

Supplemental Information

Impacts of floods on the indoor air microbial burden

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S1. Endotoxin concentrations in indoor air

Along with bacterial concentration, their by-products can also lead to many respiratory diseases (Liebers et al., 2006). The median endotoxin concentration in indoor air of flooded and control houses were 0.4 EU m⁻³ and 0.6 EU m⁻³, respectively. Endotoxin concentration in the flooded houses was comparatively lesser in most of the houses. Though the composition of gram-negative bacteria was found higher in the indoor air of flooded houses, the lower concentration of endotoxin suggest that the species might be more viable. The *Pseudomonas* genus multiplies faster at high relative humidity (80-95%) (Green et al., 1974) and was higher in this study. Thus, the higher *Pseudomonas* bacterial concentration might inhibit the promotion of endotoxin concentration (Heseltine et al., 2009; Thorne et al., 2009). Moreover, particulate matter settling down due to higher relative humidity might also have led to the lower endotoxin concentration in the air.

Non-parametric Spearman rank correlation showed indoor bacterial concentration, pets, and the number of inhabitants significantly ($p < 0.1$) correlated with indoor endotoxin concentration. As expected, a positive correlation between bacterial and endotoxin concentration was observed (Akila et al., 2020).

S2. Chlorine concentrations and dosages according to guidelines of various countries and organizations

Chlorination is the primary disinfection method followed by any natural disasters and calcium hypochlorite is widely used as a source of chlorine for the treatment. World Health Organization (WHO) and other governmental agencies prescribe the addition of chlorine in the form of calcium hypochlorite (65-70% strength). Guidelines noted in several governmental (Canada, Sri Lanka, Sudan, Australia, United States of America, India) or public health agency

documents (WHO, US Environmental Protection Agency, United Nations High Commissioner for Refugees) suggest the addition of a mass of calcium hypochlorite that reaches an equivalent chlorine concentration of 5-200 ppm.

In detail, prescribed chlorine concentrations to be achieved differ from country to country. In Canada, the recommended concentration of chlorine for flooded wells is 200 ppm to 300 ppm (“Guidelines for Disinfecting Dug and Drilled Wells - Environment and Climate Change,” n.d.). In Sri Lanka, the recommended concentration is 2 ppm to 5 ppm (RDS, n.d.). In Sudan, the recommended concentration is 50 ppm for clean water and 100 ppm for contaminated wells (Federal Ministry of Health and Ministry of Water Resources, Irrigation and Electricity, 2017). As per the Australian government guidelines, the recommended concentration for water disinfection is 5 ppm (NSW Health, 2015). Similar to Australia, the United States of America also recommends 5 ppm for water disinfection (Missouri Department Of Health and Senior Services, n.d.). Various public agencies also recommend chlorination as an immediate measure after the flood events. As per the World Health Organization (WHO), the recommended concentration of calcium hypochlorite for flooded well disinfection is 100 ppm to 200 ppm (S., 2011). The United Nations High Commissioner for Refugees (UNHCR) also recommends 100 ppm for disinfection (UNHCR, n.d.). As per the guideline in India, chlorination is to be done in two stages. The recommended concentration to be reached after a first dose is 30 ppm, followed by addition of a second dose to add a residual concentration of 0.7 ppm (Marg, n.d.).

S3. Chlorination and the persistence of ARB in the outdoor well water

Indian guidelines stipulate the addition of calcium hypochlorite to well water to reach a chlorine concentration of 30 ppm. However, our study shows while chlorination was effective against many microbes, some microbes were resistant to such treatment. Antibiotic resistant bacteria (ARB) continued to persist in chlorinated flooded water systems. Well water from 16 flooded houses and 3 control houses showed bacterial growth. The median concentration of bacteria from well water of flooded houses was 360 CFU/mL, and the control houses were 190 CFU/mL. Based on morphology, fifty-four unique colonies (41 from flooded and 13 from control houses) were picked. All colonies were resistant to calcium hypochlorite strengths of up to 1000 ppm at all incubation times. After calcium hypochlorite concentration was increased to 5000 ppm and 10000 ppm, 32 colonies from flooded houses and 4 from control houses were still resistant.

Among these 36 tested colonies, 25 from flooded wells were resistant to one or more antibiotics, but all the colonies from wells of control houses were non-resistant to any of the antibiotics. Among the 25 resistant colonies, rifampicin and penicillin resistance were the most dominant. Eighteen and seventeen colonies, respectively, were resistant to these antibiotics. Thirteen tested cultures were resistant to clindamycin and gentamycin. Ten different species belonging to the two bacterial genera, *Bacillus* and *Pseudomonas* were identified in the 25 colonies.

One colony was identified as *Pseudomonas sp.* Twelve colonies were identified as *P. putida*, five of which were resistant to four or more antibiotics. Resistance to penicillin, rifampicin, and gentamycin was found to be the most dominant (9, 8, and 7 colonies,

respectively). These colonies also showed resistance to antibiotics amoxicillin (4 colonies), tetracycline (3 colonies), and doxycycline (3 colonies). All the *P. putida* colonies were sensitive to amikacin, vancomycin, and ciprofloxacin. The *P. putida* species are known to form a biofilm in both biotic and abiotic surfaces, which requires a high concentration of antibiotics for its eradication (Molina et al., 2014).

The remaining twelve colonies belonged to eight unique species *B. subtilis*, *B. aryabhatai*, *B. altitudinis*, *B. anthracis*, *B. licheniformis*, *B. thuringiensis*, *Bacillus sp.* and *B. cerus*. Resistance to rifampicin (10 colonies) was found to be the most dominant, followed by penicillin (8 colonies). All tested Bacilli colonies were resistant to two or more antibiotics. Many species from the genus Bacilli are opportunistic pathogens and their resistance to antibiotics has health implications. For example, *B. anthracis*, which is associated with fever, was resistant to ciprofloxacin and doxycycline, two antibiotics commonly used as prescribed treatment for fever itself (Mayo Clinic, 2022).

S4. Presence of potential ARB species in indoor air of flooded houses

A few of the ARB species found in well water, such as *B. aryabhatai* and *B. anthracis* were also present in tap water and in indoor air of three flooded houses. *B. subtilis* was present in two flooded houses. With breaking water bubbles, taps connected with well water can also release ARBs and other microbes into indoor air (Massachusetts Institute of Technology, 2018). Likewise, flood water may introduce antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) directly to the indoor air through surface contamination. Previous studies conducted in Kerala after the same flood event (S et al., 2019; Shankar et al., 2021), identified antibiotic resistant *Bacillus sp.*, in outdoor environment. As per the source tracking analysis,

outdoor air and outdoor soil act as important sources of microbes to indoor air. ARB in outdoor soil could have been transported to the indoor environment by the movement of humans, for example, via soiled shoes, or ARB could have been suspended in outdoor air and carried inward via air movements. Finally, ARB was absent in all samples collected from non-flooded houses (air, tap water, and well water). Thus, floods are a likely source of ARB to the built environment.

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