1 **Validation of large-scale solar reactors for the treatment of rainwater in field trials in sub-**

2 **Saharan Africa**

3 B. Reyneke[a], T. Ndlovu[a], M.B. Vincent[b], A. Martinez-Garcia[c], M.I. Polo-López[c], P. Fernández-

4 Ibáñez[c,d], G. Ferrero[e], S. Khan[f], K.G. McGuigan[g] and W. Khan[a*]

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6 [a] Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1,

7 Stellenbosch, 7602, South Africa.

8 [b] Ecosystem Environmental Services S.A., Sant Andreu de Llavaneres, Barcelona, Spain.

9 [c] Plataforma Solar de Almeria-CIEMAT, P.O. Box 22, Tabernas, Almería, Spain.

10 [d] Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster,

11 Newtownabbey, Northern Ireland, United Kingdom.

12 [e] IHE Delft Institute for Water Education, Westvest 7, 2611, AX, Delft, the Netherlands.

13 [f] Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein, 2028, South

14 Africa.

15 [g] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin,

16 Ireland.

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18 Short title: Large-scale SODIS treatment of rainwater

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20 Abbreviations[1]

21 *Corresponding Author: Wesaal Khan; Phone: +27218085804; Fax: +27218085846; Email:

22 wesaal@sun.ac.za

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[1] ADWG – Australian drinking water guidelines; BDL – below detection limit; CFU – colony forming units; CPC – compound parabolic collector; DNA – deoxyribonucleic acid; DWAF – Department of Water Affairs and Forestry; *E. coli* – *Escherichia coli*; EMA – ethidium monoazide bromide; EU – European Union; FF – first-flush; HPC – heterotrophic plate count/heterotrophic bacteria; LB – luria bertani; PCA – principle component analysis; PET – polyethylene-terephthalate; PMA – propidium monoazide; PMMA – poly(methyl methacrylate); qPCR – quantitative polymerase chain reaction; RHRW – roof-harvested rainwater; ROS – reactive oxygen species; RWH – rainwater harvesting; SABS – South African Bureau of Standards; SODIS – solar disinfection; UV – ultraviolet radiation; WATERSPOUTT – Water Sustainable Point-Of-Use Treatment Technologies; WHO – World Health Organisation; WSP – water safety plan; Zn – zinc.

Abstract

The efficiency of two large-scale solar reactors [Prototype I (140 L) and II (88 L)] in treating rainwater on-site in a local informal settlement ~~(Site 1)~~ and farming community ~~(Site 2)~~ was assessed. Untreated ~~(Tank 1 and Tank 2 FF)~~ and treated (Prototype I and II) tank water samples were routinely collected from each site and all the measured physico-chemical parameters, anions and cations were within national and international drinking water guidelines limits. Culture-based analysis indicated that *Escherichia coli*, total and faecal coliforms, enterococci and heterotrophic bacteria counts exceeded drinking water guideline limits in 61%, 100%, 45%, 24% and 100% of the untreated tank water samples collected from both sites. However, an 8 hour solar exposure treatment for both solar reactors was sufficient to reduce these indicator organisms to within drinking water standards, with the exception of the heterotrophic bacteria which exceeded the drinking water guideline limit in 43% of the samples treated with the Prototype I reactor (1.01 log reduction). Molecular viability analysis subsequently indicated that mean overall reductions of 75% and 74% were obtained for the analysed indicator organisms (*E. coli* and enterococci) and opportunistic pathogens (*Klebsiella*, *Legionella*, *Pseudomonas*, *Salmonella* and *Cryptosporidium* oocysts) in the Prototype I and II solar reactors, respectively. The large-scale solar reactor prototypes could thus effectively provide three (88 L Prototype II) to five (144 L Prototype I) people on a daily basis with the basic water requirement for human activities (25 L). Additionally, the outlined water safety plan may aid in identifying how and where rainwater harvesting systems should be installed and maintained to ensure the quality of the treated water.

Keywords: Rainwater harvesting; solar disinfection; rainwater quality; sub-Saharan Africa

1. Introduction

The Global Risks Report released for 2019 listed water crises as one of the top ten risks in terms of likelihood (rating of 9; very likely to occur) and impact (rating of 4; severe impact) (Global Risks Report, 2019). The probability of a water crisis risk in sub-Saharan Africa is significantly increased as a high proportion of the population reside in urban informal settlements and rural areas, with limited access to a safe water supply and sanitation infrastructure (Dos Santos et al. 2017). However, as highlighted by Gwenzi and Nyamadzawo (2014) and Emenike et al. (2017), rainwater is considered an under-exploited water source in sub-Saharan Africa and may serve as an effective reserve to improve and encourage equity in water access. Roof-harvested rainwater (RHRW) can however, be contaminated with various chemicals and microorganisms, which may limit its use as a potable water source (Hamilton et al. 2019). While the chemical pollutants have not been directly associated with the incidence of disease, organic debris and faecal matter from animals and birds that have access to the catchment surface, have been identified as the primary sources of microbial contaminants such as *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium* (Hamilton et al. 2019).

Treatment strategies that may be implemented to improve the quality of rainwater include the utilisation of gutter screens or first-flush diverters for the prevention of contaminant entry into the collection tank or post-collection treatment [chemical (e.g. chlorination) and physical treatments (e.g. filtration, solar disinfection (SODIS) and thermal disinfection)] (Hamilton et al. 2019). Although various chemical and physical treatment technologies have been investigated, SODIS is considered a cost-effective treatment method and is recommended by the World Health Organisation (WHO) for the effective reduction of microbial contamination in water sources (Ubomba-Jaswa et al. 2010). In its simplest form, SODIS entails filling a transparent container [usually a 2 L or 5 L polyethylene-terephthalate (PET) bottle] with contaminated water and exposing the bottle to direct sunlight for six to eight hours to allow ultraviolet (UV) radiation and solar-mild heat to inactivate microbial contaminants

(McGuigan et al. 2012). Ultraviolet radiation directly inactivates the microbial contaminants by damaging nucleic acids and leads to the formation of reactive oxygen species (ROS), which react and damage proteins, nucleic acids and membrane lipids (Nelson et al. 2018). The water temperature will also increase as water molecules absorb the UV radiation, which leads to cell membrane damage. The major drawbacks associated with this technique are however, the small volumes of water that can effectively be treated (2 to 5 L) and decreased efficiency during overcast weather conditions (up to 48 hours of treatment). Increases in treatment volume and efficiency may then be obtained by employing various modifications (SODIS enhancement technologies) such as solar mirrors (concentrates UV radiation) and larger reactor tubes (increase treatment volume) (Ubomba-Jaswa et al. 2010; McGuigan et al. 2012).

As part of the European Union (EU) Horizon 2020 project titled Water Sustainable Point of Use Treatment Technologies (WATERSPOUTT), Polo-López et al. (2019a) investigated various enhancement technologies that may cost-effectively allow for larger volumes of water to be treated using SODIS. Results from the study indicated that the use of a static batch reactor system employing U-type solar mirrors allowed for the effective treatment of a larger volume (68% more) of water as compared to the compound parabolic collector (CPC)-type solar mirrors under the same solar exposure conditions (Polo-López et al. 2019a). In a follow-up study, the same research group designed two large-scale solar reactor prototypes (static batch systems with 88 L and 140 L treatment volumes, respectively), where multiple poly(methyl methacrylate) (PMMA) reactor tubes were positioned in the centre of U-type solar mirrors (Polo-López et al. 2019b). Preliminary assessment of the solar reactor prototypes, using spiked synthetic rainwater samples and culture-based analysis, indicated that a ≥ 6 log removal efficiency was obtained for *Escherichia coli* (*E. coli*) and *Salmonella enteriditis* after 1.5 hour natural sunlight exposure, while a 2 hour sunlight exposure was required to achieve the same log reduction for *Enterococcus faecalis* and *Pseudomonas aeruginosa* (*P. aeruginosa*).

The primary aim of the current study was to assess the efficiency of the two newly designed large-scale solar reactor prototypes (Polo-López et al. 2019b) for the treatment of

RHRW on-site in a local informal settlement (140 L Prototype I) and a rural farming community (88 L Prototype II). The chemical quality of the RHRW before and after solar reactor treatment was routinely assessed by monitoring various physico-chemical parameters (e.g. temperature, pH, and turbidity), anions and cations. Additionally, the removal of traditional indicator organisms (*E. coli*, total and faecal coliforms, enterococci and heterotrophic bacteria) and selected opportunistic pathogens (*Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp.), was assessed using culture-based analysis. Ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) assays were also used to monitor the reduction efficiency of indicator organisms (*E. coli* and enterococci) and opportunistic pathogens (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.), while propidium monoazide (PMA) qPCR assays were used to monitor *Cryptosporidium* oocyst reductions. A water safety plan (WSP) outlining guidelines for the use of rainwater harvesting combined with solar reactor treatment was also implemented.

2. Materials and methods

2.1 Description of large-scale solar reactor prototypes and sampling sites

Two large-scale solar reactor prototypes were designed and constructed as part of Work Package 1 (WP1) by the WATERSPOUTT research consortium as part of a EU Horizon 2020 project under grant agreement no. 688928 for implementation in South Africa and Uganda. Detailed information on the design and working mechanisms of the systems are outlined in Polo-López et al. (2019b), with the current study focussing on the application of these systems in field trials in South Africa. The Prototype I solar reactor (140 L treatment volume) was installed in Enkanini informal settlement (Site 1; GPS coordinates: 33°55'28.1"S 18°50'35.8"E) during July 2018 and consisted of three PMMA reactor tubes (200 mm diameter) that were positioned in the centre of a U-type solar mirror (constructed from anodized aluminium). The reactor tubes were positioned at a 34° angle (equal to the local latitude) and were inter-connected by UV-A transparent PMMA tubing (Fig. 1A). The Prototype II solar reactor (88 L

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treatment volume) was installed next to a local church building in the Skoolplaas farming community (Site 2; GPS coordinates: 33°56'38.5"S 18°46'26.3"E) during July 2018 and consisted of the same materials and design as Prototype I, with the exception that eight PMMA tubes (100 mm diameter) were used in the system (Fig. 1B). Additionally, as space was available between the gutter system and the rainwater harvesting (RWH) tank at site 2, a first-flush (FF) diverter (Superhead® rainwater filter) was installed to redirect the initial roof run-off during a rain event (Fig. 1B). A detailed description of the sampling sites and system installation is outlined in Appendix A.

2.2 Sample collection

For the microbial and chemical analysis of the water produced by the solar reactor prototypes (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)]. The respective solar reactor prototypes were filled with tank water from the RWH tanks and exposed to direct sunlight for 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the solar exposure, 10 L of each treated sample was collected directly from the solar reactor prototypes [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]. Based on the availability of rainwater in the RWH tanks, 15 sampling sessions were conducted at site 1 ($n = 30$; August 2018 to March 2019), while 18 sampling sessions were conducted at site 2 ($n = 36$; August 2018 to April 2019). For ease of presentation, sampling sessions 1 to 18 are designated as #1 (sampling session 1), #2 (sampling session 2), etc., throughout the manuscript.

The temperature, pH and total dissolved solids present in all water samples were measured using a hand-held Milwaukee Instruments MI806 meter (Spraytech, South Africa), while the dissolved oxygen was measured using a Milwaukee Instruments M600 meter (Spraytech, South Africa). Rainfall and daily ambient temperature data for the study period was obtained from the South African Weather Services, while solar irradiance data [mean ambient UV-A and UV-B radiation] was obtained from the Stellenbosch Weather Services [Stellenbosch University, Faculty of Engineering (http:// weather.sun.ac.za/)].

2.3 Chemical analysis

The chemical quality of the untreated and solar reactor treated tank water samples was determined by monitoring cation and anion concentrations and turbidity as described by Strauss et al. (2018). All samples ($n = 66$) were monitored for cations, while representative samples ($n = 22$; #1, #7, #10, #12, #15 and #18) were monitored for anions and turbidity.

2.4 Culturing of indicator organisms and opportunistic pathogens

The microbial quality of the tank water samples collected from sites 1 and 2 were monitored before (untreated) and after solar reactor treatment using various culture-based analyses. *Escherichia coli* and total coliforms were enumerated simultaneously using membrane filtration as described by Dobrowsky et al. (2015), while enterococci, faecal coliforms and the heterotrophic plate count/bacteria (HPC) were enumerated as outlined in Strauss et al. (2016), with a minor modification; Luria Bertani (LB) agar (Biolab, Merck, South Africa) replaced Reasoner's 2A agar (Oxoid, Hampshire, England) for the enumeration of HPC. For the treated samples (Prototypes I and II) where the HPC were reduced to below the detection limit [BDL; < 1 colony forming units (CFU)/1 mL], the potential regrowth of bacteria was monitored. Briefly, 20 mL of each treated sample was stored in a sterile McCartney bottle at room temperature and 100 µL of the treated water was spread plated onto LB agar (Biolab, Merck) every 24 hours for a period of 2 days. The plates were then incubated at 37 °C. Additionally, *Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp. were enumerated as outlined in Clements et al. (2019), while coliphages were enumerated as outlined by Baker et al. (2003) using *E. coli* ATCC 13706 as the target bacterial host. All culture-based analyses were performed in duplicate.

2.5 Tank water concentration, viability treatment and DNA extraction

The concentration of 1 L (Site 1) and 2 L (Site 2) samples, EMA treatment and subsequent DNA extractions were performed for each of the samples collected before and after solar

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179   reactor treatment as outlined in Reyneke et al. (2016). For the molecular quantification of

180   *Cryptosporidium* spp. within the collected samples, the same methodology was repeated with

181   the exception that a PMA treatment as described by Alonso et al. (2014) was followed.

2.6 Molecular-based enumeration of indicator organisms and opportunistic pathogens

183   Quantitative PCR was performed in order to quantify *E. coli*, enterococci, *Klebsiella* spp.,

184   *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp. in all of the collected tank water

185   samples, while *Cryptosporidium* oocysts were quantified in the samples collected from #9 to

186   #15 and #9 to #18 for sites 1 and 2, respectively. All qPCR assays were conducted using a

187   LightCycler® 96 (Roche Diagnostics, Risch-Rotkreuz, Switzerland) instrument in combination

188   with the FastStart Essential DNA Green Master Mix (Roche Diagnostics) as outlined in

189   Reyneke et al. (2017), with the primer pairs and cycling parameters presented in Table A1.

190   Standard curves for the respective qPCR assays were generated using the methodology

191   outlined in Reyneke et al. (2017), while the qPCR performance characteristics of the various

192   assays were analysed using the Roche LightCycler® 96 Software Version 1.1. Furthermore,

193   to compensate for the different sample volumes used per site for rainwater concentration [1 L

194   (Site 1) and 2 L (Site 2)] the gene copies detected in the samples utilising the qPCR assays

195   were converted to gene copies per 100 mL of the original tank water sample as outlined by

196   Waso et al. (2018). The gene copy numbers (gene copies/100 mL) were then converted to

197   cell equivalents (cells or oocysts/100 mL) by utilising the number of copies of the target gene

198   present within the target host (Table A1). All final concentrations for qPCR analyses are thus

199   presented as equivalent cells or oocysts/100 mL original tank water sample.

2.7 Maintenance of prototype reactors and water safety plan

201   Following the system installations, workshops were conducted within the respective

202   communities to outline the principle of rainwater harvesting, the working mechanism and

203   operational maintenance of the solar reactors. Information on the domestic activities (i.e.

204   laundry, cleaning, washing, etc.) the treated rainwater could be used for was also provided

(Fig. A3). Exemption from ethical clearance was obtained from the Research Ethics Committee (Humanities) Stellenbosch University (Ethics Reference no.: SU-HSD-004624), as the participating households would not be using the treated water for drinking purposes.

As outlined by the WHO (2004), the most efficient way of consistently ensuring the safety of a drinking water supply is through the utilisation of a WSP (Appendix B), which may be defined as a risk assessment and management approach that monitors the entire water supply process (e.g. collection of RHRW to utilisation of treated tank water by the consumer). The first step in the development of the WSP was to identify all potential hazards/hazardous events that may influence the quality of rainwater during the harvesting process, storage and treatment process (Appendix B), using published literature and personal observations at the respective study sites, during the study period. Additionally, various maintenance and remedial actions were identified to prevent certain water safety hazards (e.g. prevent organic debris from entering the storage tank) or to implement after a hazardous event occurred (e.g. control measure failed and organic debris washed into the storage tank) (Appendix B). Following the identification of the potential hazards, a risk assessment matrix (Appendix C) was compiled that would enable the risk characterisation associated with each hazard/hazardous event and enable the assessment of the various control measures (e.g. maintenance strategies, use of a first-flush diverter system etc.) in eliminating the identified water safety hazards.

2.8 Statistical analysis

Statistical analyses were conducted utilising either RStudio (version 1.0.153) or Microsoft Excel® Ver. 15.31. Overall differences in sample composition between site 1 and site 2 and the untreated (Tank 1 and Tank 2) and solar reactor treated (Prototype I and II) tank water samples was determined by evaluating all measured physico-chemical, chemical and microbial parameters using the parametric paired t-test (significant when $p < 0.05$). Principle component analysis (PCA) was then used to visualise the correlations between the measured

cations at both sites and identify which cations primarily influenced the sample composition at each site.

3. Results and Discussion

3.1 Physico-chemical properties and chemical analysis of the collected tank water samples

The mean ambient UV-A radiation at both sampling sites ranged from 7.16 W/m^2 (12/09/2018) to 31.29 W/m^2 (14/01/2019), while the mean ambient UV-B radiation ranged from 1.33 W/m^2 (12/09/2018) to 4.63 W/m^2 (14/01/2019) (Table A2). The untreated tank water temperature at site 1 (Tank 1) ranged from 9.0 °C (02/08/2018 and 15/08/2018) to 24.0 °C (28/01/2019), with a mean temperature of 16.3 °C recorded for all sampling days, while the tank water temperature in the samples collected from the Prototype I solar reactor ranged from 15.5 °C (12/09/2018) to 45.0 °C (28/01/2019) (mean 28.9 °C). Similarly, the untreated tank water temperature at site 2 (Tank 2-FF) ranged from 10.0 °C (15/08/2018) to 26.0 °C (25/10/2018) (mean 18.1 °C), while the tank water temperature in the samples collected from the Prototype II solar reactor ranged from 18.0 °C (12/09/2018) to 46.5 °C (28/01/2019) (mean 32.6 °C).

All measured physico-chemical parameters (pH, turbidity, electrical conductivity, total dissolved solids and dissolved oxygen) in the collected untreated and prototype treated rainwater samples adhered to the drinking water guideline limits of the South African Department of Water Affairs and Forestry (DWAF) (DWAF, 1996), South African National Standards (SANS) 241 [South African Bureau of Standards (SABS), 2005], Australian Drinking Water Guidelines (ADWG) (NHMRC and NRMMC, 2011) and WHO (2011), with no significant difference (p > 0.05) observed for the data collected for the untreated and treated (Tank 1 and Prototype I; Tank 2-FF and Prototype II) tank water samples or between sites 1 and 2 (Tank 1 and 2-FF) (Table A3).

Results for the chemical analyses of the untreated (Tank 1 and Tank 2-FF) and treated (Prototype I and Prototype II) tank water samples collected from sites 1 and 2, indicated that

257 all anions and cations (Table A3) were within the respective drinking water guideline limits

258 [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2011],

259 with the exception of the mean zinc (Zn) concentration recorded in the samples collected from

260 site 1 [Tank 1 (mean of 3044 µg/L) and Prototype I (mean of 3061 µg/L)]; which exceeded

261 (albeit not significantly) the DWAF (1996) and ADWG (NHMRC and NRMMC, 2011) limit of

262 3000 µg/L. However, these samples were within the 5000 µg/L SANS 241 (SABS, 2005) limit.

263 The increased Zn concentrations recorded at site 1 (Tank 1 and Prototype I), in comparison

264 to site 2 (Tank 2-FF and Prototype II), may primarily be attributed to the metal sheeting (e.g.

265 Zn sheeting) roofing material used to construct the catchment system, as the leaching of

266 metals from metal roofing materials (corrosion during rain events and continuous exposure to

267 sunlight) have been reported to be a major contributor of metal ions in rainwater (Chang et al.

268 2004; Reyneke et al. 2018). It should be noted, that while the catchment system at site 2 was

269 also constructed from Zn sheeting roofing material, the entire surface of the catchment system

270 was painted with a weather resistant roof paint (personal communication) which may have

271 limited the leaching of metal ions into the rainwater. Additionally, the first-flush diverter

272 connected to the rainwater tank at site 2 (Tank 2-FF) may have improved the physico-chemical

273 quality of the tank water samples. First-flush diverter systems act as a pre-treatment barrier

274 by redirecting the initial roof run-off water (at the start of a rain event), which is thought to

275 contain the highest concentration of pollutants (Sánchez et al. 2015). Gikas and Tsihrintzis

276 (2012) compared the quality of RHRW collected in the flush pipe of first-flush diverter systems,

277 with the RHRW entering the collection tanks (RWH tanks) and reported that all measured

278 mean anion and cation concentrations were higher in the collected first-flush samples. The

279 authors concluded that the diversion of the first-flush roof run-off away from the collection

280 tanks may improve the physico-chemical quality of the RHRW.

281   As no significant difference was obtained when comparing the anion and cation

282 concentrations (Table A3) recorded in the untreated tank water samples to the treated tank

283 water samples (Tank 1 vs Prototype I, Tank 2-FF vs Prototype II) and the tank water samples

284 from each site clustered together (Fig. 2), it was concluded that the solar reactor prototypes

285 (system components and the treatment mechanism) did not influence the chemical quality of

286 the tank water samples.

3.2 Removal efficiency of indicator bacteria and opportunistic pathogens

3.2.1 Culture-based analysis

289 For the untreated tank water samples collected from site 1 (Tank 1; n = 15), the *E. coli*, faecal

290 coliform, total coliform, enterococci and HPC concentrations exceeded the respective drinking

291 water guideline limits in 67%, 73%, 100%, 20% and 100% of the samples, respectively (Table

292 1). Analysis of the corresponding treated samples (Prototype I; n = 15) indicated that the

293 *E. coli* (> 0.78 log reduction), enterococci (> 3.48 log reduction) and faecal coliform (> 4.08

294 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all the collected

295 samples. Total coliforms were reduced to BDL in 63% of the treated samples collected

296 following a 6 hour solar exposure (# 1-8) (> 3.94 log reduction), with a mean of 55 CFU/100 mL

297 detected in the samples (37%) where total coliform counts above the standard were detected.

298 An increase in solar exposure to 8 hours (# 9-15) resulted in an increased treatment efficiency,

299 as total coliforms were reduced to within the 5 CFU/100 mL DWAF (1996) and

300 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits in 100% of the treated samples (4.66

301 log reduction). For the HPC analysis, 38% of the treated samples were reduced to within the

302 drinking water guideline limit of 1.0×10^4 CFU/100 mL (1.71 log reduction) after a 6 hour solar

303 exposure [mean of 2.4×10^4 CFU/100 mL detected in the remaining 63% samples (1.21 log

304 reduction)], while 57% of the treated samples were reduced to within the guideline limit (2.08

305 log reduction) after an 8 hour solar exposure [mean of 2.7×10^4 CFU/100 mL detected in the

306 remaining 43% of samples (1.01 log reduction)] (Fig. A6).

307 For the untreated tank water samples collected from site 2 (Tank 2-FF; n = 18), the

308 *E. coli*, faecal coliform, total coliform, enterococci and HPC concentrations exceeded the

309 respective drinking water guideline limits in 56%, 22%, 100%, 28% and 100% of the samples,

310 respectively (Table 1). Analysis of the corresponding treated samples (Prototype II; n = 18)

indicated that the *E. coli* (> 0.48 log reduction), enterococci (> 3.34 log reduction) and faecal coliform (> 3.04 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all collected samples, while total coliforms were reduced to within the 5 CFU/100 mL DWAF (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits (3.85 log reduction). Heterotrophic bacteria were then reduced to within the 1.0×10^4 CFU/100 mL DWAF (1996) drinking water guideline limit in 88% of the treated samples (mean of 4.6×10^3 CFU/100 mL recorded) after a 6 hour solar exposure (# 1-8) (2.11 log reduction), with a mean of 1.8×10^4 CFU/100 mL detected in the samples (12%) where HPC concentrations above the standard were detected. In comparison, 100% of the treated samples were reduced to within the 1.0×10^4 CFU/100 mL drinking water guideline limit after an 8 hour solar exposure (# 9-18) ($\geq$ 2.02 log reduction; Fig. A6).

Klebsiella spp. were detected in 100% (mean concentration of 1.9×10^4 CFU/100 mL) and *Salmonella* spp. in 60% (mean concentration of 6.3×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 1 (Tank 1); however, both organisms were reduced to BDL (> 4.28 and > 3.8 log reduction, respectively) following treatment using the Prototype I solar reactor (Table 1). *Klebsiella* spp. were also detected in 17% (mean concentration of 8.0×10^2 CFU/100 mL) and *Salmonella* spp. in 6% (mean concentration of 1.0×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 2 (Tank 2-FF), with both organisms reduced to BDL (> 2.9 and > 3 log reduction, respectively) following treatment using the Prototype II solar reactor (Table 1). *Pseudomonas* spp. and coliphages were not detected in any of the rainwater samples collected from sites 1 and 2.

Although numerous studies have investigated the use of SODIS to treat contaminated water, varying degrees of treatment efficiency (0.46 to > 6 log reductions in bacteria) have been reported depending on experimental design (McGuigan et al. 2012; Hamilton et al. 2019). However, a limitation of SODIS which has consistently been highlighted by these investigators is the small treatment volume (2 to 5 L). Ubomba-Jaswa et al. (2010) investigated the use of a 25 L SODIS reactor (methacrylate tube) situated inside a CPC and reported on the complete inactivation of *E. coli*, even during unfavourable weather conditions (cloudy with

339   low solar intensity). Polo- López et al. (2019a) then expanded on this research and

340   investigated cost-effective SODIS enhancement strategies that would enable the treatment of

341   larger volumes of water (32 L and 54 L), with the results obtained leading to the design of the

342   large-scale solar reactor prototypes (Prototype I and II) assessed in the current study. The

343   treatment efficiency of the Prototype I and II solar reactors was also assessed by Polo-López

344   et al. (2019b) under controlled conditions, by spiking synthetic rainwater with laboratory strains

345   of *E. coli*, enterococci, *Salmonella* and *Pseudomonas* ($10^5 – 10^6$ CFU/mL bacterial cells) using

346   a 6 hour solar exposure treatment time. A ≥ 6 log reduction of all the test bacteria was

347   obtained, with the system classified as "highly protective (≥ 4 log reduction)" against bacteria

348   according to the WHO (2016) household water treatment technology performance criteria. In

349   comparison, results from the current study, for both solar reactor prototypes, during a 6 hour

350   solar exposure treatment, indicated that ≥ 2.54 log reduction was obtained when monitoring

351   the removal of enterococci, faecal and total coliforms, while mean log reductions of ≥ 1.21 log

352   were obtained for the removal of HPC. Based on these results, the 6 hour solar exposure

353   treatment with the prototypes in field trials failed to meet the ≥ 2 log removal required for a

354   "protective" classification against bacteria. The Polo-López et al. (2019b) study was however,

355   conducted in a hot arid climate (Tabernas Dessert, Almería, Spain) with a mean UV radiation

356   of 28.31 W/m^2/h recorded during the 6 hour treatment trials, while the field trials of the systems

357   in the current study were conducted in a moderate Mediterranean climate (Stellenbosch,

358   Western Cape, South Africa), where a mean UV radiation of 20.82 W/m^2/h was recorded

359   during the 6 hour treatment trials (Table A2).

360   The treatment time in the current study was subsequently increased to 8 hours (Site

361   1: #9-15; Site 2: #9-18) in order to increase the overall UV dose (mean UV radiation of

362   24.72 W/m^2/h was recorded from #9-18). For both prototypes a ≥ 3.44 log reduction was

363   subsequently obtained when monitoring the removal of enterococci, faecal and total coliforms,

364   while the mean log reductions for the removal of HPC increased to ≥ 2.02 log. Based on the

365   observed treatment efficiencies obtained using the Prototype I and II solar reactors in the

366   current study (8 hour treatment), the prototypes may be classified as "protective (≥ 2 log

367   reduction)", for the removal of bacteria in the tank water (WHO, 2016). More importantly,

368   culture-based analysis indicated that both treatment systems were able to produce water that

369   adhered to the microbial parameters as stipulated in the respective drinking water guidelines

370   [DWAF, 1996; SANS 241 (SABS, 2005); ADWG (NHMRC and NRMMC, 2011); WHO, 2011],

371   with lower indicator organism counts recorded in the tank water samples collected from site 2,

372   where the first-flush diverter system was installed. The treated water collected from the large-

373   scale solar reactor prototypes could however, only be stored for a maximum of 24 hours, as

374   microbial re-growth occurred after this point.

375   **3.2.2 Molecular-based analysis**

376   The performance characteristics of the respective qPCR assays are provided in Table A4.

377   Results obtained using EMA-qPCR indicated that an overall mean decrease of 83.76% (0.79

378   log reduction) in intact *E. coli* cells was recorded after treatment using Prototype I, while an

379   overall mean decrease of 82.76% (0.76 log reduction) was recorded after treatment for

380   Prototype II (Fig. 3). Similarly, intact enterococci cells decreased by a mean of 91.68% (1.08

381   log reduction) after treatment using Prototype I, while an 84.89% (0.82 log reduction) mean

382   decrease was recorded after treatment using Prototype II (Fig. 3). In comparison,

383   quantification of intact *Klebsiella* cells indicated that this genus was more resistant to the solar

384   reactor treatment as mean decreases of 62.44% (0.43 log reduction) and 60.42% (0.40 log

385   reduction) were recorded after treatment using Prototype I and II, respectively (Fig. 3).

386   Similarly, intact *Legionella* cells decreased by 68.61% (0.50 log reduction) after treatment

387   using Prototype I and by 63.77% (0.44 log reduction) after treatment using Prototype II (Fig.

388   3). Overall mean decreases in intact *Pseudomonas* cells of 79.09% (0.68 log reduction) and

389   87.50% (0.90 log reduction) were recorded after treatment using Prototype I and II,

390   respectively, while *Salmonella* cells decreased by 78.36% (0.66 log reduction) after treatment

391   using Prototype I and 67.82% (0.49 log reduction) after treatment with Prototype II (Fig. 3).

392   Lastly, PMA-qPCR analysis indicated that *Cryptosporidium* oocysts decreased by 57.14%

(0.62 log reduction) after treatment using Prototype I, while a mean decrease of 73.81% (0.58 log reduction) was recorded after treatment using Prototype II (Fig. 3).

Overall, the EMA-qPCR and PMA-qPCR analysis indicated that the Prototype I and II solar reactors reduced the opportunistic pathogens by 74.43%. This discrepancy in the observed treatment efficiency in comparison to the results obtained using culture-based analysis, may be attributed to EMA-qPCR and PMA-qPCR detecting viable but non culturable (VBNC) cells within the water samples (Fittipaldi et al. 2012; Mansi et al. 2014). It has been reported that certain opportunistic pathogens (e.g. *Legionella pneumophila* and *P. aeruginosa*) can enter a VBNC state in which they are not detectable using standard culture-based analysis but are still viable and retain their virulence (Mansi et al. 2014). Moreover, these VBNC microorganisms may regain their ability to be cultured under favourable conditions, which corresponds to the observed bacterial re-growth observed after 24 hours (culture-based analysis). Strauss et al. (2019) then applied Illumina next-generation sequencing coupled with EMA viability treatment to identify the primary pathogenic or opportunistic pathogenic genera, capable of surviving SODIS-CPC treatment in a 10.6 L CPC-reactor (Strauss et al. 2019). Results from the study indicated that intact and potentially viable bacterial cells belonging to 11 different bacterial genera (e.g. *Acinetobacter*, *Campylobacter*, *Legionella*, *Mycobacterium* and *Pseudomonas* amongst others) were detected in the SODIS-CPC treated tank water. Monitoring for the presence of VBNC microorganisms following water treatment is thus essential as these VBNC bacteria still pose a health risk as they are potentially infectious (Mansi et al. 2014).

While the survival of the *Cryptosporidium* oocysts after SODIS treatment using the solar reactor prototypes, may be attributed to the resilient nature of the oocyst wall (Hamilton et al. 2018), the ability of the opportunistic pathogenic bacteria (*Pseudomonas* spp., *Salmonella* spp., *Legionella* spp. and *Klebsiella* spp.) to survive large-scale solar-based disinfection strategies has been attributed to their ability to initiate various stress-response mechanisms and switch to a more tolerant phenotype upon exposure to environmental stressors, such as temperature and UV exposure (Jones, 1997; Fux et al. 2005). These stress-

responses may include the production of heat shock proteins and the initiation of DNA repair mechanisms, amongst others (Fields et al. 2002; Breidenstein et al. 2011). For example, Srivastava et al. (2008) indicated that the overexpression of the sigma factor *algT*, protects *Pseudomonas* spp. from heat stress and allows these organisms to persist during unfavourable conditions, while DNA repair mechanisms may be initiated in response to UV-induced DNA damage, through the activation of the SOS-regulon (upregulation of *recA* and *lexA*) or the photolyase enzyme (Zenoff et al. 2006). Similarly, Bojer et al. (2010) attributed the heat resistance of *K. pneumoniae* to the *clpK* genetic marker, which has been shown to correlate positively with thermotolerant phenotypes observed among clinical *Klebsiella* isolates. Microorganisms have also been reported to produce pigments or structures that may enable their survival under unfavourable conditions, as has been reported for *P. aeruginosa* where the production of pyocyanin has been hypothesised to protect *P. aeruginosa* from oxidative stress (inactivation mechanism of SODIS) (Hendiani et al. 2019). It is thus evident that microorganisms may employ numerous strategies to survive disinfection treatment and that additional treatment barriers may be required to reduce the survival of these target pathogens within water treatment systems. These strategies may include the addition of a cost-effective filtration system as a pre-treatment strategy to reduce microbial load entering the large-scale solar reactor prototypes (Hamilton et al. 2019).

3.3 Water safety plan and operational sustainability of the systems

As numerous factors may influence the quality of RHRW during the harvesting and/or treatment process, a WSP (Appendix B) for the utilisation of rainwater harvesting in combination with the large-scale solar reactor prototypes was developed. As the WSP was developed concurrently with the monitoring of the large-scale solar reactor prototypes during the field trials, the effectiveness of the various control measures was assessed by comparing site 1 with site 2, as these sites were located in two distinct settings that could be influenced by different anthropogenic activities and potential pollution sources as outlined in Appendix A.

447   The application of the WSP to characterise the risk associated with RHRW collected

448   at sites 1 and 2, indicated that the external hazards at site 1 (informal settlement) posed a

449   greater risk of contamination. The increased risk was primarily attributed to the influence of

450   potential pollution sources present near the catchment system (e.g. garbage disposal site,

451   surface run-off), tree branches obstructing a section of the conveyance system, organic debris

452   (e.g. dust/soil dispersed from the dirt pathway, leaves from the tree) within the conveyance

453   system and corrosion of the metal sheeting catchment system. Correspondingly, chemical and

454   microbial analysis of the untreated tank water samples collected from sites 1 and 2 revealed

455   that the untreated tank water collected from site 1 had higher levels of chemical contaminants

456   (e.g. cations) and microbial contaminants in comparison to site 2. For example, the

457   concentration of HPC was 0.72 log [3.50×10^5 CFU/100 mL (Tank 1) vs 6.90×10^4

458   CFU/100 mL (Tank 2-FF)] greater in the untreated tank water samples from site 1 (Tank 1), in

459   comparison to site 2 (Tank 2-FF).

460   The improved tank water quality at site 2 may also be attributed to the efficiency of the

461   implemented control measures at this site. The catchment surface at site 2 was painted with

462   a weather resistant roof paint that may have reduced the leaching of metal contaminants into

463   the collected tank water. Additionally, due to space availability a first-flush diverter was

464   connected between the catchment system and Tank 2-FF, which served as a control measure

465   to reduce the introduction of organic debris into the collection tank. However, the efficiency of

466   a first-flush diverter is dependent on the maintenance of the system, which entailed

467   cleaning/emptying the first-flush diverter after each rain event. The quality of RHRW collected

468   from site 1 may then be improved by removing the obstructing tree branches (source of

469   organic debris), implementing a regular gutter cleaning regime, installing a gutter screen at

470   the inlet of the RWH tank (due to space limitation a first-flush diverter could not be connected

471   to the current catchment system) and replacing the corroded metal sheeting on the catchment

472   system or painting the catchment system with a weather resistant roof paint.

473   As previously indicated, workshops were conducted with participating households

474   within the respective communities to outline the operational maintenance of the large-scale

solar reactor prototypes and rainwater harvesting systems (Fig. A3). Subsequent monitoring
of the operational sustainability of the solar reactor prototypes at both sites indicated that
system maintenance was limited to cleaning the surface of the PMMA reactor tubes (prevent
dust accumulation that will influence UV transmittance), with no system components needing
replacement during the study period. The robustness of system components therefore also
needs to be taken into consideration when designing water treatment systems for use in rural
areas and informal settlements, where replacement components may not be readily available.
During the study period, households who had access to the treated tank water (Prototype I
and II) at sites 1 (13 households) and site 2 (5 households), primarily reported using the treated
tank water for domestic activities such as cleaning of their homes, laundry and washing.

As noted by Mahmud et al. (2007), the aim of a WSP for small community water
supplies should be to achieve an overall and sustained reduction in microbial
contaminants/sanitary risks, rather than aim for the complete removal of microbial
contaminants. The WSP outlined in the current study thus serves to reduce the contamination
of RHRW by reducing "preventable contaminant entry" (e.g. organic debris and faecal matter
containing an increased microbial load from washing into the storage tank) into the storage
tank, whereafter treatment with the large-scale solar reactor prototypes may further reduce
the microbial contaminants to within drinking water standards.

4. Conclusions

The physico-chemical and chemical quality of the Tank 1 and 2-FF and Prototype I and II
treated rainwater samples adhered to the respective drinking water guidelines, with an
improvement in quality observed at site 2 where the first-flush diverter was installed. Lower
indicator bacterial counts were also recorded in the tank water samples collected from site 2
(Tank 2-FF and Prototype II) where the first-flush diverter was installed and fewer hazards
were identified that may influence the tank water quality (WSP), in comparison to site 1 (Tank
1 and Prototype I). The installation of a first-flush diverter system may thus serve as an
inexpensive pre-treatment strategy that may improve the overall quality of RHRW, while the

establishment of a WSP may aid in identifying potential hazards/hazardous events that may influence water safety.

Although both reactor prototypes were able to significantly improve the microbial quality of the tank water after an 8 hour solar treatment, HPC exceeding the DWAF (1996) drinking water guideline limit were recorded in 43% of the Prototype I treated samples. Nevertheless, a mean 1.01 log reduction in heterotrophic bacteria was recorded for these samples, which would decrease the health risk associated with using the treated rainwater (in comparison to the utilisation of untreated rainwater). Results from the EMA-qPCR and PMA-qPCR analysis indicated that *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* oocysts were reduced by 74.43% in both reactor prototypes. While molecular analysis indicated that the target organisms in the treated rainwater samples were not reduced to below the detection limit, based on national and international drinking water guidelines, the large-scale solar reactor prototypes used in the current study may effectively treat rainwater to within drinking water standards. The 88 L and 140 L solar reactor prototype treatment systems may thus provide a viable water provision solution for the inhabitants of rural areas and urban informal settlements in sub-Saharan Africa.

Conflicts of interests

The authors have no conflicts to declare.

Acknowledgements

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662 **Table 1** Frequency of detection and mean concentrations (CFU/100 mL) of indicator organisms

663 and target bacterial pathogens in the tank water samples collected from sites 1 and 2.

Organism	Site 1		Site 2	
	Tank 1 (n = 15)	Prototype I (n = 15)	Tank 2-FF (n = 18)	Prototype II (n = 18)
E. coli	67% (6)	BDL	51% (3)	BDL
Total coliforms	100% (1.5×10^4)	27% (42)	100% (1.0×10^3)	11% (2)
Enterococci	20% (3.0×10^3)	BDL	28% (2.2×10^3)	BDL
Faecal coliforms	73% (1.2×10^4)	BDL	22% (1.1×10^3)	BDL
Heterotrophic bacteria	100% (3.5×10^5)	50% (1.8×10^4)	100% (6.9×10^4)	86% (6.5×10^3)
Klebsiella spp.	100% (1.9×10^4)	BDL	17% (8.0×10^2)	BDL
Pseudomonas spp.	ND	ND	ND	ND
Salmonella spp.	60% (6.3×10^3)	BDL	6% (1.0×10^3)	BDL
Coliphages (PFU/mL)	ND	ND	ND	ND

664 BDL – below detection limit; ND – not detected; PFU – plaque forming units

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Figure Legends:

676 **Fig. 1. (A)** The Prototype I (140 L) solar reactor installed at Site 1. **(B)** The Prototype II (88 L)

677 solar reactor installed at Site 2. The red arrow indicates the first-flush diverter which was

678 connected to Tank 2-FF.

679 **Fig. 2.** Principle component analysis of the cations affecting the tank water quality for site 1

680 (Tank 1 and Prototype I) and 2 (Tank 2-FF and Prototype II). The directionality of the arrows

681 indicate the correlation (same = positive; opposite = negative) between the different variables

682 and illustrate the predominant variables best describing the collected tank water samples.

683 **Fig. 3.** Box and whiskers plot illustrating the distribution of the intact cells or oocysts/100 mL

684 recorded for each of the target organisms using EMA-qPCR (*E. coli*, enterococci, *Klebsiella*

685 spp., *Legionella* spp., *Pseudomonas* spp. and *Salmonella* spp.) and PMA-qPCR

686 (*Cryptosporidum* oocysts) in the tank water samples collected from **(A)** site 1 and **(B)** site 2.

687 The whiskers at the end of each box indicate the minimum and maximum values, while the

688 box is defined by the lower and upper quartiles and the mean value.