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1 **Onsite Sanitation Systems and Contamination of**
2 **Groundwater: A Systematic Review of the Evidence for**
3 **Risk using the Source-Pathway-Receptor Model**

4
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9
10 **Keywords**

11 Pit Latrines

12 Septic Tanks

13 Faecal Pathogens

14 Nitrogen

15 Groundwater

16 Source-Pathway-Receptor

17
18 **Abstract**

19 The level of risk that onsite sanitation systems (OSS) pose to groundwater quality remains
20 uncertain. The link between contamination and OSS can only be proved if the source,
21 pathway, and receptor (SPR) are investigated and confirmed when assessing contamination.

22 The literature on the connection between OSS and groundwater contamination has been
23 reviewed several times. However, previous reviews have made limited assessments of the
24 extent to which the literature confirms that the source of contamination is an OSS, that a

25 pathway has been identified, and that the receptor is groundwater, mainly due to uncertainties
26 or insufficiencies in reported methodologies.

27 A systematic review was conducted on published studies with the aim of establishing the state
28 of knowledge of groundwater contamination from onsite sanitation systems that infiltrate
29 wastewater into ground, such as pit latrines and septic tank systems. The quality of the
30 methods used to establish the source, pathway and receptor was assessed. The review
31 focussed on three main contaminants: bacteria, viruses, and nitrates.

32 Scopus, PubMed, and Web of Science databases were searched, screening criterion
33 developed and applied, and 60 eligible papers were identified. 35 of these were rated as
34 having a “*strong methodology*”. The selected studies were assessed to identify areas of
35 knowledge regarding the interaction of OSS with groundwater where there is strong supportive
36 evidence and where there are knowledge gaps.

37 The review supports previous work that has concluded that the removal and transport of
38 contaminants from OSS to groundwater is complex and varies significantly according to local
39 conditions, including the nature of the ground materials, groundwater levels, ground moisture
40 and other factors. This variability means simple siting guidelines based on horizontal
41 separation are not reliable.

42 Though not always recognised in the literature, formation of a biological layer is important for
43 removal of microbial pathogens. This layer takes months to form which impacts the
44 performance of OSS that are new or subject to highly variable loading or sudden increases in
45 hydraulic load.

46 Under ideal conditions of an unsaturated zone comprising fine material (sands, silts, loams
47 with some clay content, Faecal indicator bacteria can be reduced to detection limits within 10
48 metres distance. However, ideal conditions are very often not present. Multiple studies showed
49 the presence of viruses in the absence of faecal indicator bacteria. Contamination can also
50 occur via localised pathways, but these have not been thoroughly investigated. Contribution

51 of the ingress of contaminated surface water into faulty boreholes/wells and contamination of
52 wells through the spigot or spout from users are both often significant yet not adequately
53 covered in the included literature.

54 The review established that it is extremely difficult to eliminate the risk of groundwater
55 contamination when OSS effluent is discharged into the subsurface. Generally, it seems
56 unsafe to assume that contamination can be prevented in areas where OSS occur with any
57 frequency.

58 **1. Introduction**

59 Over the last twenty years the number of people in the world using onsite sanitation systems
60 (OSS) has been growing at a rate of 4% per year (1) . It was estimated that as of 2020,
61 improved onsite sanitation was more common than offsite sewer connections (2). At the same
62 time, the use of groundwater for drinking water is on an upward trend. This is sometimes
63 fuelled by increasing water scarcity due to climatic factors and the high levels of surface water
64 contamination (3) and is exacerbated by the failure of urban water service operators to extend
65 piped water to rapidly growing low income settlements. Half of the world's population relies on
66 groundwater sources, and treatment prior to drinking may be limited. It is often therefore
67 posited that public health maybe at increasing risk due to the rising combination of onsite
68 sanitation systems and extraction of groundwater for drinking (4)

69 The term OSS has been used in literature to cover a very broad range of systems. In this
70 paper, we mean systems that contain, store and/or partially treat human excreta and
71 wastewater on the same premises they are produced, and intentionally allow the liquid portion
72 of the waste to leach into the ground. These are commonly in the form of pit latrines (including
73 dry pits and wet pits such as pour flush or flush latrines) and septic tanks connected to an
74 infiltrating system such as soak pits. Systems that allow the liquid fraction to be removed from
75 the site through methods other than ground infiltration (such as a septic tank connected to a
76 sewer or open drain) are also common but were not considered in this review.

77 The literature covering the connection between OSS and groundwater contamination has
78 been reviewed several times. An early review was conducted in 1982 with the aim of
79 understanding the state of knowledge around movement of microorganisms and nitrates
80 through both unsaturated and saturated zones surrounding pit latrines (5). The review covered
81 literature on field investigations, laboratory studies, case histories of groundwater pollution
82 and studies on the capacity of soils to remove bacteria and viruses. It aimed to use this
83 information to assess the risks to groundwater from OSS in “developing countries” (5). More
84 recently, Graham and Polizzotto (6) conducted a systematic review of empirical studies of the
85 impacts of pit latrines on groundwater quality. They found twenty-four studies that either
86 directly assessed the transport of contaminants or used statistical methods to estimate the
87 risk associated with the presence of pit latrines.

88 Both these reviews highlighted some common understanding of contaminant transport from
89 an OSS to groundwater. They showed evidence that a biological layer (variously referred to
90 as a ‘biofilm’, ‘clogging layer’, ‘scum mat’ or ‘biomat’) progressively develops around a pit
91 latrine after the initial period of use and is crucial for removal of microbial contaminants, (5, 6).
92 Further reduction in pathogens occurred when effluent passed through an unsaturated zone.
93 The presence of more than 2m of unsaturated fine soil and a hydraulic loading less than 50
94 mm/d was found to reduce microbial contaminants to minimal levels (5) . Saturated conditions
95 or fissured bedrocks over a shallow soil layer resulted in greater horizontal travel distances of
96 up to 25m for bacteria, 50m for viruses and 26m for chemicals (6). Some bacterial and viral
97 species adsorbed to various ground media may be desorbed during heavy rainfall which can
98 rapidly increase concentrations and risk of groundwater contamination (5).

99 Both reviews highlighted nitrate as the chemical contaminant of most concern. Organic
100 nitrogen in waste converts to ammonia as it breaks down in a pit or septic tank. The
101 unsaturated zone was typically aerobic, and hence this ammonia was rapidly oxidised to
102 nitrate. No evidence was found in the reviews to indicate appreciable accumulation of
103 ammonia in groundwater, though this is theoretically possible if sub-surface conditions remain

104 anaerobic (6). Nitrate may be removed through denitrification in either the unsaturated zone
105 or the aquifer given a low or zero oxygen environment and the presence of an electron donor,
106 which though often organic carbon, could be inorganic compounds such as reduced iron or
107 sulphur (5) (7). Nitrate concentrations may also be reduced by dilution in the aquifer, with the
108 amount of reduction depending on the recharge rate of the aquifer compared to the load from
109 OSS. However, denitrification does not always occur, and dilution may be limited.
110 Concentrations of nitrate in groundwater above drinking water standards have been regularly
111 found and attributed to OSS (6).

112 The reviews also concluded that it is exceedingly difficult to rely on simple siting guidelines
113 such as a horizontal distance between an OSS and a well or bore due to the high variability in
114 contaminant removal in different ground conditions and the limited understanding of the
115 mechanisms and conditions that affect contaminant removal. They concluded there is a need
116 to empirically test current siting guidelines (5); (6).

117 Another review conducted by Pang (8) reviewed the available literature on microbial removal
118 rates in different ground conditions and compiled 87 datasets covering both bacteria and virus
119 removal. Removal rates were calculated and expressed as \log_{10} organisms removed per
120 metre in order to provide a dataset that could be used to estimate safe setback distances from
121 an OSS. The data was split into removal rates for soils (defined as the first 1m of ground
122 comprising the biologically active layer), vadose zone (defined as the unsaturated zone from
123 the end of the soil to the groundwater table) and aquifers. The data show microbial removal
124 rates in the biologically active soils between 0.1 to 10 \log_{10} removal per metre, 0.1 to 1 \log_{10}
125 per metre in the vadose zone and aquifer removal rates varying from 10^{-4} per metre for karst
126 limestone to 1 \log_{10} per metre for sand aquifers (8). Such high variability of removal rates with
127 different sub-surface conditions explains how in one set of sub-surface conditions
128 investigators can find faecal indicator bacteria (FIB) are reduced to minimal levels with 2m of
129 unsaturated zone (5), while in different sub-surface conditions, groundwater contamination is
130 measured up to 50m from pit latrines (6).

131 These three previous reviews considered the different categories of pathogens: protozoa,
132 helminths, bacteria, and viruses but came to differing conclusions about their removal rates.
133 Lewis, Foster (5) assumed the large size of helminths and protozoa would ensure they are
134 effectively strained out in a short distance and found only sparse information on viruses, so
135 primarily focussed on FIB. Graham and Polizzotto (6) found no studies assessing protozoa or
136 helminths and only one study on viruses that met their systematic review criteria. They
137 concluded that there is a greater travel distance for viruses than FIB (6). However, Pang (8),
138 who used a much broader data set, concluded that virus removal rates were of the same order
139 of magnitude as bacteria, and did not report any data on helminths and limited data on
140 protozoa.

141 In 2014, a general review of groundwater contamination covering all potential sources was
142 conducted in USA and Canada. Forty-five studies were identified where the presence of
143 pathogens in groundwater was attributed to septic tanks. However, the review did not explore
144 the validity of the attribution reported (9).

145 Most of studies included in previous reviews fail to rule out indirect/localised pathways of
146 contamination seen in the aquifer. This is important as contamination could have multiple other
147 pathways such as animal or human waste entering through defects in well or bore
148 construction, or contamination of hand pumps and tube wells at the point of collection. It has
149 been argued that these are the more significant source of contamination, at least in some
150 circumstances (10). This study therefore sets out to update previous reviews but expand on
151 them by using a “source-pathway-receptor” (SPR) approach (11). The SPR is used both to
152 structure the literature search and to assess the quality of evidence in the literature. It requires
153 that a study clearly identifies OSS as a source; shows a viable pathway for contaminants to
154 be transported to the receptor; and has evidence of contamination at the receptor.

155 The review is also broader in scope than previous reviews by including all OSS discharging
156 effluent into the ground such as septic tank systems, soak pits, cess pools and pit latrines.
157 Mechanisms for contaminant transport and removal from OSS to groundwater are likely to be

158 the same regardless of the type of OSS and including these may bring in evidence from studies
159 not picked up by Graham and Polizzotto (6), who focused on pit latrines only.

160 Finally, improvements in technology since 2013 have enabled more studies to look at viruses
161 and use microbial source trackers, potentially addressing one of the findings of Graham and
162 Polizzotto (6), that the quality of experimental techniques and chosen indicators was highly
163 variable. Hence, increasing use of these novel techniques may have since generated new
164 evidence on the transport of contaminants from OSS to groundwater.

165 **2. Methodology**

166 **2.1 Source Pathway Receptor (SPR) Model**

167 Using the SPR model, the source is the point of origin of the contaminant; in this case, the
168 OSS. A wide range of terms are used to describe the various OSS and the terminology is often
169 inconsistent. For the purposes of this study, we have grouped OSS into:

170 1) Separated function OSS (Store/treat structures separated from infiltrate structures): These
171 are systems composed of a watertight container that receives excreta, flushing water and/or
172 occasionally greywater, allows for some level of primary treatment before discharging the
173 supernatant into a separate structure where infiltration into the subsurface occurs. Typically,
174 these systems are referred to as septic tanks with soak pits or leach fields.

175 2) Combined function OSS (store/treat/infiltrate in same structure): These are systems
176 composed of a lined/unlined pit that receives human excreta, flushing water, cleansing
177 materials and/or grey water and allows direct infiltration into the subsurface. This covers
178 systems such as pit latrines, VIP latrines, pour flush latrines, aqua privy. In some papers these
179 systems that receive flushing water are also referred to as septic tanks.

180 Our reason for using these two categories is that there might be a difference in how they
181 interact with groundwater on the basis that separated function systems should in theory
182 provide some greater level of treatment prior to effluent being infiltrated and would usually
183 handle a higher volume of water as they include flushing water and/or grey water (12).

184 The pathway is the route through which the pollutant travels. Pathways are broadly
 185 categorized into two as follows (11) and as shown in Figure 1:

- 186 • Aquifer pathway – Direct movement of contaminants from the OSS into the groundwater
 187 through the pores in the soil/rock structure
- 188 • Localised pathway – Indirect movement of contaminants from the OSS into groundwater
 189 as a result of failures in the design and/or construction of the groundwater supply system
 190 (e.g., well, borehole, spring etc.).

191

192 The receptor is the groundwater located within a borehole or a well.

193 *Figure 1 Conceptual diagram of OSS, pathways and receptors*

194 Insert Figure 1

195 **2.2 Literature search strategy**

196 The search terms were grouped according to the source-pathway-receptor model as shown
 197 in Table 1. Words used interchangeably to mean “latrine,” “septic tank” and “soak pit” were
 198 used to describe the source of the contaminants. The pathway and receptor were described
 199 using variations of the terms, “groundwater”, “borehole” and “well”. Words that were
 200 associated with non-human contamination sources such as animals, mines and fertilizers
 201 were used to exclude articles.

202 *Table 1 Search terms using the SPR model*

Source - Onsite containment in the ground	
1	“Pit latrine*” OR pit-latrine* OR latrine* OR toilet* OR “septic tank*” OR “soak* tank*” OR “soak* pit” OR “leach pit*” OR “cess pit” OR cesspit OR “cess pool” OR cesspool
2	(onsite OR on-site OR “onsite”) W/3 sanitation
3	Fecal OR faecal OR Feces OR Faeces OR excret*
Pathway/Receptor - Passage mechanism for pathogens	

4	Groundwater OR "ground water" OR "ground-water" OR aquifer
5	"Shallow well*" OR "shallow borehole*" OR borehole* OR hydro-geologic* OR "hydro geologic*" OR underground OR hydrogeochemical
6	Well OR Wells W/5 Water
7	Contamin* OR pollut*
Exclusion terms – non-human source	
8	animal OR bird OR manure OR irrigat* OR "surface water" OR river OR marine OR landfill OR "land fill" OR "solid waste" OR oil OR gas OR mining OR agriculture
	Combined Searches
9	(1 OR 2 OR 3) AND (4 OR 5 OR 6) AND 7 AND NOT 8

203

204 Searches were made using Scopus, PubMed, and Web of Science databases covering the
 205 period 1900 to April 2022. The search was done in two phases, one covering 1900 to June
 206 2020 by the lead author followed by an update covering 2020 to April 2022. Key word strings
 207 are presented in Table 2.

208 *Table 2 Search Key word strings*

Database	Search string
Scopus	(TITLE-ABS-KEY ("Pit latrine*" OR pit-latrine* OR latrine* OR toilet* OR "septic tank*" OR "soak* tank*" OR "soak* pit" OR "leach pit*" OR "cess pit" OR cesspit OR "cess pool" OR cesspool OR fecal OR faecal OR feces OR faeces OR excret*) OR TITLE-ABS-KEY ((onsite OR on-site OR "onsite") W/3 sanitation) AND TITLE-ABS-KEY (groundwater OR "ground water" OR "ground-water" OR aquifer OR "shallow well*" OR "shallow borehole*" OR borehole* OR hydro-geologic* OR "hydro geologic*" OR underground OR hydrogeochemical OR ((well OR wells) W/5 water)) AND TITLE-ABS-KEY (contamin* OR pollut*) AND NOT

	TITLE-ABS-KEY (animal OR bird OR manure OR irrigat* OR "surface water" OR river OR marine OR landfill OR "land fill" OR "solid waste" OR oil OR gas OR mining OR arsenic OR agriculture))
Web of Science	("Pit latrine*" OR pit-latrine* OR latrine* OR toilet* OR "septic tank*" OR "soak* tank*" OR "soak* pit" OR "leach pit*" OR "cess pit" OR cesspit OR "cess pool" OR cesspool OR Fecal OR faecal OR Feces OR Faeces OR excret* OR (onsite OR on-site OR "onsite") NEAR/3 sanitation) AND TS=(Groundwater OR "ground water" OR "ground-water" OR aquifer OR "shallow well*" OR "shallow borehole*" OR borehole* OR hydro-geologic* OR "hydro geologic*" OR underground OR hydrogeochemical OR ((Well OR Wells) NEAR/5 Water)) AND TS=(contamin* OR pollut*) NOT TS=(animal OR bird OR manure OR irrigat* OR "surface water" OR river OR marine OR landfill OR "land fill" OR "solid waste" OR oil OR gas OR mining OR agriculture)
PubMed	((((("Pit latrine*" OR pit-latrine* OR latrine* OR toilet* OR "septic tank*" OR "soak* tank*" OR "soak* pit" OR "leach pit*" OR "cess pit" OR cesspit OR "cess pool" OR cesspool OR Fecal OR faecal OR Feces OR Faeces OR excret* OR ((onsite OR on-site OR "onsite") N/3 sanitation)))) AND (Groundwater OR "ground water" OR "ground-water" OR aquifer OR "shallow well*" OR "shallow borehole*" OR borehole* OR hydro-geologic* OR "hydro geologic*" OR underground OR hydrogeochemical OR ((Well OR Wells) N/5 Water)))) AND (contamin* OR pollut*) NOT (animal OR bird OR manure OR irrigat* OR "surface water" OR river OR marine OR landfill OR "land fill" OR "solid waste" OR oil OR gas OR mining OR agriculture)

210 **2.3 Eligibility and Review**

211 **2.3.1 Title & Abstract Screen**

212 The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) protocol
 213 was used to conduct the systematic review (13). Articles were screened on title and abstract
 214 by two reviewers using Rayyan software (14) and based on the criteria presented in Table 3.

215 *Table 3 Title and Abstract Screening Criteria*

Item	Inclusion Criteria	Exclusion Criteria
Article type	Published – lab, field, and observational papers	Unpublished documents and modelling papers that had no real-world data
Language	English	Non-English
Source	Human faeces and urine contained underground in a structure termed as or similar to a “pit latrine, septic tank or soakaway”	Animal faecal or urine containment Human excreta in off-site systems e.g., sewage Note: Mixed sources with both animal and human excreta were included at this stage.
Pathway	Aquifer and localized pathways by confirmation or indication	Pathway not linked to OSS Note: This assessment was often minimal as insufficient information on pathways was presented in the abstract
Receptor	Groundwater	Surface water, coastal/marine, recreational water bodies with no groundwater data
Contaminants	Microbial and nitrogen	No contaminants tested, or exclusively tested pharmaceutical products, and contaminants of emerging concern e.g.,

Item	Inclusion Criteria	Exclusion Criteria
		nicotine, caffeine surfactants & industrial additives.

216

217 **2.3.2 Full Text Screen**

218 The screening was done against the criteria in Table 4. Criteria 'C4', 'C5', and 'C6' represent
 219 the application of the source-pathway-receptor model to screen out papers that did not track
 220 contaminants from an OSS via a pathway into the receptor.

221 *Table 4 Full Text Screening Criteria*

Criteria	Explanation / Reason to exclude papers
C1. Does the scope of the paper extend beyond testing of OSS modifications?	Field or laboratory trials of the performance of different OSS. While these provide useful information on performance, they were not aimed at showing an OSS-groundwater contamination link.
C2. Is the full text paper accessible?	Full text could not be obtained.
C3. Is specific data on microorganisms (pathogens) and/or nitrogen included?	Abstract & title review may not have been clear that the paper did not have data and so this was screened again at full text review.
C4. Is the contaminant tracked through a pathway?	Did not provide any information on ground conditions / aquifer or other information to show it is possible for contaminants to move from OSS to receptor. For example, paper looked at data from wells and tried to correlate with OSS (location, density). Although a statistical correlation

	may be shown, paper did not provide a plausible pathway or any other evidence to show causation.
C5. Is the contaminant tracked at the receptor?	No data on groundwater at a point of extraction. For example, may have sampled soils around an OSS but not sampled groundwater/aquifer.
C6. Were other sources of the contaminant excluded?	Conducted in locations with multiple potential sources (e.g., livestock, open defecation) and did not have any means to exclude other sources or quantify OSS contribution compared to other sources. Did not clearly establish the presence of OSS systems (e.g., might have been assessing well water quality and mentioned OSS as likely source without any data confirming they are present.)

222

223 **2.4 Quality Assessment**

224 Papers that passed through the full text screening were then given a quality rating by
 225 assessing against the criteria illustrated in Figure 3 and explained in Table 5 and Table 6.
 226 Studies were rated “weak methodology” if they answered “No” to any of the questions (1-4)
 227 and “strong methodology” if they answered “Yes” in all questions presented in the flow diagram
 228 in Figure 3. The purpose of this quality assessment is to provide a set of papers we consider
 229 to have strong evidence that we can rely on as the primary source for drawing conclusions.
 230 The process worked as a series of decision points, with a paper categorised as weak once it
 231 failed one decision point, hence we did not assess all papers against all quality criteria. A full
 232 list of papers, strong and weak, is provided in the supplementary information.

233 *Table 5 Quality Assessment Criteria*

Question	Examples of studies answering	Examples of studies answering
	“Yes”	“No”

<p>1) Is it a plausible real-life situation?</p>	<ul style="list-style-type: none"> • Field studies of existing OSS in use • Constructed test sites that used realistic household excreta loads and patterns 	<ul style="list-style-type: none"> • A laboratory experiment in columns; • Constructed test sites with artificial wastewater and/or dosing regimens.
<p>2) Is the methodology strong in determining the pathway?</p>	<ul style="list-style-type: none"> • Used a tracer for pathway – dyes, SF6, Li, etc. The tracer may have been injected as part of a test or may have been a substance unique to OSS and tracked to receptor. • Had multiple sampling points that tracked contaminants along the pathway from source to receptor • Developed a groundwater flow model to track contaminant pathways and the model was calibrated with real world measurements • Contained information on the nature of the soils, ground conditions, groundwater levels, movement and direction 	<ul style="list-style-type: none"> • Statistical correlation of density of OSS with concentration of contaminants without information on the nature of the soils, ground conditions, groundwater levels, movement and direction.
<p>3) Is the methodology strong in</p>	<ul style="list-style-type: none"> • Purpose-built sampling wells or piezometers and which 	<ul style="list-style-type: none"> • Looked at fecal indicator bacteria and used samples from groundwater sources but did not

tracking the receptor?	<p>described methods used to prevent cross contamination.</p> <ul style="list-style-type: none"> • Groundwater sources if study used some means to ensure samples represented groundwater quality and were not cross contaminated in sampling. 	provide a method to ensure that the samples were representative of groundwater quality and not affected by contamination from sources such as birds, prior use of contaminated buckets, ropes, etc.
4) Is the methodology strong in excluding other sources?	Refer table 6	Refer table 6

234

235 For criteria 4, there is a wide range of techniques that studies have used to exclude other
 236 potential sources of groundwater contamination. These techniques were analysed and rated
 237 as presented in Table 6.

238 *Table 6 Methods used for Excluding Other Sources of Contamination*

Method for excluding other sources or demonstrating contamination is human source.	Method Rating	Explanation	Examples of studies that used this method
Statistical correlation	<u>Weak</u>	Statistical correlation alone leaves open many other possible sources of	(15)

between distance to OSS or density of OSS's and well FIB and NO ₃		contamination and does not show a pathway. Studies that solely relied on this method were considered weak.	
Network of bores or sampling points surrounding the OSS being studied.	<u>Strong</u>	Provided it is clear other sources are excluded within the network & that upstream groundwater is not contaminated.	(16) (17); (18); (19)
Cl:Br ratio	<u>Weak</u>	Basis is that anthropogenic sources have more NaCl and so higher Cl:Br ratio but doesn't easily differentiate between human/animal and whether human is OSS or other source.	(20)
Stable isotopes - ¹⁵ N and ¹⁸ O in NO ₃	<u>Weak</u>	Based on some data that sources of nitrate (e.g., chemical fertilizer, sewage) have different ratios of these isotopes. However method has challenges when multiple sources are present (21).	(22, 23)
Microbial Source Trackers	<u>Strong-</u>	A range of substances are used that are intended to solve the problem of E Coli, F Coli or NO ₃ not being unique to human sources. <ul style="list-style-type: none"> - HF183 (qPCR test for the 16S rRNA gene of human associated bacteroid) 	(24); (25)

		<ul style="list-style-type: none"> - qPCR used to match virus samples of source and receptor 	
Pepper Mild Mottle Virus	<u>Weak</u>	Pepper mild mottle virus is a virus of plants such as pepper which has been detected in human excreta in many countries. It is proposed as a viral indicator in much the same way as E Coli is a bacterial indicator of possible human contamination. However, its presence is not unique to human excreta, so detection doesn't exclude other sources.	(26)
Other chemicals likely to be human specific	<u>Strong if the chemical is human specific.</u>	<p>Some of the chemicals used were:</p> <ul style="list-style-type: none"> • Pharmaceutical products • Aspartame • Caffeine • Acesulfame & Sucrose (artificial sweeteners) 	(27); (28)
DNA analysis to characterize bacterial community	<u>Weak</u>	The argument is that if waste from an OSS is the source, the community of bacteria in impacted wells will be different to wells without OSS influence.	(29)

240 **3. Results and discussion**

241 **3.1 Results**

242 A total of 2,956 results were identified from the search in Scopus (1,576), Web of Science
243 (1,062) and PubMed (318). A further 22 articles were added through citation searches. 1,918
244 articles remained on removal of duplicates. 242 papers remained after assessment of the titles
245 and abstract. Further review by content screening resulted in 60 papers that met the eligibility
246 criteria. There were three studies where two separate papers were published covering
247 different aspects of the same study. The results of the PRISMA screening criteria are shown
248 in Figure 2 (13).

249 *Figure 2 Prisma Diagram*

250 Insert Figure 2

251 **3.2 Classification of included papers**

252 58% (35 Nos.) of the 60 included studies were rated “strong methodology” while the remaining
253 42% (25 Nos.) were rated as having a “weak methodology” on analysis using the quality
254 assessment flow diagram (Figure 3).

255 *Figure 3 Quality Assessment Results*

256 Insert Figure 3

257 Eight studies were rated weak because they did not pass the plausible real-world situation
258 test, typically they involved experimental setups with artificial dosing regimens. Six papers
259 were rated weak because they provided limited information on the pathways that contaminants
260 moved along. Six papers were rated weak in tracking the receptor, commonly because they
261 used data from wells or bores where other potential contamination sources such as animals
262 and dirty collection buckets/ropes could not be ruled out. Of the studies that made it through
263 to the fourth test, a further five were assessed as having a weak method of excluding other
264 sources.

265 Strong studies included in our review are summarised in Table 7.

266 *Table 7 Summary of Strong Studies*

Authors	Country	OSS Type	Contaminants	Subsurface Conditions	Distance to groundwater	Findings
Caldwell & Parr, 1937	United States	P	FIB, N	Unconsolidated fine gravel to clayey soils with impervious stratum underlying the latrine bore and flow	Latrine extended to groundwater	After 3 months biofilm formed, and FIB retreated so that by 7 months FIB was only detected practically at the latrine wall.
Caldwell, 1938	United States	P	FIB	Fine gravel to clayey unconsolidated soils over rock formation	0.6m	3 log FIB removal at 0.3m. No FIB detected at horizontal distance of 1.2m from latrine.
Dyer, 1941	India	P	FIB	Alakaine soils with between 12% and 23% clay content	Latrine extended to groundwater	Pollution initially extended beyond 5 foot, then retreated to between 2.5 to 5 foot horizontally.
Reneau, 1977	United States	S	N	Sandy loam	water table mounds around drainpipes	Tracked Nitrogen transformations and showed nitrification and denitrification
Viraraghavan, 1978	Canada	S	FIB	Sandy clay & Clay	0 to 0.15m	3 log FIB removal after 15m horizontal travel
Starr and Sawhney, 1979	United States	S	N	Coarse Sandy well drained soils	0.9m max depth measured, groundwater probably lower	Complete nitrification in a dry year, but no nitrification in a wet year when subsurface seems to have been saturated
Lewis et al., 1980	Botswana	P	FIB, N	Saturated and unsaturated clayey soils to weathered granite	3m	High nitrate > 500 mg/L in some locations. E Coli detected 5m horizontal. Observed cracks in clay
Stewart and Reneau, 1981	United States	S	FIB	Poorly drained fine to coarse loamy soils	Level of water table varied with seasons	In dry season FIB not detected at 10m horizontal, 5 orgs/100mL at 5m. In wet season FIB >10,000 orgs/100mL
Vaughn et al., 1983	United States	S	Virus, FIB, N	Sandy soils - shallow aquifer distance between static GW level and bottom of leaching pool was 0.6m	3.6m	Detected virus in a well 18m deep, 67m horizontal from septic, but FIB rarely detected beyond 1.5m

Authors	Country	OSS Type	Contaminants	Subsurface Conditions	Distance to groundwater	Findings
Reddy and Dunn, 1984	United States	S	N	Fine sandy loams	Not stated	Nitrate reduced with depth
Chen, 1987	United States	S	FIB, N	Sandy loams, Silty Loams, Loamy sands and gravelly silty loams	n/a	The depth to the ground water from ground surface and the distance of the ground water from the discharge point of the sewage system are main factors influencing pattern and severity of ground water contamination
Alhajjar et al, 1988	United States	S	FIB, Virus	Sands, loamy sands and sandy loams	About 2m	Limited (1 log) reduction in virus at 6m horizontal, FIB largely below detection by 0.3m horizontal
Cogger et al, 1988	United States	S	FIB, N, virus	Sandy soils	0.3 - 0.6m	0.6m unsaturated zone provides effective FIB removal (4 log) and 3 log virus removal. Saturated conditions resulted in significantly worse performance
Postma et al., 1992	United States	S	FIB, N	Coarse grained beach soils- well sorted medium sands	1.6 - 1.7m	4 log FIB reduction at 2m horizontal, 5 to 6 log removal by 6m horizontal. Seasonally used septic, found biological layer to be absent
Gondwe et al., 1997	Tanzania	U	FIB, N	Silty sands underlain by hard clay and clayey sand layers	Pits below groundwater level	10 ⁵ to 10 ⁶ F coli in all shallow bores
DeBorde et al., 1988	United States	S	Virus	Sand and gravel medium sand in vadose zone- Unconfined sand & gravel aquifer	2.8m from leach pipe to groundwater	Dosed coliphages reduced 6 Logs to below detection level by 38m horizontal
Smith et al., 1999	Indonesia	U	FIB, N	Porous volcanic soils	0.38m	Nitrate over drinking water standard attributed to OSS
Chen et al., 2001	Taiwan	U	FIB	Not indicated	Not specified	Disease outbreak attributed to OSS 10m horizontal from well. Submersible pump whose outlet pierced the lining of the well near

Authors	Country	OSS Type	Contaminants	Subsurface Conditions	Distance to groundwater	Findings
						the ground allowing entry route for contaminants.
Geary, 2004	Australia	S	N	Sandy soils	0.4 - 0.8m	Ammonia converted rapidly to nitrate, nitrate lost through denitrification and plant uptake
Geary, 2005	Australia	S	N	Sandy soils	0.4 - 0.8m	As above
Alexander et al., 2008	United States	S	FIB, Virus	fine sandy loam over fractured bedrock (Karst)	Not stated	New septic system identified as cause of waterborne illness in restaurant. A leak prior to leach pits possible cause, but leach pit also shown to be hydraulically connected to water supply.
Harden et al., 2008	United States	S	FIB, N	Sandy soils, underlying karst aquifer	1.3 - 4.5m	0.4–4 m thick sandy surficial soils and underlying karst aquifer allow rapid contaminant transport and limit the ability to attenuate NO ₃
Katz et al., 2010	United States	S	FIB, virus, N	Thin sands and clays overlying a karst aquifer	2.6 - 4.4m	Indicator bacteria and human enteric viruses were only intermittently in groundwater. Contaminant movement to groundwater beneath each septic tank system also was related to water use and differences in lithology at each site.
Banerjee, 2010	India	P	FIB	Clayey silty soils & Sandy gravel soils	varied - 0.5 - 5m, some pits saturated	Maximum horizontal travel of FIB was 6m, a clay barrier effectively stopped FIB transport
Sonbul et al., 2011	Saudi Arabia	S	FIB, N	Coarse sand to gravel soils with traces of silt and clay	7 - 12m	FIB not detected in receptor wells, but nitrate up to 193 mg/L
Borchardt et al., 2011	USA	S	FIB, virus	sandy loam topsoil, sandy clay to 0.76m. Glacial till with large cobbles to at least 3m depth then fractured dolomite, often with dissolution having	35m	Virus attributed to septic source detected at well 85m deep and 188m horizontal distance from septic

Authors	Country	OSS Type	Contaminants	Subsurface Conditions	Distance to groundwater	Findings
				enlarged fracture opening and pores		
Keegan et al., 2014	Ireland	S	FIB, N	6 sites covering low permeability clay/bedrock to high permeability sandstone till	not specified	Performance varies greatly with permeability and composition of subsoil, with high permeability sites presenting a risk to groundwater
Islam et al., 2016	Bangladesh	U	FIB	Sandy Loams, Loam, Loamy sands, Clayey Loams	7 - 20m	the safe distance from the tube well to the pit latrine varied from site to site depending on the horizontal and vertical distances of the tube well as well as hydrogeological conditions of a particular area.
Van Ryneveld et al., 2016	South Africa	P	FIB	1m silty sand, dense gravel and stiff sandy clay soil (residual granite)	15m	At horizontal distances of 3 m from the soak away, levels of the FIB and nitrogen had dropped to values consistent with the background levels
Ravenscroft et al., 2017	Bangladesh	P	FIB	2 sites - on sandy, more permeable sediments, deep water tables and 2 sites - on river, fine sediment, internal sedimentary structures & shallow water tables - All sites have 3-5m thick aquitards where latrines were constructed/Jajjira-silt & clay	Up to 30m	leakage from pit latrines is a minor contributor to faecal contamination of drinking water in alluvial-deltaic terrains
Higgins et al., 2020	USA	S	FIB, N	glacial fluvial sand & gravel	4.5m	Highly likely that domestic wells have elevated nitrate arising from local septic leach fields, not farm fertiliser. FIB not detected in wells.
Murphy et al., 2020	United States	S	FIB, virus	Not indicated	Unclear	Evidence of human faecal contamination in the private wells located 9 to 54m horizontal from household septic systems.

Authors	Country	OSS Type	Contaminants	Subsurface Conditions	Distance to groundwater	Findings
						Evidence that rainfall events play a key role in transport.
Sabina, et al., 2020	Canada	S	N	fine sand	not stated	Tracks nitrogen plume through groundwater and shows nitrogen removal at groundwater-lake interface
Mattioli et al, 2021	USA	S	FIB, virus	Fine sand, silt, clay on surface overlying sandstone \ siltstone \ shale bedrock, slopes towards well	unknown	An overloaded septic system was the likely source of human faecal and norovirus contamination in a well
Wiegner et al., 2021	USA	M	FIB, N, virus	Fractured basalt with unconfined aquifer, little soil	Not specified	Sewage is entering the groundwater at Puako and the underlying geology, rather than OSS type, primarily controls the speed at which sewage reaches the shoreline

267 Notes: OSS type: P = pit or “combined function OSS”; S = septic or “separated function OSS”; U = unknown; M =
 268 Mixed of separated and combined function OSS. Contaminants: FIB = Faecal Indicator Bacteria, N = nitrogen
 269 compounds, at least nitrate

270 Papers were grouped by the income status of the country as of 2022 using World Bank
 271 definitions (Table 8). Most of the included studies were undertaken in HICs. Studies from HIC
 272 or UMIC were more likely to be rated “strong methodology” presumably because more
 273 resources are available in these countries to use more advanced techniques to investigate the
 274 sources, pathways and receptors.

275 Table 8 Country income, technology characteristics and method of studies included in the review

	High income	Upper middle income	Lower middle income	Low income	Total
	N°(%)	N°(%)	N°(%)	N°(%)	N°(%)
Technology Type					

Combined Functions ¹	2 (3%)	3 (5%)	7 (12%)	0 (0%)	12 (20%)
Separated Functions ²	32 (53%)	1 (2%)	0 (0%)	0 (0%)	33 (55%)
Uncertain	2 (3%)	1 (2%)	6 (10%)	2 (3%)	11 (18%)
Mixed technologies	1 (2%)	0 (0%)	3 (5%)	0 (0%)	4 (7%)
Method					
Strong studies	26 (43%)	4 (7%)	5 (8%)	0	35 (58%)
Weak studies	11 (18%)	1 (2%)	11 (18%)	2 (3%)	25 (42%)
Total	37 (62%)	5 (8%)	16 (27%)	2 (3%)	60 (100%)

276

277 Table 8 also shows the split of technology types in the included papers according to the income
 278 status of the country where the study was located. Many papers provided only a minimal
 279 description of the type of technology used and so for 11 of the included papers we were not
 280 able to be confident which of the two broad categories we developed was being used in the
 281 study. Separated function structures are the most used OSS technology in HICs and therefore
 282 formed most of the studies (32Nos.) in the category. The majority of combine function
 283 structures studies (6Nos.) were in LMICs.

284 The included papers are presented by year of publication in Figure 4. The number of papers
 285 that were considered suitable for inclusion has increased in the last ten years with a maximum
 286 of 11 papers included between 2017-2021. This may reflect both increased interest in this
 287 topic and/or improved approaches resulting in less papers being excluded in more recent
 288 years.

289 *Figure 4 Papers by year of publication (in 5-year blocks, year is first year of block)*

290 Insert Figure 4

291 Table 9 shows aspects of the included studies. Studies were either cross-sectional which
 292 meant that they looked at a situation at a particular point in time or longitudinal meaning that
 293 they looked at the variability over time, often with the aim of looking for seasonal impacts. Any
 294 study that covered at least a whole year of data was classified as longitudinal and nearly half
 295 of studies did this as shown in Table 9. A total of seven papers also looked at the impact of
 296 rainfall events, usually by recording daily rainfall and measuring contaminants with sufficient
 297 frequency to allow some conclusions to be drawn about the impact of rainfall events.

298 *Table 9 Characteristics of studies included in the review with strength of method*

	Strong studies N°(%)	Weak studies N°(%)	Total N°(%)
<i>Type of Study</i>			
Cross Sectional	20 (33%)	13 (22%)	32 (55%)
Longitudinal	15 (25%)	12 (20%)	27 (45%)
<i>Pathways considered</i>			
Aquifer only	33 (5%)	18 (30%)	51 (85%)
Aquifer and localised	2 (3%)	7 (12%)	9 (15%)
<i>Contaminant observed</i>			
Faecal indicator bacteria	27	17	44
Viruses	9	4	13
Nitrogen	18	13	31

299

300 Most papers (51) only looked at the aquifer pathway and of the few (9) that considered
301 localised pathways, most (7) had a weak methodology. Faecal indicator bacteria were most
302 widely used (44) studied, followed by nitrogen (31). Viruses were the least investigated (13)
303 although they are receiving increasing attention as analytical methods improve.

304 **3.3 Discussion**

305 Of the studies we reviewed, those with strong methodologies in particular provide some new
306 insights into the interaction between OSS and groundwater. Processes of pathogen removal
307 have been quite well described. The formation of the biological layer is important and variable
308 depending on conditions. Pathogen removal processes are influenced by ground conditions;
309 in special cases pathogens have been detected at relatively long distances from OSS. The
310 relationships between bacteria removal compared to virus removal and nitrate removal are
311 complex.

312 These points are expanded below. We refer primarily to the papers rated as strong. When we
313 draw on evidence from weak or not included studies this is stated in the text.

314 *Biological layer*

315 Although previous reviews stated the importance of the biological layer, it is only mentioned
316 in seven of the strong studies (16, 17, 30-34), though its existence has been confirmed in
317 several studies outside the scope of this review, such as a recent paper by Knappe, Somlai
318 (35) and is discussed by Beal, Gardner (36) in their review of soil absorption systems. None
319 of the studies attempted to directly measure pathogen reduction across the biological layer,
320 although several authors provided indirect evidence that it is important. Caldwell (16) observed
321 increased pathogen removal after a period of seven months operation and attributed that to
322 development of the biological layer, and similarly Dyer (30) observed contamination extending
323 several metres from a new latrine then retreating after some months and attributed this to

324 biological layer formation / soil clogging. In a case where septic tanks were only used
325 seasonally, elevated faecal indicator bacteria (FIB) was detected in groundwater. Upon
326 digging up the leach field no biological layer could be observed, and the authors concluded
327 that seasonal use meant a biological layer did not adequately form and the lack of this layer
328 reduced pathogen removal compared to what should have been expected given 1.7m of
329 unsaturated sand and gravel (32). The data compiled by Pang (8) shows higher removal rates
330 in the soil layer which was defined as the biologically active ground layer so might support the
331 importance of the biological layer. In 2008 and 2011, disease outbreaks affecting hundreds of
332 people were reported in the United States following the use of newly constructed septic
333 systems (37, 38). The possibility that the biological layer may not have had adequate time to
334 form was not considered in these studies but is a plausible explanation.

335 *Conditions for effective pathogen removal*

336 We examined the strong papers to understand the conditions where effective pathogen
337 removal was achieved. An unsaturated zone of varying depths and soil characteristics caused
338 attenuation of microbial organisms to negligible quantities in most of the strong studies. Soil
339 conditions where this was achieved were described in ten of the strong studies as: fine gravel
340 to clayey unconsolidated soils over rock formation (16); fine to coarse loamy soils (34); sandy
341 soils, loamy sands and sandy loams (31, 39); thin sands and clays overlying a karst aquifer
342 (22); clayey silty and sandy gravel soils (40); coarse sand to gravel soils with traces of silt and
343 clay (41); silty sand, dense gravel and stiff sandy clay soil (residual granite) (42); sandy
344 permeable sediments and fine river sediments (10, 16, 22, 31, 34, 39-42). The strong papers
345 do not use a standard method of reporting soil structure. Most studies describe the structure
346 using the relative content of clay, sand, silt and loam with little or no data provided on other
347 soil properties that affect contaminant movement such as hydraulic conductivity, permeability
348 and porosity. These studies all report reduction of FIB to below or near detection limits after
349 travel distances ranging from as little as 1.2m to 10m (16, 30, 34, 40).

350 Two strong studies seemed to suggest longer travel distances in an unsaturated vadose zone
351 than reported in the majority of the strong studies. The two studies were conducted in soils
352 described as coarse-grained beach soils with well sorted medium sands (32) and sandy
353 loams, silty loams, loamy sands and gravelly silty loams (43). Chen (43) found high FIB
354 concentrations were detected at 30.5m horizontal distance in 4 of 17 boreholes while
355 investigating septic tank disposal systems, although limited data was provided on ground
356 conditions. Postma, Gold (32) found a travel distance of about 6m in a seasonally used septic
357 absorption system but as discussed above, attributed this to a lack of a biological layer due to
358 seasonal use.

359 *Conditions that impaired pathogen removal*

360 Groundwater levels near, at or above the bottom of the pit or leach field were shown to
361 increase the horizontal travel distances of pathogens when compared to unsaturated
362 conditions in seven studies (16, 17, 34, 39, 40, 44, 45). The effect of saturated conditions was
363 mainly examined by sampling contaminants over wet and dry seasons and/or sampling at
364 locations with varying groundwater levels. Cogger, Hajjar (39) reported high bacterial counts
365 while the water table was within 0.3m of a septic system and low counts during the dry season
366 when the water table was 0.9m deep. There were cases where faecal indicator bacteria were
367 only detected during the periods when the water table was high (34) indicating impairment of
368 the OSS performance under these conditions. This observation was further emphasized by
369 another study conducted on very shallow aquifers where the groundwater quality was found
370 to be similar to soak pit effluent quality (44).

371 Water contained in the pores of saturated soils appears to be the medium for travel of soluble
372 contaminants resulting in greater travel velocities and distances. Movement occurs in the
373 direction of the groundwater flow which is likely to follow the gradient of the land surface in
374 high water table areas (17). The occurrence of a very thin vadose layer (<0.5m) under septic
375 systems resulted in a lower vertical movement of bacteria compared to lateral movement. A
376 study conducted over a 0.15m thick vadose zone in sandy clay soils reported lateral bacteria

377 travel distances of 15.25m in the direction of the water gradient (45). Where there is no water
378 gradient, vertical percolation dominates (34). Maximum travel velocity of 0.7m/d for
379 contaminants was reported during the monsoon season in sandy silty soils where the ground
380 was completely saturated (40).

381 Varying ground conditions caused by seasonal weather changes directly impact removal of
382 contaminants. Frequently, though not always, authors reported increased concentrations of
383 pathogens in groundwater receptors during wet seasons. The possible reasons for this
384 include:

- 385 • Raised groundwater table reducing (or eliminating) the thickness of the unsaturated zone
- 386 • Increased water volumes flowing through the ground material causing flushing of
387 pathogens from ground material and/or higher velocities leading to longer travel distances
388 before complete pathogen die off.

389 Of the five strong papers that looked more specifically at rainfall events rather than seasonal
390 variation, three showed a correlation between events and increased pathogen levels in
391 monitoring bores, whereas two found no such correlation. One stated rainfall had caused a
392 temporary increase in FIB but did not provide data (39). Keegan, Kilroy (33) found in one site
393 rainfall increased pathogens while in their other site it decreased pathogens, though only in
394 nearby sampling sites and no effect was seen in more distant groundwater bores. They
395 suggested that the site where pathogens increased was one with a higher proportion of clay
396 particles and rainfall may have washed out some pathogens that were attached to the clay.
397 The other site was gravel with very little silt/clay and so may have had little pathogen removal
398 occurring due to adsorption and so dilution from rainfall events was a bigger factor. This
399 highlights the complexity of pathogen removal in differing ground conditions making it very
400 difficult to be definitive about the impact of rainfall events in any given location.

401 Where the soils are highly permeable (such as in coarse sands and gravels), bedrocks are
402 fractured or karst formations, pathogen transportation occurs over much longer distances as
403 shown in seven strong papers. Evidence of flow in karst aquifers was found to be complex. It

404 can be through the conduits in karst formation, or through unconsolidated sediments also
405 known as matrix flow. Flow appeared to bypass one well in close proximity to the OSS while
406 tracer was found in a well further down showing that there are possibilities of arrival at a well
407 through multiple pathways (46). There are cases where attenuation to negligible levels was
408 achieved in a karst aquifer but in this case the karst was overlain by 2-3m of clayey loam soil
409 which likely was enough of a barrier to pathogen transport (22). Another study found that a
410 fractured karst bedrock under a layer of sandy loam allowed bacterial travel velocity of more
411 than 8.2m/d to a 60m deep well (37). Greater travel distances have been reported in a study
412 done on fractured dolomite subsurface where a tracer injected into a septic system was
413 observed to travel at a velocity of 31m/d (38). In another study in fractured bedrock underlying
414 clayey soils movement of about 30m/d was reported in saturated conditions (19). The
415 orientation of the bedrock also was also reported to affect the movement of contaminants. The
416 existence of viral contamination in a well was attributed to a steep dip of bedrock beneath a
417 septic leach field sloping towards the well under unsaturated conditions (25).

418 The occurrence of pure clay soils presents a unique situation. In wet conditions it acts as an
419 aquitard meaning it will hold moisture and restrain movement, which can lead to overland flow
420 of effluent (33). In dry conditions, the clay shrinks and forms cracks which increase the risk of
421 contamination (5). Banerjee (40) tested two pits with a 0.5m clay envelope (51% clay, 44%
422 silt, 5% sand) and found this prevented faecal coliform transmission. Across 12 sites, Banerjee
423 (40) found increasing clay content in a sand/silt/clay soil decreased the horizontal travel
424 distance of faecal coliforms.

425 *Localised Pathways*

426 Quantifying the significance of localised pathways is challenging and this remains a major gap
427 in the literature. Only two studies comment on localised pathways at all. Ravenscroft, Mahmud
428 (10) observed lower levels of bacterial contamination in purposely-constructed monitoring
429 wells constructed between and downstream of the OSS, when compared to the levels found
430 on the spouts of an in-use hand pump. They argued that this showed that contamination was

431 arising at the pump spout due to localised pathways. Ravenscroft, Mahmud (10) estimated
432 that the localised pathways were a more significant source of disease burden than the aquifer
433 pathway. The only other strong paper to discuss localised pathways was Keegan, Kilroy (33)
434 who simply observed effluent from a soakaway running across the surface during heavy
435 rainfall and potentially entering a bore or well through gaps in the apron or pump.

436 *Impact of OSS technology type*

437 The type of OSS studied may have an impact on levels of contamination especially for
438 microbial contaminants. The biological layer forms in response to organic matter in the
439 effluent. Therefore, improving removal of organics through, for example, a more effective
440 septic tank design or using an aerobic treatment stage before the leach field, likely reduces
441 formation of the biological layer and this has been shown to be the case in a study not included
442 in this review (35). However, none of the included studies set out to establish microbial and
443 nitrogen removal between different types of OSS under similar conditions and there is too
444 much variability in the conditions of the various studies for us to be able to discern any
445 difference due to technology type.

446 *OSS density*

447 It can be argued that an increase in OSS density increases the risks of groundwater
448 contamination, even if ground conditions are near ideal such as a thick unsaturated zone and
449 fine ground material. However, the correlation between OSS density and level of microbial
450 contamination has not been strongly established in the strong studies included in this review.
451 While several papers do suggest a correlation, causality cannot be inferred as there is an
452 absence of information on the active pathways, and other sources were not excluded. The
453 most that can be said is, given the large pathogen concentrations in OSS effluent, a small
454 percentage of failing systems in dense areas are likely to result in contamination of
455 groundwater above safe drinking water standards.

456 Higher density OSS is also likely to occur in areas of higher density housing, and often in
457 conjunction with other, non-sanitation related, sources of contamination to shallow aquifers,
458 via rainwater runoff and leaching both of which may be carrying industrial and urban
459 contaminants.

460 *Hydraulic load fluctuations*

461 None of the studies supported the idea that a sudden increase in hydraulic load might force
462 effluent flows beyond the biofilm later resulting in increased pathogen concentrations in the
463 groundwater. Mattioli, Benedict (25) investigated a norovirus outbreak affecting 179 people at
464 a camp whose toilets were discharging effluent to a nearby leach field. The drinking water well
465 they were using tested positive for norovirus and human microbial source tracking (MST)
466 genetic marker. Fluctuations in hydraulic loading before and during the camping events could
467 have influenced contaminant movement but the idea was not considered in the study. One
468 study attempted to look at the impact of different sustained hydraulic loading rates by
469 comparing two septic leach fields, but was ultimately inconclusive as other site factors
470 confounded the results (39).

471 In 1938, Caldwell conducted another study where the moisture content in pit latrines was
472 varied by the addition of water. The result was an increase in the distance of travel of bacteria
473 to 2m compared to a dry pit distance of 0.3m (16, 17).

474 *FIB versus Virus transport*

475 Given that the review by Pang (8) suggested virus and bacteria removal rates were similar,
476 we examined the included studies to see if there was evidence of good correlation between
477 FIB and virus transport and detection. Nine strong studies included virus data and the
478 consensus is that there is no correlation between occurrence of viruses and FIB (22, 24, 31,
479 47, 48). Viruses are smaller in size compared to FIB and this is assumed to be the reason
480 they can pass more easily through the biological layer (31) and achieve greater distances in
481 sub-surface material (48) (47).

482 *Nitrate*

483 The included papers are consistent with the view that, provided aerobic conditions in the sub-
484 surface, ammonia will rapidly oxidise to nitrate. Nitrogen transformations were examined in 32
485 of the included papers, 19 of which were considered to have a strong methodology. Only one
486 strong paper (49) had an example where nitrification did not occur and the authors attributed
487 this to very wet conditions beneath the leach field resulting in inadequate aeration.
488 Groundwater nitrate concentrations exceeded WHO drinking water standards (11.3 mg/L as
489 N or 50 mg/L as NO₃) in twelve of the 19 strong papers studying nitrogen.

490 We sought to assess the included literature for evidence that denitrification was the most
491 significant cause of nitrate removal and thus whether it can be relied upon to eventually reduce
492 groundwater nitrate concentrations. However, resolving whether observed reductions in
493 nitrate as effluent moves away from an OSS are due to dilution in surrounding groundwater,
494 denitrification or potentially uptake by surface vegetation is not straightforward and most
495 papers did not attempt to do this. Only five of the strong papers made a clear case that they
496 have observed nitrate removal through denitrification, while two strong papers showed nitrate
497 persisting in groundwater with no observable removal by denitrification or any other means.

498 Even where the source of nitrate inputs is reduced or ceased, if the aquifer has a long
499 residence time and does not have the conditions to enable denitrification, nitrates may persist
500 at high levels. For example, one study outside the scope of our review looked at data spanning
501 over 20 years for nitrates in the aquifers beneath Urânia, Brazil and developed a calibrated
502 model which predicted it will take 30 to 40 years for nitrate concentrations in the aquifer to fall
503 to drinking water standards in a situation where cesspits have now largely been replaced by
504 sewers (50).

505 **4. Significance and Limitations**

506 This is an update to previous reviews but the first to use the source-pathway-receptor (SPR)
507 method. This robust search methodology provides a new systematic understanding of the

508 current state of the literature. Some studies which provide useful contributions to our
509 understanding but whose methods preclude the confirmation of the SPR are excluded (for
510 example Knappe, Somlai (35) and Beal, Gardner (36)). Some research on this topic may be
511 outside of the academic sphere (for example in technical reports and feasibility studies) and
512 there may be some published in languages other than English.

513 **5. Conclusions**

514 The source-receptor-pathway model is important in understanding the dynamics of
515 contaminant movement from OSS to groundwater. It enables a more precise understanding
516 of the contamination that arises directly from OSS as compared to contamination that may be
517 associated with other sources and pathways. The dynamics of contaminant travel are
518 complex and highly context specific. Ground conditions including soil structure, moisture,
519 porosity, permeability, tortuosity and continuity, groundwater level, velocity and direction,
520 excreta/wastewater hydraulic loading rate and composition, are some of the factors that affect
521 movement of effluent in the subsurface (5, 39). This variability means simple siting guidelines
522 based on horizontal separation are not reliable.

523 Though not always recognised in the literature, formation of a biological layer is important for
524 removal of microbial pathogens. This layer takes months to form which impacts the
525 performance of OSS that are new or subject to highly variable loading or sudden increases in
526 hydraulic load.

527 Under ideal conditions of an unsaturated zone comprising fine material (sands, silts, loams
528 with some clay content) with effluent moving along the aquifer pathway, and an OSS greater
529 than around 6 months old with a consistent hydraulic load, FIB can be reduced to detection
530 limits within under 10 metres distance. However, ideal conditions are very often not present.
531 If the groundwater level is close to the OSS, certainly under 1m, FIB can be expected to travel
532 in the direction of the groundwater movement for greater distances, though it is hard to predict
533 how far. Fissured rocks and karst formations can allow FIB to travel much further with
534 distances of 60m or more recorded.

535 Contamination can occur via localised pathways, but these were not well investigated.
536 Contribution of the ingress of contaminated surface water into faulty boreholes/wells is
537 significant yet not adequately covered in the included literature. There is a need more research
538 on localised pathways as it has been argued they cause more diarrhoeal disease burden
539 compared to aquifer pathways (10).

540 The use of FIB to detect viral contamination remains risky as achieving FIB below detection
541 level doesn't mean viruses are reduced to "safe" levels. Multiple studies showed the presence
542 of viruses in the absence of FIB.

543 The review established that it is extremely difficult to eliminate the risk of groundwater
544 contamination when OSS effluent is discharged into the subsurface. Generally, it seems
545 unsafe to assume that contamination can be prevented in areas where OSS occur with any
546 frequency. In such cases, it would seem prudent to consider a shift to sanitation technologies
547 with significantly lower risks of contaminating groundwater coupled with the provision of viable
548 and convenient alternatives to the use of shallow groundwater for drinking.

549 **Reflections on systematic reviews**

550 The extraction of data on a specific topic from existing published literature through a detailed
551 screening and eligibility process allows creation of a detailed summary on what is known about
552 the topic. Further, by critically examining the collected evidence, gaps in literature are easily
553 identified and presented to researchers for further investigation. Although systematic reviews
554 are extensively used in the medical discipline, they are very useful tools in building the body
555 of knowledge in all academic disciplines including the engineering and environmental sciences
556 as presented in this paper.

557 **Declaration of competing interests**

558 The authors declare that they do not have any competing financial or personal interest with
559 people or organizations that could create a bias in their work.

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562 not available in the general search.

563 Supporting Information

564 A Prisma Checklist is included in supporting information.

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688

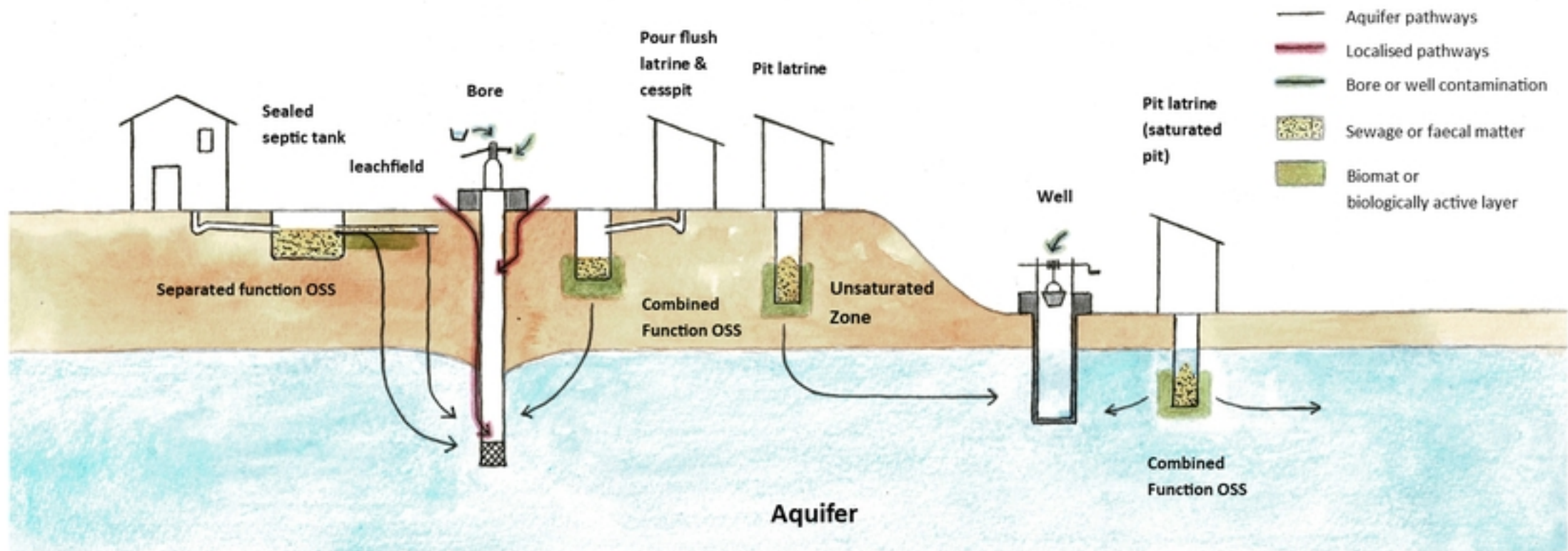


Figure 1

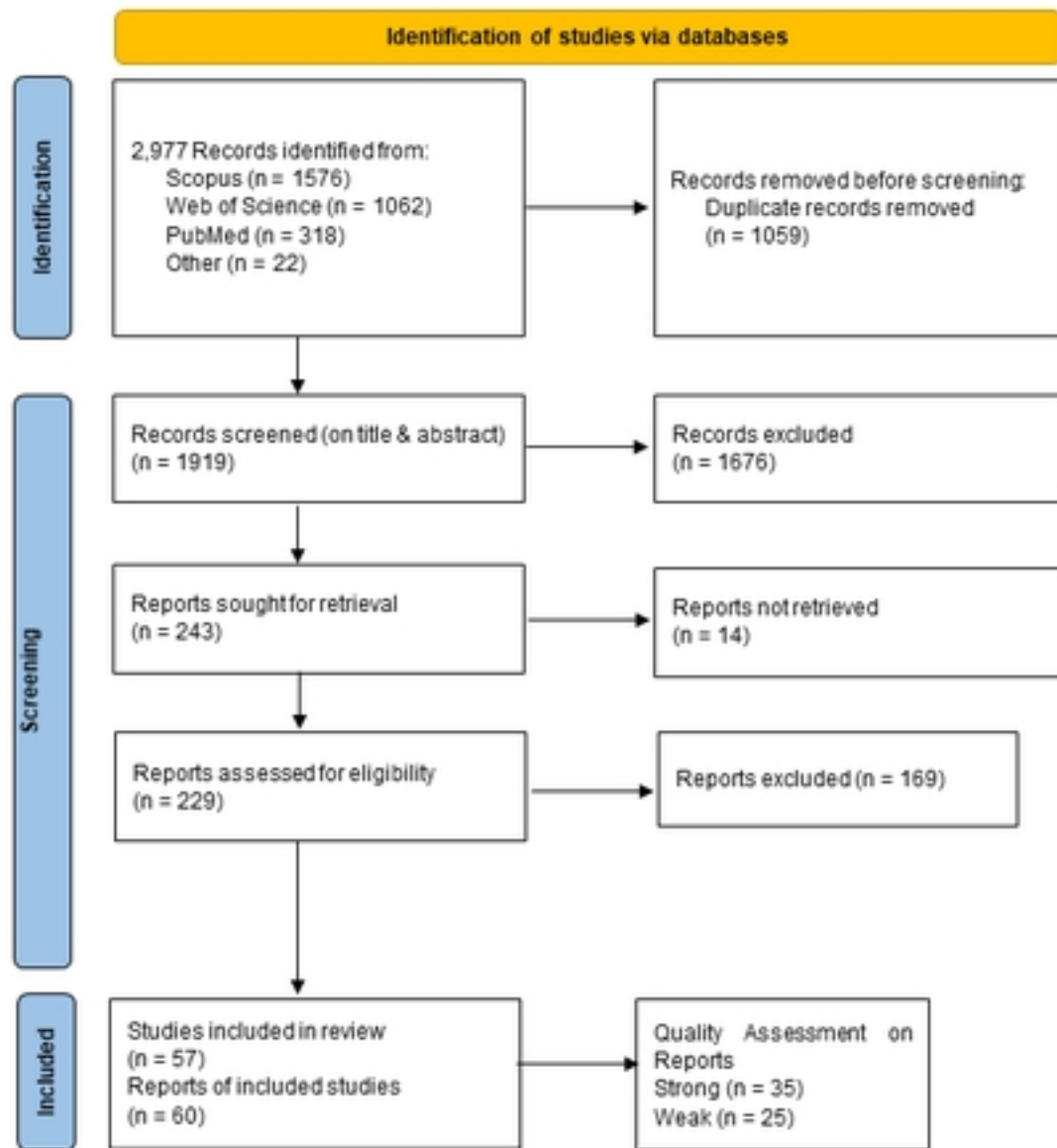


Figure 2 PRISMA diagram

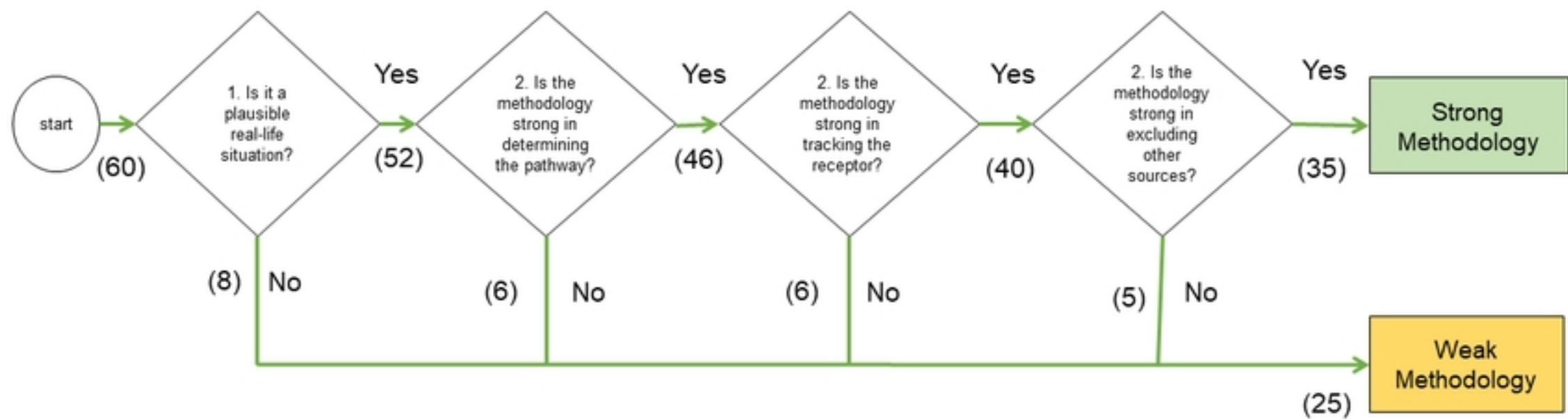


Figure 3

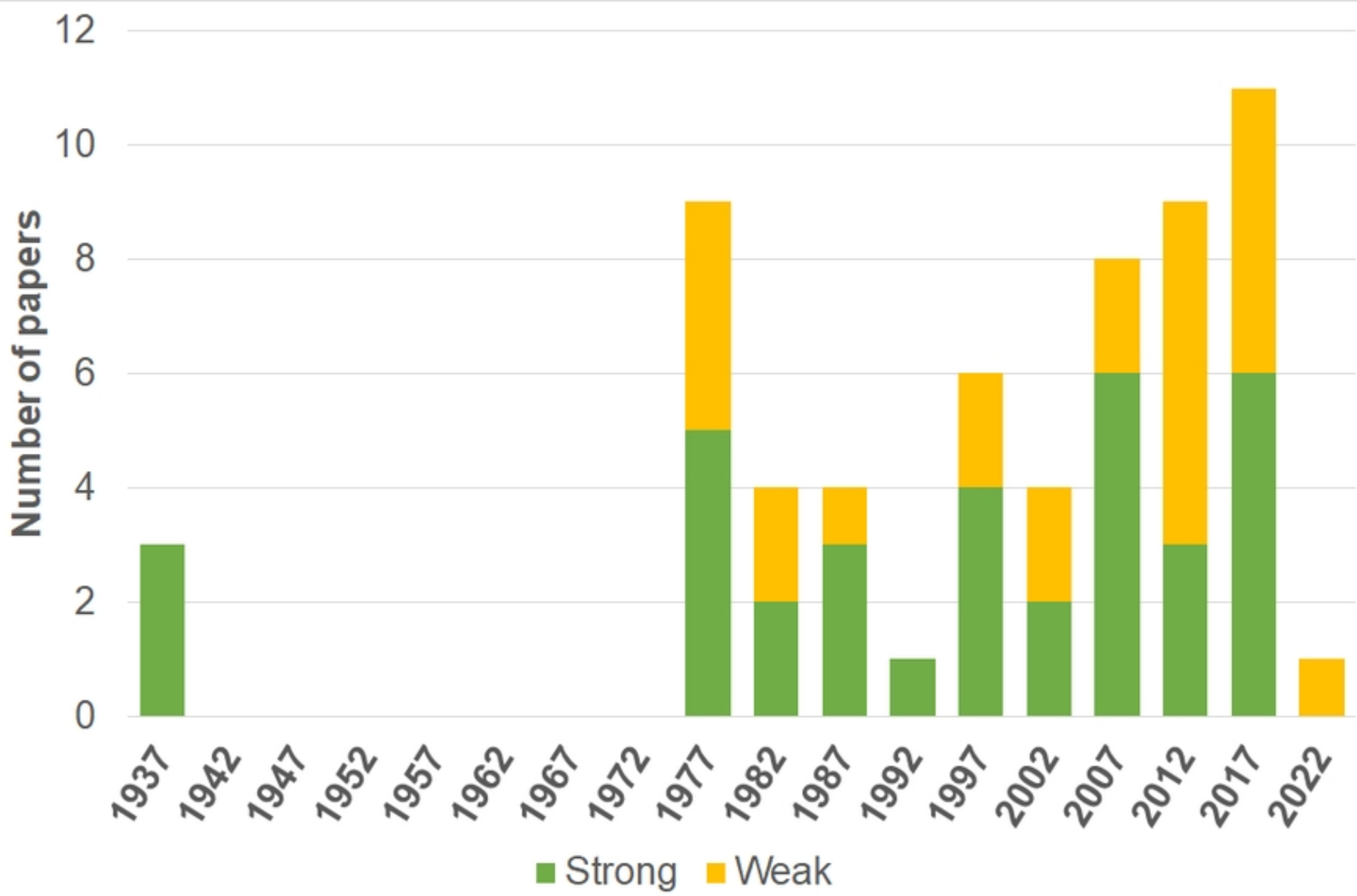


Figure 4