Effect of High Concentration of Nevirapine on the Growth of E. Coli in Wastewater Treatment

Lawrence Obidike and Jean Mulopo

Abstract - With over 3 million HIV+ people on Anti-Retroviral drugs (ARVD), South Africa has the highest number of patients on treatment in the world. This anticipated overload of its wastewater with the compounds of these ARVDs and their metabolic products originating from the urine and faeces of these patients is expected to create new process problems for Wastewater Treatment Plants (WWTPs) and the organisms normally found in wastewaters. The objective of this study is to investigate the effect of nevirapine, one of the ARVDs used in the treatment of HIV, on Escherichia coli. For this experiment, a laboratory-scale wastewater treatment plants of 30 l volume was constructed, modeling the 3-Phase Phoredox process which. The growth of the E Coli is monitored from inception of the process with 20, 40, and 80 mg/l concentrations of nevirapine introduced into the wastewater. The pH, electrical conductivity and the e. coli presence in the wastewater were checked every two hours. For the 20 mg/l, 40 mg/l and 80 mg/l of nevirapine spiced wastewaters, the pH increased from 6.93 to 7.69, 6.93 to 7.30, and 6.77 to 6.87, respectively. The e. coli persisted in the wastewater as the nevirapine concentration increased.

***Index Terms* —, antiretroviral, escherichia coli, nevirapine, wastewater treatment.**

Introduction

South Africa (SA) is mostly semi-arid and endures regular droughts of varying degrees. Water, therefore becomes a precious commodity that needs to be fervently sourced and prudently managed. With only 94.7% of the population having access to clean drinking water, and about 710 000 households having no access to safe drinking water [12], wastewater has become a treasured source of freshwater that needs to be properly treated before use. However, the presence of pharmaceuticals and personal care products (PPCPs), pesticides, household detergents, fragrances, flavourings, steroids, hormones, nanoparticles, flame retardants, perfluorinated compounds and endocrine disrupting compounds in various environmental compartments in water supplies have raised concern recently. Researchers have attributed their presence to the inadequacy of the wastewater treatment [46; 45], and the improvement of analytical techniques [15; 36]. These are chemicals which have been in use for decades in consumer goods, but are now being discovered in water, and also newly synthesized substances, are collectively referred to as emerging contaminants (ECs) [11].

Many believe that, of all the emerging contaminants, antibiotics are of greatest concern. However, other compounds, especially polar metabolites and complex mixtures which include Antiretroviral drugs (ARVD), also pose great challenges to toxicologists [8]. This stems from the challenge of detecting them as well as predicting their long-term effect on living beings and organisms. Incidentally, these organisms in wastewater are critical in wastewater treatment plants utilizing the biological process. Unfortunately, there hasn't been any comprehensive national survey undertaken on the presence of ECs in drinking water in South Africa [42].

Like many other pharmaceuticals, ARVDs may not be completely metabolized by treated human and animals, but are subsequently excreted and discharged into wastewaters [35]. As a result, ARVD may find their way into the environment if they are not effectively eliminated during wastewater treatment [1-2]. Since wastewater treatment plants (WWTP) are not specifically designed to remove them, they are likely to be present in effluents from WWTPs even at trace levels [37], thereby acting as major point sources of ARV active pharmaceutical ingredients (API) to the aquatic environment. This is of concern as toxicity studies have shown that new emerging contaminants can cause adverse effects on the microbial populations, including those useful for effluent treatment in the WWTPs. On the other hand, reports have highlighted the effects of emerging contaminants in the aquatic systems as largely influenced by fate and behavior processes. Therefore, to elucidate the potential impacts and implications of ARV APIs for safe and efficient operation of WWTPs, require an understanding of the pollutants' fate and behavior in such systems. This is the motivation behind this research. The aim of this study is to:

(1) investigate the stability of API of Nevirapine in wastewater as a function of selected parameters such pH, Electrical Conductivity, etc. This is critical in order to elucidate the dissolution, persistence, fate, and potential toxicity of API of Nevirapine in wastewater;
(2) evaluate the fate and behavior of API of Nevirapine upon short term exposure to wastewater in simulated WWTP.

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(3) assess and examine bacterial viability in wastewater upon exposure to API of nevirapine using e coli as the focus bacteria.

Nevirapine API's toxicity may cause adverse effects to the microbial populations useful for effluent treatment in the WWTP. In this regard, sludge collected from both the test (WWTP with Nevirapine) and control units (WWTP without Nevirapine) will be used for bacterial viability assessments. Nevirapine was chosen for its high daily consumption by HIV+ patients in SA, as well as its stubborn persistency and high concentration in wastewater [29].

E Coli

Escherichia coli is a rod-shaped, Gram-negative bacterium in the family Enterobacteriaceae. It is one of the predominant facultative aerobic bacterium in the intestinal tract, even though the anaerobic bacteria outnumber it bountifully [9]. It was initially believed to mainly inhabit the lower intestinal tract of warm-blooded animals, including humans, and gets discharged into the environment through faeces and wastewater treatment plants [10] through which contamination of the environment may occur [7]. They can grow rapidly under optimal growth conditions, replicating in about 20 min. Pathogenic e. coli cause a variety of human diseases that result in >2 million deaths each year [18].

In the intestines of warm-blooded animals, e. coli is subjected to frequent encounters with antibiotics, leading to resistance against antibiotics consumed by its host [22]. Likewise, the consumption of ARVD by AIDS patients in SA, which is lifelong, unlike antibiotics, is believed to have the capacity to lead to a higher probability of ARVD resistance through build up in the intestine.

Both biotic and abiotic factors can influence the growth and survival of E. coli in natural environments [30]. Abiotic factors include temperature, water and nutrient availability, pH, and solar radiation, while biotic factors include the presence of other micro-organisms, and the ability of E. coli to acquire nutrients, compete with other micro-organisms and form biofilms in natural environments. Of all these factors, temperature and the availability of nutrients such as carbon, nitrogen and phosphorus are probably the most important factors influencing E. coli survival and growth in the environment.

Escherichia coli populations grew much better in sterile vs nonsterile soils, indicating that microbiota has a crucial effect on E. coli survival [41; 17]. The challenge for scientists is how these bacteria influence current water quality monitoring. In tracking the behavior of e. coli and the water quality through the wastewater treatment process, we can gain insight into the significant roles that organisms play, as well as evaluate their behavior when varying concentrations of Nevirapine are introduced into the wastewater.

Table 1: Specification requirement of the wastewater effluent issued by SA's DWS [14]:

Variable	Limit
pH	6.0 – 9.0
Electrical Conductivity	80 mS/m
Nitrates (as N)*	4 mg/l
Ammonia (as N)	1.5 mg/l
Chemical Oxygen Demand (mg/COD)	30 mg/l after removal
Typical (Faecal) coli (CFU/100ml)	0
Orthophosphate (as P)	0.5 mg/l
Suspended Solids (mg/l)	30 mg/l
Magnesium (Mg)	30
Chloride	75 mg/l
Fluoride	0.7 mg/l

Pharmaceuticals and Personal Care Product (PPCP)

PPCPs occur in the environment at low concentrations but their continuous presence leads to long term detrimental effects on aquatic and terrestrial organisms. They undergo mostly anaerobic and aerobic degradation, and very few undergo sorption. PPCPs include a wide variety of products, including prescription and non-prescription drugs, veterinary drugs, fragrances, cosmetics, soaps, sunscreens, antiseptics and antiperspirants. The main sources of PPCP in the environment are hospitals, veterinary clinics, industries, domestic wastewater and WWTP.

With SA's high rate of HIV/AIDS, it is expected that a growing concentration of ARV compounds will be in their river [40; 45] analyzed samples from almost every major river and dam in SA and found Nevirapine, Lopinavir and Zidovudine to be the most commonly occurring compound among their focus 12 ARVD. Stavudine, Nevirapine and Zidovudine had the highest averages, though their concentrations were in the low ng/L range. [38] study shows that some of the contaminants survive conventional drinking water treatment processes and appear in potable water whereas others are reduced. This is of grave concern as continuous ingestion of these contaminated waters will cause a toxin tolerance of the compounds, and which leads to drug resistance when such drugs are used in future for medical treatment.

Anti-retrovirals

ARVD are widely used in the treatment and prophylaxis of various viral infections including influenza, hepatitis, herpes and HIV (13; 19; 20; 26; 34]. The high number of HIV infected people in SA, about 6.3 million [44], naturally leads to high consumption of ARVD which are distributed at no cost to the patients by the government. According to World Health Organization (WHO), about 2 150 800 people in SA received ARVs in 2012, which contrasts sharply with the approximate 199 000 people on ARV therapy in Eastern Europe [43]. In December 2014, SA had, with just over 3 million people on treatment, the world's largest antiretroviral therapy programme. This figure is expected to

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increase by up to a million people in the next few years as the government rolls out its test-and-treat campaign. Some of the drugs presently being used in SA to treat HIV AIDS are Abacavir, Atazanavir, Darunavir, Didanosine, Efavirenz, Emtricitabine, Etravirine, Lamivudine, Lopinavir, Nevirapine, Raltegravir, Ritonavir, Stavudine, Tenofovir and Zidovudine.

There hasn't been much research done nor written about the presence of ARVs, and those of their transformation products in surface water [45] but being ranked as the eighth predicted most toxic drugs to typical aquatic organisms [31] elicits enough concern. Since the discovery of HIV as causative agent of AIDS, no less than twenty-five anti-HIV compounds have been formally approved for clinical use in the treatment of AIDS [4; 3]. These compounds fall into six categories: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs): protease inhibitors (PIs), cell entry inhibitors or fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs) [4; 23]. Nevirapine falls under the umbrella of NNRTI.

Nevirapine as an Emerging Contaminant

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) ARVD which is commonly given to pregnant women to inhibit transfer of HIV to the unborn baby. Its excretion is at 2.7% via urine after ingestion. Nevirapine's K_{OW} is less than 4 and indicates medium sorption potential and is less likely to bind to the PST sludge. This is supported by absence of nevirapine in the PST sludge.

Material

The following equipment will be used in the simulation experiments:

1. Two laboratory scale Wastewater Treatment Plants, each containing the following:
    a. 1 Primary Settling Tank (50 l)
    b. 1 Anaerobic tank (9 l)
    c. 1 Anoxic tank (7.84 l)
    d. 1 Aerobic tank (16 l)
    e. 1 Clarifier (19 l)
    f. 2 Transfer pumps (260 l/min)
    g. 1 Air flow meter.
    h. 1 Air Blower
    i. 1 Collection Vessel (20l)

Methods

A model wastewater treatment plant is constructed as shown in Figure 1 above. The model comprised of four tanks: anaerobic, anoxic, and aerobic tanks and a clarifier. Primary sewage (wastewater) is collected. The wastewater and activated sludge are collected after the primary screening with the removal of grit from Bushkoppie Wastewater Treatment Plant (Johannesburg South Africa: 26°18'40"S 27°56'6"E) and transported to the laboratory while kept in a cooler at 4^{o}C to reduce bacterial activity. In the laboratory, the wastewater and the activated sludge are fed into the

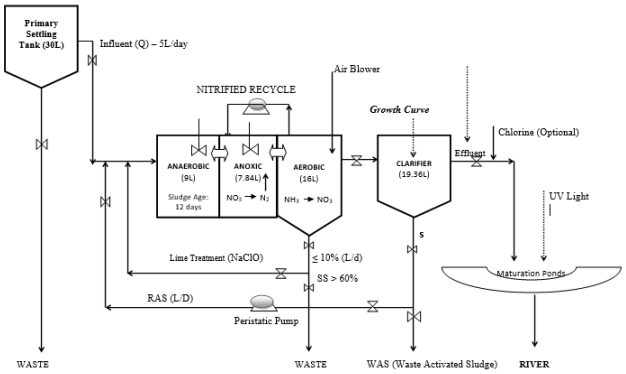

Figure 1: Wastewater Treatment Process

WWTP at a ratio of 1:1 to simulate biological treatment. Nevirapine (Aspen Pharmacare) obtained from a local pharmacy, which came in tablets of 200mg each was introduced into the anaerobic tank of the WWTP at concentrations of 20, 40, 60 and 80 mg/l, and samples were taken every 2 hours to monitor the presence of e. coli in the effluent. The growth of E Coli is measured using Merck Millipore's Samplers and Swabs Test Kit. The pH and electrical conductivity were also measured for each sample.

Discussions and Results

E Coli

In general, the impact of Nevirapine on the properties of the wastewater being treated, and E. Coli in the wastewater is investigated. Nevirapine is introduced into the wastewater and the concentration of the e coli in the effluent recorded regularly.

Figure 3: Impact of 20 mg/l of nevirapine on the e. coli

After 2 hrs	50/ml (Faint presence of e. coli)
After 4 hrs	Absence of e. coli
After 6 hrs	Absence of e. coli
After 8 hrs	Absence of e. coli

Figure 4: Impact of 40 mg/l of nevirapine on the e. coli

At 2 hrs	300/ml (Reasonable presence of e. coli)
At 4 hrs	500/ml (Overwhelming presence of e. coli)
At 6 hrs	30/ml (Trace presence of e. coli)
At 8 hrs	Absence of e. coli

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The impact of the different concentrations of the nevirapine on the e. coli is given above.

With the 20 mg/l of nevirapine wastewater, the e. coli disappeared after the 2 hours mark but before the 4 hour mark (Figure 3). But with the 40 mg/l of nevirapine wastewater, the e. coli until after the 6 hour mark, and only disappeared before the 8 hour mark. This shows the persistence of the e. coli with increasing concentration of nevirapine in the wastewater.

pH

This is expected to stay between 6 to 9 and it did so but there was an increase in the values as treatment time increased.

Figure 2: pH of Effluent of WWTP

	0hrs	2hrs	4hrs	6hrs	8 hrs
20 mg/l	6.93	7.09	7.17	7.44	7.69
40 mg/l	6.95	6.96	7.01	7.28	7.30
80 mg/l	6.72	6.81	7.05	6.87	6.87

Also, increase in the concentration of nevirapine increased the acidity of the wastewater effluent. An odd trend is the decline in alkalinity with the wastewater of 80 mg/l concentration. The alkalinity increased on inception of the experiment but started declining after the 4 hour mark but remained steady at the 6 hour mark.

Electrical Conductivity

This is a measure of the wastewater's ability to conduct an electrical current. It is frequently monitored and recorded. Through it, the ionic strength of the water will be calculated, thus:

$I = 1.6 \text{ X } 10^{-5} \text{ X EC } (\mu S/cm)$

Figure 2: Electrical Conductivity of Effluent of WWTP

	0hrs	2hrs	4hrs	6hrs	8hrs
20 mg/l	2280	535	408	184	200
40 mg/l	2265	197	215	208	211
80 mg/l	407	310	460	310	334

There is an immediate decline in the values both for the 20 mg/l and 40 mg/l of nevirapine spiced wastewater. For the 80 mg/l of nevirapine spiced wastewater, it initially rose to 460 µS/cm at the 4 hour mark but declined and returned to its original value of 310 µS/cm. This aberration still needs explanation.

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