhang, Y. Luo and J. Song, 2012. Occurrence and dissipation of veterinary antibiotics in two typical swine wastewater treatment systems in east China. Environ. Monit. Assess, 184: 2205-2217.

Collado, N., S. Rodriguez-Mozaz, M. Gros, A. Rubirola and D. Barceló *et al*., 2014. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. Environ. Pollution, 185: 202-212.

Conley, J.M., S.J. Symes, M.S. Schorr and S.M. Richards, 2008. Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. Chemosphere, 73: 1178-1187.

Derwich, E., Z. Beziane, L. Benabidate and D. Belghyti, 2008. Evaluation de la qualité des eaux de surface des Oueds Fès et Sebou utilisées en agriculture maraîchère au Maroc. LARHYSS J.

Dinh, Q.T., F. Alliot, E. Moreau-Guigon, J. Eurin, M. Chevreuil and P. Labadie, 2011. Measurement of trace levels of antibiotics in river water using on-line enrichment and triple-quadrupole LC–MS/MS. Talanta, 85: 1238-45.

Drechsel, P. and B. Keraita, 2014. Irrigated urban vegetable production in Ghana: characteristics, benefits and risk mitigation. IWMI.

Focazio, M.J., D.W. Kolpin, K.K. Barnes, E.T. Furlong and M.T. Meyer *et al*., 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States. II. Untreated drinking water sources. Sci. Total Environ., 402: 20-1216.
Gracia-Lor, E., J.V. Sancho, R. Serrano and F. Hernández, 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere, 87: 453-62.

Gros, M., S. Rodríguez-Mozaz and D. Barcelo, 2013. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr A, 1292: 173-188.

Gulkowska, A.L., H.W. So, M.K. Taniyasu, S. Yamashita and N. Leo et al., 2008. “Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. Water Res., 42: 395-403.

Hektoen, H., J.A. Berge, V. Hormazabal and M. Yndestad, 1995. Persistence of antibiotic agents in marine sediments. Aquaculture, 133: 175e184.

Inouss, H., S. Ahid, A. Belaiche and Y. Cherrah, 2015. Évolution de la consommation des antibiotiques au Maroc (2003–2012). EPI-CLIN 2015. Revue d’Épidémiologie et de Santé Publique, 63S: S61-S89.

Jiang, M., L. Wang and R. Ji, 2011. Biotic and abiotic degradation of four cephalosporin antibiotics in a lake surface water and sediment. Chemosphere, 80: 1399e1405.

Jin-Lin, L. and M.H. Wong, 2013. Pharmaceuticals and Personal Care Products (PPCPs): A review on environmental contamination in China. Environ. Int., 59: 208-224.

Karci, A. and I.A. Balcioglu, 2009. Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Science Total Environ., 407: 4652-4664.

Kasprzyk-Hordern, B., R.M. Dinsdale and A.J. Guwy, 2007. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J. Chromatography, 1161: 132-45.

Khan, G.A., B. Berghlund, K.M. Khan, P.E. Lindgren and J. Fick, 2013. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities – a study in Pakistan. J. Pone, 0062712: e62712.

Kim, S.C. and K. Carlson, 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environ. Sci. Technol., 41: 50-57.

Lerbech, A.M., J.A. Opintan, S.O. Bekoe, M.A. Ahiaiu and B.P. Tersbøl, 2014. Antibiotic exposure in a low-income country: Screening urine samples for presence of antibiotics and antibiotic resistance in coagulase negative staphylococcal contaminants. PLoS ONE, 9: e113055.

Leung, H.W., T.B. Minh, M.B. Murphy, J.C.W. Lam and M.K. So et al., 2012. Distribution, fate and risk assessment of antibiotics in sewage treatment plants in Hong Kong, South China. Environ. International, 42: 753 1-9.

Li, C., J.Y. Chen, J.H. Wang, Z.H. Ma and P. Han et al., 2016. Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci. Total Environ. 521: 101e107.

Liang, X., B. Chen, X. Nie, Z. Shi and X. Huang et al., 2013. The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. Chemosphere, 92: 1410-1416.

Liao, X., B. Li, R. Zou, S. Xie and B. Yuan, 2017. Antibiotic sulfanilamide biodegradation by aclimated microbial populations. Appl. Microbiol. Biotechnol., 100: 2439e2447.

Lien, L., N. Hoa, N. Chuc, N. Thoa and N. Phuc et al., 2016. Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment and the relationship with antibiotic use—a one-year study from Vietnam. Int. J. Environ. Research Public Health, 13: 588.

Locatelli, M.A.F., F.F. Sodré and W.F. Jardim, 2011. Determination of antibiotics in brazilian surface waters using liquid chromatography–electrospray tandem mass spectrometry. Archives Environ. Contamination Toxicology, 60: 385-93.

Lombard-Latune, R., N. Chahinian, J.L. Perrin, L. Benaabidate and A. Lahrach, 2010. Hydrological processes controlling flow generation in a Mediterranean urbanized catchment. IAHS Publication n° 340, pp: 69-76.

Moreno-Bondi, M.C., M.D. Marazuela, S. Herranz and E. Rodriguez, 2009. An overview of sample preparation procedures for LC-MS multiclass antibiotic determination in environmental and food samples. Analytical Bioanalytical Chemistry, 395: 921-946.

Nazer, D.W., R.M. Al-Sa’ed and M.A. Siebel, 2006. Reducing the environmental impact of the unhairing–liming process in the leather tanning industry. J. Clean. Prod., 14: 65-74.

Perrin, J.L., N. Raïs, N. Chahinian, P. Moulin and M. Ijaali, 2014. Water quality assessment of highly polluted rivers in a semi-arid Mediterranean zone Oued Fez and Sebou River (Morocco). J. Hydrology, 510: 26-34.
Petrović, M., B. Škrbić, J. Živančev, L. Ferrando-Climent and D. Barcelo, 2014. Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole–linear ion trap in different types of water in Serbia. Science Total Environ., 468-469: 415-28.

Prabhavathy, C.D.S., 2010. Treatment of fatliquoring effluent from tannery using membrane separation process: Experimental and modelling. J. Hazard. Mater., 176: 434-443.

Proia, L., D. von Schiller, A. Sanchez-Melsio, S. Sabater and C.M. Borrego et al., 2016. Occurrence and persistence of antibiotic resistance genes in river biofilms after wastewater inputs in small rivers. Environ. Pollut., 210: 121-128.

Raschid-Sally, L. and P. Jayakody, 2008. Drivers and characteristics of wastewater agriculture in developing countries: Results from a Global Assessment. Colombo, Sri Lanka. International Water Management Institute (IWMI Research Report 127).

Ruixue, H., P. Ding, D. Huang and F. Yang, 2015. Antibiotic pollution threatens public health in China. Lancet, 387: 773-774.

Sarmah, A.K., M.T. Meyer and A.B.A. Boxall, 2006. A Global perspective on the use, sales, exposure pathways, occurrence, fate and effects of Veterinary Antibiotics (VAs) in the Environment. Chemosphere, 65: 725-59.

Shishir, K.B., H.W. Kim, J.E. Oh and H.S. Park, 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Science Total Environ., 409: 4351-4360.

Smith, P. and O.B. Samuelson, 1996. Estimates of the significance of out-washing of oxytetracycline from sediments under Atlantic salmon sea-cages. Aquaculture, 144: 17e26.

Simon, N.S., 2005. Loosely bound oxytetracycline in riverine sediments from two tributaries of the Chesapeake bay. Environ. Sci. Technol., 39: 3480e3487.

Sørensen, L.K. and T.H. Elbk, 2004. Simultaneous determination of trimethoprim, sulfadiazine, florfenicol and oxolinic acid in surface water by liquid chromatography tandem mass spectrometry. Chromatographia, 60: 5-6.

Szekeres, E., A. Baricz, C.M. Chiriac, A. Farkas and O. Obris et al., 2017. Abundance of antibiotics, antibiotic resistance genes and bacterial community composition in wastewater effluents from different Romanian hospitals. Environ. Pollution, 225: 304-315.

Tamtam, F., F. Mercier, B. Le Bot, J. Eurin and Q.T. Dinh et al., 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Science Total Environ., 393: 84-95.

Tang, X., C. Lou, S. Wang, Y. Lu and M. Liu et al., 2015. Effects of long-term manure applications on the occurrence of antibiotics and Antibiotic Resistance Genes (ARGs) in paddy soils: Evidence from four field experiments in south of China. Soil Biol. Biochem., 90: 179-187.

Verlicchi, P.M., A. Al Aukidy, M. Jelic and D.B. Petrović, 2014. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: A case study of a catchment area in the po valley (Italy).” Science Total Environ., 470-71: 844-54.

Yan, C., Y. Yang, J. Zhou, M. Liu and M. Nie et al., 2013. Antibiotics in the surface water of the Yangtze estuary: Occurrence, distribution and risk assessment. Environmental Pollution, 175: 22-29.

Zhang, X. and T. Zhang, 2011. Occurrence abundance and diversity of tetracycline resistance genes in 15 sewage treatment plants across China and other global locations. Environ. Sci. Technol., 45: 2598-2604.

Zhang, X., H.X. Zhao, J. Du, Y.X. Qu and C. Shen et al., 2017. Occurrence, removal and risk assessment of antibiotics in 12 wastewater treatment plants from Dalian, China. Environ. Sci. Pollution Res., 24: 16478-16487.

Zhou, J.L. and K. Chen, 2013. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere, 95: 604-612.