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While skin lesions led to social stigma, the most serious consequences of long-term arsenic exposure are cancers, diabetes, cardiovascular disease and impaired intellectual development in children.

Despite intensive efforts to reduce arsenic exposure, an estimated 39 million people in Bangladesh are still consuming contaminated water. In the worst affected areas around 20% of all deaths can be attributed to arsenic poisoning and it claims 43,000 lives every year. Whilst Bangladesh has endured the greatest burden, arsenic contamination at levels above WHO guidelines is a global issue, affecting at least 140 million people in 50 countries.

Researchers from the Centre for Synthetic and Systems Biology at the University of Edinburgh are finding new ways to tackle this threat. Our group has developed a user-friendly bacteria-based biosensor to detect unsafe arsenic levels. The portable device could prove a game changer for resource-limited countries, costing just 30 pence per test.

### Arsenic: smartphone-friendly biosensor to tackle an insidious global threat

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#### Background

It was the largest mass poisoning of a population in history – bigger even than Chernobyl. This alarming assessment by the WHO in the 1990s marked a dark period in Bangladesh. The country faced a silent killer threatening the lives of millions. Nearly 30 years later they are still struggling with its devastating consequences.

The tragedy is that this was the unintended consequence of a project designed to bring safe drinking water to millions. In the 1970s, one of the biggest killers of children in Bangladesh was drinking stagnant water contaminated by microorganisms. To tackle this, an internationally funded effort installed tube wells across the country, tapping into underground water sources. The private sector soon stepped in to install millions more. By 1997, 80% of the population had direct access to ‘safe’ water that had travelled through rocks and sediments, filtering out the most harmful microorganisms.

But in the years that followed tube well installations, people began to display worrying symptoms. Skin lesions were the first sign of many potentially fatal health problems. The cause was arsenic, a natural but highly toxic component of the earth’s crust. Arsenic has a long history as a poison, used by the ruling classes during the Middle Ages and Renaissance to murder rivals. Odourless and tasteless, it became known as the ‘Poison of Kings and the King of Poisons’. Its insidious nature contributed to the crisis in Bangladesh. The lower levels typically found in contaminated water meant that it did not kill quickly – the first symptoms could take 10 years to appear.

It was the private installation of shallow and more affordable tube wells in many Bangladeshi households that raised the risk of arsenic contamination compared with deeper government and community wells. Half of the 10 million tube wells were found to be contaminated.

#### Nikon | USB | Cell phone | [As$^{3+}$]

| 0 ppb | 1 ppb | 10 ppb | 50 ppb | 100 ppb |

Microfluidic encapsulation-enabled microbial sensor array for monitoring arsenic contamination, showing different output patterns on various arsenic (As$^{3+}$) levels. Left, middle, right panels: images acquired by a Nikon microscope, USB fluorescence microscope and cell phone camera.

While skin lesions led to social stigma, the most serious consequences of long-term arsenic exposure are cancers, diabetes, cardiovascular disease and impaired intellectual development in children.
In Bangladesh, arsenic monitoring is challenging and expensive. Water samples need to be analysed in specialist laboratories by atomic absorption spectroscopy (AAS). There is typically only one laboratory for every five districts. Remote communities face long journeys to drop off their samples and the success of public awareness campaigns has led to backlogs as long as 2 months. Although portable test kits are available, they are expensive and produce toxic chemicals. Household filtering systems are a promising solution, but water must be regularly tested to ensure they work correctly.

Biosensors have long been championed as a potential solution to meet the urgent need for affordable, on-site testing. Many bacteria, such as Escherichia coli, grow easily in the presence of arsenic and have genetic machinery encoding metabolic processes that detect and pump out this toxin. By altering their genetic circuit to produce visual pigments in the presence of arsenic, bacteria could provide a simple and self-renewing form of detection.

However, the technology has struggled to make it out of the lab – biosensors are often difficult to use and rarely sensitive enough for real-world conditions. Attempts to
improve biosensors have often focused on one feature over another leading to trade-offs.

To create a biosensor that tackled these market barriers we combined several approaches. To improve sensitivity we needed to adjust the biosensor’s sensing module. In *E. coli* the presence of arsenic functions as an ‘on switch’. It binds to the surface of particular protein receptors that repress the genetic circuit, disrupting their function. This activates the genetic circuit, producing an output – in our biosensor, green fluorescence protein (GFP). The threshold for this switch depends on the amount of ‘binding’. By simply reducing the number of arsenic receptors we increased sensitivity 5000-fold, allowing detection of arsenic levels below WHO guidelines of 10 parts per billion (ppb).

Whilst improving sensitivity was important, we needed to ensure that enough fluorescent protein was produced to be visible to the human eye. To achieve this we introduced genetic parts that function as biological amplifiers. Similar to boosting the horsepower of a car’s engine, these amplifiers act as turbo boosters converting the signal received by the bacteria’s sensing module into a stronger output. We tested the performance of a number of these genetic amplifiers and then created a cascade, installing them one after another, to boost GFP production.

The downside of increasing sensitivity and output is that it increases background noise. For genetic machinery to work effectively it is never fully switched off. This means that small quantities of GFP are produced in the absence of arsenic and could result in a false-positive reading.

To tackle this trade-off we used two approaches. By altering the genetic circuit to include an extra binding site for the repressor proteins we created a ‘roadblock’ that reduced background GFP production. We also modified the fluorescent protein reporter, including a degradation tag that triggered it to be broken down by the bacteria. However, we didn’t want GFP to be continuously degraded in the presence of arsenic so we added another protein, which acts as ‘biological scissors’, cutting off the degradation tag as arsenic levels rise.

These advances provided precise mechanisms for controlling sensitivity, but we also needed to translate GFP production into an accurate measurement of arsenic levels. To do this we created bacteria with different levels of arsenic sensitivity and placed them inside a clear plastic device – seeding them in patterns, similar to volume bars, displaying the level of contamination. After allowing time for GFP to be produced, the device is attached to a smartphone, using the camera to illuminate the volume bars.

Working with local partners in Khulna University and monitoring offices allowed us to test the biosensor. We travelled to Bangladesh to collect well water samples from villages in some of the worst affected regions, with arsenic levels up to 20 times higher than WHO guidelines. The arsenic levels reported by the biosensor were consistent with lab-based standard tests, proving its accuracy.

However, there are still hurdles in the journey to market. Devices using genetically modified bacteria face an uncertain path through Europe’s regulatory environment. We used microfluidic devices and hydrogel to trap bacteria and tackle safety concerns. But we are also exploring cell-free systems. If successful, the genetic circuits, floating in a cytoplasmic soup, could be freeze-dried onto paper with a hydrophobic barrier that prolongs its shelf life. When ready to use it is simply rehydrated with a water sample. We also aim to improve speed as the current biosensor requires 24 h of incubation. Increasing bacterial cell densities and exploring alternatives to GFP, such as enzymes that produce a faster, colorimetric output, could help to achieve this.

The real advantage of our approach is that it’s not limited to arsenic. It opens the door to a new generation of ultrasensitive biosensors with many uses, including detecting other environmental toxins, disease diagnosis or even detecting landmines.
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