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Letter to the Editor

Letter to the Editor RE: High levels of faecal contamination in drinking groundwater and recreational water due to poor sanitation, in the sub-rural neighbourhoods of Kinshasa, Democratic Republic of the Congo by Kayembe et al. 2018

Dear Dr. Calafat,

We have read with great interest the manuscript entitled "High levels of faecal contamination in drinking groundwater and recreational water due to poor sanitation, in the sub-rural neighbourhoods of Kinshasa, Democratic Republic of the Congo" by Kayembe et al. published very recently in the International Journal of Hygiene and Environmental Health (2018) (Kayembe et al., 2018). In the study, the authors measured physicochemical and microbiological parameters of water from shallow wells and an urban river during dry and wet seasons to assess both the water quality and to identify the source of water contamination. While the manuscript provides insightful information and contribute greatly to the sources of contamination in the Democratic Republic of Congo, we encountered several issues and inconsistencies in the manuscript which we would like to raise.

To indicate the pollution source, Kayembe et al. first isolated faecal indicator bacteria (FIB), namely *Escherichia coli* (*E. coli*) and Enterococcus, from water and surface sediment, using the selective culture media. The isolated colonies were confirmed for their *E. coli* species or Enterococcus genus using PCR primers as stated in Table 3. Subsequently, a total of 505 isolated *E. coli* and Enterococcus colonies were evaluated by human-specific Bacteroides PCR assays (utilizing the widely cited HF183/Bac708R and HF134/Bac708R primer pairs) after being taken directly from colonies (and without genome extraction). In Table 5 of the manuscript, the authors presented the results of testing the HF primers with genomic material from each single colony of *E. coli* and enterococci, as opposed to the common approach of using total DNA of filtrated water samples. Even though inaccurately captioned, Table 5 stated that 'PCR presence/absence assays for detection of human-specific bacteroides in water samples from wells, river and hospital outlet pipe and sediment samples from river (Kokolo Canal).' Both the method and results presented are doubtful because genomic material originating from *E. coli* and Enterococcus isolates cannot be amplified by the HF183/Bac708R or HF134/Bac708R primers. Both HF primers were designed from 16 s rRNA gene of Bacteroides-Prevotella genus (Bernhard and Field, 2000a,b) and sequence similarity search conducted by us using the BLAST algorithm at the NCBI homepage showed no match with DNA sequences of *E. coli* and Enterococcus (access date 19 April 2018). As reference for this approach, Kayembe et al. cited their two previous studies for the direct human-specific Bacteroides PCR assay to *E. coli* and Enterococcus colonies. However, we found incorrect information in their citations; the first article (Thevenon et al., 2012) in fact performed the HF183/Bac708R and HF134/Bac708R assays from DNA material that has been extracted from total bacteria remaining on the membrane after vacuum filtration, and not on the colonies of *E. coli* and Enterococcus that had grown in the selective media. The second reference (Tshibanda et al., 2014) also stated in the Materials and Methods section that the HF primers were used for amplification of total DNA extracted from the samples; however, its abstract conveyed misleading information as 'The FIB characterization was performed for general *E. coli*, *Enterococcus faecalis* (*E. faecalis*) and human-specific bacteroides by PCR, using specific primers.'

With no total DNA extracted from water and sediment samples for human-specific Bacteroides PCR assay, the Kayembe et al. article published in IJHEH fails to specifically indicate that the water is contaminated with human faecal pollution. Therefore, the third objective of this study, which was – as the authors stated – to identify the source of water contamination, could not be achieved. Ultimately, PCR results utilizing those primers cannot be used to indicate the presence of 'human FIB' as stated by the authors, and the conclusion drawn by the authors that the surface water and well water were contaminated with human sewage pollution is erroneous. High FIB concentrations may be attributed to regrowth in the environment, especially in tropical climates, and might not be necessarily linked to faecal matter or anthropogenic pollution levels (Desmarais et al., 2002; Fujioka et al., 2015). Consequently, the title of Kayembe et al. paper that stated 'High levels of faecal contamination in drinking groundwater, (…)' misrepresented the results and conclusion of the study.

While statistical analysis was performed for the physicochemical parameters between wet and dry seasons, no such analysis was conducted for microbiological parameters. Therefore, there was no data to support the conclusion that the concentration of FIB in wet season was higher than the dry season. The only parameter that showed significantly higher values in wet compared to dry seasons is the electrical current (EC). Furthermore, the statement claimed by the authors that *E. coli* and ENT concentrations in shallow wells during the wet season could increase by 2–3 orders of magnitude compared to those measured during the dry season is not supported by the data provided in Table 4. We also found that similar sets of data are redundantly presented in Fig. 4, Table 4, and the text. Additionally, the effect of wet and dry seasons is problematic to evaluate especially when no precipitation data was provided, and only one sample was collected during each season at each sampling site.

Moreover, we encounter some inconsistent or insufficient details as provided in the Materials and Methods section of the article. First, while the authors mentioned that they took the sediment samples labelled as KCS4 from the hospital effluent outlet pipe (HOP) as listed in Table 1, it is unclear how the sediment in the outlet pipe was collected as said sediment sampling is merely described for river sediments in the manuscript. Second, the dissolved oxygen parameter is widely abbreviated as 'DO', instead of 'O_2' as used in this manuscript. Third, the authors stated that positive controls at 520 bp size for HF183/Bac708 amplified from sewage was used for each PCR assay. This is problematic at least as such positive controls should only be used for the HF183/Bac708 assay, while other PCR assays require their own positive

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controls with different primers, annealing temperatures, and product sizes. The reference for the said statement (Pote et al., 2009) is incorrect as the article cited did not describe any PCR assay in their study. Fourth, Table 3 provided wrong information about primer sequences and references: 1) the primer pairs are mismatched, where HF183 forward primer should be paired with Bac708R reverse primer to make a 520-bp product, and HF134 forward primer should be paired with Bac708R reverse primer to make a 570-bp product. The table listed three primers even though it showed four primer sequences, one of which is a duplicate (ATCARGTCACATGTCCCG); 2) the sequence for HF134 is incorrect. The correct sequence is 5′-GCCGTCTACTCTTG GCC-3′ while the manuscript listed it as 5′-ATCARGTCACATGTC-CCG-3′; 3) while the correct reference is cited for the primer HF183 (Bernhard and Field, 2000a), the primer Bac708R requires another reference (Bernhard and Field, 2000b) as it is absent from the first.

Lastly, there are minor issues that are worth improving. First, the last line of the section 2.5, an ‘s’ should be removed from ‘each PCR assays’. Second, in the section 2.6 there is a misspelling of the word ‘plate count’. Third, in Table 3 the last number in the column ‘Cond. dry’ should be presented with one decimal digit as others. Fourth, in the Results and Discussion section 3.2, the unit for FIB values in sediment samples is wrong and should be changed to ‘CFU $100\,g^{-1}$’. Fifth, the numbers for ENT in river water samples (section 3.2) and in well water samples (section 3.3) during wet and dry seasons incorrectly listed as $\times 10^5$ rather than $\times 10^4$ CFU $100\,mL^{-1}$. Sixth, the caption of Fig. 4 contains an asterisk (*) but no definition was provided. Seventh, Fig. 4 appears before Fig. 3 when cited in text. Lastly, in some places the figures are written as ‘Figure’ but in others they are called ‘Fig.’; this has to be changed for consistency.

We hope that these remarks and suggestions can help clarifying the inconsistencies in the article by Kayembe et al. (2018) and help maintain the high quality of the International Journal of Hygiene and Environmental Health.

Kind Regards,

Dr. Mats Leifels, Dr. Skorn Mongkolsuk, Dr. Kwanrawee Sirikanchana.

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