



HAL
open science

Village Settlements in Mountainous Tropical Areas, Hotspots of Fecal Contamination as Evidenced by Escherichia coli and Stanol Concentrations in Stormwater Pulses

Laurie Boithias, Emilie Jardé, Keoudone Latsachack, Chanthanousone Thammahacksa, Norbert Silvera, Bounsamay Souleuth, Mose Xayyalart, Marion Viguiet, Alain Pierret, Emma Rochelle-Newall, et al.

► To cite this version:

Laurie Boithias, Emilie Jardé, Keoudone Latsachack, Chanthanousone Thammahacksa, Norbert Silvera, et al.. Village Settlements in Mountainous Tropical Areas, Hotspots of Fecal Contamination as Evidenced by Escherichia coli and Stanol Concentrations in Stormwater Pulses. *Environmental Science and Technology*, 2024, 58 (14), pp.6335-6348. <10.1021/acs.est.3c09090>. <insu-04532170>

HAL Id: insu-04532170

<https://insu.hal.science/insu-04532170v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1 Village settlements in mountainous tropical areas,
2 hotspots of fecal contamination as evidenced by
3 *Escherichia coli* and stanol concentrations in storm
4 water pulses
5

6 Laurie BOITHIAS^{1,*}, Emilie JARDÉ², Keooudone LATSACHACK³, Chanthanousone THAMMAHACKSA³,
7 Norbert SILVERA³, Bounsamay SOULILEUTH³, Mose XAYYALART³, Marion VIGUIER³, Alain PIERRET¹,
8 Emma ROCHELLE-NEWALL⁴, Olivier RIBOLZI¹

9

10 **Affiliations**

11 ¹ GET, Université de Toulouse, CNRS, IRD, UPS, 31400 Toulouse, France

12 ² Univ Rennes, CNRS, Géosciences Rennes, UMR 6118, 35000 Rennes, France

13 ³ IRD, Department of Agricultural Land Management (DALaM), P.O. Box 4199, Ban Nongviengkham,
14 Xaythany District, Vientiane, Lao PDR

15 ⁴ Institute of Ecology and Environmental Sciences of Paris (iEES-Paris), Sorbonne Université, Univ Paris
16 Est Créteil, IRD, CNRS, INRAE, 4 place Jussieu, 75005 Paris, France

17 * corresponding author: laurie.boithias@get.omp.eu

18

19 **Abstract**

20 Fecal bacteria in surface water may indicate threats to human health. Our hypothesis is that village
21 settlements in tropical rural areas are major hotspots of fecal contamination, because of the number
22 of domestic animals usually roaming in the alleys, and the lack of fecal matter treatment before
23 entering river network. By jointly monitoring the dynamics of *Escherichia coli* (*E. coli*) and of seven
24 stanol compounds during four flood events (July-August 2016) at the outlet of a ditch draining
25 sewage and surface runoff out of a village of Northern Lao PDR, our objectives were (1) to assess the
26 range of *E. coli* concentration in the surface runoff washing off from a village settlement, and (2) to
27 identify the major contributory sources of fecal contamination using stanol compounds during flood
28 events. *E. coli* pulses ranged from $4.7 \cdot 10^4$ to $3.2 \cdot 10^6$ MPN 100 mL⁻¹, with particle-attached *E. coli*
29 ranging from 83 to 100 %. Major contributory feces sources were chicken and human (about 66 and
30 29 %, respectively), the highest percentage switching from the human pole to the chicken pole
31 during flood events. Concentrations indicate a severe fecal contamination of surface water during
32 flood events and suggest that villages may be considered as major hotspots of fecal contamination
33 pulses into the river network, and thus as point-sources in hydrological models.

34

35 **Keywords**

36 Fecal biomarkers; Fecal Indicator Bacteria; Microbial Source Tracking; EMMA; Freshwater; *E. coli*
37 partition; Hot moments; Tropical rural areas.

38

39 **Synopsis**

40 Little is known about the contribution of villages to the fecal contamination of surface water in
41 tropical rural areas. This study documents *E. coli* and stanol concentration levels in surface runoff,
42 and tracks the origin of the fecal contamination during flood events.

43

44 1. Introduction

45 Livestock droppings or human feces, where open defecation is practiced or where sanitation systems
46 are lacking or deficient, are primary sources of fecal bacteria in the environment¹⁻³. Fecal pathogenic
47 bacteria may threaten human health if present in surface water^{4,5}, especially in least developed
48 countries where about 40 % of the population still use unimproved water sources for domestic needs
49 ⁶.

50 Fecal Indicator Bacteria (FIB), such as *Escherichia coli* (*E. coli*), are transported in water as free cells or
51 cells attached to soil particles, manure, or sediment⁷⁻¹⁰. In tropical humid rural areas such as the
52 mountainous areas of Southeast Asia, the ranges of *E. coli* concentrations have already been
53 investigated during both low flow and high flow periods at the outlet of mixed land use catchments
54 of various spatial scales^{11,12}, highlighting the leading role of surface runoff and soil erosion in *E. coli*
55 dissemination^{13,14}.

56 In rural areas of Southeast Asia, villages concentrate population and domestic animals most often
57 without wastewater treatment facilities, and the ranges of FIB exported during storms from village
58 settlements towards river network have not been yet quantified. Similarly, the dynamics of FIB
59 during storms on impervious surfaces such as compacted soils in village settlements, where surface
60 runoff is the only vector of diffuse fecal bacteria dissemination, and the attachment of FIB to
61 suspended sediment in surface runoff, remain to be fully documented. This information is needed to
62 design and feed catchment-scale hydrological models capable of predicting FIB concentrations during
63 flood events.

64 The sources of fecal contamination and their relative contributions to the overall fecal contamination
65 during a flood event are seldom known, although they represent essential information to (1)
66 understand and model the transfers of fecal bacteria on the catchment scale¹⁵, and (2) better
67 manage and mitigate microbial dissemination in the environment^{16,17}. Microbial source tracking with
68 lipid biomarkers such as stanol compounds^{18,19} can be used to identify the origin of the fecal
69 contamination in the environment²⁰⁻²⁶, including stream water^{16,21,27-34}. The stanol fingerprint of
70 feces depends on the animals' diet, their ability to biosynthesize endogenous sterols, and the
71 composition of the intestinal flora responsible for sterol biohydrogenation into stanols¹⁷. Unlike
72 other less persistent, source-specific markers such as caffeine³⁵, and similarly to methods using
73 ratios of fecal source-associated genetic markers³⁶, the analysis of a number of stanol compounds
74 can help identifying different fecal sources and their apportionment. Several ratios of stanol
75 compounds have been proposed to identify the origin of fecal matter^{28,34,37-43}. However, the use of
76 such ratios has been questioned because they could result in overlaps for a number of animal

77 sources⁴⁴. Multivariate analyses such as principal component analyses (PCA)⁴⁵, possibly associated
78 to end-member mixing analyses (EMMA), circumvent the problem of ratio specificity to identify the
79 origin and spatial patterns of fecal matter^{17,20,27,34,44,46–49}. However, PCA-based EMMA has not been
80 tested in a tropical context, although it has the potential to serve as a global method²⁷, and it has not
81 been used to quantify the relative contribution of each fecal contamination source. A number of
82 studies measured stanol compound concentration in stream suspended sediment^{16,21,28,31}, both at
83 low and high flow^{29,30}, but the concentration dynamics and the switch in fecal contamination sources
84 during flood events, have not been yet investigated.

85 In tropical areas such the mountainous Northern Lao PDR, about 62% of the population lives in rural
86 areas⁵⁰, in villages with little or no infrastructure for the containment and treatment of human and
87 animal feces, and of wastewater⁶. Our hypothesis is that village settlements are major hotspots of
88 fecal contamination because of the number of domestic animals usually roaming in the alleys and the
89 lack of fecal matter treatment before entering river network. By jointly monitoring the dynamics of *E.*
90 *coli* and stanol compounds during four flood events (July-August 2016) at the outlet of a ditch
91 draining sewage and surface runoff out of a village of Northern Lao PDR, our objectives were thus (1)
92 to assess the range of concentrations of *E. coli* in the surface runoff washing off from a village
93 settlement, and (2) to identify the major contributory source of fecal contamination using stanol
94 compounds.

95 2. Materials and methods

96 2.1. Study design

97 In this study, we quantified the range of *E. coli* concentration in water samples collected at the outlet
98 of a ditch draining sewage and surface runoff out of a village of Northern Lao PDR during four flood
99 events. We measured the concentration of *E. coli* in both raw water and filtered water to assess the
100 percentage of particle-attached *E. coli*. We quantified the concentrations and the relative
101 abundances of 7 stanol compounds (coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-
102 ethylepicoprostanol, cholestanol, campestanol, and sitostanol) in three feces sources, namely
103 human, porcine, and chicken feces, as well as in the suspended sediment of water samples. We
104 applied PCA-based EMMA on stanol compounds relative abundances in feces to calculate the relative
105 contribution of the three feces sources to surface runoff fecal contamination during flood events.
106 Finally, we calculated the amount of *E. coli* originating from the three sources during flood events.

107 2.2. Study area

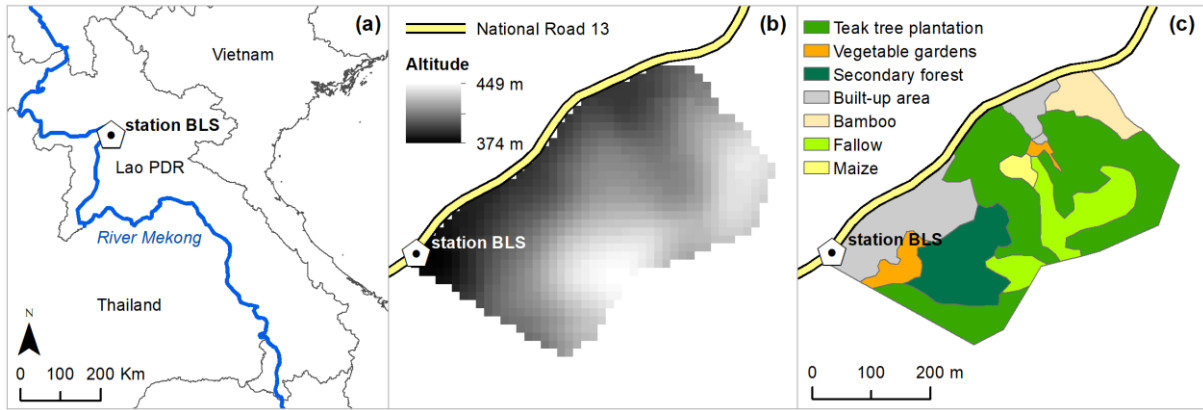
108 The study area is the 13.3-ha south-east part of the Laksip village (Ban Laksip), located 10-km
109 southeast of Luang Prabang in Northern Lao PDR (Fig. 1a). This study area is part of the 800,000 km²

110 Mekong basin. The climate is sub-tropical humid and is characterized by a monsoon regime with a
111 dry season from November to May, and a rainy season from June to October. The mean annual
112 temperature is 23.4 °C. The mean annual rainfall is 1,366 mm (CV=0.23), about 71 % (CV=0.09) of
113 which falls during the rainy season ⁵¹.

114 The sampling and gauging station (latitude: 19.848421; longitude: 102.168295), named BLS after
115 village name, is located at the outlet of the village drainage area, in a ditch alongside the National
116 Road 13 (NR13); this ditch collects surface runoff from the drainage area and sewage from several
117 pipes (piped latrines, bathroom, and kitchen outflows). The drainage area considered in this study is
118 located between two concrete tubes evacuating water beneath the NR13. Altitude in the considered
119 drainage area ranges from 374 to 449 m (Fig. 1b). Soil is classified as Acrisol.

120 In the study area, around 5 % of households have no latrines and practice open defecation. Around
121 80 % of households are equipped with pit latrines that are not connected to the ditch. When these
122 latrines are full, they are emptied using a pump truck, or they are condemned and new pit latrines
123 are built. Around 15 % of households are equipped with piped latrines connected to the ditch. These
124 are the houses built along the ditch, in the downstream part of the study area. Residents take
125 advantage of certain intense and prolonged rainfall events to open the tap and drain off the
126 supernatant liquid. However, during the four flood events monitored in our study, we did not
127 observe flushes from piped latrines. We do not expect sewage piped from bathroom and kitchen to
128 carry fecal contamination. As for animal droppings, they are sometimes stored as manure or applied
129 in vegetable gardens, but no manure is imported from outside the village for fertilization. There is
130 virtually no wildlife due to hunting pressure. Therefore, the fecal contamination in the ditch mostly
131 comes from surface runoff during rainfall events, through human open defecation and domestic
132 animal droppings. The ditch is dry during non-storm events, except some intermittent discharge from
133 sinks and showers.

134 In August 2016, we conducted a detailed land-use survey within the drainage area with a handheld
135 GPS (Fig. 1c). We classified land use into seven classes, namely: teak tree plantation (20 to 22 years
136 old monospecific teak tree plantations, predominantly without understory), vegetable gardens,
137 secondary forest, built-up area, bamboo, fallow (2 to 8 years old spontaneously regrown vegetation
138 following annual crops), and maize ⁵². The built-up area is characterized by impervious surfaces such
139 as roofs and compacted anthropized soil between dwellings. We also conducted a survey among the
140 24 households of the built-up area to qualify the possible feces sources (120 humans, 23 pigs, and
141 111 chickens). Humans, occasionally practicing open defecation ⁶, and domestic animals, may traffic
142 in the surroundings of the built-up area.



143

144 Fig. 1. (a) Location of the BLS gauging and sampling station in Northern Lao PDR, at the outlet of the
 145 Laksip village (Ban Laksip) drainage area; (b) Elevation within the drainage area (in meters above
 146 mean sea level; ALOS PALSAR ©JAXA, METI); (c) Land use in August 2016 within the drainage area.

147 **2.3. Sampling of feces sources**

148 To quantify stanol compounds in chicken feces, we collected chicken feces within six randomly
 149 chosen households of the Laksip village. We mixed equivalent masses of the feces from the six
 150 households to get a composite sample. The same method was applied to quantify stanol compounds
 151 in porcine feces. To quantify stanol compounds in human feces, we collected human feces from ten
 152 randomly chosen inhabitants of the Laksip village. We mixed equivalent masses of the feces from the
 153 ten inhabitants to get a composite sample. Composite feces samples were dried and kept in the
 154 refrigerator until the stanol compounds were analyzed. We chose to analyze composite samples of
 155 feces sources to measure the average concentration of each stanol compound in each feces source,
 156 and to incorporate the variability of each feces source, with a limited number of analyses of stanol
 157 compounds.

158 Study procedures for human sample collection and laboratory analysis were reviewed and approved
 159 by the National Ethics Committee for Health Research, Lao Ministry of Health Council of Medical
 160 Sciences (reference no. 51/NECHR)^{53,54}.

161 **2.4. Flood event monitoring and water sampling**

162 **Rainfall**

163 Rainfall was measured using an automatic weather station (Campbell ARG100, 0.2 mm capacity
 164 tipping-bucket) located at station BLS (Fig. 1b). Rainfall data was recorded at 1-min time intervals.

165 **Flow rate measurements and water sampling**

166 We measured ditch water level at the BLS gauging station (Fig. 1) with a water level recorder (OTT
 167 Thalimedes) connected to a data logger, with 1-mm vertical precision at a minimum of 3-min time

168 interval. To relate water level to surface runoff discharge (Q), a control rating curve was determined
169 using both the velocity area method and the salt dilution method by slug injection.

170 We collected samples of ditch water at BLS sampling station in clean, plastic bottles using an
171 automatic sampler for the measurement of total suspended sediment concentration ([TSS]), *E. coli*
172 concentration in raw water ($[E. coli]$), *E. coli* concentration in filtered water ($[E. coli]_{FREE}$), and stanol
173 compound concentrations. The automatic sampler⁵⁵ was triggered by the water level recorder to
174 collect water every time the water level rose by 2-cm and every time it fell by 4-cm. The bottles were
175 new empty bottles, supplied by a plastic bottle plant producing bottles for mineral water packaging.
176 We regularly verified that the bottles were free from *E. coli*⁵⁶.

177 2.5. Laboratory analyses of water samples

178 Data of [TSS], $[E. coli]$, $[E. coli]_{FREE}$, and of stanol compound concentrations and percentages, are
179 available on a public repository⁵⁷.

180 *E. coli*

181 We removed water samples from the water sampler immediately after the flood event, and stored
182 them in a cool-box until analysis, which took place within one hour of sampling. We measured $[E.$
183 $coli]$ with the standardized microplate method (ISO 9308-3)⁵⁶. A water sub-sample was incubated at
184 four dilution rates (i.e. 1:2, 1:20, 1:200 and 1:2000) in a 96-well microplate (MUG/EC, BIOKAR
185 DIAGNOSTICS) for 48 h at 44 °C. Ringers' Lactate solution was used for the dilutions and one plate
186 was used per sample. The number of positive wells for each microplate was noted and the Most
187 Probable Number (MPN) per 100 mL was determined using the Poisson distribution. The upper limit
188 of quantification of $[E. coli]$ using dilution rates 1:2, 1:20, 1:200, and 1:2000, is $3.2 \cdot 10^6$ MPN 100 mL⁻¹.
189 We measured $[E. coli]_{FREE}$ by first filtering 5 mL raw water with polycarbonate filters (3 µm porosity;
190 Whatman) and then applying the standardized microplate method to filtered water.

191 We calculated the percentage of *E. coli* in the particle-attached fraction, i.e. *E. coli* attached to
192 suspended sediment ($[E. coli]_{ATT\%}$) with equation Eq. 1:

$$[E. coli]_{ATT\%} = \frac{[E. coli] - [E. coli]_{FREE}}{[E. coli]} \times 100 \quad \text{Eq. 1}$$

193 where $[E. coli]$ is the concentration of *E. coli* in raw water (MPN 100 mL⁻¹), $[E. coli]_{FREE}$ is the
194 concentration of *E. coli* in the free-living fraction, measured in the filtered water (MPN 100 mL⁻¹), and
195 $[E. coli]_{ATT\%}$ is the percentage of *E. coli* in the particle-attached fraction.

196 Total suspended sediment

197 We measured [TSS] in each sample after filtration of a 200 mL water sub-sample on polycarbonate
198 filters (0.2 μm porosity; Whatman) followed by evaporation at ambient temperature for 48 h. Dry
199 filters were kept in the refrigerator until the analysis of dry suspended sediment for stanol
200 compounds.

201 Stanol compounds

202 We measured the concentrations and the relative abundances of seven stanol molecules in both the
203 dried feces and the dried suspended sediment: coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-
204 ethylepicoprostanol, cholestanol, campestanol, and sitostanol.

205 We extracted the total lipid fraction with dichloromethane using an accelerated solvent extractor
206 (ASE 200, Dionex). Extraction conditions were described previously⁴⁴. The total lipid extract was then
207 fractionated using solid/liquid chromatography (silica column) to separate the polar fraction. The
208 polar fraction was derivatized with a mixture of N,O-bis-(trimethylsilyl)trifluoroacetamide and
209 trimethylchloro-silane (BSTFA + TMCS, 99/1, v/v, Supelco) after addition of 5 α -cholestane (CDN
210 isotope) as an internal standard. Derivatized samples were analyzed by gas chromatography-mass
211 spectrometry (GC-MS) using a Shimadzu QP2010plus equipped with a capillary column (Supelco, 60
212 m x 0.25 mm ID, 0.25 μm film thickness). The temperature of the transfer line was set at 280 $^{\circ}\text{C}$, and
213 molecules were ionized by electron impact at an energy of 70 eV. The temperature of the ionization
214 source was set at 200 $^{\circ}\text{C}$. Samples were injected in splitless mode at 310 $^{\circ}\text{C}$. The oven temperature
215 was programmed from an initial temperature of 200 $^{\circ}\text{C}$ (held for 1 min) then rising to 310 $^{\circ}\text{C}$ at 15 $^{\circ}\text{C}$
216 min^{-1} (held for 35 min). Helium was used as the carrier gas, with a flow rate of 1.0 mL min^{-1} . The
217 analyses were made in Selective Ion Monitoring (SIM) mode. The compound identification was
218 performed based on the comparison of the retention times and the mass spectra with available
219 standards or the literature data (Table SI1)²⁰. The quantification was achieved with a 5-point internal
220 calibration curve (0.1, 0.3, 0.5, 0.7, and 1 ppm) of stanol standards with a constant internal standard
221 concentration at 0.5 ppm. To ensure the absence of potential contaminants and to verify system
222 performance during GC-MS measurements⁵⁸, we analyzed two to five blank samples (empty vials)
223 when beginning the GC-MS sequence, and we ran the 5-point internal calibration curve of stanol
224 standards at the start, middle and end of each GC-MS sequence (one sequence = 20 samples). A
225 blank was also analyzed after each real sample during the GC-MS sequence.

226 Limit of quantification (LQ) was 0.01 $\mu\text{g g}^{-1}$. Limit of detection (LD) ranged from 0.005 to 0.01 $\mu\text{g g}^{-1}$
227 depending on the stanol compound (Table SI1). For the stanol compounds for which we found
228 concentrations below the LQ (here epicoprostanol and 24-ethylepicoprostanol), we replaced the

229 concentrations values by the value of the LD. Concentrations of stanol compounds were expressed in
230 $\mu\text{g g}^{-1}$ of dry matter and converted to $\mu\text{g L}^{-1}$ by multiplying the concentrations of stanol compounds
231 by [TSS] in the case of water samples. Hereafter, we refer to [copros]_F, [epicop]_F, [24etco]_F, [24etep]_F,
232 [choles]_F, [campes]_F, and [sitost]_F, and to [copros]_{WS}, [epicop]_{WS}, [24etco]_{WS}, [24etep]_{WS}, [choles]_{WS},
233 [campes]_{WS}, and [sitost]_{WS} as the concentration of coprostanol, epicoprostanol, 24-ethylcoprostanol,
234 24-ethylepicoprostanol, cholestanol, campestanol, and sitostanol in feces and water samples,
235 respectively. We calculated the concentration of total stanol compounds in feces ([stanol]_F) and
236 water samples ([stanol]_{WS}) as the sum of the concentrations of the seven stanol compounds in feces
237 and water samples, respectively. We analyzed separately coprostanol and the other three 5 β -stanol
238 compounds (epicoprostanol, 24-ethylcoprostanol, and 24-ethylepicoprostanol) because we expected
239 coprostanol to be the major stanol compound in human feces¹⁷. We thus calculated the
240 concentration of 5 β -stanol compounds without coprostanol ([5 β -w/ocop]_F) by summing
241 concentrations of epicoprostanol, 24-ethylcoprostanol, and 24-ethylepicoprostanol in both feces and
242 water samples ([5 β -w/ocop]_{WS}). We calculated the concentration of 5 α -stanol compounds in feces
243 ([5 α -stanol]_F) and water samples ([5 α -stanol]_{WS}) as the sum of the concentrations of 5 α -stanol
244 compounds, namely cholestanol, campestanol, and sitostanol, in feces and water samples,
245 respectively.

246 Finally, we calculated the relative abundance, or distribution, in percentage, of the stanol compounds
247 in the three feces sources and in each water sample by measuring the relative abundance of each
248 stanol compound compared to the internal standard from the GC-MS characteristic peaks. For each
249 stanol compound, we integrated the peak area of the selected m/z (Table SI1). We then calculated
250 the relative abundance of each stanol compound by dividing the area of the compound by the sum of
251 the peak areas of all the analyzed compounds, and expressed the ratio as a percentage. This way, we
252 could compare the relative abundances of stanol compounds in both feces sources and water
253 samples by avoiding stanol concentrations gaps between feces sources (more concentrated) and
254 water samples (less concentrated), even when concentrations were below the limit of quantification.
255 Similar to concentrations, we refer to %copros_F, %epicop_F, %24etco_F, %24etep_F, %choles_F, %campes_F,
256 and %sitost_F, and to %copros_{WS}, %epicop_{WS}, %24etco_{WS}, %24etep_{WS}, %choles_{WS}, %campes_{WS}, and
257 %sitost_{WS} as the relative abundance of coprostanol, epicoprostanol, 24-ethylcoprostanol, 24-
258 ethylepicoprostanol, cholestanol, campestanol, and sitostanol in feces and water samples,
259 respectively. We calculated the sum of the relative abundances of epicoprostanol, 24-
260 ethylcoprostanol, and 24-ethylepicoprostanol in both feces (%5 β -w/ocop_F) and water samples (%5 β -
261 w/ocop_{WS}). We calculated the sum of the relative abundances of cholestanol, campestanol, and
262 sitostanol in both feces (%5 α -stanol_F) and water samples (%5 α -stanol_{WS}).

263 2.6. Correlation analysis

264 We calculated Spearman's rank correlation coefficients between Q, [TSS], [*E. coli*], [copros]_{WS},
265 [epicop]_{WS}, [choles]_{WS}, [24etco]_{WS}, [24etep]_{WS}, [campes]_{WS}, [sitost]_{WS}, [stanol]_{WS}, [5 β -w/ocop]_{WS}, and
266 [5 α -stanol]_{WS}, with R statistical package version 4.0.3. We considered a correlation to be statistically
267 significant when its p-value adjusted for multiple comparisons (adjustment method "fdr") was lower
268 than 0.05.

269 2.7. End-member mixing analysis

270 We performed an end-member mixing analysis (EMMA) based on a principal component analysis
271 (PCA)^{45,59} to estimate the relative contributions of the three feces sources (i.e., human, porcine, and
272 chicken) at the time of each water sampling at the outlet of the Laksip village⁴⁴ during the four flood
273 events. We applied PCA-based EMMA on stanol compounds relative abundances to attenuate the
274 dilution effect of stanol compounds between feces source samples and water samples, and to
275 include stanol compound concentrations below LQ.

276 We first built mixing diagrams⁶⁰ of stanol compound relative abundances, and checked that the
277 stanol signatures of the water samples were included in the triangles defined by the three end-
278 members, i.e., the three feces sources. We discarded stanol compounds for which none of the points
279 representing a water sample were included in the triangles (here cholestanol): these compounds are
280 suspected of not having a conservative behavior.

281 We then derived eigenvalues and eigenvectors using PCA (performed with XLSTAT 20.1.1, Addinsoft,
282 2010) by considering the three end-members as individuals, along with six variables, i.e., the stanol
283 compound relative abundances in human, porcine, and chicken feces sources, after discarding
284 cholestanol. We confirmed the *a priori* choice of the three end-members using eigenvalues, and we
285 used eigenvectors to orthogonally project stanol compound relative abundances in end-members
286 and in water samples, the latter as supplementary individuals⁴⁷. We used orthogonal projections
287 along with mixing equations⁶¹ to estimate the relative contributions of the feces sources at each
288 sampling time.

289 We assessed the uncertainty within the end-members using a Monte-Carlo method⁶¹: the
290 mathematical expectation and the standard deviation of the relative abundance of each stanol
291 compound were estimated for each end-member, based on a literature review of stanol compound
292 concentration in human^{20,27}, chicken^{17,46}, and pig^{20,27} feces. For each compound, 1000 relative
293 abundance values were inferred by randomly sampling without replacement a normal distribution
294 corresponding to both the estimated expectation and the estimated standard deviation. These 1000

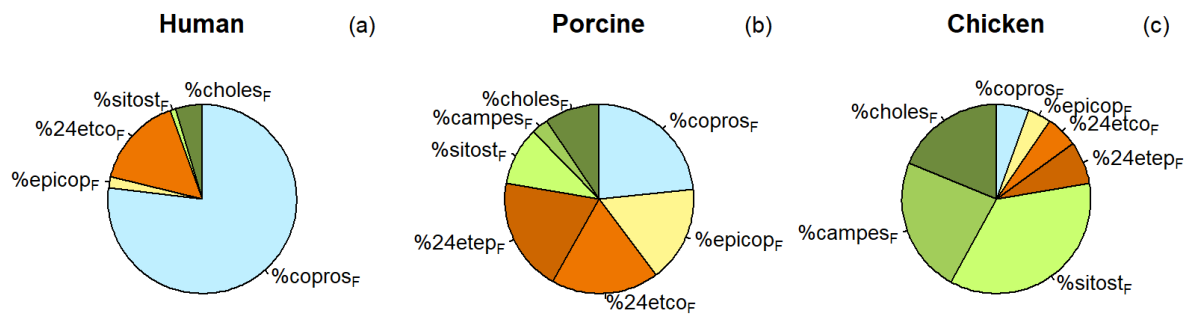
295 new individuals were then included in the PCA to derive the corresponding 1000 eigenvectors and to
296 calculate the mean and the standard deviation.

297 3. Results and discussion

298 3.1. Concentration and relative abundance of stanol compounds in feces

299 As for concentrations, $[\text{stanol}]_F$ in human, porcine, and chicken feces, were 9642.9, 956.2, and 375.4
300 $\mu\text{g g}^{-1}$, respectively (Table SI2). As for relative abundances of stanol compounds (Fig. 2), the relative
301 abundance of 5β -stanol compounds (i.e., $\% \text{copros}_F$ and $\%5\beta\text{-w/ocop}_F$) in human feces was 94 %,
302 while $\%5\alpha\text{-stanol}_F$ was 6 %. Coprostanol was the major compound ($\% \text{copros}_F=77\%$), followed by 24-
303 ethylcoprostanol ($\%24\text{etco}_F=16\%$). In porcine feces, the relative abundance of 5β -stanol compounds
304 was 78 %, while $\%5\alpha\text{-stanol}_F$ was 22 %. Among 5β -stanol compounds, the relative abundance of 5β -
305 stanol compounds derived from the hydrogenation of cholesterol (coprostanol and epicoprostanol),
306 was similar to the relative abundance of 5β -stanol compounds derived from the hydrogenation of
307 sitosterol (24-ethylcoprostanol and 24-ethylepicoprostanol) at 40 and 38 %, respectively. 5β -stanol
308 compounds without coprostanol ($\%5\beta\text{-w/ocop}_F$) was 54 %. In chicken feces, the relative abundance
309 of 5α -stanol compounds ($\%5\alpha\text{-stanol}_F$) was 78 %, with sitostanol being the major compound
310 ($\% \text{sitost}_F=36\%$). 5β -stanol compounds were 22 %, with $\% \text{copros}_F$, $\% \text{epicop}_F$, $\%24\text{etco}_F$, and $\%24\text{etep}_F$,
311 representing less than 10 % each.

312 Our results are consistent with the relative abundances of stanol compounds documented in the
313 literature for human, porcine, and chicken feces. Coprostanol is known to be the major stanol
314 compound in human feces, while other 5β -stanol compounds such as 24-ethylcoprostanol (19 %) and
315 24-ethylepicoprostanol (20 %) are often major stanol compounds in porcine feces^{17,20,62}. 24-
316 ethylcoprostanol and 24-ethylepicoprostanol are also found in human feces but are less abundant
317 compared to coprostanol. 5α -stanol compounds are often major stanol compounds in chicken feces
318^{17,39,46}, although stanol profiles of birds feces are known to greatly vary depending on the animals'
319 diet^{16,17}. These results allow to infer that coprostanol, 5β -stanol compounds without coprostanol,
320 and 5α -stanol compounds, mainly originate from human, porcine, and chicken fecal sources,
321 respectively, and use such inference for further analysis hereafter.



322

323 Fig. 2. Pie charts of the relative abundances of stanol compounds in (a) human feces, (b) porcine
 324 feces, and (c) chicken feces. %copros_F: relative abundance of coprostanol (%); %epicop_F: relative
 325 abundance of epicoprostanol (%); %24etco_F: relative abundance of 24-ethylcoprostanol (%);
 326 %24etep_F: relative abundance of 24-ethylepicoprostanol (%); %sitost_F: relative abundance of
 327 sitostanol (%); %campe_{SF}: relative abundance of campestanol (%); %choles_F: relative abundance of
 328 cholesterol (%).

329 3.2. Concentration of TSS, *E. coli*, and stanol compounds in water samples

330 The four flood events sampled in July and August 2016 covered wide ranges of rainfall duration,
 331 depth, and intensity (Fig. S11). Rainfall depths on July 20, July 23, August 8, and August 19 were 27,
 332 51, 25, and 40 mm, respectively, while rainfall durations were 101, 102, 105, and 233 minutes.
 333 Maximum rainfall intensities were 108, 252, 84, and 132 mm h⁻¹, respectively. The rainfall event of
 334 August 8 actually was a sequence of two rainfall events with maximum intensities of 84 and 72 mm h⁻¹,
 335 respectively. According to rainfall time-series analysis for the synoptic station of Luang Prabang⁶³,
 336 the flood events of July 20 and of August 8 correspond to a cumulative probability lower than 0.01
 337 (return period of 1 year) and can be classified as “small” events. The flood event of August 19
 338 corresponds to a cumulative probability ranging between 0.01 and 0.1 and can be classified as a
 339 “medium” event. The flood event of July 23 corresponds to a cumulative probability ranging between
 340 0.1 and 0.5 (return period of 2 years) and can be classified as a “large” event. The four flood events
 341 were thus representative of the common rainfall conditions prevailing during the rainy season in
 342 northern Lao PDR. Q ranged from 10 to 3491 L s⁻¹, with maxima of 410, 3491, 561, and 3085 L s⁻¹ on
 343 July 20, July 23, August 8, and August 19, respectively. A total of 29 water samples were collected
 344 during the four flood events, including five, five, nine, and ten water samples on July 20, July 23,
 345 August 8, and August 19, respectively (Fig. S11).

346 [TSS] ranged from 1.0 to 20.2 g L⁻¹ (Fig. 3a), with maximum [TSS] of 9.1, 6.3, 11.7, and 20.2 g L⁻¹ on
 347 July 20, July 23, August 8, and August 19, respectively. These concentrations are in the range of [TSS]
 348 measured during twelve flood events sampled from 2011 to 2015 at the outlet of the adjacent 60-ha

349 mixed land use Houay Pano catchment ($0.1\text{-}25.7\text{ g L}^{-1}$)¹³. As in the case of these previous [TSS]
350 measurements, eroded suspended particles were detached from bare soil surfaces found in the
351 alleys of the village and in the teak tree plantations grown without understory (Fig. 1). Bare soils
352 most often display a high rate of crusting, which induces high surface runoff^{64,65} and, in turn,
353 generates high soil erosion in areas of lower surface shear-stress resistance⁶⁶.

354 [*E. coli*] ranged from $4.7\cdot 10^4$ to $3.2\cdot 10^6$ MPN 100 mL⁻¹ (Fig. 3b). Maximum [*E. coli*] were $1.8\cdot 10^6$,
355 $3.2\cdot 10^6$, $1.6\cdot 10^6$, and $2.9\cdot 10^5$ MPN 100 mL⁻¹ on July 20, July 23, August 8, and August 19,
356 respectively. The two first water samples collected on July 23 resulted in [*E. coli*] above the limit of
357 quantification, to be related to the *a priori* choice of the four dilution rates with the standardized
358 microplate method. This implies that [*E. coli*] are under-estimated for these two time points. Overall,
359 the concentrations are higher than the range of [*E. coli*] measured at the outlet of the adjacent
360 Houay Pano catchment ($1.6\cdot 10^2\text{-}7.4\cdot 10^4$ MPN 100 mL⁻¹)¹³, or during high flow in a range of Mekong
361 tributaries in Lao PDR ($8.0\cdot 10^4$ MPN 100 mL⁻¹)¹¹, but they are in the range of the maximum [*E. coli*]
362 measured in the Mekong Delta at the end of the rainy season ($1.1\cdot 10^6$ MPN 100 mL⁻¹)²¹. [*E. coli*] are
363 also within the range of [*E. coli*] measured in the influent of wastewater treatment plants (WWTP)
364 ranging from 10^4 to 10^7 CFU or MPN 100 mL⁻¹⁶⁷⁻⁷¹. All these values exceed the 500 cells per 100 mL⁻¹
365 FIB threshold provided by the World Health Organization for freshwater⁷², associated to a 10 %
366 chance of gastroenteritis per single exposure⁷³. Bathing in such running water, as children often do
367 to refresh themselves from the heat during the hot and rainy season⁴, may thus pose a serious
368 health risk.

369 [*E. coli*]_{ATT%} ranged from 83 to 100 %, with a median of 97 % (Fig. 3c). These percentages are similar
370 to the ranges measured in *in situ* mesocosms installed within the Houay Pano catchment (91-99 %),
371 for [TSS] up to 0.5 g L^{-1} ⁷⁴. The range of [*E. coli*]_{ATT%} is narrower than the range measured in grab
372 samples along the Red River during one year (9.7-100 %), for [TSS] up to 0.6 g L^{-1} ⁹. Hence, [*E. coli*] at
373 the outlet of the ditch may be mostly driven by the detachment of soil-associated human and animal
374 feces with soil erosion during flood events, and most of the fecal contamination is attached to
375 particles, as are stanol compounds^{21,23}.

376 None of the stanol compound concentrations measured in the particulate phase during the four
377 flood events was below the detection limit. Two stanol compounds were often below the
378 quantification limit: epicoprostanol and 24-ethylepicoprostanol. [stanol]_{WS} ranged from 1.3 to 591.0
379 $\mu\text{g L}^{-1}$, [copros]_{WS} ranged from 0.3 to 293.0 $\mu\text{g L}^{-1}$, [5 β -w/ocop]_{WS} ranged from 0.4 to 222.3 $\mu\text{g L}^{-1}$,
380 while [5 α -stanol]_{WS} ranged from 0.4 to 75.7 $\mu\text{g L}^{-1}$ (Fig. 3d,e,f,g).

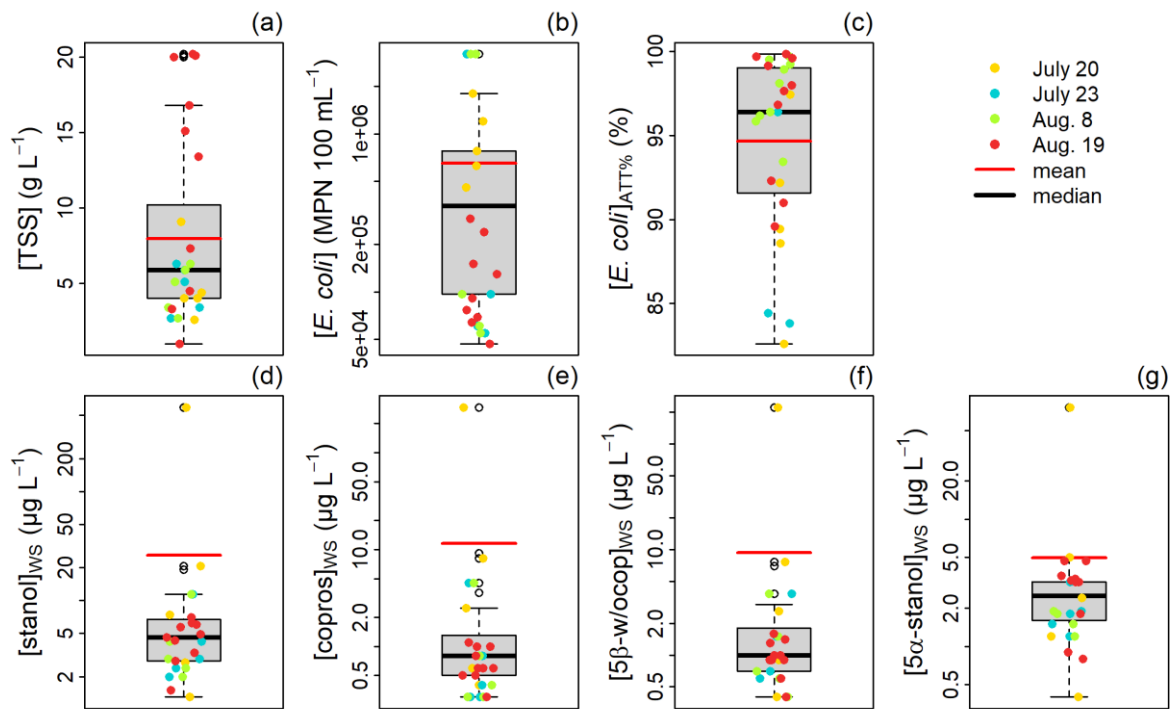
381 The range of [copros]_{WS} is higher than the ranges measured in the Mekong Delta (0.001-97.1 µg L⁻¹)
382 ²⁹, in Western Malaysia rivers and in the Mekong Delta (2*10⁻⁵-13.5 µg L⁻¹) ²¹, in streams of Manaus
383 area in Brazil (0.03-46.92 µg L⁻¹) ³⁴, and in Solo and Serayu rivers in Indonesia (<LD-0.68 µg L⁻¹) ³¹, but
384 in general lower than the range of [copros]_{WS} reported for WWTP influent in Japan (290.03-327.8 µg
385 L⁻¹) ²¹ and in the USA (385 µg L⁻¹) ⁷⁵.

386 The range of [epicop]_{WS} (<LQ-12.8 µg L⁻¹) is higher than the ranges measured in Western Malaysia
387 rivers and in the Mekong Delta (5*10⁻⁶-1.579 µg L⁻¹) ²¹, and in streams of Manaus area in Brazil (<LD-
388 2.28 µg L⁻¹) ³⁴, but in general lower than the range of [epicop]_{WS} reported for WWTP influents in
389 Japan (5.08-12.18 µg L⁻¹) ²¹. [24etep]_{WS} ranged from <LQ to 3.9 µg L⁻¹ while [24etco]_{WS} ranged from
390 0.4 to 205.6 µg L⁻¹ (Fig. SI2). [24etco]_{WS} reported for the streams of Manaus area in Brazil ranged
391 from <LQ to 9.03 µg L⁻¹ ³⁴. [24etco]_{WS} reported for the WWTP influent in the USA was 140 µg L⁻¹ while
392 [epicop]_{WS} and [24etep]_{WS} were below the limit of detection ⁷⁵. In our study, given the number of
393 values below the quantification limit for both [epicop]_{WS} and [24etep]_{WS}, [5β-w/ocop]_{WS} mostly
394 reflects [24etco]_{WS}.

395 The range of [choles]_{WS} (0.2-53.0 µg L⁻¹; Fig. SI2) is higher than the ranges measured in Western
396 Malaysia rivers and in the Mekong Delta (6*10⁻⁴-3.485 µg L⁻¹) ²¹, in streams of Manaus area in Brazil
397 (0.02-2.67 µg L⁻¹) ³⁴, and in Solo and Serayu rivers in Indonesia (0.052-0.468 µg L⁻¹) ³¹. [choles]_{WS} was
398 in the range reported for WWTP influent in Japan (23.95-31.27 µg L⁻¹) ²¹. The ranges of [campes]_{WS}
399 and of [sitost]_{WS} (0.1-7.7 µg L⁻¹ and 0.1-15.0 µg L⁻¹, respectively; Fig. SI2) are higher than the
400 [campes]_{WS} and [sitost]_{WS} measured in Solo and Serayu rivers in Indonesia (<LD-0.261 µg L⁻¹ and <LD-
401 0.476 µg L⁻¹, respectively) ³¹. [sitost]_{WS} measured in streams of Manaus area in Brazil ranged from <LQ
402 to 1.54 µg L⁻¹ ³⁴. [choles]_{WS}, [campes]_{WS}, and [sitost]_{WS} reported for the WWTP influent in the USA
403 were 40, 55, and 23 µg L⁻¹, respectively ⁷⁵. Hence, the range of [5α-stanol]_{WS} is higher than the [5α-
404 stanol]_{WS} measured in Solo and Serayu rivers in Indonesia (0.072-1.206 µg L⁻¹) ³¹, but lower than the
405 [5α-stanol]_{WS} measured in the USA WWTP influent (118 µg L⁻¹) ⁷⁵.

406 The concentrations of *E. coli* and of stanol compounds measured at the outlet of the Laksip village
407 during the four flood events were generally higher than the concentrations measured in the other
408 tropical rivers, whatever the hydrological conditions, while they were lower or in the same range
409 than the concentrations measured in the WWTP influent. Since concentrations are measured in
410 surface runoff and are thus diluted by rainfall, the range of both [*E. coli*] and [stanol]_{WS} can be
411 considered high. In the environment, the highest stanol compound concentrations are often found at
412 sampling sites located near domestic outfalls, and concentrations tend to decrease with distance
413 from the sources, involving mixing and dilutions processes ^{23,34,76-78}. Hence, lower concentrations of

414 stanol compounds found in e.g. the Mekong Delta ^{21,29} are consistent with higher concentrations at
 415 the outlet of a village located within a headwater catchment such as the Laksip village. A common
 416 feature in rural areas of developing countries is that although some families have individual
 417 treatment systems (septic tanks), surface runoff water and most of wastewater are not stored nor
 418 treated before entering the river network. Besides, [*E. coli*] in surface runoff and at the soil surface
 419 are positively associated ⁷⁹, and urbanized areas are known to increase FIB concentration due to
 420 impervious surfaces and failing septic systems ⁸⁰, two features common to our study area. Hence,
 421 both high [stanol]_{ws} and high [*E. coli*] suggest that ditch water is highly contaminated by fecal matter
 422 and that villages in rural areas can be considered as hotspots of fecal contamination during flood
 423 events. Villages may thus be described as point-sources of fecal contamination in distributed or semi-
 424 distributed hydrological models.



425
 426 Fig. 3. Water quality during four flood events sampled in 2016 at the BLS sampling station, Laksip
 427 village, Northern Lao PDR. (a) [TSS]: concentration of total suspended sediment (g L^{-1}); (b) [*E. coli*]:
 428 concentration of *Escherichia coli* ($\text{MPN } 100 \text{ mL}^{-1}$); (c) [*E. coli*]_{ATT%}: percentage of *Escherichia coli*
 429 attached to suspended sediment (%); (d) [stanol]_{ws}: sum of the concentrations of seven stanol
 430 compounds (coprostanol, epicoprostanol, cholestanol, 24-ethylcoprostanol, 24-ethylepicoprostanol,
 431 campestanol, and sitostanol) ($\mu\text{g L}^{-1}$); (e) [copros]_{ws}: concentration of coprostanol ($\mu\text{g L}^{-1}$); (f) [5β-
 432 w/ocop]_{ws}: sum of the concentrations of epicoprostanol, 24-ethylcoprostanol, and 24-
 433 ethylepicoprostanol ($\mu\text{g L}^{-1}$); (g) [5α-stanol]_{ws}: sum of the concentrations of cholestanol,
 434 campestanol, and sitostanol ($\mu\text{g L}^{-1}$). Y-axes for [*E. coli*] and stanol compounds concentration are

435 shown on log scales. Each boxplot contains the extreme of the lower whisker (dashed line), the lower
436 hinge (thin line), the median (bold line), the upper hinge (thin line), and the extreme of the upper
437 whisker (dashed line). The whiskers extend to the most extreme data point which is no more
438 than 1.5 times the interquartile range from the box.

439 3.3. Spatial origin of TSS, *E. coli*, and stanol compounds measured in water samples

440 [TSS] was positively correlated to [campes]_{WS} ($r=0.46$, $p\text{-value}=0.026$) and [sitost]_{WS} ($r=0.54$, $p\text{-}$
441 $\text{value}=0.007$) while [*E. coli*] was positively correlated to [copros]_{WS} ($r=0.81$, $p\text{-value}<0.001$), [epicop]_{WS}
442 ($r=0.55$, $p\text{-value}=0.007$), [choles]_{WS} ($r=0.53$, $p\text{-value}=0.009$), [24etco]_{WS} ($r=0.83$, $p\text{-value}<0.001$),
443 [24etep]_{WS} ($r=0.64$, $p\text{-value}=0.001$), and to [5 β -w/ocop]_{WS} ($r=0.81$, $p\text{-value}<0.001$) (Fig. S13).
444 Concentrations of the stanol compounds were in general positively correlated among each other. Q
445 was positively correlated to [TSS] ($r=0.76$, $p\text{-value}<0.001$) and negatively correlated to [*E. coli*] ($r=-$
446 0.55 , $p\text{-value}=0.006$). No significant correlation was found between [*E. coli*] and [TSS].

447 Soil erosion is expected to drive both [*E. coli*], *E. coli* being mostly transported attached to suspended
448 particles, and [stanol]_{WS}, stanol compounds being mostly bound to particulate matter^{21,23}. The
449 discrepancy in correlations suggests that the sources of suspended sediment, *E. coli*, and stanol
450 compounds, may not always coincide: suspended sediment may come either closely from the alleys
451 of the village or more remotely from the teak tree plantations grown without understory⁶⁵, while *E.*
452 *coli* may preferentially come from the built-up area⁸¹ (Fig. 1). *E. coli*-free suspended sediment may
453 get enriched with attached *E. coli* while transferred from remote areas towards the built-up area and
454 eventually to the ditch, or conversely, dilute the concentration of suspended sediment loaded with
455 attached *E. coli* originating from soil eroded in the built-up area. The absence of significant
456 correlation between [*E. coli*] and [5 α -stanol]_{WS} suggests that the origin of [5 α -stanol]_{WS} may be not
457 only the chicken contamination source in the built-up area^{17,31}, but also teak tree plantations where
458 soil erosion is known to be high⁶⁴. Indeed, campesterol and sitosterol have been commonly used as
459 plant biomarkers or as markers of terrigenous organic matter^{17,20,25,46}, and if chicken were roaming in
460 the teak tree plantations, the correlation between [*E. coli*] and both [TSS] and Q would have been
461 stronger and positive. In addition, [*E. coli*] and [stanol]_{WS} generally decreased when reaching the
462 flood event peak (see detail provided in Supporting Information, including Fig. S11 and Fig. S12),
463 indicating a dilution of the concentration by surface runoff. Hence, the correlation between [*E. coli*]
464 and both [copros]_{WS} and [5 β -w/ocop]_{WS} suggests that fecal contamination is mostly associated to
465 human and porcine feces sources⁷⁹ and seems to imply that fecal matter production is concentrated
466 in the area close to the ditch outlet, i.e., in the built-up area (Fig. 1).

467 The complementary information given by both the microbial indicator and the biomarkers provides a
468 multi-metrics evaluation of water quality^{19,82,83}. In our study, [*E. coli*] was correlated to [copros]_{WS}
469 ($r=0.81$; $r^2=0.66$) and to [24etco]_{WS} ($r=0.83$; $r^2=0.69$) (Fig. SI3). Strong positive associations between
470 [*E. coli*] and [copros]_{WS} were already highlighted in the Mekong Delta ($r^2=0.86$ and 0.91 during rainy
471 and dry seasons, respectively)^{21,29}. Besides, reported half-lives of dissolved-phase coprostanol, 24-
472 ethylcoprostanol, and sitostanol were 3.8, 4.4, and 5.4 days in aerobic and dark conditions at 18 °C³⁵,
473 while reported half-lives of *E. coli* near the study area were 0.5-5 days depending on the suspended
474 sediment settling and sunlight exposure conditions⁷⁴. The interval between each monitored flood
475 event and the previous one of similar return period was 1.8-5.7 days. Since the production of fecal
476 matter is virtually constant in the Laksip village, and considering the half-lives of both stanol
477 compounds and *E. coli*, no decay of neither *E. coli* nor stanol compounds is expected between fecal
478 matter production and flood events, and during flood events (typically 1-2 hours for the four flood
479 events). However, positive correlation between [*E. coli*] and [stanol]_{WS} ($r=0.67$, $p\text{-value}<0.001$) (Fig.
480 SI3) imply that the source of *E. coli* likely coincides with most of the sources of stanol compounds,
481 and that fecal input in ditch water is fresh¹⁷. Hence, the dynamics of [copros]_{WS}, [5 β -w/ocop]_{WS}, and
482 to a lesser extent [stanol]_{WS} are consistent with the dynamics of [*E. coli*], and confirm that *E. coli* is a
483 relevant FIB in the study area.

484 3.4. Relative abundance of stanol compounds in water samples

485 The relative abundance of stanol compounds in water samples varied during flood events (Fig. SI4):
486 %copros_{WS} ranged from 7 to 56 %, while %5 β -w/ocop_{WS} ranged from 13 to 28 %, and %5 α -stanol_{WS}
487 ranged from 19 to 79 %. %copros_{WS} decreased from the onset of the flood event until a minimum
488 occurring after the discharge peak, i.e., when %5 α -stanol_{WS} reached its maximum, while %5 β -
489 w/ocop_{WS} remained relatively stable. Maximum %copros_{WS} and %5 α -stanol_{WS} indicate that the
490 contribution of both human and chicken feces sources, along with possible terrigenous stanol
491 compounds, are high in the surface runoff washing off the alleys of the Laksip village^{17,20}.
492 Epicoprostanol is usually used as an indicator of the level of treatment or age of the fecal matter.
493 High %copros_{WS} (7-56 %) and low %epicop_{WS} (3-8 %) suggest that the fecal matter is little or not
494 treated before entering the river water network^{37,38}, confirming that villages in rural areas contribute
495 to decrease surface water quality.

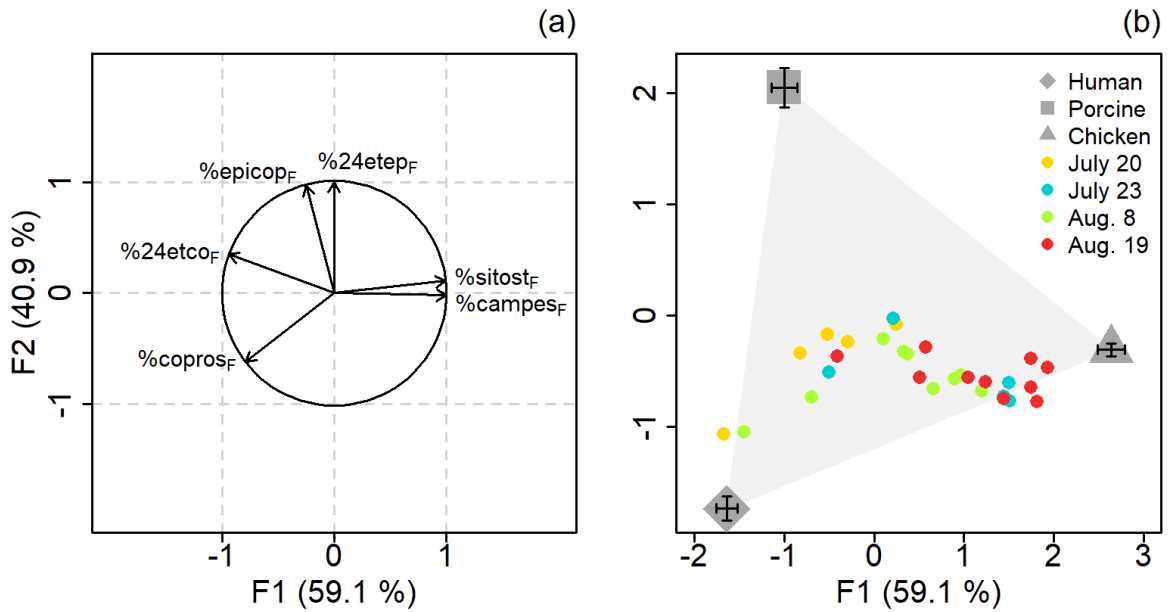
496 3.5. Separation of feces sources with PCA in a tropical context

497 Mixing diagrams show that the relative abundances of the stanol compounds in water samples were
498 mostly included in the triangles defined by the relative abundances of the stanol compounds in feces
499 sources, except for %choles_{WS}, for which none of the relative abundances were included in the
500 triangles (Fig. SI5). Hence, we discarded cholestanol before computing PCA.

501 Stanol compounds in water samples not included in the triangles defined by the relative abundance
502 of the stanol compounds in feces sources may mean that a source, represented by a pole of the
503 triangle, is inaccurately defined, highly variable, or even missing. In this study, a source other than
504 the chicken could contribute to the concentration of cholestanol, campesterol, and sitosterol, in
505 surface runoff: these three compounds have been commonly used as plant biomarkers or as markers
506 of terrigenous organic matter^{17,20,25,46}, but they may also originate from domestic vegetable oils
507 discharged in sewage^{76,84}. As for %24etco_{WS} and %24etep_{WS}, a source other than the porcine could
508 contribute, such as caprine²⁰. This source could have been missed during the survey because
509 possibly present in a small number, or not owned by the 24 households located in the built-up area.
510 Similarly, uncounted dogs could contribute to %copros_{WS}²⁰.

511 The first (F1) and second (F2) components of the PCA explained 59 % and 41 %, respectively, of the
512 total variance (Fig. 4a). The main contributive variables on the axis F1, in decreasing order of
513 importance, were %campes_F (28 %), %sitost_F (28 %), %24etco_F (25 %), and %copros_F (18 %), while the
514 main contributive variables on the axis F2 were %24etep_F (41 %), %epicop_F (38 %), and %copros_F (16
515 %) (Table SI3). The PCA analysis clearly separated the 5 α -stanol compounds and the 5 β -stanol
516 compounds without coprostanol along axis F1, and revealed that %copros_F discriminated the human
517 feces source, while %epicop_F and %24etep_F discriminated the porcine feces source, and %sitost_F and
518 %campes_F discriminated the chicken feces source (Fig. 4b). The case of %24etco_F is ambiguous, since
519 it is located between the poles defined by the porcine and the human feces sources: %24etco_F in
520 both human and porcine feces sources represents 16 and 19 % of the stanol compounds,
521 respectively, thus a similar percentage that prevents the discrimination of the two feces sources by
522 using %24etco_F only²⁰. It confirms that PCA based on six conservative stanol compounds measured in
523 human, porcine, and chicken feces, was more powerful to separate the three feces sources than
524 using a single compound, and that PCA was successful to separate feces sources in a tropical context
525²⁷.

526 When plotted within the stanol signature of the feces sources (Fig. 4b), the stanol signatures of the
527 29 water samples collected during the four flood events were mostly discriminated along axis F1,
528 with most of the samples located near the pole defined by the chicken feces source, and to a lesser
529 extent towards the pole defined by the human feces source.



530

531 Fig. 4. Principal component analysis (PCA) applied to three individuals (human, porcine, and chicken
 532 feces sources) and to six variables (%copros_F, %epicop_F, %24etco_F, %24etep_F, %campes_F, %sitost_F): (a)
 533 PCA correlation circle; (b) PCA score plot of stanol compounds relative abundance in feces sources,
 534 with the stanol signature in dry suspended sediment of 29 water samples collected during four flood
 535 events in 2016 at the BLS sampling station, Laksip village, Northern Lao PDR. F1: principal component
 536 1; F2: principal component 2. The numbers between brackets are the variance explained by each
 537 component. Standard deviation is shown as error bars.

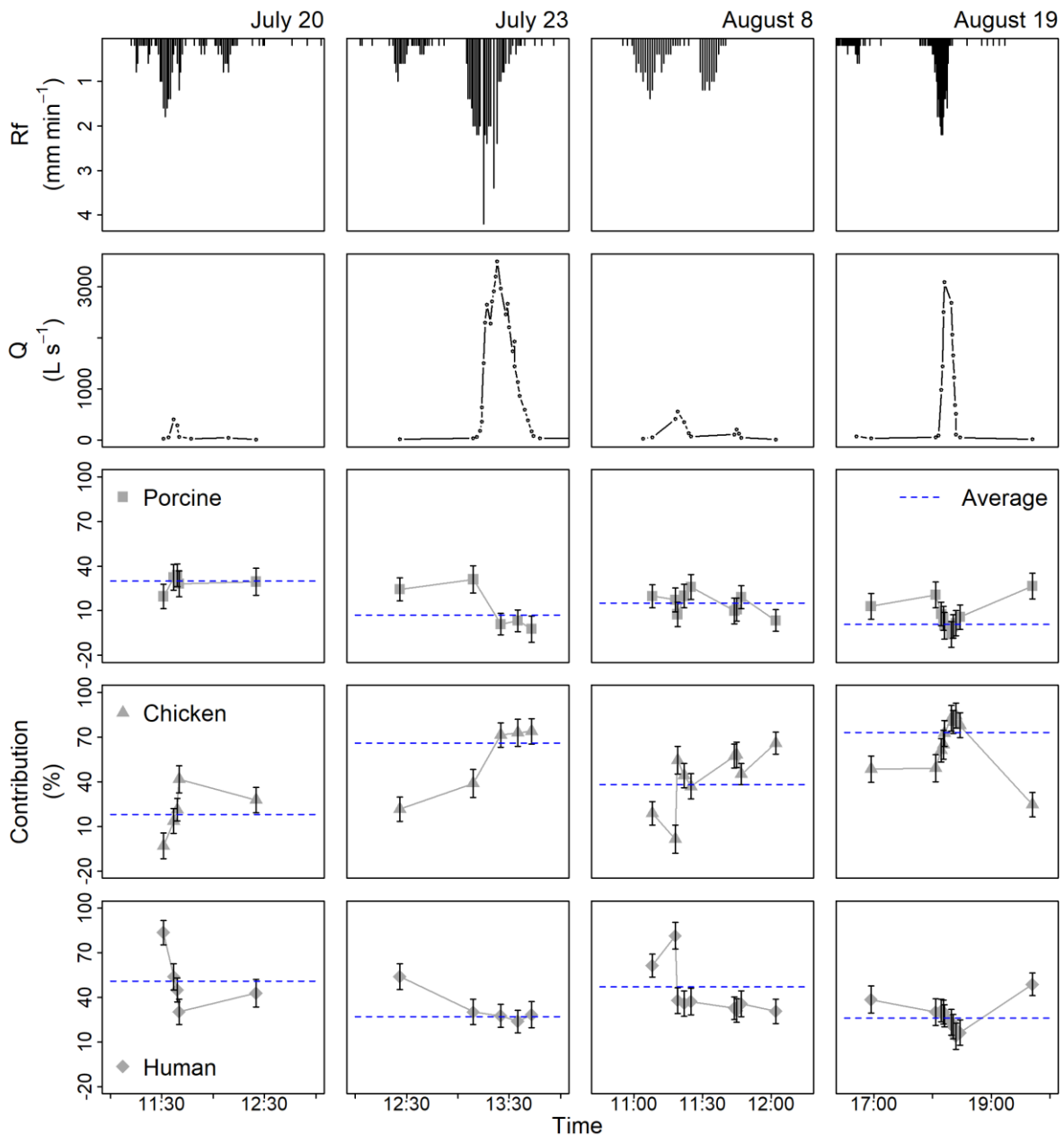
538 3.6. Dynamics of the contributions of the fecal contamination sources during flood events

539 The relative contributions of human, porcine, and chicken feces sources at each sampling time during
 540 flood events ranged from 14±9 to 84±8 %, -6±9 to 34±8 %, and -3±9 to 84±8 %, respectively (Fig. 5).
 541 Negative values reflect an inaccurately defined pole, the variability of the pole, or the absence of a
 542 pole in the analysis, or stanol compounds that are not fully conservative. Here however, these
 543 negative values are within the confidence interval, the average standard deviation of the relative
 544 contributions of the three feces sources being ±8 %. The contribution of the human feces source
 545 generally decreased from flood event onset to shortly after flood event peak, while the contribution
 546 of the chicken feces source generally increased during flood event. The increasing contribution of the
 547 chicken source may be explained by the fact that (1) human sources are generally closer to the ditch
 548 outlet than the chicken source; with a shorter travel time to the outlet, human sources are thus first
 549 detected and exhausted, and (2) 5α-stanol compounds may not only originate from the chicken
 550 source, but also from terrigenous organic matter originating from agricultural and forest areas
 551 located more remotely than the built-up area.

552 Discharge-weighted average contributions per flood event of human, porcine, and chicken feces
553 sources ranged from 26 to 51 %, 1 to 30 %, and 18 to 73 %, respectively (Fig. 5). The average
554 contribution per flood event of porcine feces sources was in general the smallest. The highest
555 average contributions of the chicken feces source were found for the highest values of rainfall depth,
556 rainfall maximal intensity, and maximal discharge, while the highest average contributions of both
557 human and porcine feces sources were found for the lowest values of rainfall depth, rainfall maximal
558 intensity, and maximal discharge. Discharge-weighted average contributions over the four flood
559 events of human, porcine, and chicken feces sources were 29, 5, and 66 %, respectively.

560 The three feces sources produce different concentrations of *E. coli*: humans produce about $1.6 \cdot 10^{11}$
561 *E. coli* cap⁻¹ day⁻¹, while pigs and chickens produce about $1.3 \cdot 10^{10}$ and $6.8 \cdot 10^8$ *E. coli* cap⁻¹ day⁻¹,
562 respectively⁷⁹. Multiplied by the units of each feces source in the village (see Section 2.2), the
563 amount of *E. coli* produced is $1.92 \cdot 10^{13}$, $2.99 \cdot 10^{11}$, and $7.55 \cdot 10^{10}$ *E. coli* day⁻¹ for human, pigs, and
564 chickens, respectively. The proportions of *E. coli* load from each feces source calculated from the
565 latter, i.e. the *E. coli* input into the system from literature review, is therefore 98.1, 1.5, and 0.4 % for
566 human, pigs, and chickens, respectively. The discrepancies between the proportions calculated from
567 *E. coli* input and from stanol-based discharge-weighted average contributions can be explained by (1)
568 preferential surface runoff paths (e.g., is the area convex or concave), and (2) human habits and
569 animal management (e.g., surface runoff flow into areas favored for open defecation and/or animal
570 droppings; animals free-ranging or restricted to a fenced area; surface runoff flow into fenced areas).
571 As an example, for the human source, the difference between the *E. coli* load calculated from human
572 density and the *E. coli* load calculated from stanol-based average contributions depends in part on
573 human access to sanitation, i.e., latrines that could store fecal matter which would therefore not be
574 measured at the village outlet during the four flood events; however, having latrines at home does
575 not imply that open defecation is not practiced from time to time.

576 *E. coli* exports at the ditch outlet, calculated over the flood events defined as the period lasting from
577 the first sampling to the last sampling, ranged from $2.6 \cdot 10^7$ to $6.4 \cdot 10^7$ MPN. Using the above-
578 mentioned stanol-based contributions for each sampling time, *E. coli* originating from human,
579 porcine, and chicken feces sources ranged from $1.2 \cdot 10^7$ to $2.8 \cdot 10^7$ MPN, $0.1 \cdot 10^7$ to $1.0 \cdot 10^7$ MPN,
580 and $0.6 \cdot 10^7$ to $3.3 \cdot 10^7$ MPN, respectively, during flood events. Surface water fecal contamination of
581 human origin is especially a concern in terms of risk assessment, since the risk posed to human
582 health appears substantially higher when ingested water is contaminated by human sources
583 compared to other animal sources such as birds, poultry, or pigs⁸⁵. One explanation for this is the
584 host-specific nature of viruses such as noroviruses^{86,87}.



585

586 Fig. 5. Rainfall, discharge, and contribution in percentage (%) of porcine, chicken, and human feces
 587 sources in dry suspended sediment during four flood events sampled in 2016 at the BLS sampling
 588 station, Laksip village, Northern Lao PDR. Rf: rainfall (mm min⁻¹); Q: discharge (L s⁻¹). Standard
 589 deviation is shown as error bars. Dotted lines are the average contributions of the feces source
 590 calculated over the flood event defined as the period lasting from the first sampling to the last
 591 sampling.

592 3.7. Summary

593 To our knowledge, this study is the first one to document the concentrations of fecal bacteria
 594 exported out of a village settlement in a tropical mountainous area of Southeast Asia. It is also the

595 first one to describe the dynamics of in-stream fecal contamination using biomarkers such as stanol
596 compounds during flood events.

597 We showed that *E. coli* pulses in surface runoff ranged from $4.7 \cdot 10^4$ to $3.2 \cdot 10^6$ MPN 100 mL⁻¹: such
598 concentrations indicate a severe fecal contamination of surface water, and suggest that village
599 settlements in tropical rural areas may be considered as major hotspots of fecal contamination
600 pulses into the river network. Major contributory sources of fecal contamination were human and
601 chicken (up to 51 and 73 %, respectively), the highest percentage switching from the human pole to
602 the chicken pole during flood events. The chicken source may be overestimated since chicken-
603 associated stanol compounds may also be terrigenous or plant-related.

604 In addition, we showed that the range of *E. coli* attached to suspended sediment in surface runoff
605 was 83-100 %. Concentration of total stanol compounds ranged from 1.3 to 591.0 µg L⁻¹ during flood
606 events. Concentrations of 5β-stanol, including coprostanol and 24-ethylcoprostanol, were consistent
607 with the concentrations of *E. coli*, confirming that *E. coli* is a relevant FIB in the context of tropical
608 village settlements.

609 The transferability of these results to other tropical humid catchments or village areas is ensured
610 provided (1) the production of fecal matter is virtually constant, (2) there is no contributions from pit
611 latrines with aged sewage, and (3) the travel time of fecal contaminants during flood events is short.

612 Our results concerning the export of *E. coli* and its attachment to suspended sediment will make it
613 possible to better parameterize village settlements as point-sources in distributed or semi-
614 distributed hydrological models. Such models can be used to test scenarios (improved hygiene
615 practices and sanitation ⁶, improved land use management to increase rain water infiltration and
616 decrease surface runoff and soil erosion ⁶⁴, improved management of livestock and manure ⁸⁸), and
617 to suggest better ways of managing and mitigating microbial dissemination in the environment.

618

619 Supporting Information

620 Details regarding hysteresis loops analysis from the concentration of TSS, *E. coli*, and stanol
621 compounds measured in water samples (Pages S2-S3); Rainfall, discharge, and water quality (Fig. S11,
622 page S4); [TSS]-Q, [E. coli]-Q, [copros]_{WS}-Q, [choles]_{WS}-Q, [24etco]_{WS}-Q, [24etep]_{WS}-Q, [campes]_{WS}-Q,
623 and [sitost]_{WS}-Q hysteresis loops (Fig. S12, page S5); Spearman's rank correlation coefficient matrix of
624 log-transformed discharge and concentrations of water quality variables in water samples (Fig. S13,
625 page S6); Rainfall, discharge, and relative abundance of stanol compounds in water samples (Fig. S14,
626 page S7); Mixing diagrams of the relative abundance of seven stanol compounds in feces sources and

627 in water samples (Fig. S15, page S8); Names of the stanol compounds analyzed in this study, m/z
628 values used for the identification and quantification, and information on quantification compounds
629 (Table S11, page S9); Concentration and relative abundance of the seven stanol compounds measured
630 in porcine, chicken, and human feces (Table S12; page S10); Factorial coordinates and contribution of
631 variables on the F1 and F2 axes calculated from the Principal Component Analysis (PCA) based on the
632 relative abundance of six stanol compounds measured in human, porcine, and chicken feces sources
633 (Table S13, page S11); [TSS]-Q, [*E. coli*]-Q, [copros]_{WS-Q}, [choles]_{WS-Q}, [24etco]_{WS-Q}, [24etep]_{WS-Q},
634 [campes]_{WS-Q}, and [sitost]_{WS-Q} hysteresis loops (Table S14, page S12).

635

636 Acknowledgments

637 The authors sincerely thank the Lao Department of Agricultural Land Management (DALaM) for its
638 support, including granting the permission for field access, and the Multiscale TROPical CatchmentS
639 Critical Zone Observatory (M-TROPICS CZO; <https://mtropics.obs-mip.fr/>) for data access and
640 logistical support. This study was funded by the French National Research Agency (TecltEasy project;
641 ANR-13-AGRO-0007). LB thanks the French National Research Institute for Sustainable Development
642 (IRD) for her postdoctoral grant 2016-2017 (*Accueil de post-doctorants / Campagne 2015*;
643 www.ird.fr). The authors thank Yves AUDA for processing the ALOS PALSAR DEM shown in Fig. 1.

644

645 **References**

- 646 (1) Exley, J. L. R.; Liseka, B.; Cumming, O.; Ensink, J. H. J. The Sanitation Ladder, What Constitutes
647 an Improved Form of Sanitation? *Environ. Sci. Technol.* **2015**, *49* (2), 1086–1094.
648 <https://doi.org/10.1021/es503945x>.
- 649 (2) Tong, Y.; Yao, R.; He, W.; Zhou, F.; Chen, C.; Liu, X.; Lu, Y.; Zhang, W.; Wang, X.; Lin, Y.; Zhou,
650 M. Impacts of Sanitation Upgrading to the Decrease of Fecal Coliforms Entering into the
651 Environment in China. *Environ. Res.* **2016**, *149*, 57–65.
652 <https://doi.org/10.1016/j.envres.2016.05.009>.
- 653 (3) Rochelle-Newall, E.; Nguyen, T. M. H.; Le, T. P. Q.; Sengtaeuanhoun, O.; Ribolzi, O. A Short
654 Review of Fecal Indicator Bacteria in Tropical Aquatic Ecosystems: Knowledge Gaps and Future
655 Directions. *Front. Microbiol.* **2015**, *6*, 308. <https://doi.org/10.3389/fmicb.2015.00308>.
- 656 (4) Boithias, L.; Choisy, M.; Souliyaseng, N.; Jourden, M.; Quet, F.; Buisson, Y.; Thammahacksa, C.;
657 Silvera, N.; Latsachack, K.; Sengtaeuanhoun, O.; Pierret, A.; Rochelle-Newall, E.; Becerra,
658 S.; Ribolzi, O. Hydrological Regime and Water Shortage as Drivers of the Seasonal Incidence of
659 Diarrheal Diseases in a Tropical Montane Environment. *PLoS Negl. Trop. Dis.* **2016**, *10* (2),
660 e0005195. <https://doi.org/10.1371/journal.pntd.0005195>.
- 661 (5) IHME. *Global Burden of Disease (GDB) Results*. Seattle, WA: IHME, University of Washington;
662 2020. <https://vizhub.healthdata.org/gbd-results/> (accessed 2022-08-12).
- 663 (6) WHO-UNICEF. *Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG*
664 *Baselines*; World Health Organization (WHO) and the United Nations Children’s Fund
665 (UNICEF): Geneva, 2017; p 116. <https://apps.who.int/iris/handle/10665/258617>.
- 666 (7) Garcia-Armisen, T.; Servais, P. Partitioning and Fate of Particle-Associated *E. Coli* in River
667 Waters. *Water Environ. Res.* **2009**, *81* (1), 21–28.
668 <https://doi.org/10.2175/106143008X304613>.
- 669 (8) Krometis, L.-A. H.; Characklis, G. W.; Simmons, O. D.; Dilts, M. J.; Likirdopulos, C. A.; Sobsey,
670 M. D. Intra-Storm Variability in Microbial Partitioning and Microbial Loading Rates. *Water Res.*
671 **2007**, *41* (2), 506–516. <https://doi.org/10.1016/j.watres.2006.09.029>.
- 672 (9) Nguyen, H. T. M.; Le, Q. T. P.; Garnier, J.; Janeau, J.-L.; Rochelle-Newall, E. Seasonal Variability
673 of Faecal Indicator Bacteria Numbers and Die-off Rates in the Red River Basin, North Viet
674 Nam. *Sci. Rep.* **2016**, *6*, 21644. <https://doi.org/10.1038/srep21644>.
- 675 (10) Soupier, M. L.; Mostaghimi, S.; Dillaha, T. Attachment of *Escherichia Coli* and Enterococci to
676 Particles in Runoff. *J. Environ. Qual.* **2010**, *39* (3), 1019–1027.
677 <https://doi.org/10.2134/jeq2009.0296>.
- 678 (11) Nakhle, P.; Ribolzi, O.; Boithias, L.; Rattanavong, S.; Auda, Y.; Sayavong, S.; Zimmermann, R.;
679 Soullileuth, B.; Pando, A.; Thammahacksa, C.; Rochelle-Newall, E.; Santini, W.; Martinez, J. M.;
680 Gratiot, N.; Pierret, A. Effects of Hydrological Regime and Land Use on In-Stream *Escherichia*
681 *Coli* Concentration in the Mekong Basin, Lao PDR. *Sci. Rep.* **2021**, *11*, 3460.
682 <https://doi.org/10.1038/s41598-021-82891-0>.
- 683 (12) Rochelle-Newall, E. J.; Ribolzi, O.; Viguier, M.; Thammahacksa, C.; Silvera, N.; Latsachack, K.;
684 Dinh, R. P.; Naporn, P.; Sy, H. T.; Soullileuth, B.; Hmimum, N.; Sisouvanh, P.; Robain, H.;
685 Janeau, J.-L.; Valentin, C.; Boithias, L.; Pierret, A. Effect of Land Use and Hydrological Processes
686 on *Escherichia Coli* Concentrations in Streams of Tropical, Humid Headwater Catchments. *Sci.*
687 *Rep.* **2016**, *6*, 32974. <https://doi.org/10.1038/srep32974>.
- 688 (13) Boithias, L.; Ribolzi, O.; Lacombe, G.; Thammahacksa, C.; Silvera, N.; Latsachack, K.; Soullileuth,
689 B.; Viguier, M.; Auda, Y.; Robert, E.; Evrard, O.; Huon, S.; Pommier, T.; Zouiten, C.;
690 Sengtaeuanhoun, O.; Rochelle-Newall, E. Quantifying the Effect of Overland Flow on
691 *Escherichia Coli* Pulses during Floods: Use of a Tracer-Based Approach in an Erosion-Prone
692 Tropical Catchment. *J. Hydrol.* **2021**, *594*, 125935.
693 <https://doi.org/10.1016/j.jhydrol.2020.125935>.
- 694 (14) Ribolzi, O.; Evrard, O.; Huon, S.; Rochelle-Newall, E.; Henri-des-Tureaux, T.; Silvera, N.;
695 Thammahacksa, C.; Sengtaeuanhoun, O. Use of Fallout Radionuclides (⁷Be, ²¹⁰Pb) to

- 696 Estimate Resuspension of *Escherichia Coli* from Streambed Sediments during Floods in a
 697 Tropical Montane Catchment. *Environ. Sci. Pollut. Res.* **2016**, *23* (4), 3427–3435.
 698 <https://doi.org/10.1007/s11356-015-5595-z>.
- 699 (15) Cho, K. H.; Pachepsky, Y. A.; Oliver, D. M.; Muirhead, R. W.; Park, Y.; Quilliam, R. S.; Shelton, D.
 700 R. Modeling Fate and Transport of Fecally-Derived Microorganisms at the Watershed Scale:
 701 State of the Science and Future Opportunities. *Water Res.* **2016**, *100*, 38–56.
 702 <https://doi.org/10.1016/j.watres.2016.04.064>.
- 703 (16) Devane, M. L.; Wood, D.; Chappell, A.; Robson, B.; Webster-Brown, J.; Gilpin, B. J. Identifying
 704 Avian Sources of Faecal Contamination Using Sterol Analysis. *Environ. Monit. Assess.* **2015**,
 705 *187* (10), 625. <https://doi.org/10.1007/s10661-015-4800-3>.
- 706 (17) Leeming, R.; Ball, A.; Ashbolt, N.; Nichols, P. Using Faecal Sterols from Humans and Animals to
 707 Distinguish Faecal Pollution in Receiving Waters. *Water Res.* **1996**, *30* (12), 2893–2900.
 708 [https://doi.org/10.1016/S0043-1354\(96\)00011-5](https://doi.org/10.1016/S0043-1354(96)00011-5).
- 709 (18) Simpson, J. M.; Santo Domingo, J. W.; Reasoner, D. J. Microbial Source Tracking: State of the
 710 Science. *Environ. Sci. Technol.* **2002**, *36* (24), 5279–5288. <https://doi.org/10.1021/es026000b>.
- 711 (19) Field, K. G.; Samadpour, M. Fecal Source Tracking, the Indicator Paradigm, and Managing
 712 Water Quality. *Water Res.* **2007**, *41* (16), 3517–3538.
 713 <https://doi.org/10.1016/j.watres.2007.06.056>.
- 714 (20) Harrault, L.; Milek, K.; Jardé, E.; Jeanneau, L.; Derrien, M.; Anderson, D. G. Faecal Biomarkers
 715 Can Distinguish Specific Mammalian Species in Modern and Past Environments. *PLOS ONE*
 716 **2019**, *14* (2), e0211119. <https://doi.org/10.1371/journal.pone.0211119>.
- 717 (21) Isobe, K. O.; Tarao, M.; Zakaria, M. P.; Chiem, N. H.; Minh, L. Y.; Takada, H. Quantitative
 718 Application of Fecal Sterols Using Gas Chromatography-Mass Spectrometry To Investigate
 719 Fecal Pollution in Tropical Waters: Western Malaysia and Mekong Delta, Vietnam. *Environ. Sci.*
 720 *Technol.* **2002**, *36*, 4497–4507. <https://doi.org/10.1021/es020556h>.
- 721 (22) White, A. J.; Stevens, L. R.; Lorenzi, V.; Munoz, S. E.; Lipo, C. P.; Schroeder, S. An Evaluation of
 722 Fecal Stanols as Indicators of Population Change at Cahokia, Illinois. *J. Archaeol. Sci.* **2018**, *93*,
 723 129–134. <https://doi.org/10.1016/j.jas.2018.03.009>.
- 724 (23) Hatcher, P. G.; McGillivray, P. A. Sewage Contamination in the New York Bight. Coprostanol as
 725 an Indicator. *Environ. Sci. Technol.* **1979**, *13* (10), 1225–1229.
 726 <https://doi.org/10.1021/es60158a015>.
- 727 (24) Pratt, C.; Warnken, J.; Leeming, R.; Arthur, J. M.; Grice, D. I. Detection of Intermittent Sewage
 728 Pollution in a Subtropical, Oligotrophic, Semi-Enclosed Embayment System Using Sterol
 729 Signatures in Sediments. *Environ. Sci. Technol.* **2007**, *41* (3), 792–802.
 730 <https://doi.org/10.1021/es061450f>.
- 731 (25) Venkatesan, M. I.; Kaplan, I. R. Sedimentary Coprostanol as an Index of Sewage Addition in
 732 Santa Monica Basin, Southern California. *Environ. Sci. Technol.* **1990**, *24* (2), 208–214.
 733 <https://doi.org/10.1021/es00072a009>.
- 734 (26) White, A. J.; Stevens, L. R.; Lorenzi, V.; Munoz, S. E.; Schroeder, S.; Cao, A.; Bogdanovich, T.
 735 Fecal Stanols Show Simultaneous Flooding and Seasonal Precipitation Change Correlate with
 736 Cahokia's Population Decline. *Proc. Natl. Acad. Sci.* **2019**, *116* (12), 5461–5466.
 737 <https://doi.org/10.1073/pnas.1809400116>.
- 738 (27) Derrien, M.; Jardé, E.; Gruau, G.; Pourcher, A. M.; Gourmelon, M.; Jadas-Hécart, A.; Pierson
 739 Wickmann, A. C. Origin of Fecal Contamination in Waters from Contrasted Areas: Stanols as
 740 Microbial Source Tracking Markers. *Water Res.* **2012**, *46* (13), 4009–4016.
 741 <https://doi.org/10.1016/j.watres.2012.05.003>.
- 742 (28) Grimalt, J. O.; Fernandez, P.; Bayona, J. M.; Albaiges, J. Assessment of Fecal Sterols and
 743 Ketones as Indicators of Urban Sewage Inputs to Coastal Waters. *Environ. Sci. Technol.* **1990**,
 744 *24* (3), 357–363. <https://doi.org/10.1021/es00073a011>.
- 745 (29) Isobe, K. O.; Tarao, M.; Chiem, N. H.; Minh, L. Y.; Takada, H. Effect of Environmental Factors on
 746 the Relationship between Concentrations of Coprostanol and Fecal Indicator Bacteria in

- 747 Tropical (Mekong Delta) and Temperate (Tokyo) Freshwaters. *Appl. Environ. Microbiol.* **2004**,
748 *70* (2), 814–821. <https://doi.org/10.1128/AEM.70.2.814-821.2004>.
- 749 (30) Jardé, E.; Gruau, G.; Mansuy-Huault, L. Detection of Manure-Derived Organic Compounds in
750 Rivers Draining Agricultural Areas of Intensive Manure Spreading. *Appl. Geochem.* **2007**, *22*
751 (8), 1814–1824. <https://doi.org/10.1016/j.apgeochem.2007.03.037>.
- 752 (31) Li, W.; Dagaut, J.; Saliot, A. The Application of Sterol Biomarkers to the Study of the Sources of
753 Particulate Organic Matter in the Solo River System and and Serayu River, Java, Indonesia.
754 *Biogeochemistry* **1995**, *31* (3), 139–154. <https://doi.org/10.1007/BF00004046>.
- 755 (32) Segura, P. A.; Takada, H.; Correa, J. A.; El Saadi, K.; Koike, T.; Onwona-Agyeman, S.; Ofosu-
756 Anim, J.; Sabi, E. B.; Wasonga, O. V.; Mghalu, J. M.; dos Santos, A. M.; Newman, B.; Weerts, S.;
757 Yargeau, V. Global Occurrence of Anti-Infectives in Contaminated Surface Waters: Impact of
758 Income Inequality between Countries. *Environ. Int.* **2015**, *80*, 89–97.
759 <https://doi.org/10.1016/j.envint.2015.04.001>.
- 760 (33) Jardé, E.; Jeanneau, L.; Harrault, L.; Quenot, E.; Solecki, O.; Petitjean, P.; Lozach, S.; Chev e, J.;
761 Gourmelon, M. Application of a Microbial Source Tracking Based on Bacterial and Chemical
762 Markers in Headwater and Coastal Catchments. *Sci. Total Environ.* **2018**, *610–611*, 55–63.
763 <https://doi.org/10.1016/j.scitotenv.2017.07.235>.
- 764 (34) de Melo, M. G.; dos Anjos, O. C.; Pinheiro Nunes, A.; dos Santos Farias, M. A.; Val, A. L.; da
765 Silva Chaar, J.; Anceski Bataglion, G. Correlation between Caffeine and Coprostanol in
766 Contrasting Amazonian Water Bodies. *Chemosphere* **2023**, *326*, 138365.
767 <https://doi.org/10.1016/j.chemosphere.2023.138365>.
- 768 (35) Jeanneau, L.; Solecki, O.; W ery, N.; Jard e, E.; Gourmelon, M.; Communal, P.-Y.; Jadas-H ecart,
769 A.; Caprais, M.-P.; Gruau, G.; Pourcher, A.-M. Relative Decay of Fecal Indicator Bacteria and
770 Human-Associated Markers: A Microcosm Study Simulating Wastewater Input into Seawater
771 and Freshwater. *Environ. Sci. Technol.* **2012**, *46* (4), 2375–2382.
772 <https://doi.org/10.1021/es203019y>.
- 773 (36) Wang, D.; Farnleitner, A. H.; Field, K. G.; Green, H. C.; Shanks, O. C.; Boehm, A. B. Enterococcus
774 and Escherichia Coli Fecal Source Apportionment with Microbial Source Tracking Genetic
775 Markers - Is It Feasible? *Water Res.* **2013**, *47*, 6849–6861.
776 <https://doi.org/10.1016/j.watres.2013.02.058>.
- 777 (37) Froehner, S.; Martins, R. F.; Errera, M. R. Assessment of Fecal Sterols in Barigui River
778 Sediments in Curitiba, Brazil. *Environ. Monit. Assess.* **2009**, *157* (1–4), 591–600.
779 <https://doi.org/10.1007/s10661-008-0559-0>.
- 780 (38) Bull, I. D.; Lockheart, M. J.; Elhmmali, M. M.; Roberts, D. J.; Evershed, R. P. The Origin of
781 Faeces by Means of Biomarker Detection. *Environ. Int.* **2002**, *27* (8), 647–654.
782 [https://doi.org/10.1016/S0160-4120\(01\)00124-6](https://doi.org/10.1016/S0160-4120(01)00124-6).
- 783 (39) Shah, V. G.; Hugh Dunstan, R.; Geary, P. M.; Coombes, P.; Roberts, T. K.; Von Nagy-Felsobuki,
784 E. Evaluating Potential Applications of Faecal Sterols in Distinguishing Sources of Faecal
785 Contamination from Mixed Faecal Samples. *Water Res.* **2007**, *41* (16), 3691–3700.
786 <https://doi.org/10.1016/j.watres.2007.04.006>.
- 787 (40) Chan, K.-H.; Lam, M. H. W.; Poon, K.-F.; Yeung, H.-Y.; Chiu, T. K. T. Application of Sedimentary
788 Fecal Stanols and Sterols in Tracing Sewage Pollution in Coastal Waters. *Water Res.* **1998**, *32*
789 (1), 225–235. [https://doi.org/10.1016/S0043-1354\(97\)00175-9](https://doi.org/10.1016/S0043-1354(97)00175-9).
- 790 (41) Jard e, E.; Gruau, G.; Mansuy-Huault, L.; Peu, P.; Martinez, J. Using Sterols to Detect Pig Slurry
791 Contribution to Soil Organic Matter. *Water. Air. Soil Pollut.* **2007**, *178* (1–4), 169–178.
792 <https://doi.org/10.1007/s11270-006-9188-9>.
- 793 (42) Solecki, O.; Jeanneau, L.; Jard e, E.; Gourmelon, M.; Marin, C.; Pourcher, A. M. Persistence of
794 Microbial and Chemical Pig Manure Markers as Compared to Faecal Indicator Bacteria Survival
795 in Freshwater and Seawater Microcosms. *Water Res.* **2011**, *45* (15), 4623–4633.
796 <https://doi.org/10.1016/j.watres.2011.06.012>.

- 797 (43) Prost, K.; Birk, J. J.; Lehndorff, E.; Gerlach, R.; Amelung, W. Steroid Biomarkers Revisited –
798 Improved Source Identification of Faecal Remains in Archaeological Soil Material. *PLOS ONE*
799 **2017**, *12* (1), e0164882. <https://doi.org/10.1371/journal.pone.0164882>.
- 800 (44) Derrien, M.; Jardé, E.; Gruau, G.; Pierson-Wickmann, A.-C. Extreme Variability of Steroid
801 Profiles in Cow Feces and Pig Slurries at the Regional Scale: Implications for the Use of
802 Steroids to Specify Fecal Pollution Sources in Waters. *J. Agric. Food Chem.* **2011**, *59* (13),
803 7294–7302. <https://doi.org/10.1021/jf201040v>.
- 804 (45) Liu, F.; Williams, M. W.; Caine, N. Source Waters and Flow Paths in an Alpine Catchment,
805 Colorado Front Range, United States. *Water Resour. Res.* **2004**, *40* (9), W09401.
806 <https://doi.org/10.1029/2004WR003076>.
- 807 (46) Biache, C.; Philp, R. P. The Use of Sterol Distributions Combined with Compound Specific
808 Isotope Analyses as a Tool to Identify the Origin of Fecal Contamination in Rivers. *Water Res.*
809 **2013**, *47* (3), 1201–1208. <https://doi.org/10.1016/j.watres.2012.11.037>.
- 810 (47) Harrault, L.; Jardé, E.; Jeanneau, L.; Petitjean, P. Are Fecal Stanols Suitable to Record and
811 Identify a Pulse of Human Fecal Contamination in Short-Term Exposed Shellfish? A Microcosm
812 Study. *Mar. Pollut. Bull.* **2014**, *89* (1–2), 40–48.
813 <https://doi.org/10.1016/j.marpolbul.2014.10.037>.
- 814 (48) Mudge, S. M.; Norris, C. E. Lipid Biomarkers in the Conwy Estuary (North Wales, U.K.): A
815 Comparison between Fatty Alcohols and Sterols. *Mar. Chem.* **1997**, *57* (1–2), 61–84.
816 [https://doi.org/10.1016/S0304-4203\(97\)00010-8](https://doi.org/10.1016/S0304-4203(97)00010-8).
- 817 (49) Roslev, P.; Bastholm, S.; Iversen, N. Relationship Between Fecal Indicators in Sediment and
818 Recreational Waters in a Danish Estuary. *Water, Air, Soil Pollut.* **2008**, *194* (1–4), 13–21.
819 <https://doi.org/10.1007/s11270-008-9715-y>.
- 820 (50) The World Bank. *Rural population (% of total population) - Lao PDR*.
821 <https://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=LA> (accessed 2023-08-01).
- 822 (51) Boithias, L.; Auda, Y.; Audry, S.; Bricquet, J.; Chanhphengxay, A.; Chaplot, V.; de Rouw, A.;
823 Henry des Tureaux, T.; Huon, S.; Janeau, J.; Latsachack, K.; Le Troquer, Y.; Lestrelin, G.;
824 Maeght, J.; Marchand, P.; Moreau, P.; Noble, A.; Pando-Bahuon, A.; Phachomphon, K.;
825 Phanthavong, K.; Pierret, A.; Ribolzi, O.; Riotte, J.; Robain, H.; Rochelle-Newall, E.; Sayavong,
826 S.; Sengtaheuanghoung, O.; Silvera, N.; Sipaseuth, N.; Soullileuth, B.; Souliyavongsa, X.;
827 Sounyaphong, P.; Tasaketh, S.; Thammahacksa, C.; Thieboux, J.; Valentin, C.; Vigiak, O.;
828 Viguier, M.; Xayyathip, K. The Multiscale TROPICAL Catchments Critical Zone Observatory M-
829 TROPICS Dataset II: Land Use, Hydrology and Sediment Production Monitoring in Houay Pano,
830 Northern Lao PDR. *Hydrol. Process.* **2021**, *35* (5), e14126. <https://doi.org/10.1002/hyp.14126>.
- 831 (52) Soullileuth, B.; Boithias, L.; Ribolzi, O.; Xaiyalard, M.; Pierret, A. Land Use of the Laksip Village
832 South of the National Road 13, Northern Lao PDR (2016) [Data Set]. *DataSuds* **2023**.
833 [https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=40a3dc7f-ec8d-4029-9db8-](https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=40a3dc7f-ec8d-4029-9db8-b063896429e3)
834 [b063896429e3](https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=40a3dc7f-ec8d-4029-9db8-b063896429e3).
- 835 (53) Olaitan, A. O.; Thongmalayvong, B.; Akkhavong, K.; Somphavong, S.; Paboriboune, P.;
836 Khounsy, S.; Morand, S.; Rolain, J.-M. Clonal Transmission of a Colistin- Resistant *Escherichia*
837 *Coli* from a Domesticated Pig to a Human in Laos. *J. Antimicrob. Chemother.* **2015**, *70*, 3402–
838 3404. <https://doi.org/10.1093/jac/dkv252>.
- 839 (54) Ribas, A.; Jollivet, C.; Morand, S.; Thongmalayvong, B.; Somphavong, S.; Siew, C.-C.; Ting, P.-J.;
840 Suputtamongkol, S.; Saensombath, V.; Sanguankiat, S.; Tan, B.-H.; Paboriboune, P.;
841 Akkhavong, K.; Chaisiri, K. Intestinal Parasitic Infections and Environmental Water
842 Contamination in a Rural Village of Northern Lao PDR. *Korean J. Parasitol.* **2017**, *55*, 523–532.
843 <https://doi.org/10.3347/kjp.2017.55.5.523>.
- 844 (55) Silvera, N.; Jeanneau, J. L. *Technical Note #3/2016 - PASS - Automatic Sediment Sampler*; IRD,
845 2016; pp 1–12.
- 846 (56) Boithias, L.; Ribolzi, O.; Rochelle-Newall, E.; Thammahacksa, C.; Nakhle, P.; Soullileuth, B.;
847 Pando-Bahuon, A.; Latsachack, K.; Silvera, N.; Sounyafong, P.; Xayyathip, K.; Zimmermann, R.;
848 Rattanavong, S.; Oliva, P.; Pommier, T.; Evrard, O.; Huon, S.; Causse, J.; Henry-des-Tureaux, T.;

- 849 Sengtaheuanghoung, O.; Sipaseuth, N.; Pierret, A. *Escherichia Coli* Concentration, Multiscale
850 Monitoring over the Decade 2011–2021 in the Mekong River Basin, Lao PDR. *Earth Syst. Sci.*
851 *Data* **2022**, *14* (6), 2883–2894. <https://doi.org/10.5194/essd-14-2883-2022>.
- 852 (57) Boithias, L.; Jardé, E.; Latsachack, K.; Thammahacksa, C.; Silvera, N.; Rochelle-Newall, E.;
853 Ribolzi, O. *Escherichia Coli*, Stanol, and Total Suspended Sediment Concentrations at the
854 Outlet of a Ditch Draining Sewage and Surface Runoff out of a Village, Northern Lao PDR
855 (2016) [Data Set]. *DataSuds* **2023**.
856 [https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=55950a09-a7cc-4a07-8abb-](https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=55950a09-a7cc-4a07-8abb-6eecd2f5ab34)
857 [6eecd2f5ab34](https://doi.org/dataverse.ird.fr/privateurl.xhtml?token=55950a09-a7cc-4a07-8abb-6eecd2f5ab34).
- 858 (58) Chapman, E. A.; Baker, J.; Aggarwal, P.; Hughes, D. M.; Nwosu, A. C.; Boyd, M. T.; Mayland, C.
859 R.; Mason, S.; Ellershaw, J.; Probert, C. S.; Coyle, S. GC-MS Techniques Investigating Potential
860 Biomarkers of Dying in the Last Weeks with Lung Cancer. *Int. J. Mol. Sci.* **2023**, *24*, 1591.
861 <https://doi.org/10.3390/ijms24021591>.
- 862 (59) Christophersen, N.; Hooper, R. P. Multivariate Analysis of Stream Water Chemical Data: The
863 Use of Principal Components Analysis for the End-Member Mixing Problem. *Water Resour.*
864 *Res.* **1992**, *28* (1), 99–107. <https://doi.org/10.1029/91WR02518>.
- 865 (60) Christophersen, N.; Neal, C.; Hooper, R. P.; Vogt, R. D.; Andersen, S. Modelling Streamwater
866 Chemistry as a Mixture of Soilwater End-Members — A Step towards Second-Generation
867 Acidification Models. *J. Hydrol.* **1990**, *116* (1–4), 307–320. [https://doi.org/10.1016/0022-](https://doi.org/10.1016/0022-1694(90)90130-P)
868 [1694\(90\)90130-P](https://doi.org/10.1016/0022-1694(90)90130-P).
- 869 (61) Ribolzi, O.; Andrieux, P.; Valles, V.; Bouzigues, R.; Bariac, T.; Voltz, M. Contribution of
870 Groundwater and Overland Flows to Storm Flow Generation in a Cultivated Mediterranean
871 Catchment. Quantification by Natural Chemical Tracing. *J. Hydrol.* **2000**, *233*, 241–257.
872 [https://doi.org/10.1016/S0022-1694\(00\)00238-9](https://doi.org/10.1016/S0022-1694(00)00238-9).
- 873 (62) Tyagi, P.; Edwards, D. R.; Coyne, M. S. Fecal Sterol and Bile Acid Biomarkers: Runoff
874 Concentrations in Animal Waste-Amended Pastures. *Water. Air. Soil Pollut.* **2009**, *198*, 45–54.
875 <https://doi.org/10.1007/s11270-008-9824-7>.
- 876 (63) Bricquet, J. P.; Boonsaner, A.; Bouahom, B.; Toan, T. D. Statistical Analysis of Long-Term Series
877 Rainfall Data: A Regional Study in Southeast Asia. In *From soil research to land and water*
878 *management: harmonizing people and nature. Proceedings of the IWMI-ADB Project Annual*
879 *Meeting and 7th MSEC Assembly.*; IWMI, 2003; pp 83–89.
- 880 (64) Ribolzi, O.; Evrard, O.; Huon, S.; de Rouw, A.; Silvera, N.; Latsachack, K. O.; Souleuth, B.;
881 Lefèvre, I.; Pierret, A.; Lacombe, G.; Sengtaheuanghoung, O.; Valentin, C. From Shifting
882 Cultivation to Teak Plantation: Effect on Overland Flow and Sediment Yield in a Montane
883 Tropical Catchment. *Sci. Rep.* **2017**, *7* (1), 3987. <https://doi.org/10.1038/s41598-017-04385-2>.
- 884 (65) Song, L.; Boithias, L.; Sengtaheuanghoung, O.; Oeurng, C.; Valentin, C.; Souksavath, B.;
885 Sounyafong, P.; de Rouw, A.; Souleuth, B.; Silvera, N.; Lattanavongkot, B.; Pierret, A.; Ribolzi,
886 O. Understory Limits Surface Runoff and Soil Loss in Teak Tree Plantations of Northern Lao
887 PDR. *Water* **2020**, *12*, 2327. <https://doi.org/10.3390/w12092327>.
- 888 (66) Ziegler, A. D.; Giambelluca, T. W. Importance of Rural Roads as Source Areas for Runoff in
889 Mountainous Areas of Northern Thailand. *J. Hydrol.* **1997**, *196* (1–4), 204–229.
890 [https://doi.org/10.1016/S0022-1694\(96\)03288-X](https://doi.org/10.1016/S0022-1694(96)03288-X).
- 891 (67) Mascher, F.; Mascher, W.; Pichler-Semmelrock, F.; Reinthaler, F.; Zarfel, G.; Kittinger, C.
892 Impact of Combined Sewer Overflow on Wastewater Treatment and Microbiological Quality
893 of Rivers for Recreation. *Water* **2017**, *9* (11), 906. <https://doi.org/10.3390/w9110906>.
- 894 (68) Barrios-Hernández, M. L.; Pronk, M.; Garcia, H.; Boersma, A.; Brdjanovic, D.; van Loosdrecht,
895 M. C. M.; Hooijmans, C. M. Removal of Bacterial and Viral Indicator Organisms in Full-Scale
896 Aerobic Granular Sludge and Conventional Activated Sludge Systems. *Water Res. X* **2020**, *6*,
897 100040. <https://doi.org/10.1016/j.wroa.2019.100040>.
- 898 (69) Truchado, P.; Gil, M. I.; López, C.; Garre, A.; López-Aragón, R. F.; Böhme, K.; Allende, A. New
899 Standards at European Union Level on Water Reuse for Agricultural Irrigation: Are the Spanish
900 Wastewater Treatment Plants Ready to Produce and Distribute Reclaimed Water within the

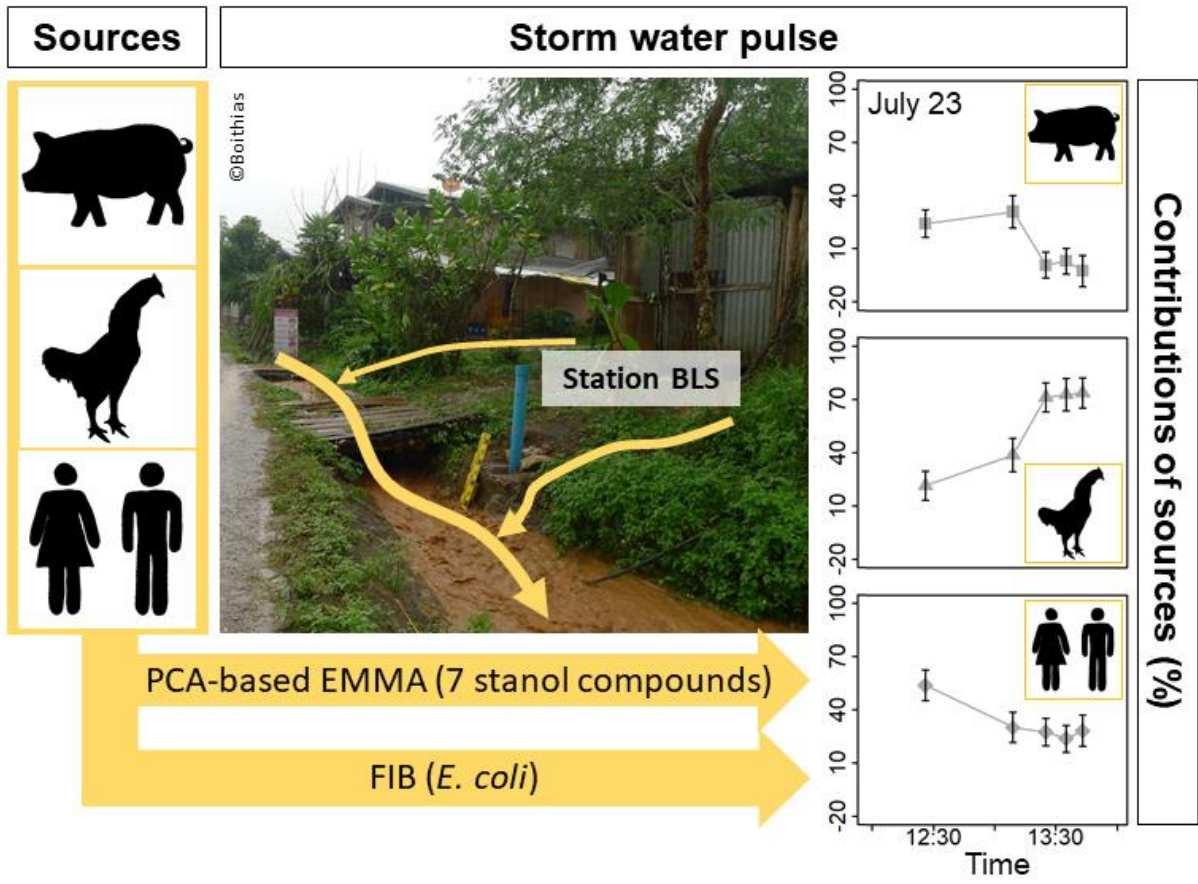
- 901 Minimum Quality Requirements? *Int. J. Food Microbiol.* **2021**, *356*, 109352.
 902 <https://doi.org/10.1016/j.ijfoodmicro.2021.109352>.
- 903 (70) Mbanga, J.; Abia, A. L. K.; Amoako, D. G.; Essack, Sabiha. Y. Quantitative Microbial Risk
 904 Assessment for Waterborne Pathogens in a Wastewater Treatment Plant and Its Receiving
 905 Surface Water Body. *BMC Microbiol.* **2020**, *20* (1), 346. [https://doi.org/10.1186/s12866-020-](https://doi.org/10.1186/s12866-020-02036-7)
 906 [02036-7](https://doi.org/10.1186/s12866-020-02036-7).
- 907 (71) Edokpayi, J.; Odiyo, J.; Msagati, T.; Popoola, E. Removal Efficiency of Faecal Indicator
 908 Organisms, Nutrients and Heavy Metals from a Peri-Urban Wastewater Treatment Plant in
 909 Thohoyandou, Limpopo Province, South Africa. *Int. J. Environ. Res. Public Health* **2015**, *12* (7),
 910 7300–7320. <https://doi.org/10.3390/ijerph120707300>.
- 911 (72) WHO. *WHO Recommendations on Scientific, Analytical and Epidemiological Developments*
 912 *Relevant to the Parameters for Bathing Water Quality in the Bathing Water Directive*
 913 *(2006/7/EC)*; World Health Organization (WHO): Geneva, 2018; p 96.
 914 [https://www.who.int/publications/m/item/who-recommendations-on-scientific-analytical-](https://www.who.int/publications/m/item/who-recommendations-on-scientific-analytical-and-epidemiological-developments-relevant-to-the-parameters-for-bathing-water-quality-in-the-bathing-water-directive-(2006-7-ec))
 915 [and-epidemiological-developments-relevant-to-the-parameters-for-bathing-water-quality-in-](https://www.who.int/publications/m/item/who-recommendations-on-scientific-analytical-and-epidemiological-developments-relevant-to-the-parameters-for-bathing-water-quality-in-the-bathing-water-directive-(2006-7-ec))
 916 [the-bathing-water-directive-\(2006-7-ec\)](https://www.who.int/publications/m/item/who-recommendations-on-scientific-analytical-and-epidemiological-developments-relevant-to-the-parameters-for-bathing-water-quality-in-the-bathing-water-directive-(2006-7-ec)).
- 917 (73) WHO. *Guidelines for Safe Recreational Water Environments. Volume 1: Coastal and Fresh*
 918 *Waters*; World Health Organization (WHO): Geneva, 2003; p 253.
 919 <https://apps.who.int/iris/handle/10665/42591>.
- 920 (74) Nakhle, P.; Boithias, L.; Pando-Bahuon, A.; Thammahacksa, C.; Gallion, N.; Sounyafong, P.;
 921 Silvera, N.; Latsachack, K.; Soulileuth, B.; Rochelle-Newall, E. J.; Marcangeli, Y.; Pierret, A.;
 922 Ribolzi, O. Decay Rate of *Escherichia Coli* in a Mountainous Tropical Headwater Wetland.
 923 *Water* **2021**, *13* (15), 2068. <https://doi.org/10.3390/w13152068>.
- 924 (75) Biache, C.; Navarro Frómata, A. E.; Czechowski, F.; Lu, Y.; Philp, R. P. Thiosteranes in Samples
 925 Impacted by Fecal Materials and Their Potential Use as Marker of Sewage Input. *Environ.*
 926 *Pollut.* **2015**, *196*, 268–275. <https://doi.org/10.1016/j.envpol.2014.10.010>.
- 927 (76) Martins, C. de C.; Fillmann, G.; Montone, R. C. Natural and Anthropogenic Sterols Inputs in
 928 Surface Sediments of Patos Lagoon, Brazil. *J. Braz. Chem. Soc.* **2007**, *18* (1), 106–115.
 929 <https://doi.org/10.1590/S0103-50532007000100012>.
- 930 (77) Writer, J. H.; Leenheer, J. A.; Barber, L. B.; Amy, G. L.; Chapra, S. C. Sewage Contamination in
 931 the Upper Mississippi River as Measured by the Fecal Sterol, Coprostanol. *Water Res.* **1995**, *29*
 932 (6), 1427–1436. [https://doi.org/10.1016/0043-1354\(94\)00304-P](https://doi.org/10.1016/0043-1354(94)00304-P).
- 933 (78) Araújo, M. P.; Hamacher, C.; Farias, C. de O.; Soares, M. L. G. Fecal Sterols as Sewage
 934 Contamination Indicators in Brazilian Mangroves. *Mar. Pollut. Bull.* **2021**, *165*, 112149.
 935 <https://doi.org/10.1016/j.marpolbul.2021.112149>.
- 936 (79) Causse, J.; Billen, G.; Garnier, J.; Henri-des-Tureaux, T.; Olasa, X.; Thammahacksa, C.;
 937 Latsachak, K. O.; Soulileuth, B.; Sengtaeuanghoung, O.; Rochelle-Newall, E.; Ribolzi, O. Field
 938 and Modelling Studies of *Escherichia Coli* Loads in Tropical Streams of Montane Agro-
 939 Ecosystems. *J. Hydro-Environ. Res.* **2015**, *9* (4), 496–507.
 940 <https://doi.org/10.1016/j.jher.2015.03.003>.
- 941 (80) Strauch, A. M.; Mackenzie, R. A.; Bruland, G. L.; Tingley, R.; Giardina, C. P. Climate Change and
 942 Land Use Drivers of Fecal Bacteria in Tropical Hawaiian Rivers. *J. Environ. Qual.* **2014**, *43* (4),
 943 1475–1483. <https://doi.org/10.2134/jeq2014.01.0025>.
- 944 (81) Ribolzi, O.; Boithias, L.; Thammahacksa, C.; Silvera, N.; Latsachack, K.; Soulileuth, B.; Arnoux,
 945 M.; Evrard, O.; Huon, S.; Pommier, T.; Sengtaeuanghoung, O.; Rochelle-Newall, E. *Escherichia*
 946 *Coli* Concentration in Overland Flow and in Groundwater in the Houay Pano Catchment,
 947 Northern Lao PDR (2012-2014) [Data Set]. *DataSuds* **2022**. <https://doi.org/10.23708/ZPRFHR>.
- 948 (82) Pachepsky, Y. A.; Allende, A.; Boithias, L.; Cho, K.; Jamieson, R.; Hofstra, N.; Molina, M.
 949 Microbial Water Quality: Monitoring and Modeling. *J. Environ. Qual.* **2018**, *47* (5), 931–938.
 950 <https://doi.org/10.2134/jeq2018.07.0277>.

- 951 (83) Lu, Y.; Philp, R.; Biache, C. Assessment of Fecal Contamination in Oklahoma Water Systems
952 through the Use of Sterol Fingerprints. *Environments* **2016**, 3 (4), 28.
953 <https://doi.org/10.3390/environments3040028>.
- 954 (84) Furtula, V.; Liu, J.; Chambers, P.; Osachoff, H.; Kennedy, C.; Harkness, J. Sewage Treatment
955 Plants Efficiencies in Removal of Sterols and Sterol Ratios as Indicators of Fecal Contamination
956 Sources. *Water. Air. Soil Pollut.* **2012**, 223 (3), 1017–1031. [https://doi.org/10.1007/s11270-](https://doi.org/10.1007/s11270-011-0920-8)
957 [011-0920-8](https://doi.org/10.1007/s11270-011-0920-8).
- 958 (85) Soller, J. A.; Schoen, M. E.; Bartrand, T.; Ravenscroft, J. E.; Ashbolt, N. J. Estimated Human
959 Health Risks from Exposure to Recreational Waters Impacted by Human and Non-Human
960 Sources of Faecal Contamination. *Water Res.* **2010**, 44 (16), 4674–4691.
961 <https://doi.org/10.1016/j.watres.2010.06.049>.
- 962 (86) Campos, C. J. A.; Lees, D. N. Environmental Transmission of Human Noroviruses in Shellfish
963 Waters. *Appl. Environ. Microbiol.* **2014**, 80 (12), 3552–3561.
964 <https://doi.org/10.1128/AEM.04188-13>.
- 965 (87) Le Guyader, F.; Atmar, R. L.; Maalouf, H.; Pendu, J. L. Shellfish Contamination by Norovirus :
966 Strain Selection Based on Ligand Expression? *Clin. Virol.* **2013**, 41, 3–18.
- 967 (88) Mügler, C.; Ribolzi, O.; Viguier, M.; Janeau, J.-L.; Jardé, E.; Latsachack, K.; Henry-Des-Tureaux,
968 T.; Thammahacksa, C.; Valentin, C.; Sengtaheuanghoung, O.; Rochelle-Newall, E. Experimental
969 and Modelling Evidence of Splash Effects on Manure Borne Escherichia Coli Washoff. *Environ.*
970 *Sci. Pollut. Res.* **2021**. <https://doi.org/10.1007/s11356-021-13011-8>.
- 971

972

973 Graphical abstract

974 For Table of Contents only



975