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River toxicity assessment using molecular biosensors: heavy metal contamination in the turag-balu-buriganga river systems, dhaka, bangladesh

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C P. N. Rampley³, P. G. Whitehead^{1, 3}, L. Softley¹, M.A. Hossain², L. Jin⁵, J. David¹, S. Shawal², P. Das², I. P. Thompson⁴, W. E. Huang⁴, R. Peters¹, P. Holdship⁶, R. Hope¹ and G. Alabaster⁷

¹ School of Geography and the Environment, University of Oxford, Oxford, OX1 3QY, UK

² Institute of Water and Flood Management (IWFM), Bangladesh University of Engineering and Technology (BUET) Dhaka 1000, Bangladesh

³ Oxford Molecular Biosensors, Centre for Innovation and Enterprise, Begbroke, Oxford OX5 1PF, UK

⁴ Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PF

⁵ Geology Department, State University of New York College at Cortland, Cortland, NY 13045, USA

⁶ Department of Earth Sciences, University of Oxford, Parks Road, OX1 3AN

⁷ United Nations Human Settlements Programme, Waste Management and Sanitation Division, Palais des Nations, Avenue de la Paix, 8-14, 1211 Genève, Switzerland

Corresponding author: Paul Whitehead email paul.whitehead@ouce.ox.ac.uk

ABSTRACT

Pollution in rapidly urbanising cities and in delta systems is a serious problem that blights the lives and livelihoods of millions of people, damaging and restricting potable water supply and supplies to industry (Whitehead et al, 2015, 2018). Employing new technology based on luminescent molecular biosensors, the toxicity in the rivers around Dhaka in Bangladesh, namely the Turag, Tongi, Balu and Buriganga, has been assessed. Samples taken at 36 sites during medium and low flow conditions and during the Bishwa Ijtema Festival revealed high levels of cell toxicity, as well as high concentrations of metals, particularly aluminium, cadmium, chromium, iron, zinc, lithium, selenium and nickel. Chemical analysis also revealed low dissolved oxygen levels and anoxic conditions in the rivers at certain sites. The bacterial molecular biosensors were demonstrated to be fast, with results in 30 minutes, robust and a highly sensitive method for the assessment of water toxicity in the field. Furthermore, the biosensor toxicity analysis correlated with the metals data, and a multivariate regression relationship was developed relating toxicity to key metals, such as selenium, zinc and chromium. The resulting model has been validated against split samples and the Bishwa Ijtema Festival data. The combination of modelling and the molecular biosensor technology provides a new approach to detecting and managing pollution in urban river systems.

KEY WORDS

Biosensors, toxicity, Bangladesh, Buriganga River, metals, pollution, Bishwa Ijtema

INTRODUCTION

In many countries undergoing rapid economic growth from industrialization, surface water pollution is a major problem. Poor water quality affects people's health and livelihoods, and limits the availability of water for fisheries, agriculture, and public water supply. Pollution can also limit long-term economic growth for water intensive industries such as textiles, which need a high quantity and quality of water for production. At the same time, improving water quality is a critical element of the United Nations 2030 Sustainable Development Goals (SDGs), fulfilling an essential role in reducing poverty and disease and promoting sustainable growth. SDG 6 is aimed at ensuring the availability and sustainable management of water and sanitation for all. It is underpinned by target 6.3, to improve water quality by reducing pollution, eliminating dumping, and minimising the release of hazardous chemicals, by halving the proportion of untreated wastewater (UN, 2019).

Pollution is extremely serious in Bangladesh where the rivers in and around the capital city of Dhaka (Figure 1) are heavily impacted largely due to untreated waste. In this area alone there are over 30,000 factories discharging into the rivers. Whitehead *et al* (2018) demonstrated that rivers in the Greater Dhaka Watershed have high levels of organic pollution levels, pathogens, and ammonia with consequently low dissolved oxygen (DO) levels which are close to zero in the low flow periods. The low DO levels create anoxic conditions, resulting in metals mobilisation and the release of toxic gases such as hydrogen sulphide. In this paper we review additional sampling data from rivers around the Greater Dhaka Watershed and evaluate both the chemistry of the metals and toxicity of the water samples, in order to provide useful water quality information and demonstrate a new method to rapidly assess toxicity and pollution in urban river systems.

As with other Asian mega-cities, economic growth in Dhaka depends on the health of its rivers which support communities, industry, and agriculture. However, as economic activity in Dhaka grows, water demand increases while wastewater treatment lags. Only 25% of households are serviced by sewage treatment facilities, and industrial pollution contributes 60% of total pollution in the Dhaka watershed (Islam *et al.* 2015). Further pollution control is required to improve this situation and, in this paper, we explore a new technology of enhanced bacterial biosensors to speed up pollution detection and relate measured toxicity to metal concentrations. Previously, numerous species of naturally luminescent bacteria have been successfully exploited to inform on environmental toxicity (Gu, 2005). Hassan *et al* (2017) report on biosensors used to monitor pesticides and Long *et al* (2013) discuss the use of biosensors to measure metals in the environment. Justino *et al* (2017) give a very thorough review of the applications of biosensors in the environmental monitoring. However, modern synthetic biology techniques have allowed the development of specifically designed, tightly controlled biosensors, which may be more sensitive to the toxicity of complex environmental media. This engineered biosensor technology is robust and can provide a rapid assessment of the toxicity of water supplies, groundwaters or river waters (Cui *et al.*, 2018). Field analysis is possible by using a portable device, which eliminates the time-consuming need to transport samples back to laboratories for chemical analysis. The biosensors offer a means of improved pollution assessment and can be coupled with water quality models to inform optimal strategies to improve water quality.

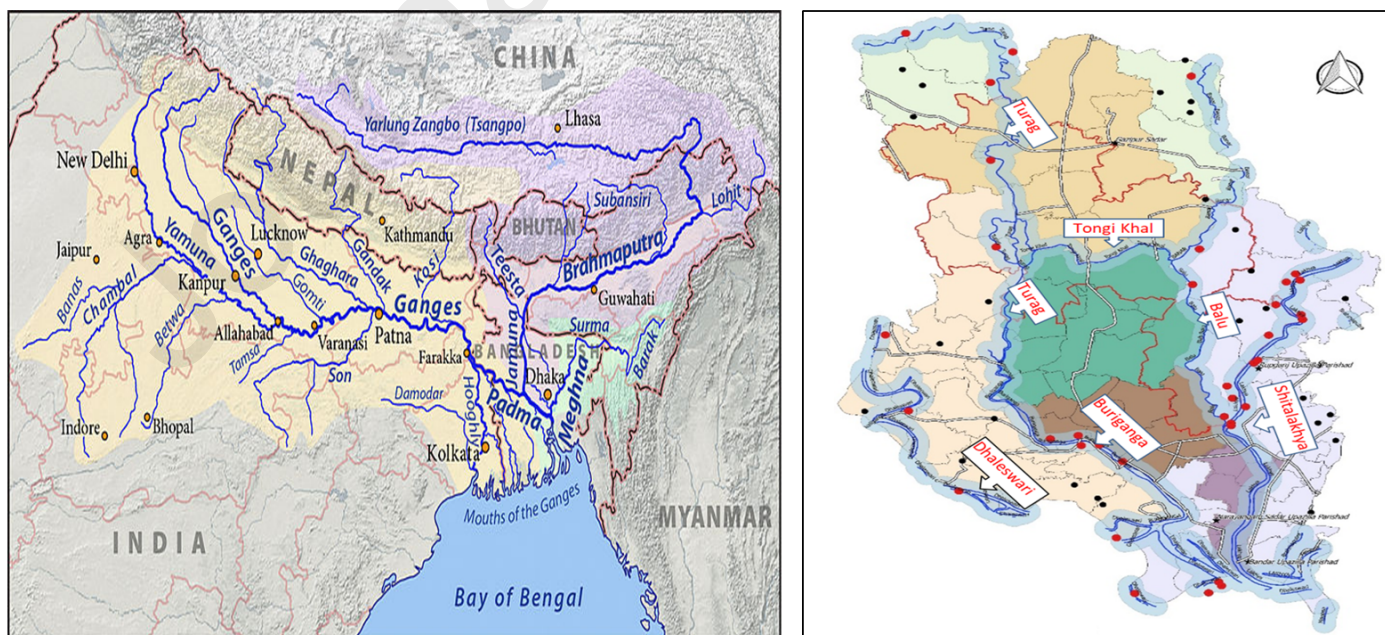


Figure 1 Maps of Bangladesh showing Dhaka at the junction of the Brahmaputra, Ganges (Padma) and the Meghna, with details of the Turag, Tongi Khal, Balu and Buriganga rivers surrounding Dhaka

WATER QUALITY DATA AND METALS CHEMISTRY

Water quality data for Dhaka rivers is sparse and so a new set of data has been derived by Bangladesh University of Engineering and Technology (BUET) and University of Oxford from field sampling and measurement of water quality parameters, plus laboratory analysis for key parameters. The rivers included were the Turag-Tongi-Balu River System and the Buriganga System, as shown in Figure 1. Sites were selected following geographic information systems (GIS) mapping tools (ARC GIS) and satellite image analysis as well as local visits. All locations are listed in the supplementary material, together with the details of the field and laboratory analysis undertaken. Metals analysis was undertaken using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a PerkinElmer NexION 350D ICP-MS at the Department of Earth Sciences, Oxford University. A total of 78 water samples from the Turag-Tonga-Balu, and Turag-Buriganga were sampled during December 2017 and January 2018, and a standalone study was conducted for the Bishwa Ijtema Festival in January 2018.

Figure 2 shows the samples sites where water quality data has been collected with samples being analysed for temperature, turbidity, dissolved oxygen (DO), ammonia, nitrate, total coliforms (TC), and *E. coli*, all of which are shown in Table 1. Whilst Bangladesh national water quality regulations are striving to meet global standards, many of the water samples do not meet the minimum biological, chemical, or physical parameters requirements (Table 1). Water quality for the rivers around Dhaka was found to be poor, with very low DO, high organic loading, and high levels of pathogens such as total coliforms and *E. coli* which greatly exceed national standards as set by the Department of Public Health Engineering (DPHE). Several water quality parameters tested at sample sites exceeded national standards in the dry season, including DO, ammonia, TC, *E. coli* and suspended solids (SS). High total dissolved solids, nitrate, ammonia, phosphates and pathogens are observed with very high ammonia levels which can cause very low DO levels. The DO levels are so low that the waters and sediments in the observed Turag-Tongi-Balu rivers may be presumed to be anaerobic, with metals and gases such as methane and hydrogen sulphide being released. This is very poor from a public health perspective, with significant impact on people working or living close to the river.

Table 1: Range of water quality parameters for Turag-Buriganga-Tongi Khal – Balu river system

Parameter	Observed December 2017	Observed January 2018	Observed Bishwa Ijtema 2018	Dept. Public Health Engineering Standards (2019)
Temp (°C)	22.5 - 25	20.3 - 24.1	20.1 - 24.1	20 - 30
pH	7.5 - 8.01	7.21 - 8.26	7.12 - 7.7	
EC (µS/cm)	277 - 679	593 - 1115	765 - 977	
TDS (mg/L)	184.1 - 327	301 - 580	406 - 490	1000
DO (mg/L)	0.21 - 2.36	0.16 - 0.45	0.22 - 1.78	6
Turbidity (NTU)	12.15 - 42.1	16.93 - 85.9		10
Color (Pt-CO)	34 - 180	77 - 457		15
Suspended Solids (mg/L)	7 - 48	5 - 127		10
Alkalinity (mg/L as CaCO ₃)	45 - 215	28 - 79		
Ammonia-N (mg/L)	0.17 - 8.4	1.94 - 21.12		0.5
Nitrate (mg/L)	0.3 - 78.1	0.6 - 5.8		10
Phosphate (mg/L)	1.2 - 7.9	1.9 - 13.7		6

Sulphide ($\mu\text{g/L}$)	0 – 15	5 – 70		
Sulphate (mg/L)	20 – 30	30 – 35		400
Chloride (mg/L)	120 – 270	74 – 325		150 - 600
TOC (mg/L)	0.99 - 6.46	0.19 - 13.2		
TC count/100ml	90000 - 870000	120000 - 1040000		
E. coli count/100ml	10000 - 420000	30000 – 490000		

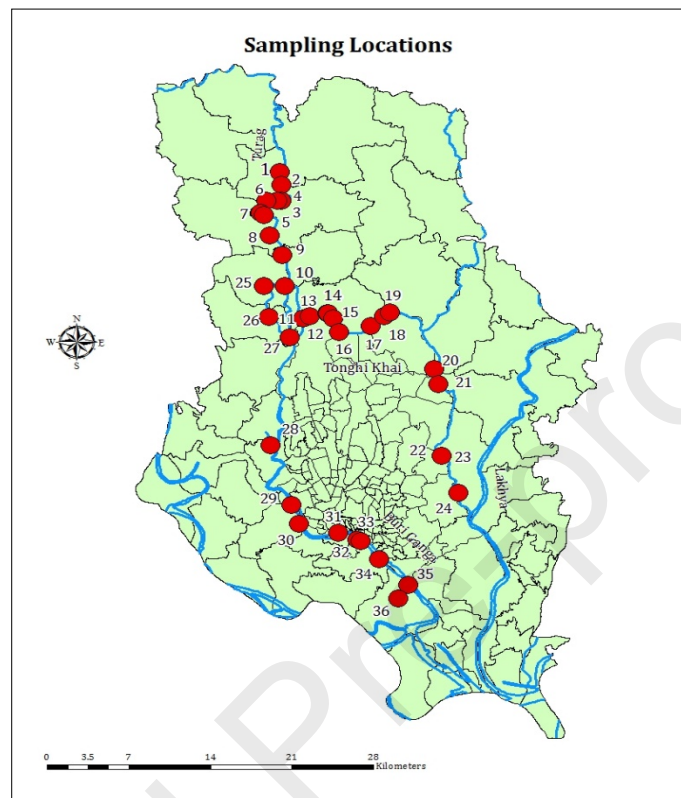


Figure 2 Sampling sites for chemical and metals analysis in the study area. Sample sites 1-24 are in the Turag-Tongi-Balu river system and sample sites 25-36 are in the Buriganga river system.

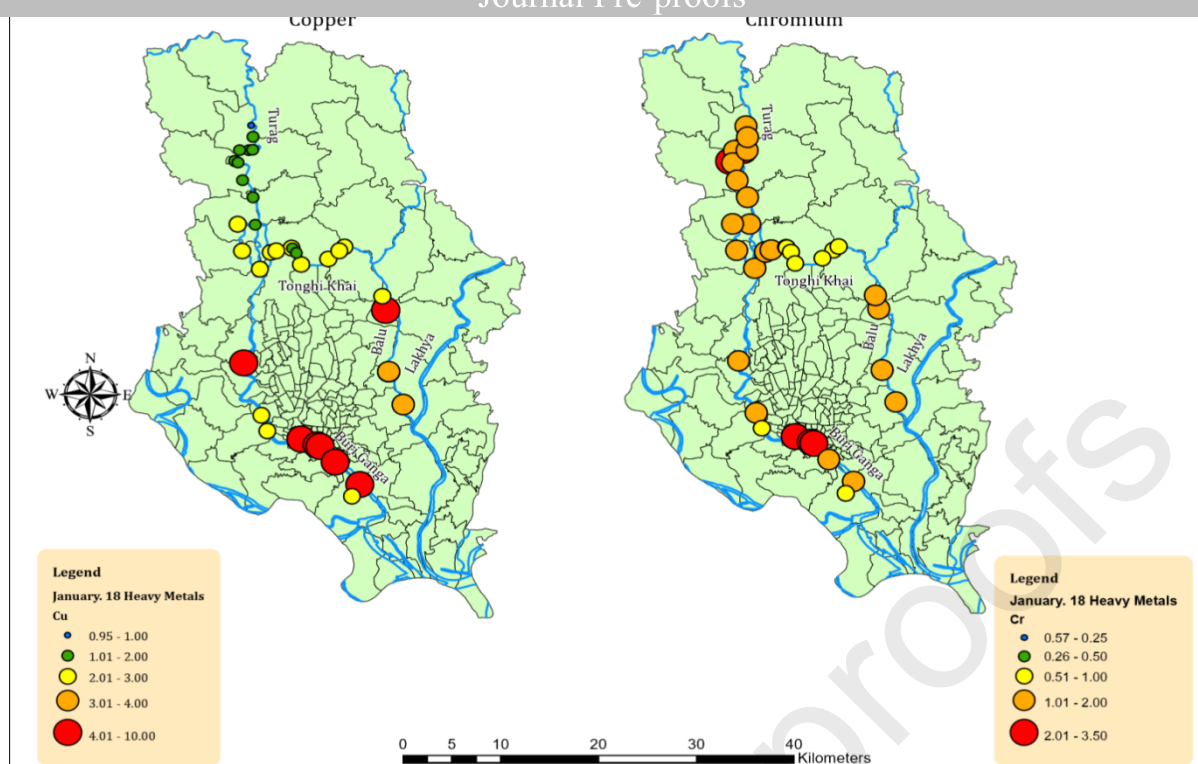


Figure 3 Sampling sites for chemical and metals analysis and concentrations of Copper and Chromium (units are ppb or $\mu\text{g/L}$) at key reach boundary points from January 2018 samples.

Metals Chemistry and industrial context

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a PerkinElmer NexION 350D ICP-MS at the Department of Earth Sciences, Oxford University, was used to determine the concentration of heavy metals in the samples collected from 36 sites in the Turag and Buriganga Rivers in both December 2017 and January 2018. In addition, 6 samples were collected from the Tongi Khal immediately after the Bishwa Ijtema Festival in January 2018. Table 2 lists 20 selected metals, the corresponding observed concentrations.

Table 2 Observed ranges for 20 selected heavy metals and arsenic metalloid; all values quoted in ppb ($\mu\text{g/L}$)

Metal	Observed December 2017	Observed January 2018	Observed Bishwa Ijtema 2018
Aluminium	2.863 – 12.151	6.481 – 53.226	6.093 – 41.475
Arsenic	0.722 – 5.834	2.696 – 5.226	2.474 – 3.594
Barium	9.314 – 42.018	22.935 – 43.967	35.726 – 43.618
Cadmium	<i>BDL</i> – 0.028	0.004 – 0.072	0.007 – 0.018
Caesium	<i>BDL</i> – 0.055	0.022 – 0.077	0.060 – 0.092
Chromium	0.099 – 1.561	0.570 – 3.175	1.162 – 6.470
Cobalt	0.022 – 0.883	0.066 – 0.194	0.095 – 0.332
Copper	0.375 – 6.669	0.947 – 9.053	0.383 – 7.450
Iron	0.678 – 5.024	1.911 – 12.779	20.649 – 92.734
Lead	0.004 – 0.158	0.004 – 0.0824	0.0462 – 0.108
Lithium	1.369 – 5.212	4.508 – 7.891	5.712 – 6.517
Manganese	<i>BDL</i> – 9.378	0.111 – 96.590	180.338 – 532.978
Mercury	<i>BDL</i> – 0.024	<i>BDL</i> – 0.024	<i>BDL</i> – 1.69

Nickel	0.218 – 2.849	1.219 – 3.177	1.164 – 2.914
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Selenium	0.175 – 0.813	0.661 – 2.071	0.970 – 1.681
Tin	BDL – 0.048	BDL – 0.079	0.051 – 1.787
Tungsten	BDL – 0.468	0.020 – 0.180	0.069 – 0.241
Uranium	0.085 – 1.459	0.465 – 1.408	0.404 – 0.971
Zinc	2.269 – 7.603	3.148 – 19.916	2.548 – 13.335

Note BDL-Below Detection Limits

Water quality in the river systems around Dhaka are driven by effluent discharges from the thousands of factories discharging into the rivers as well as largely untreated domestic waste (Islam et al, 2016). The concentrations are also affected by flows in the rivers which are highly variable with very low flows in the dry season and vary high flows in the wet season. The high flows significantly dilute the effluents and pollution runoff, but low flows exacerbate the pollution loads and create extremely poor water quality. The three data sets shown in Table 2 indicate the water quality in medium flow conditions (December 2017), low flow conditions (January 2018) and during the Bishwa Ijtema festival. The data shows that the water quality deteriorates from December to January as the river flows fall and deteriorate further during the Bishwa Ijtema festival.

GIS plots for the 20 key metals have been produced using the ARC GIS software to locate pollution hotspots along the river system (see Figure 3 for Copper and Chromium). The plots identified four key areas where the observed concentrations of multiple heavy metals were highest:

1. Upper reaches of the Turag River (north of Dhaka City) – has high concentrations of Al, Ba, Cd, Cr, Co, Cu, Fe, Pb, Hg, Ni, Se, Sn, U, Zn
2. Tongi Khal – has high concentrations of Al, As, Ba, Cs, Co, Cu, Li, Rb, Se, Sn, W, U
3. Lower reaches of the Buriganga River – has high concentrations of all metals
4. Lower reaches of Balu River – Al, As, Cs, Cu, Se, Sn, W, Zn

Waste effluent from industries in Dhaka contains many chemicals used in the processing of textiles, leathers, bricks, steel, paper, and fertilizers. The upper reaches of the Turag River are located near the Dhaka Export Processing Zone (DEPZ), which houses over 100 factories, many of which produce and export textiles and garments. All units within the DEPZ are required to have Effluent Treatment Plants (ETP). However only a few industries comply with these rules and many do not run their ETP to save on costs (Islam et al, 2016). Similarly, the Konabari Kashimpur industrial cluster, located south of the DEPZ, and Ashulia, which is situated at the junction of the Turag River with the Tongi Khal and Buriganga and contains over 300 textile factories, will also contribute to the levels of heavy metal pollution in the upper reaches of the Turag River (Rouf et al, 2013, Shams et al, 2009). The Tongi Khal runs through a highly urbanised and industrialised area of Dhaka, where hundreds of factories are discharging their untreated waste directly or indirectly into the canal from both sides (Hossen et al, 2017).

From 1960 until 2017, approximately 90% of the tanneries in Bangladesh were located in Hazaribagh, which is situated in central Dhaka along the lower reaches of the Buriganga River. Tanneries use many heavy metals to process hides to form leather, including aluminium, arsenic (metalloid), cadmium, chromium, copper, iron, lead, mercury, nickel, and zinc. According to the Bangladesh Department of Environment (DoE), 22,000m³ of untreated liquid waste from the tanneries was indirectly discharged into the Buriganga River (Hossen et al., 2017, Rouf et al., 2017). Hazaribagh was regarded as one of the most polluted areas on the planet in 2013 (Chowdury et al., 2013), driving the government to order the tanneries to close and relocate to Savar, an area north of Dhaka less affected by industrial pollution. Despite the closure of the

Industrial and built-up areas account for over 75% of the land use in the western side of Dhaka, and 65% is classed as medium or high density (Shams et al., 2009). Conversely, the eastern side of Dhaka is only 35% developed with most of the land in scattered rural settlements, water bodies, agricultural land and woodlands. The Balu River runs through the eastern side of Dhaka and is subjected to less industrial effluent compared to the rivers flowing through the western side. However, the presence of notable levels of heavy metal pollution along this stretch of the river suggests that significant transportation downstream is occurring. This results in increased health and environmental hazards, despite distance from industrial pollution sources. Similarly, some of the highest concentrations of metals were observed in the Buriganga, where the river is widest (over 100m in places). This could be a combined result of significantly higher quantities of industrial waste being discharged into the river and an accumulation of metals from industrial effluents further upstream. The Buriganga also has a number of tributaries from other industrialised zones, which may introduce additional pollutants into this stretch of the river.

Bishwa Ijtema festival

Samples were collected immediately after the second phase of the Bishwa Ijtema (BI) festival, held on 19th – 21st January 2018. Samples from 6 sites along the Tongi Khal: upstream, in the middle of, and downstream of the event site. The concentrations of heavy metals at these locations were compared with the samples from the Turag – Tongi – Balu river system that were collected in January 2018, a week prior to the festival. A significant increase in concentration at 1 or more of the 6 sample sites was detected for 12 of the 20 heavy metals examined (Al, Cr, Co, Cu, Fe, Pb, Mn, Hg, Se, Sn, W, and Zn).

The increase in heavy metals and toxicity in the river around the Bishwa Ijtema Festival site was most likely the consequence of the millions of people congregating in this area over three consecutive days. Levels of heavy metals in the river could be impacted by garbage disposal, insufficient sanitary provisions, and increased erosion of soils around the riverbanks. Trace amounts of most heavy metals, in particular essential metals such as chromium, copper, iron, manganese, nickel, selenium and zinc, can be found in urine, suggesting that human waste in the river could be a contributing factor to the increased concentrations of these metals around the festival site (Bird et al., 2018). Levels of aluminium and tin may be increased due to the discarding of packaging into the river in the absence of sufficient waste disposal facilities. Soils and sediments on the riverbanks may contain adsorbed heavy metals which could re-enter the river due to erosion from the crowds of people gathering as well as from resuspension of contaminated river sediments due to turbulence from many boats crossing the river at that time and other activities. This in turn could contribute to the increase in acute toxicity of the river samples.

BIOSENSORS AND TOXICITY ANALYSIS

For sustainable development studies, it is necessary to know the pollution loadings (e.g. COD, BOD), impacts on river quality (e.g. DO), metal concentrations and other key contaminants such as pathogens, and how these combine to create toxic water conditions. A recent technology to measure toxicity is now available from Oxford University, which uses synthetic biology to produce biosensors which can detect metals, organics and biological toxins at very low concentrations. These can be deployed to monitor and manage environmental pollution.

Bacterial biosensors are bacterially derived sensors employed to determine the presence, concentration and, crucially, the bioavailability of both specific chemicals and the overall toxic effect. This technology is being made available by Oxford Molecular Biosensors, a spinout company from Oxford University (www.omb.co.uk). The biosensor employed in this study is an acute metabolic-based toxicity sensor, *Acinetobacter baylyi* ADP1 Tox2, which has previously

been successfully utilised to detect cell damage or cytotoxicity in heavy metal contaminated sea

and light output decreases in the presence of cell or cytotoxicity, as shown in Figure 4. The design allows it to respond at very low concentrations of a toxic contaminant, with sensitivity over several orders of magnitude. Naturally bioluminescent bacteria such as *Vibrio fischeri* have been utilised commercially in freshwater toxicity detection for many years by companies such as Microtox (Johnson, 2005), however biosensors that are synthetically designed for increased sensitivity and specificity of detection are new to the market. Testing of OMB biosensors is undertaken in conjunction with positive and negative controls, allowing a semi-quantitative result proportional to toxicity, which can be calibrated against known cytotoxicants for comparison.

A separate measurement can be made for genotoxicity and this test can measure the extent to which the sample damages double-stranded DNA (Figure 4), which is very important from both an ecology viewpoint and of particular use if the water is being used for public supplies. The advantage of the biosensors is that they are fast and so can be used as an early warning of toxic pollution. The biosensor has previously been compared with the traditional toxicity model fish, *Mugilogobius chulae* (yellowstripe goby), and results found to be comparable, demonstrating that the biosensor is a good indicator of ecotoxicity, and can function as a proxy for animal models (Cui et. al., 2018). The biosensor reports on presence and bioavailability of toxicity rather than requiring the use of multiple sensors to detect individual chemical compounds, and hence can be used as an early screen to assess whether further testing is required. It can be deployed in both fresh and saline water with no additional pre-treatment required, allowing great flexibility of testing. The biosensor data collected in this study was analysed in the context of the extensive metals data collected, in order to attempt to identify any trends or patterns that might be important in the assessment of pollution around Dhaka.

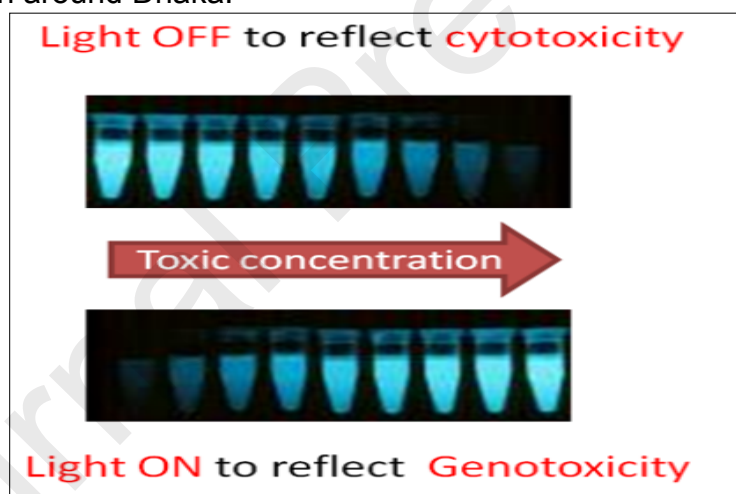


Figure 4 Luminescence from the bacterial biosensors reflecting cyto- or geno toxicity

An interesting feature of the biosensor technology is that it is relatively fast and accurate compared to conventional chemical methods. So, the analysis can be undertaken in the field or a local laboratory and the cytotoxicity results can be generated in approximately 30 minutes. The genotoxicity is longer at 2-3 hours. However, this is much faster than transporting a sample to a remote laboratory where the analysis may last days to get a result back to the site or back to key water quality managers or stakeholders. With the biosensors, faster results may aid with more immediate control, in the case of protection of a water supply, or the improved ability to control a water treatment plant or an effluent treatment plant to ensure either low risk water supply or improved effluent treatment processes.

The biosensor employed in this study was *Acinetobacter baylyi* ADP1 Tox3, containing medium copy number plasmid pWH1274_lux, as described in Cui *et.al.* (2018), with the only difference being the resistance gene used was kanamycin instead of ampicillin. The luminescence reporter cassette *luxCDABE* was under control of a constitutively expressed promoter P_{tet} . This construction results in luminescent light output, which is proportional to acute cytotoxicity, and can be quantified in a luminescent plate reader. *A. baylyi* ADP1 Tox3 was cultured at 37 °C overnight from glycerol stocks on LB agar containing 10 µg/ml kanamycin (km10), prior to being inoculated in sterile LB km10 broth and cultured for 16 h in a shaking incubator at 180 rpm at 30 °C (OD₆₀₀ nm=1.0). The resulting culture was then centrifuged at 3500 g for 10 minutes, the supernatant discarded, and fresh LB broth added. The culture was then incubated at 30 °C for 30 minutes prior to use, to enable the luminescence to reach the maximum value. Water samples were pipetted into each well of a 96 well plate in triplicate with 180 µl in each well, to which 20 µl of bacterial culture was added. Luminescence (RLU) and optical density at 600 nm (OD₆₀₀) were measured using a Tecan Spark plate reader after 15 minutes. The 15 minutes is required for the light signal to reach a stable level at which point it can be measured. The following calculation is then used to determine the percentage of negative control values:

$$\% \text{ of Control} = \left(\frac{RLU \text{ sample}}{OD_{600}} \right) / \left(\frac{RLU \text{ control}}{OD_{600}} \right) \times 100\% \quad (1)$$

Biosensor Toxicity Results

The results of acute toxicity for Turag-Tongi-Balu Rivers and the Turag-Buriganga Rivers over the December 2017 (medium flow – at the end of wet season) and January 2018 (low flow during dry season) periods are shown in Figure 5. In total, 32 out of 78 samples exhibited significantly different toxicity to the negative control ($p < 0.05$), which amounted to 41% of all samples. It was noted that overall toxicity differed between the December 2017 and January 2018 samples, with January samples exhibiting higher toxicity in general, reflecting the lower flows from the dry season and, hence, reduced dilution in January.

When the toxicity results are plotted on a map (Figure 6) it is possible to see the spatial variation in toxicity along the river system and locate the hotspots of increased toxicity, especially in the low flow January sampling period. Interestingly the toxicity is high in the same locations as those found in the heavy metals' analysis above, namely the upper reaches of the Turag River (north of Dhaka City, the Tongi Khal, the lower reaches of the Buriganga and the lower reaches of the Balu River. This suggests a significant association with the effluents high in metals being discharged. All these reaches are close to many industrial units close to river as well as municipal settlements discharging high amount of untreated wastewater.

Figure 7 shows the toxicity results during the Bishwa Ijtema Festival. Toxicity testing revealed that 5 of the 6 sites along this reach of river were significantly more toxic than the control, with site 6 exhibiting the highest toxicity of all samples in the study (71% of control, $p < 0.01$). This suggests the Bishwa Ijtema Festival is having a significant impact on the water quality as site 6 is below the festival site. However, there is significant toxicity along the whole reach derived by industrial and domestic effluents entering the river system.

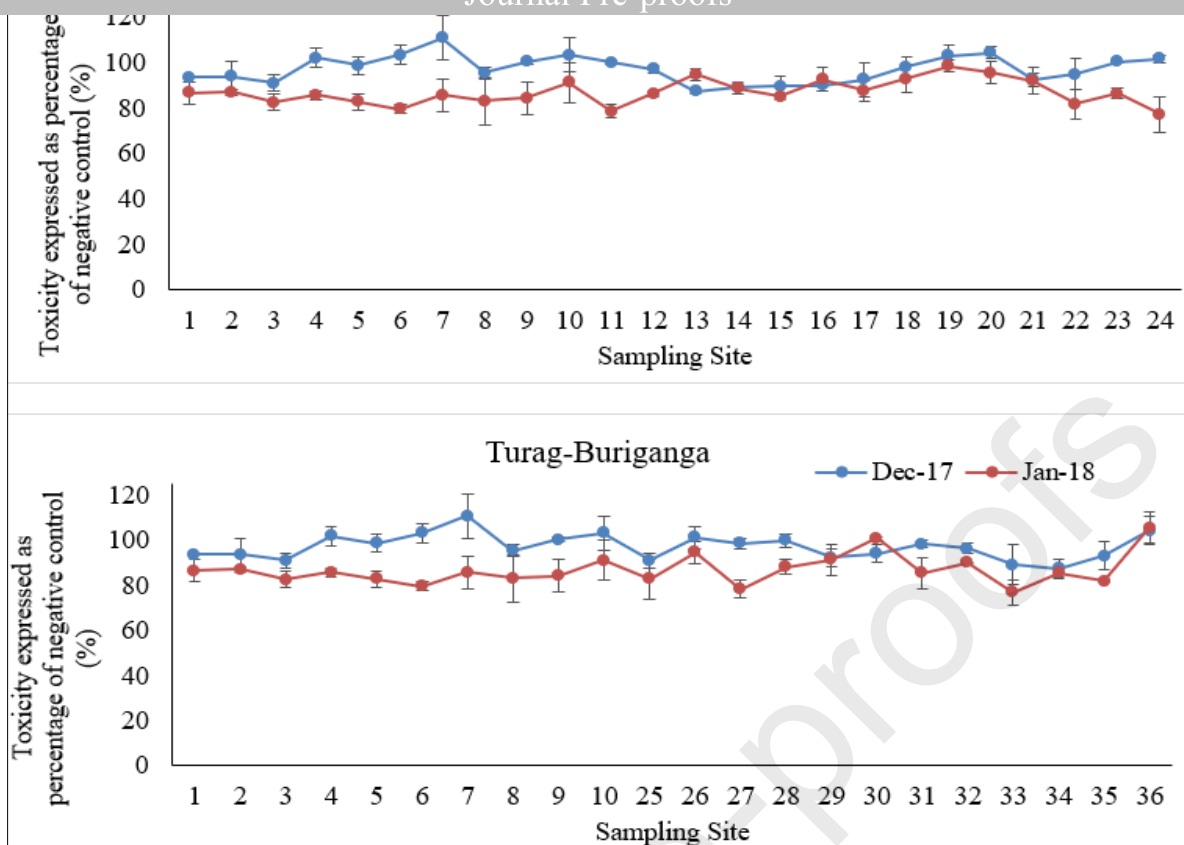


Figure 5 Graphs showing acute toxicity along the Turag-Tongi-Balu (top) and Turag-Buriganga (bottom). Toxicity expressed as a percentage of the negative control i.e. DI water, with error bars denoting 1 standard deviation above and below the mean (n=3). Note that the lower the percentage relative to negative control, the higher the toxicity.

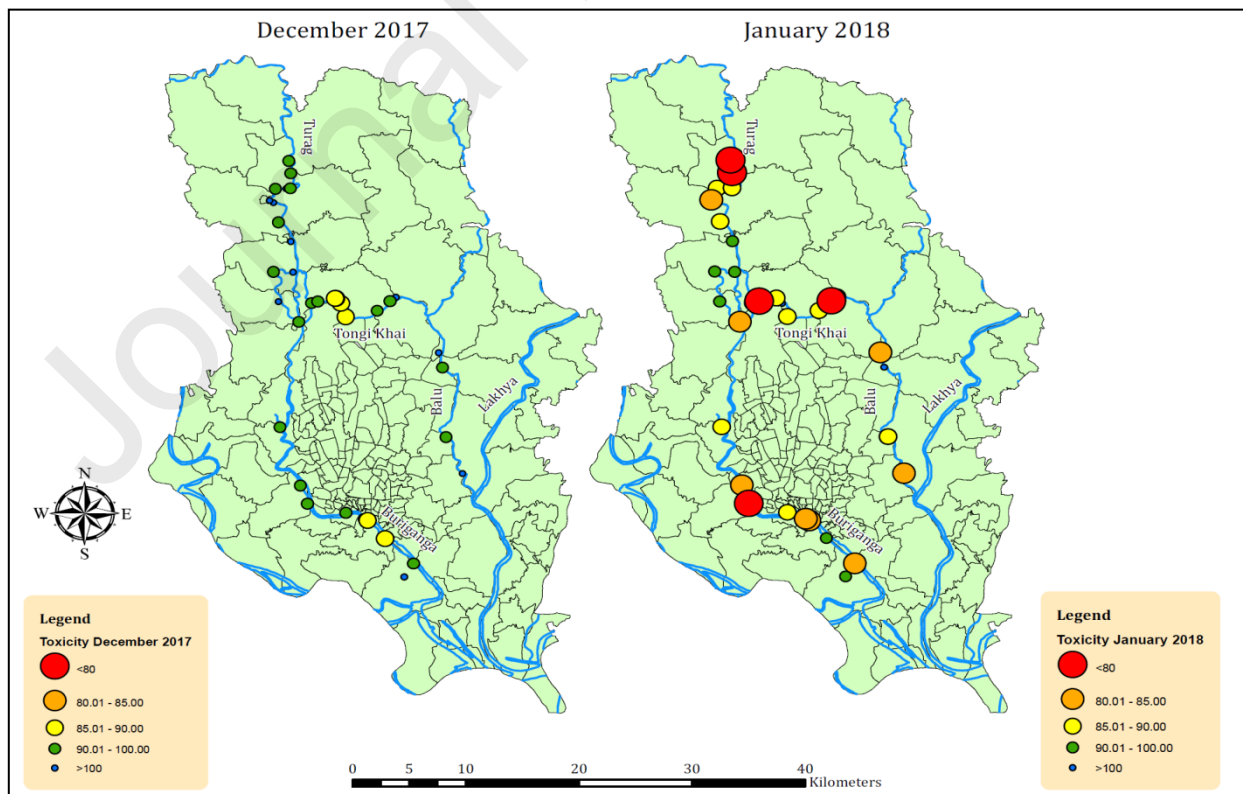


Figure 6 Toxicity, expressed as a percentage of the negative control, at sampling sites around the Turag, Tongi, and Buriganga Rivers in December 2017 and January 2018.

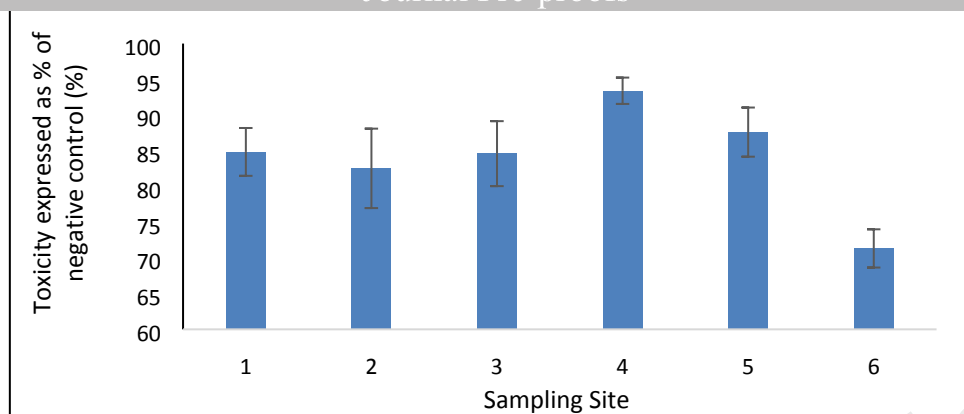


Figure 7 Toxicity at the Bishwa Ijtema Festival sites showing all sites to be significantly more toxic.

TOXICITY AND METAL CORRELATION AND MODELLING

Given the high likelihood of the toxicity being driven by the metal pollution, the data were analysed using multiple statistical techniques in order to evaluate the relationships between metals and toxicity. Firstly, the Pearson correlation coefficient was calculated using the R statistics software package for each metal in relation to average toxicity using the data across the river systems (Table 3). The p-values of less than 0.05 indicate that the correlation is significantly different from zero, as highlighted in Table 3. Table 3 shows that the correlation coefficients and associated p-values vary significantly between metals, with the correlation coefficients for Chromium, Lithium, Selenium, Nickel, Aluminium, Caesium, Zinc, Rubidium, Tin and Copper being significantly different from zero.

A Principal Components Analysis (PCA) was performed using the R stats software package to identify the key metals linked to toxicity. All ten significant metals, as highlighted in Table 3, were represented as a vector, and the direction and length of the vector indicate how each metal contributes to the two principal components. The first principal component had positive coefficients for all ten metals, with the two largest coefficients in the first principal component are Se and Li. The second principal component was found to have positive coefficients for the metals Sn, Al, Cr, Se and Cs, and negative coefficients for the remaining five metals (Li, Ni, Rb, Zn, Cu). These results are particularly interesting as certain metals such as chromium, zinc, tin and copper are associated with the metal working industries and tanneries in Bangladesh whereas selenium and lithium are associated with battery production or battery recycling.

Table 3 Metals and Toxicity Correlation Coefficients

Metal	Pearson Correlation Coefficient	p-value
Cr	-0.6962	1.32E-14
Li	-0.6485	2.76E-12
Se	-0.6444	4.20E-12
Ni	-0.5836	1.02E-09
Al	-0.4433	9.60E-06
Cs	-0.3695	0.00029
Zn	-0.303	0.00333
Rb	-0.2949	0.00432
Sn	-0.2465	0.01788
Cu	-0.2371	0.02288

Co	-0.1995	0.05653
La	-0.1787	0.08837
As	-0.159	0.13002
Fe	-0.1418	0.17746
Ba	-0.1209	0.25087
Mn	-0.0927	0.37944
U	-0.0814	0.44066
Eu	-0.0484	0.64699
Hg	-0.0449	0.67075
W	-0.0325	0.75876
Pb	0.0304	0.77387

Biosensor Toxicity and Metal Relationships

In order to obtain an acceptable mathematical model relating metals to toxicity, a set of different regression methodologies including linear models (i.e. stepwise, interactions), Gaussian process regression models (i.e. rational quadratic, Matern 5/2), support vector machines (i.e. linear, quadratic and Gaussian) and ensembles of trees (i.e. bagged and boosted) were applied to the data sets, as discussed in the supplementary material. The ensemble of bagged trees regression method provided the best fit against the relevant response variables. Bagged trees regression is a combination of bagging (or bootstrap aggregating) and a form of ensemble/decision tree learning (Breiman, 1996) in order to obtain a predictive model. Bagging is a machine learning ensemble algorithm used to improve regression classification by combining classifications of randomly generated training sets. In the context of ensemble bagged trees, multiple decision trees are built by repeatedly resampling the training data with replacement. An average response of the trained ensemble can thus be derived by taking an average over all predictions from individual trees. Typically, the number of bootstraps can be modified to improve the predictive power of the model, although an optimum number of bootstraps exists for any given regression model. A useful outcome of the statistical analysis is a model relating the toxicity and metals values, with aim to model and predict toxicity based on measurable metals concentrations.

The model was trained using 75% of the sample data and validated using the remaining 25% and then tested using sample data from the Bishwa Ijtema festival. It performed well, without over-fitting, particularly given the small number of samples and relatively large number of predictive variables. Table 4 provides key model performance statistics for the training, validation and test data, and Figure 8 shows the fits for the calibration and the validation data sets, showing a highly predictive model. The Bishwa Ijtema festival data provides a fully independent test data set, and as shown in Table 4, and the results suggest that the model adequately captures the relationship between the metals and the toxicity data. However, several points need to be considered, including that the predictors were reduced to 10 key metals and then subsequently subjected to PCA to remove co-linearity and potential over-fitting of the model. A variety of regression methods were tested, with ensemble of bagged trees proving most effective, with model parameters optimised using an iterative approach. The performance of the model could be improved by increasing the number of samples available, particularly at other times of the year to capture more variability. Anomalous metal concentrations cause significant alterations to model predictive capacity with such a low sample size. This was somewhat countered through standardisation of metal concentrations prior to PCA, but metal concentrations orders of magnitude above baseline levels should be noted for their impact on model performance. Small size of samples used as a test for the festival provides a relatively basic and perhaps misleading approximation of modelled toxicity values. It would be necessary to have more data available to form better predictions of toxicity.

Model data		R ²	RMSE	SSE
Training		0.82	2.68	502.87
Validation		0.82	2.56	143.71
Bishwa Festival	Ijtema	0.69	2.84	40.42

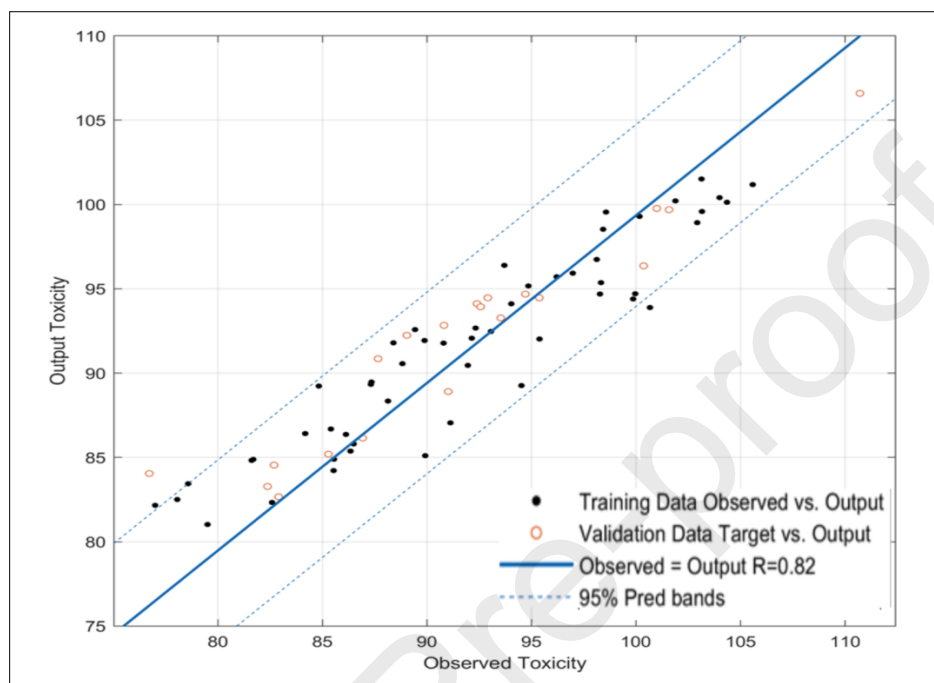


Figure 8 Observed and Simulated Model Fit for the training and validation data sets

Simplified Approach to Toxicity and Metal Concentration Modelling

In order to further explore the relationships between biosensor toxicity and trace metals using a simpler regression approach such as linear and non-linear (e.g. quadratic and logarithm) functions. Standard regression techniques were applied to the dataset for the sampling locations in Turag-Tongi-Balu river system in Bangladesh using both the December 2017 and the January 2018 datasets. Three trace metals were selected based on their high Pearson correlation coefficients with toxicity, as shown in Table 3, with the relationship between toxicity, chromium, selenium and lithium explored.

Table 5 summarises the results of fitting linear, logarithmic and quadratic functions to the data, indicating that linear and quadratic functions perform better than the logarithm function. Also, linear and quadratic functions produced nearly identical R² and residual sum of squares (Table 5). Among 20 analysed trace metals, Chromium (Cr), Lithium (Li), Selenium (Se), Nickel (Ni), Aluminium (Al), Caesium (Cs), Zinc (Zn), Rubidium (Rb), Tin (Sn) and Copper (Cu) have correlation coefficients that are greater than 0.2 (Table 3) and are statistically significant ($p < 0.05$). These ten trace metals have been considered for inclusion in a multiple linear regression model to predict toxicity using trace metal concentrations. Stepwise linear regression was used to regress these ten variables while nonsignificant variables simultaneously removed from the model. Results show that model could be reduced to just three metals, namely Se, Zn and Cr and these generated a regression relationship shown in equation 2.

$$\text{Toxicity} = -11.61 \text{ Se} + 1.61 \text{ Zn} - 4.74 \text{ Cr} - 2.279 \quad (2)$$

The model gave a reasonable predication of toxicity using trace metal concentrations with R^2 of 0.611) and Cr ($p=0.027$) are also statistically significant and account for the variance in the toxicity. This simple multiple linear model can be used for predicting toxicity in Turag-Tongi-Balu-Buriganga River systems.

Table 5 Statistical summary of relationships between Cr, Li and Se metals and toxicity.

	Linear		Logarithmic		Quadratic	
	R^2	Residual Sum of Squares	R^2	Residual Sum of Squares	R^2	Residual Sum of Squares
Tox-Cr	0.443	1548	0.403	1660	0.445	1543
Tox-Li	0.414	1630	0.384	1711	0.418	1618
Tox-Se	0.500	1389	0.081	2555	0.505	1375

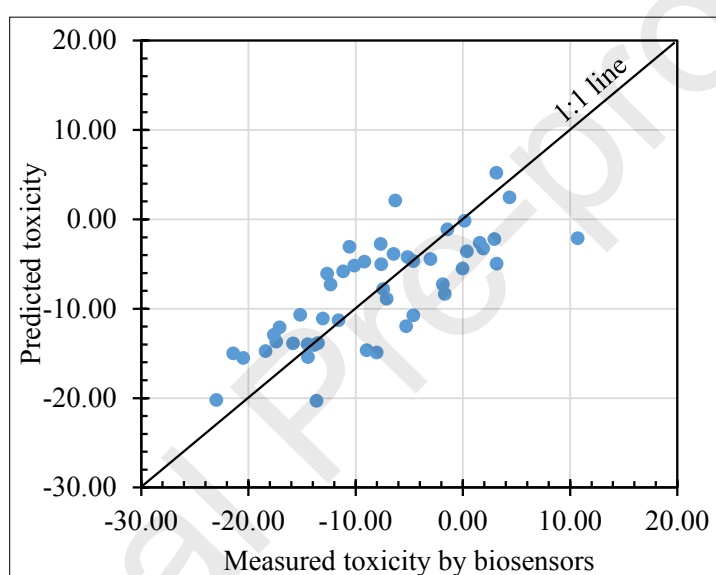


Figure 9 Toxicity measured by biosensors versus toxicity predicted by Selenium, Zinc and Chromium

DISCUSSION AND CONCLUSIONS

This research linked biosensor technology with metals modelling to provide a new approach to detect and manage pollution in urban river systems. The data shows that pollution in the rivers surrounding Dhaka is extremely high during the low flow periods. The research also showed that concentration of heavy metals in the rivers in Dhaka city is highly variable. Pollution hotspots, where concentrations of heavy metals are routinely highest, were found to occur in areas of dense urbanisation and industrial activity, supporting the numerous reports that waste effluents are discharged into the rivers with little or no treatment. The presence of some heavy metals downstream of industrial zones suggests that transportation is significant, leading to increased health and environmental hazards even in non-industrial rural areas. Some of the highest levels of heavy metals were observed near the Hazaribagh area of central Dhaka, a year after the tanneries discontinued production in the area, suggesting slow recovery of the river despite reduction of the hazardous tannery effluents.

Despite most heavy metal concentrations falling within water quality guidelines, the concentration of ten out of twenty metals increased significantly between December 2017 and January 2018. This implies that the concentration of these metals - Chromium (Cr), Lithium (Li), Selenium (Se), Nickel (Ni), Aluminium (Al), Caesium (Cs), Zinc (Zn), Rubidium (Rb), Tin (Sn) and Copper (Cu) -

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will continue to increase towards the end of the dry season and especially following the Bishwa Ijtema. It is shown to be another significant source of heavy metal pollution by comparing samples taken after the Bishwa Ijtema Festival with the Turag– Tongi – Balu river system. Significant concentration increases were observed for more than half of the 20 metals analysed, which is likely to be a result of increased effluent disposal, erosion of soils around the riverbanks and resuspension of contaminated riverbed sediment during the Bishwa Ijtema Festival.

Consequently, the local population may be exposed to serious possible health issues relating to water contamination and atmospheric pollution. The convergence of intense industrial and urban expansion with low regulation contributes to a significant association of poverty and exposure to water pollution as elaborated in the *Lancet* Commission on Pollution and Health (2018). The GoB is presently developing a new industrial wastewater policy and strengthening the environmental quality standards for industry to advance the achievement of SDG 6.3. These findings also suggest the benefit of wider ongoing surface water quality monitoring. The value of a monitoring system and rapid assessment of water quality is evident from the high toxicity and metals concentration (Whitehead et al. 2019). The current lack of a robust national monitoring program means that water quality data is limited.

The biosensors proved to be a rapid and valuable method to evaluate the toxicity of these waters, demonstrating a cost-effective and timely method to monitor pollution in river systems. In-depth metals analysis such as ICP-MS is expensive and requires laboratory facilities. In instances such as these where metals concentrations correlate with toxicity, biosensors may be a less expensive, rapid proxy for informing on water quality. A new portable kit is in development for the biosensors which will enable field or remote lab analysis of toxicity *in situ*. One main driver of toxicity is the concentration of various metals, and this study has indicated that selenium, zinc and chromium might be particularly toxic in the river system. These derive from numerous industries including tanneries, metal processing and battery factories.

If these conditions persist, the excess of metals and resulting toxicity presents increased health risks for people living in proximity to impacted areas (Tchounwou et al., 2012). The toxicity analysis from this study has demonstrated the need for the widespread adoption of clean-up technologies and strengthened environmental regulation in order to reduce the impact of polluting effluents, as well as organic loading from domestic sources. To further the applicability of the bio-physical findings of this research, future studies could extend the application of the biosensor technology to other impacted urban river systems. Additional research may consider the temporal and spatial dimensions of metals pollution in complex river systems and usefully link these aspects with public health considerations to improve human welfare and attain SDG 6.

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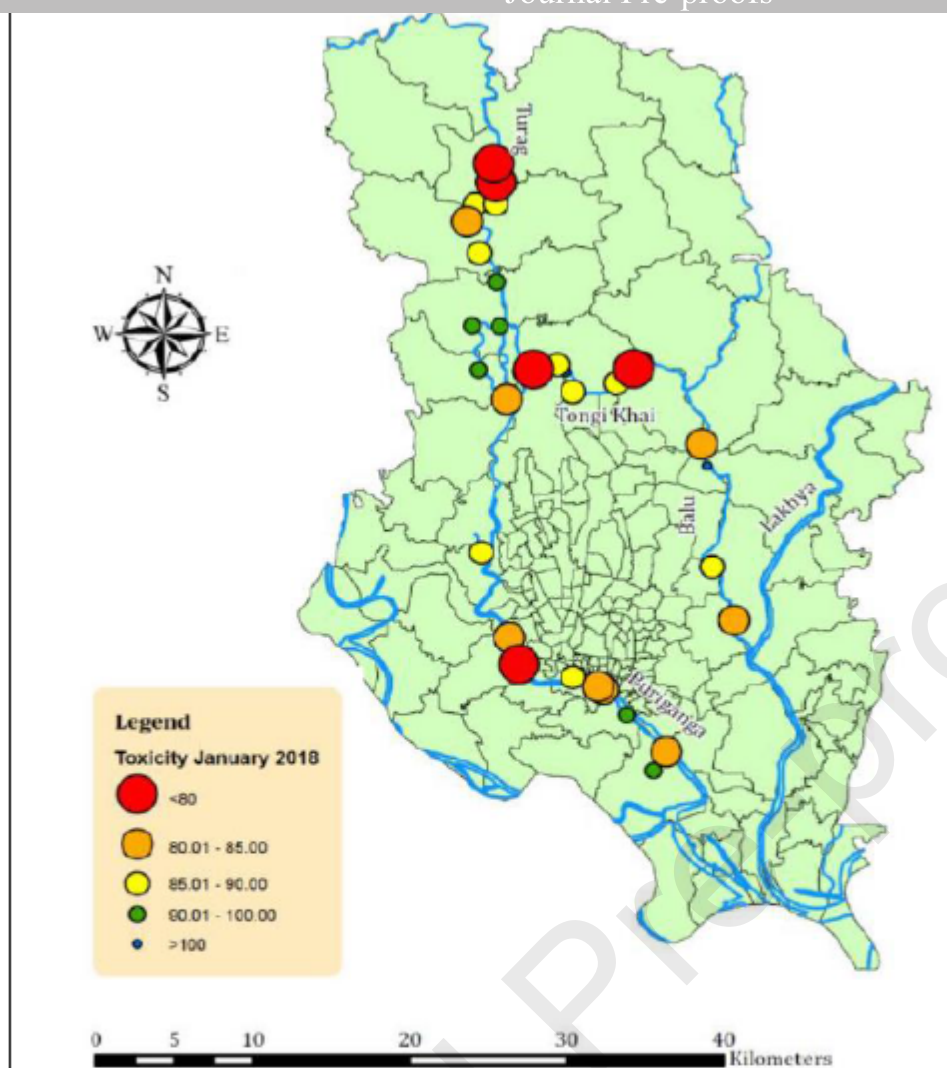
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Toxicity Map of the Rivers Around Dhaka, Bangladesh (January 2018)

1. New biosensor technology applied to assess toxicity in the Turag-Tongi-Balu and Buriganga rivers in Dhaka, Bangladesh
2. Results show highly toxic waters around Dhaka, with key metals of chromium, zinc and selenium driving high observed levels
3. Model developed relating metals to toxicity to captures the relationship between metals concentration and average toxicity
4. Model indicates that where metals concentrations correlate with toxicity, biosensors may be a less expensive, rapid proxy to provide water quality data
5. Bishwa Ijtema festival shown to have significant impacts on downstream river toxicity levels