# EMERGING CONTAMINANTS

## 'If it's brown, flush it down and if it's yellow, let it mellow': Exploring the issues surrounding untreated wastewater

*South Africa's rivers are under silent threat from pharmaceuticals and antibiotic-resistant bacteria escaping wastewater treatment works. New research using environmental DNA and global modelling reveals the extent of this pollution and its danger to ecosystems and health. Article by Nick Rivers-Moore, Isabella Gosetto, Miracle Osoh and Leo Quayle.*

Whether we thank them or blame them, it is reasonable to say that the Romans probably invented waterborne sewers and that the Victorians perfected them. Their legacy means that our sewage is flushed away, and we no longer need to think about it. However, we should be thinking about it, because through the city sewerage systems (the piping and reticulation systems that take the sewage away), millions of litres of wastewater are concentrated at single points within catchments across South Africa. Even if this wastewater has been treated to meet compliance standards, which, according to the latest Green Drop report, only equates to 36% of wastewater treatment works (WWTWs), this does not automatically mean that the water discharged back into rivers is 'clean'.

Dr Miracle Osoh, an environmental scientist working on wastewater pollution, explains: "Not all the medications we ingest are completely metabolised by our bodies." This means that every time we go to the toilet, we're also flushing a unique mix of chemicals and bacteria, defined by our gut microflora, the medications we take, and even the probiotics in our diets. Globally, while negligible in small amounts from a single household, the buildup of these products in wastewater can be harmful to freshwater ecosystems, particularly the active components found in most medicines, known as Active

Pharmaceutical Ingredients (APIs). Added to the mix are bacteria from our digestive systems, some of which are antibiotic-resistant and have the potential to negatively alter their receiving environment. The real issue? WWTWs around the world have not been designed to deal with eliminating this cocktail of additives in wastewater, including in South Africa.

As part of a broader Environmental Pollution Programme, funded by UK International Development from the UK government and delivered by the Joint Nature Conservation Committee (JNCC) in collaboration with South African partners, we were able to dig deeper into this challenge.  Our action-based research focused on emerging contaminants – pollutants that are only now starting to gain attention but are already affecting our rivers and aquatic life. Studies show that pharmaceutical pollution is an emerging yet major growing threat to freshwater environmental health. A recent study of pollution of the world's rivers due to APIs found that the highest concentrations of sites were in low- to middle-income countries, including sub-Saharan Africa.  Most of these occurrences were associated with poor wastewater and waste management infrastructure.  Combine this mix of harmful chemicals in freshwater ecosystems, with potentially antibiotic-resistant strains of human bacteria, and we are left with a ticking time-bomb of a breeding ground for strains of bacteria that have become resistant to antibiotics, commonly referred to as "superbugs".

**What we did**

We approached this research using two methods: Environmental DNA (or eDNA) sampling and modelling the concentration of pharmaceutical residues downstream of WWTWs. eDNA refers to the genetic material present in the environment, which is found in sediment, water, and air. It can include everything, from whole cells and free-floating DNA to potentially whole organisms. Collecting and analysing eDNA is an efficient method to provide a snapshot of the living organisms present in each location, and this method is becoming a vital tool in modern environmental monitoring.

The aim of our study is to investigate the extent to which a WWTW impacts aquatic life downstream in rivers, especially bacterial communities, due to untreated pharmaceutical pollution such as antibiotics. To explore this, we had two main goals:

- Compare the diversity of aquatic species upstream and downstream of WWTWs by analysing eDNA samples, looking at changes in the makeup of aquatic species, such as invertebrates, fish, bacteria and eukaryotes (organisms with complex cells).
- Link these changes to water quality data, measured upstream and downstream of a WWTW.

We also wanted to answer two key questions: (1) does the level of WWTW compliance (i.e. how well it meets treatment standards) influence the types of bacteria and other life found downstream; (2) given the widespread use of pharmaceuticals and antibiotics in particular, where is the risk to aquatic life highest form these pollutants highest relative to WWTW servicing urbanised areas?.  eDNA sampling points were selected from two sites in KwaZulu-Natal, upstream and downstream of the Howick sewage works discharge point on the uMngeni River, and upstream and downstream of the Mooi River WWTW discharge point on the Mooi River.

eDNA samples were collected according to the sampling methods developed by NatureMetrics, a UK-based environmental DNA lab. This included taking a mixed sample from a variety of locations at the site, and syringing the water samples through filters sized to collect the appropriate DNA material for analysis. Three samples were taken at each site: one sample targeting invertebrates and vertebrates in the water column, one sample targeting bacteria and eukaryotes in the water column, and one sample specifically targeting organisms in the sediment. The sediment sample was not filtered and was collected manually from areas where the sediment type differs and put into a sealed and sterilised plastic container. The filters and sediment samples were preserved, sealed and labelled and couriered to the NatureMetrics laboratory in the UK.


Leo Quayle

*Sample collection through syringing sample through a filter.*

Nick Rivers-Moore)

*Filter labelled, sealed and preserved.*

Nick Rivers-Moore)

*Sediment sample labelled, preserved and sealed.*

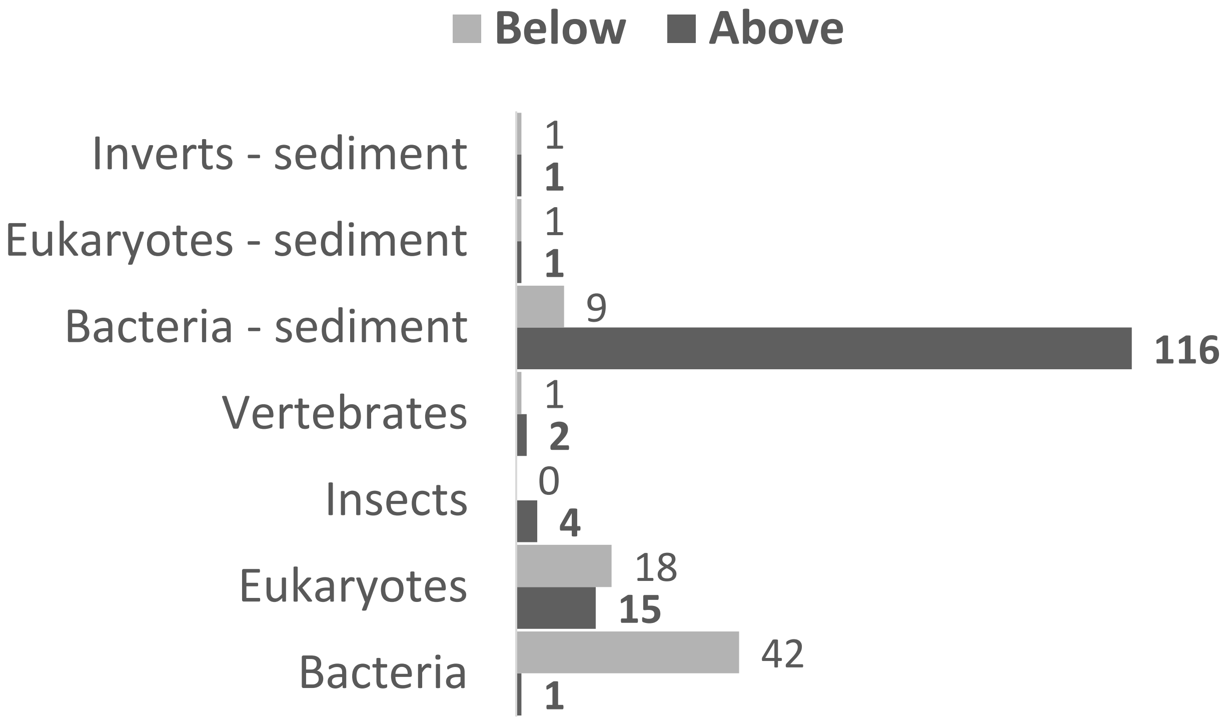
A complementary study has mapped out which rivers in South Africa are most at risk from a widely used antibiotic called sulfamethoxazole (SMX). This drug, commonly prescribed to treat bacterial infections, has become one of the most frequently detected antibiotics in rivers and streams across the world. The problem? Even at very low concentrations, SMX can fuel antimicrobial resistance (AMR) – a growing global health threat where bacteria evolve to resist medicines that once killed them.

To understand how and where SMX enters South Africa's waterways, researchers turned to a powerful global modelling tool called HydroFATE. This system pulls together a wide range of information: river maps, wastewater treatment performance, medicine use, population data, and river flow patterns. By combining these layers, the model can estimate how much of the antibiotic is being released into rivers and how long it lingers there.

The approach is detailed but intuitive: it looks at how much SMX people consume, how much passes through their bodies into sewage, how well local treatment plants filter it out, how sensitive aquatic life is to the drug, and how quickly it breaks down once it enters a river. The results show which stretches of river are most vulnerable, both under normal flow conditions and during dry periods, when low water levels make pollution problems worse.

**What we found**

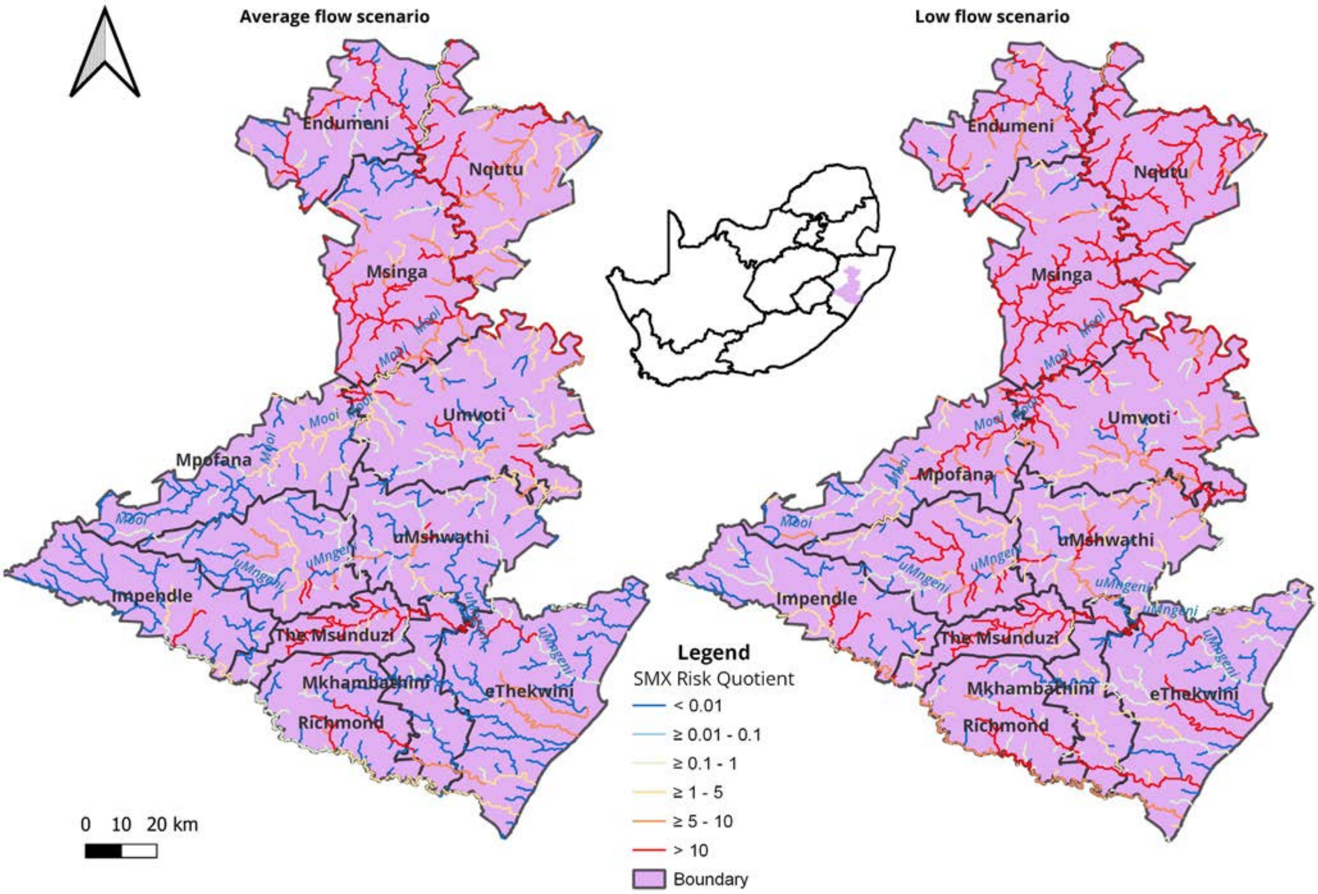
The number of observable taxonomic units (OTUs) that occurred either exclusively above or below the WWTWs in both river systems showed several differences at the lowest levels of the food chain. In the aquatic samples, there were similar numbers of OTUs that occurred exclusively downstream and upstream of the WWTWs (18 and 15, respectively), whereas only one species of bacteria occurred exclusively upstream of both WWTWs, compared to 42 species of bacteria that only occurred downstream of the WWTWs. In contrast, only the bacterial communities from the riverbed samples provided any discriminating power of the impacts of the WWTW on aquatic communities at different levels of the food chain. Here, the pattern was the inverse of what was reflected from the water samples, in that there were 116 OTUs of bacteria that only occurred upstream of the WWTWs, compared to nine species that occurred exclusively below both WWTWs.



*The number of OTUs that occurred exclusively above or below WWTWs for the Mooi and Umgeni rivers across a range of living groups for the water and riverbed samples.*

Of the 59 122 rivers assessed, about 38% were potentially at risk of sulfamethoxazole during average flow conditions and about 52% were at risk of SMX during low flow conditions. About 116 km of the Mooi River was predicted to be at risk of SMX impact at both average and low flow scenarios, and risk generally increased by about four orders of magnitude higher during low flow conditions.

For the uMgeni River, about 121,2 km of river were at risk of SMX during average flow conditions. The length of the uMgeni River at risk of SMX increased to 168.6 km during low flow conditions. The concentration of SMX and associated risk along the uMgeni River varied significantly depending on the proximity of the river to WWTW effluent, urban centres and input from rural water washing off the land. In various rivers across South Africa, the risk of SMX can be associated with areas remarkable for high human population densities. This calls for further investigation of the socioecological dimensions of pharmaceutical pollution in South Africa.



***Maps of Mooi and uMgeni River catchments showing risk of SMX under average and low flow scenarios.***

## Conclusions

Dr Nick Rivers-Moore, a freshwater ecologist, warns: "Wastewater treatment generally fails to remove antibiotics from entering river systems, where there is the potential for the development of antibiotic-resistant bacteria." For example, studies in the Msunduzi River in South Africa found high concentrations of several antibiotics downstream of a wastewater treatment plant. This raises concern not only for the environment, where different species may be affected in different ways, but also for human health, as there is a higher presence of antibiotic resistance genes in these areas.

The good news is that these findings don't just highlight a problem, they point the way to solutions. Thanks to cutting-edge tools like environmental DNA (eDNA) and global models such as HydroFATE, scientists can now "see" pollution in ways that weren't possible before. This means we can identify which rivers are most at risk, understand what's driving the problem, and start developing smarter, more effective ways to protect our water.

"Both of these studies highlight exciting future research avenues," says Dr Rivers-Moore. "They also show that we finally have the scientific tools to turn awareness into action." With this new knowledge, South Africa, and indeed the world, has a chance to get ahead of the problem, safeguarding rivers, wildlife, and human health for the future.