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

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18 Short title: Large-scale SODIS treatment of rainwater

19

20 Abbreviations¹

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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

46 **1. Introduction**

47 The Global Risks Report released for 2019 listed water crises as one of the top ten risks in
48 terms of likelihood (rating of 9; very likely to occur) and impact (rating of 4; severe impact)
49 (Global Risks Report, 2019). The probability of a water crisis risk in sub-Saharan Africa is
50 significantly increased as a high proportion of the population reside in urban informal
51 settlements and rural areas, with limited access to a safe water supply and sanitation
52 infrastructure (Dos Santos et al. 2017). However, as highlighted by Gwenzi and Nyamadzawo
53 (2014) and Emenike et al. (2017), rainwater is considered an under-exploited water source in
54 sub-Saharan Africa and may serve as an effective reserve to improve and encourage equity
55 in water access. Roof-harvested rainwater (RHRW) can however, be contaminated with
56 various chemicals and microorganisms, which may limit its use as a potable water source
57 (Hamilton et al. 2019). While the chemical pollutants have not been directly associated with
58 the incidence of disease, organic debris and faecal matter from animals and birds that have
59 access to the catchment surface, have been identified as the primary sources of microbial
60 contaminants such as *Legionella*, *Klebsiella*, *Pseudomonas* and *Cryptosporidium* (Hamilton
61 et al. 2019).

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

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

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

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

101 RHRW on-site in a local informal settlement (140 L Prototype I) and a rural farming community
102 (88 L Prototype II). The chemical quality of the RHRW before and after solar reactor treatment
103 was routinely assessed by monitoring various physico-chemical parameters (e.g. temperature,
104 pH, and turbidity), anions and cations. Additionally, the removal of traditional indicator
105 organisms (*E. coli*, total and faecal coliforms, enterococci and heterotrophic bacteria) and
106 selected opportunistic pathogens (*Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp.),
107 was assessed using culture-based analysis. Ethidium monoazide bromide quantitative
108 polymerase chain reaction (EMA-qPCR) assays were also used to monitor the reduction
109 efficiency of indicator organisms (*E. coli* and enterococci) and opportunistic pathogens
110 (*Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Salmonella* spp.), while propidium
111 monoazide (PMA) qPCR assays were used to monitor *Cryptosporidium* oocyst reductions. A
112 water safety plan (WSP) outlining guidelines for the use of rainwater harvesting combined with
113 solar reactor treatment was also implemented.

114 **2. Materials and methods**

115 **2.1 Description of large-scale solar reactor prototypes and sampling sites**

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

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

135 **2.2 Sample collection**

136 For the microbial and chemical analysis of the water produced by the solar reactor prototypes
137 (Fig. 1), an untreated 10 L sample was collected directly from the RWH tank at each site
138 [hereafter referred to as Tank 1 (Site 1) and Tank 2-FF (Site 2)]. The respective solar reactor
139 prototypes were filled with tank water from the RWH tanks and exposed to direct sunlight for
140 6 hours (sampling sessions 1 to 8) or 8 hours (sampling sessions 9 to 18). Following the solar
141 exposure, 10 L of each treated sample was collected directly from the solar reactor **prototypes**
142 [hereafter referred to as Prototype I (Site 1) and Prototype II (Site 2)]. Based on the availability
143 of rainwater in the RWH tanks, 15 sampling sessions were conducted at site 1 ($n = 30$; August
144 2018 to March 2019), while 18 sampling sessions were conducted at site 2 ($n = 36$; August
145 2018 to April 2019). For ease of presentation, sampling sessions 1 to 18 are designated as
146 #1 (sampling session 1), #2 (sampling session 2), etc., throughout the manuscript.

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

154 **2.3 Chemical analysis**

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

159 **2.4 Culturing of indicator organisms and opportunistic pathogens**

160 The microbial quality of the tank water samples collected from sites 1 and 2 were monitored
161 before (untreated) and after solar reactor treatment using various culture-based analyses.
162 *Escherichia coli* and total coliforms were enumerated simultaneously using membrane
163 filtration as described by Dobrowsky et al. (2015), while enterococci, faecal coliforms and the
164 heterotrophic plate count/bacteria (HPC) were enumerated as outlined in Strauss et al. (2016),
165 with a minor modification; Luria Bertani (LB) agar (Biolab, Merck, South Africa) replaced
166 Reasoner's 2A agar (Oxoid, Hampshire, England) for the enumeration of HPC. For the treated
167 samples (Prototypes I and II) where the HPC were reduced to below the detection limit [BDL;
168 < 1 colony forming units (CFU)/1 mL], the potential regrowth of bacteria was monitored. Briefly,
169 20 mL of each treated sample was stored in a sterile McCartney bottle at room temperature
170 and 100 μ L of the treated water was spread plated onto LB agar (Biolab, Merck) every 24
171 hours for a period of 2 days. The plates were then incubated at 37 °C. Additionally, *Klebsiella*
172 spp., *Pseudomonas* spp. and *Salmonella* spp. were enumerated as outlined in Clements et
173 al. (2019), while coliphages were enumerated as outlined by Baker et al. (2003) using *E. coli*
174 ATCC 13706 as the target bacterial host. All culture-based analyses were performed in
175 duplicate.

176 **2.5 Tank water concentration, viability treatment and DNA extraction**

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

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.

182 **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.

200 **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

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

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

224 **2.8 Statistical analysis**

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

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

233 **3. Results and Discussion**

234 **3.1 Physico-chemical properties and chemical analysis of the collected tank water** 235 **samples**

236 The mean ambient UV-A radiation at both sampling sites ranged from 7.16 W/m² (12/09/2018)
237 to 31.29 W/m² (14/01/2019), while the mean ambient UV-B radiation ranged from 1.33 W/m²
238 (12/09/2018) to 4.63 W/m² (14/01/2019) (Table A2). The untreated tank water temperature at
239 site 1 (Tank 1) ranged from 9.0 °C (02/08/2018 and 15/08/2018) to 24.0 °C (28/01/2019), with
240 a mean temperature of 16.3 °C recorded for all sampling days, while the tank water
241 temperature in the samples collected from the Prototype I solar reactor ranged from 15.5 °C
242 (12/09/2018) to 45.0 °C (28/01/2019) (mean 28.9 °C). Similarly, the untreated tank water
243 temperature at site 2 (Tank 2-FF) ranged from 10.0 °C (15/08/2018) to 26.0 °C (25/10/2018)
244 (mean 18.1 °C), while the tank water temperature in the samples collected from the Prototype
245 II solar reactor ranged from 18.0 °C (12/09/2018) to 46.5 °C (28/01/2019) (mean 32.6 °C).

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

255 Results for the chemical analyses of the untreated (Tank 1 and Tank 2-FF) and treated
256 (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 NRMCC, 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 NRMCC, 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.

287 **3.2 Removal efficiency of indicator bacteria and opportunistic pathogens**

288 **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; $n = 18$)

311 indicated that the *E. coli* (> 0.48 log reduction), enterococci (> 3.34 log reduction) and faecal
312 coliform (> 3.04 log reduction) concentrations were reduced to BDL (< 1 CFU/100 mL) in all
313 collected samples, while total coliforms were reduced to within the 5 CFU/100 mL DWAF
314 (1996) and 10 CFU/100 mL SANS 241 (SABS, 2005) guideline limits (3.85 log reduction).
315 Heterotrophic bacteria were then reduced to within the 1.0×10^4 CFU/100 mL DWAF (1996)
316 drinking water guideline limit in 88% of the treated samples (mean of 4.6×10^3 CFU/100 mL
317 recorded) after a 6 hour solar exposure (# 1-8) (2.11 log reduction), with a mean of
318 1.8×10^4 CFU/100 mL detected in the samples (12%) where HPC concentrations above the
319 standard were detected. In comparison, 100% of the treated samples were reduced to within
320 the 1.0×10^4 CFU/100 mL drinking water guideline limit after an 8 hour solar exposure (# 9-
321 18) (≥ 2.02 log reduction; Fig. A6).

322 *Klebsiella* spp. were detected in 100% (mean concentration of 1.9×10^4 CFU/100 mL)
323 and *Salmonella* spp. in 60% (mean concentration of 6.3×10^3 CFU/100 mL) of the untreated
324 rainwater samples collected from site 1 (Tank 1); however, both organisms were reduced to
325 BDL (> 4.28 and > 3.8 log reduction, respectively) following treatment using the Prototype I
326 solar reactor (Table 1). *Klebsiella* spp. were also detected in 17% (mean concentration of
327 8.0×10^2 CFU/100 mL) and *Salmonella* spp. in 6% (mean concentration of
328 1.0×10^3 CFU/100 mL) of the untreated rainwater samples collected from site 2 (Tank 2-FF),
329 with both organisms reduced to BDL (> 2.9 and > 3 log reduction, respectively) following
330 treatment using the Prototype II solar reactor (Table 1). *Pseudomonas* spp. and coliphages
331 were not detected in any of the rainwater samples collected from sites 1 and 2.

332 Although numerous studies have investigated the use of SODIS to treat contaminated
333 water, varying degrees of treatment efficiency (0.46 to > 6 log reductions in bacteria) have
334 been reported depending on experimental design (McGuigan et al. 2012; Hamilton et al.
335 2019). However, a limitation of SODIS which has consistently been highlighted by these
336 investigators is the small treatment volume (2 to 5 L). Ubomba-Jaswa et al. (2010) investigated
337 the use of a 25 L SODIS reactor (methacrylate tube) situated inside a CPC and reported on
338 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 $28.31 \text{ W/m}^2/\text{h}$ 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 $20.82 \text{ W/m}^2/\text{h}$ 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 $24.72 \text{ W/m}^2/\text{h}$ 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 NRMCC, 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%

393 (0.62 log reduction) after treatment using Prototype I, while a mean decrease of 73.81% (0.58
394 log reduction) was recorded after treatment using Prototype II (Fig. 3).

395 Overall, the EMA-qPCR and PMA-qPCR analysis indicated that the Prototype I and II
396 solar reactors reduced the opportunistic pathogens by 74.43%. This discrepancy in the
397 observed treatment efficiency in comparison to the results obtained using culture-based
398 analysis, may be attributed to EMA-qPCR and PMA-qPCR detecting viable but non culturable
399 (VBNC) cells within the water samples (Fittipaldi et al. 2012; Mansi et al. 2014). It has been
400 reported that certain opportunistic pathogens (e.g. *Legionella pneumophila* and
401 *P. aeruginosa*) can enter a VBNC state in which they are not detectable using standard
402 culture-based analysis but are still viable and retain their virulence (Mansi et al. 2014).
403 Moreover, these VBNC microorganisms may regain their ability to be cultured under
404 favourable conditions, which corresponds to the observed bacterial re-growth observed after
405 24 hours (culture-based analysis). Strauss et al. (2019) then applied Illumina next-generation
406 sequencing coupled with EMA viability treatment to identify the primary pathogenic or
407 opportunistic pathogenic genera, capable of surviving SODIS-CPC treatment in a 10.6 L CPC-
408 reactor (Strauss et al. 2019). Results from the study indicated that intact and potentially viable
409 bacterial cells belonging to 11 different bacterial genera (e.g. *Acinetobacter*, *Campylobacter*,
410 *Legionella*, *Mycobacterium* and *Pseudomonas* amongst others) were detected in the SODIS-
411 CPC treated tank water. Monitoring for the presence of VBNC microorganisms following water
412 treatment is thus essential as these VBNC bacteria still pose a health risk as they are
413 potentially infectious (Mansi et al. 2014).

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

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

439 **3.3 Water safety plan and operational sustainability of the systems**

440 As numerous factors may influence the quality of RHRW during the harvesting and/or
441 treatment process, a WSP (Appendix B) for the utilisation of rainwater harvesting in
442 combination with the large-scale solar reactor prototypes was developed. As the WSP was
443 developed concurrently with the monitoring of the large-scale solar reactor prototypes during
444 the field trials, the effectiveness of the various control measures was assessed by comparing
445 site 1 with site 2, as these sites were located in two distinct settings that could be influenced
446 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

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

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

493 **4. Conclusions**

494 The physico-chemical and chemical quality of the Tank 1 and 2-FF and Prototype I and II
495 treated rainwater samples adhered to the respective drinking water guidelines, with an
496 improvement in quality observed at site 2 where the first-flush diverter was installed. Lower
497 indicator bacterial counts were also recorded in the tank water samples collected from site 2
498 (Tank 2-FF and Prototype II) where the first-flush diverter was installed and fewer hazards
499 were identified that may influence the tank water quality (WSP), in comparison to site 1 (Tank
500 1 and Prototype I). The installation of a first-flush diverter system may thus serve as an
501 inexpensive pre-treatment strategy that may improve the overall quality of RHRW, while the

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

504 Although both reactor prototypes were able to significantly improve the microbial
505 quality of the tank water after an 8 hour solar treatment, HPC exceeding the DWAF (1996)
506 drinking water guideline limit were recorded in 43% of the Prototype I treated samples.

507 Nevertheless, a mean 1.01 log reduction in heterotrophic bacteria was recorded for these
508 samples, which would decrease the health risk associated with using the treated rainwater (in
509 comparison to the utilisation of untreated rainwater). Results from the EMA-qPCR and PMA-
510 qPCR analysis indicated that *E. coli*, enterococci, *Klebsiella* spp., *Legionella* spp.,
511 *Pseudomonas* spp., *Salmonella* spp. and *Cryptosporidium* oocysts were reduced by 74.43%
512 in both reactor prototypes. While molecular analysis indicated that the target organisms in the
513 treated rainwater samples were not reduced to below the detection limit, based on national
514 and international drinking water guidelines, the large-scale solar reactor prototypes used in
515 the current study may effectively treat rainwater to within drinking water standards. The 88 L
516 and 140 L solar reactor prototype treatment systems may thus provide a viable water provision
517 solution for the inhabitants of rural areas and urban informal settlements in sub-Saharan
518 Africa.

519 **Conflicts of interests**

520 The authors have no conflicts to declare.

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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) |
| <i>E. coli</i> | 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) |
| <i>Klebsiella</i> spp. | 100% (1.9 × 10 ⁴) | BDL | 17% (8.0 × 10 ²) | BDL |
| <i>Pseudomonas</i> spp. | ND | ND | ND | ND |
| <i>Salmonella</i> 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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675 **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 (*Cryptosporidium* 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.