ARTICLE IN PRESS
DES-09960; No of Pages 7
Desalination xxx (2010) xxx–xxx
Contents lists available at ScienceDirect
Desalination
journal homepage: www.elsevier.com/locate/desal

Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests

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ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 4 May 2010
Accepted 4 May 2010
Available online xxxx

Keywords:
Leachate
Septic tank
Bacteria
Tracer test
Groundwater contamination

ABSTRACT

We reported on the transit and survival of 6 potentially pathogenic bacterial populations in a schist aquifer beneath the Etueffont landfill (France). Total coliforms, *Escherichia coli*, *Enterococci*, *Pseudomonas aeruginosa*, *Salmonella* and *Staphylococcus aureus* were monitored for 15 months in groundwater and leachate and coupled to tracer tests in an attempt to identify the source of contamination. The results showed the absence of *S. aureus* and *Salmonella*. The monitoring of piezometer 30 (PZ30) located downstream from the landfill highlighted leachate infiltrations into the substrate. Groundwater analysis showed high levels of faecal bacteria in the underground environment (20,000 CFU 100 mL^{-1} for total coliforms, 15,199 CFU 100 mL^{-1} for *E. coli* and 3290 CFU 100 mL^{-1} for *Enterococci*). Data from tracer tests indicated that bacteria originated from the septic tank of the transfer station and part of these bacteria transited through waste. Bacterial density was lower in leachates than in groundwater sampled from PZ30, except for *P. aeruginosa* which seemed to take advantage of adverse environmental conditions. The landfill, closed since 2002, was not a source of faecal bacteria which appeared to be able to survive in the schist substrate, and may be considered as good markers of recent faecal contamination.

1. Introduction

According to the French law 92-646 of July 13th 1992 [1], municipal solid waste disposal in landfill sites is no longer allowed in France since 2002. This legislation also requires continued monitoring of the environmental impact of landfills over a 30-year period after landfill closure. While most studies on leachate have focused on the physical and chemical sources of the pollutant and its impact on groundwater quality [2–4], little is known about the composition of pathogenic bacterial populations in landfill leachate. Yet, pathogenic bacteria may contaminate drinking water and their presence must thus be controlled in order to avoid potential health hazards. This is even more important since the new active waste treatment centers are generally located in the vicinity of old landfills. The purpose of this work is to identify the origin of pathogenic bacteria found in the Etueffont (northeastern France) landfill leachates and groundwater by means of bacterial analysis and dye tracer test experiments. Seasonal variations in abundance of total coliforms, *Escherichia coli*, *Enterococci*, *Salmonella*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* which may develop in leachate are investigated. The presence of the latter two bacterial populations is not considered a public health issue, but rather an indication of the presence of wastewater. Total coliforms are frequently measured as an indicator of contamination. Among total coliforms, non-faecal coliforms are capable of proliferation under normal environmental conditions. On the other hand, the presence of total coliforms may indicate the presence of faecal coliforms, common indicators of faecal pollution [5]. The other bacteria are more systematically involved in sanitary problems. *E. coli*, commensal of numerous animal digestive tracts, is not a saprophyte in temperate climate and is the most appropriate coliform bacteria to indicate warm blooded animal faecal pollution [6–8]. *Enterococci* bacteria are found under a variety of environmental conditions, especially in wastewater. Their life span is longer than coliforms [5,9–11] and may be equivalent to viruses [12] but they can not multiply [13]. These features make them not only excellent indicators of faecal contamination but also indicators of the presence of viruses. *P. aeruginosa* is the bacterial species with the widest range of habitats; it lives and proliferates as a saprophyte in water and humid soils. Its presence is generally abundant in wastewater [14] but it is not a specific indicator of recent faecal contamination. *Salmonella* sp. are widespread in the environment and they can survive for several weeks in dry conditions and up to several months in water. *S. aureus* is a skin commensal of human and animal mucous. It may survive for long periods under a wide range of environmental conditions and, it is resistant to antibiotics.

The Etueffont landfill is surrounded by a moderate urban activity which may exacerbate bacterial contamination of groundwater

Abbreviations: E, Eosin; FL, Former landfill; N, Naphthionate; NC, New cell; PZ30, Piezometer 30; SWTS, Selective waste collection facility and transfer station; UC, Under cell.

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doi:10.1016/j.desal.2010.05.007

through existing septic tanks. The health hazards associated with bacterial survival from septic tanks have often been investigated in alluvial aquifers [15–17] but rarely in discontinuous schist aquifers. Injections of dye tracers were performed in the vicinity of the septic tanks in order to evaluate their potential impact on groundwater and leachate quality [18,19].

2. Materials and methods

2.1. Site description

The Etueffont landfill, located in northeastern France, opened in 1976 in order to receive municipal solid waste from approximately 50,000 inhabitants (Fig. 1a). The site extended over an area of 4 ha. The climate was continental and very humid, with an average annual rainfall of 1418 mm, an average annual temperature of 8 °C and, on average, 120 frost days per year.

The waste was shredded and successively deposited in 1-m-thick layers, without compaction after a 2–3-month period of maturation between each layer. The degradation was mainly provided by aerobic processes. From 1976 to 1998, the waste was directly deposited onto the soil surface, which was underlain by a schist bedrock considered impermeable. This dump zone was referred as the former landfill (FL). After 1998, the waste was dumped in a new watertight cell (NC). Waste disposal stopped in 2002 in accordance with the French legislation. Part of the leachates originated from the FL and the NC were collected and then treated by a natural lagooning system [20]. After treatment, the effluents were discharged into the Gros Prés brook.

On the western side of the landfill stood a platform with a selective waste collection facility and a transfer station (SWTS) (Fig. 1b), which were both equipped with toilet facilities connected to a septic tank. Rainwater runoff originated from the platform was collected and mixed with the septic tank effluents. The resulting wastewater was then channelled to an oil separator before being discharged into the Gros Prés brook. A series of piezometers was installed within the landfill area, in order to monitor the quality of groundwater and the potential pollution from leachate infiltrations. Piezometer 30 (PZ30), which was located downstream from the landfill and the SWTS platform, was 6 m deep and screened between 3 and 6 m below ground surface. PZ30 was the sole piezometer which showed significant levels of bacteria. The other piezometers, all located downstream and/or downgradient from the landfill and the platform, presented concentrations of bacteria below the detection limits.

2.2. Geological and hydrogeological contexts

The site was located at the southeastern end of the Vosges mountains in the Permian basin of Giromagny [21]. However, the landfill was established on an eroded horst formed by the Devono-Dinantian schists of Etueffont (silt and sandstone). The horst was bounded on its southeastern part by a NE–SW fault, which put the schists into contact with the Permian formations (Fig. 2). During our field investigations we noted that the schists dipped at a high angle (>75°) and were at some places nearly vertical with an average direction of N75–N80. The network of piezometers enabled to determine the groundwater flow direction, which was NE to SW. Two perpendicular fracture families

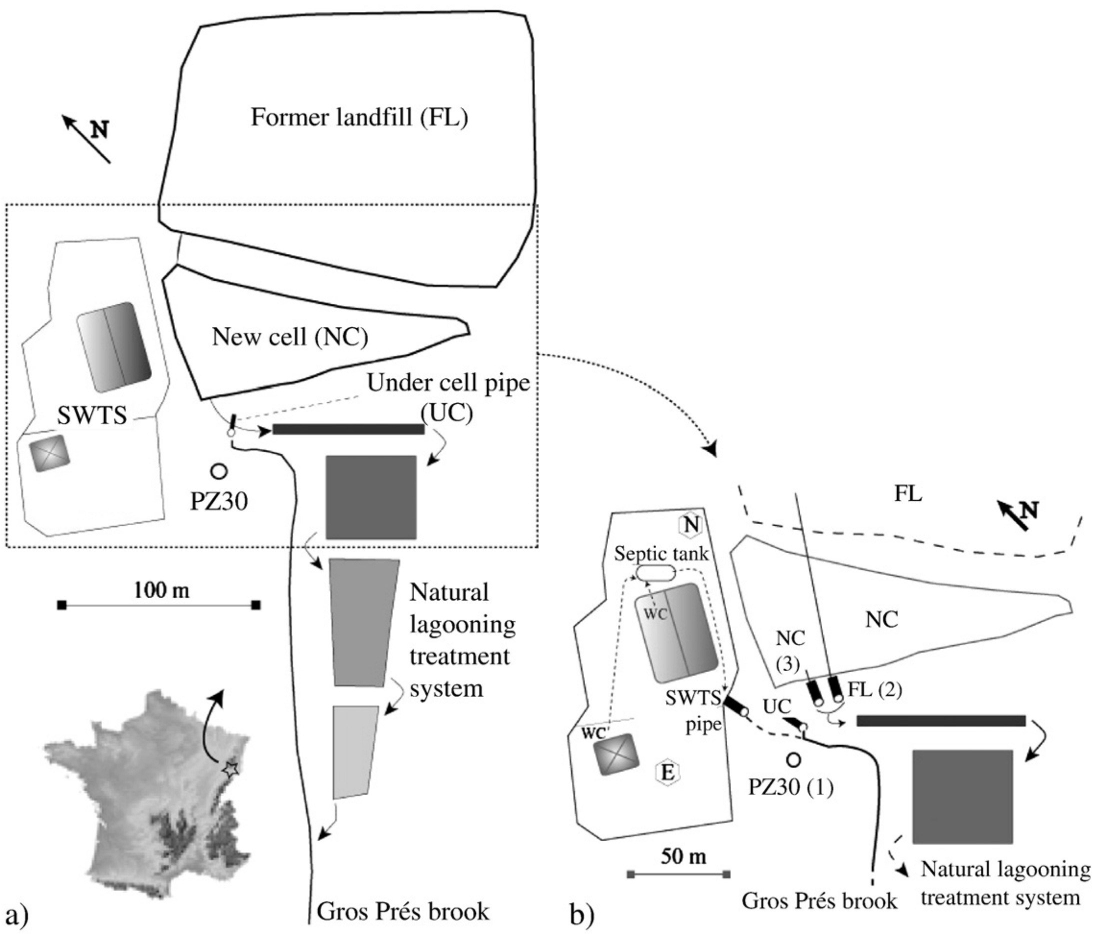

Fig. 1. a) Schematic map of the site; b) location of sampling points for bacterial analysis: PZ30 (1), former landfill (FL) leachate collection pipe (2) and new cell (NC) leachate collection pipe (3) and injection points of eosin (E) and naphthionate (N).

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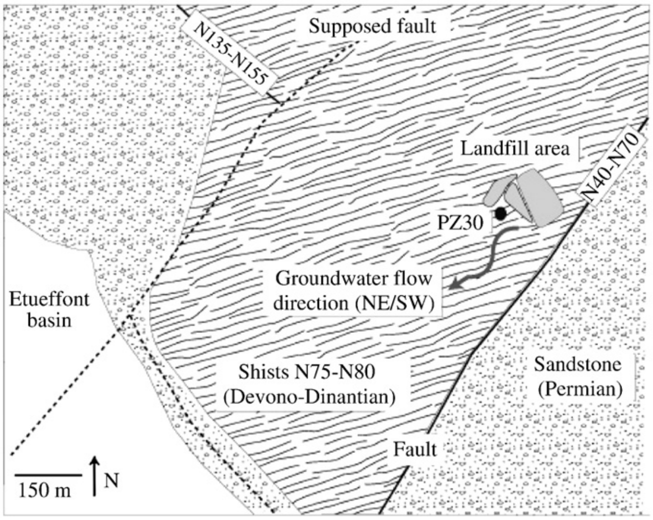

Fig. 2. Structural map of the landfill area.

(N40–N70/subvertical and N135–N155/75–80W) may influence the groundwater flow (personal observation).

2.3. Sample collection

Groundwater was manually sampled from the piezometer PZ30 (1) with a 1 L polyethylene bailer and stored in 1 L sterile polyethylene bottles containing thiosulfate (Fig. 1b). Leachate samples were directly collected at the end of the drainage pipes of the FL (2) and the NC (3) in 1 L sterile polyethylene bottles containing thiosulfate (Fig. 1b). All samples were placed in a cooler until analysis, within 24 h.

The SWTS discharge pipe was not used as a permanent sampling point because it was entirely dependent on rainfall intensity. Consequently, its flow was unsteady.

2.4. Quantitative analysis of bacteria

Samples were taken from PZ30 and the FL pipe for bacterial analysis on a monthly basis for 15 months (June 2004–August 2005). The NC pipe was monthly sampled over a 6-month period (March–August 2005).

We looked for the presence in leachate and groundwater of the following bacterial pathogens and microorganisms commonly found in wastewater.

Total coliforms were enumerated by the membrane filtration (MF) method on Lactose 0.025 TTC agar with 0.1 Tergitol®7, according to [22]. The lactose-positive colonies were recultured on non-selective tryptic soy agar medium for the oxidase test.

The most probable number (MPN) method in EC-MUG medium was used for the detection of *E. coli* (specific substrate for *E. coli*: EC, MUG: 4-methylumbelliferyl-β-D-glucuronide) [23].

The number of *Enterococci* was determined by the MPN method on MUD/SF microplates (MUD: 4-methylumbelliferyl-β-D-glucopyranoside, specific substrate for faecal *Streptococci:* SF) [23,24].

P. aeruginosa were counted by the MF method, with sub-culture on CNA (cetrimide nalidixic acid) agar [25].

S. aureus were enumerated by the MF method, with sub-culture on Chapman agar [26,27].

Salmonella were detected by the presence–absence test, following the ISO 6340 standard [28].

All results were expressed as colony-forming units (CFU 100 mL^{-1}).

2.5. Tracer tests

Two simultaneous fluorescent tracer tests were performed on January 19th 2006. Excessive quantities of tracers were voluntarily injected (1 kg) to avoid a potential adsorption of the dyes on organic materials.

Naphthionate (N) was diluted in 50 L of local freshwater and injected into the storm sewer of the transfer station platform (Fig. 1b) along the NC. Eosin (E) was injected with 150 L of local freshwater into the storm sewer located in the middle of the selective waste collection platform (Fig. 1b). Be it reminded that each storm sewer was connected to the septic tank.

Theoretically, both tracers (N and E) should be discharged into the natural environment through the same pipe, after oil separation. The aim of this experiment was to check if the total amount of water collected from the SWTS platform would indeed flow through the SWTS discharge pipe.

Three water autosamplers were installed as follows:

- At the end of the NC discharge pipe, to find out if there was any relationship between the SWTS platform and the NC,
- At the end of the UC (under cell) discharge pipe, which collected water circulating underneath the NC, to see if it drained water from the SWTS platform and,
- In PZ30, to determine if discharged water from the SWTS pipe had an impact on groundwater quality.

Monitoring was carried out over a 69-day period with an adaptative sampling frequency (daily basis at the beginning of the monitoring period and weekly basis towards the end).

The presence of E and N was determined in each water sample by a Perkin-Elmer LS 50B fluorescence spectrometer, which measured the fluorescence intensity of samples.

3. Results

3.1. Bacterial dynamics

It must first be noted that during the experimental period, *S. aureus* and *Salmonella* were never detected.

3.1.1. Total coliforms and E. coli

In PZ30 (Table 1), the total coliforms density ranged from 15 to 20,000 CFU 100 mL^{-1} and the *E. coli* density from 15 to 15,199 CFU 100 mL^{-1}. Two maximum values are measured in September 2004 (20,000 and 15,199 CFU 100 mL^{-1}, for total coliforms and *E. coli* respectively) and in July 2005 (10,000 and 1295 CFU 100 mL^{-1}, for total coliforms and *E. coli* respectively). A decrease in bacterial loads is observed in August and October 2004 (Fig. 3) while high amounts of cumulative rain were reported (226

Table 1
Monitoring of bacterial concentrations for 15 months (1, 2) and for 6 months (3).

CFU 100 mL^{-1}		PZ30 (1)	FL (2)	NC (3)
Total coliforms	Min–max	15–20,000	15–3000	<15–500
	Mean	3842	308	237
	σ	5447	771	229
E. coli	Min–max	15–15,199	<15–38	15
	Mean	2046	15	<15
	σ	4008	7	?
Enterococci	Min–max	15–3290	<15–116	15–349
	Mean	740	28	80
	σ	907	34	132
P. aeruginosa	Min–max	0–35	1–300	5–320
	Mean	11	59	70
	σ	12	83	124

σ: Standard deviation.
The minimum concentration of total coliforms, *E. coli* and *Enterococci*, is 15 CFU 100 mL−[1].

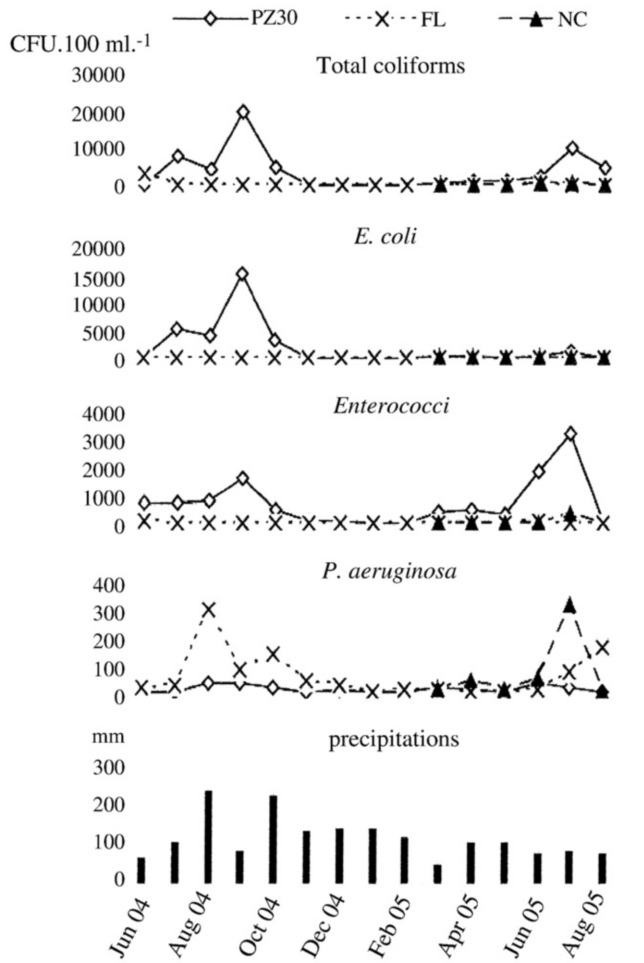

Fig. 3. Bacterial concentrations in piezometer 30 (PZ30), leachates from the former landfill (FL) and the new cell (NC), with monthly rainfall.

and 222 mm, in the order given). The maximum abundance of both bacteria measured in September 2004 coincides with a smaller amount of rain (80 mm).

The variation in bacterial densities seemed to be related to rainfall events at the beginning of the monitoring period. The increase in concentrations of bacteria was opposed to the amount of rainfall: weak rainfall induced high concentrations of total coliforms and *E. coli* whereas heavy rainfall generated low concentrations (August and October 2004).

Contrary to the general trend, *E. coli* was not, in July 2005, the dominant coliform in groundwater according to the ratio of *E. coli* (EC) densities to total coliforms (TC) densities (EC/TC ratio = 12.95%).

Concentrations of total coliforms and *E. coli* were low in leachate and remained quite stable over the monitoring period (mean values in FL: 308 and 237 CFU 100 mL^{-1}; in NC: 15 and <15 CFU 100 mL^{-1}, for total coliforms and *E. coli* respectively).

3.1.2. Enterococci

The *Enterococci* densities in PZ30 varied from 15 CFU 100 mL^{-1} in January and February 2005 to 3290 CFU 100 mL^{-1} in July 2005 (Table 1). The PZ30 curve shows two peaks in September 2004 (1673 CFU 100 mL^{-1}) and in July 2005 (3290 CFU 100 mL^{-1}). The similar trend is previously observed for total coliforms and *E. coli* concentrations with, nevertheless, a more pronounced peak in July 2005 for *Enterococci* (Fig. 3).

The concentrations of *Enterococci* were lower in the leachates than in PZ30, with a maximum of 116 CFU 100 mL^{-1} for the FL and 349 CFU 100 mL^{-1} for the NC.

3.1.3. P. aeruginosa

P. aeruginosa was low (max. 35 CFU 100 mL^{-1}) in groundwater (Fig. 3). Two peaks are however observed in September 2004 and June 2005, which coincide with an abrupt decline in rainfall amount.

P. aeruginosa concentrations were more important in the leachates. In the FL leachate, the maximum abundance (300 CFU 100 mL^{-1}) is in August 2004, which is followed by a sharp decrease to a second maximum in October 2004. These maximum concentrations of *P. aeruginosa* were observed during heavy rain episodes. In the NC leachate (no measurement prior to March 2005), the colony numbers vary from 3 in August 2005 to 320 CFU 100 mL^{-1} in July 2005.

3.2. Tracer test monitoring

3.2.1. Groundwater (PZ30)

Tracer breakthrough curves show two peaks which are synchronous for both tracers (Fig. 4). A significant problem arose at the beginning of the experiment: the monitoring started only on January 24th 2006 (D + 5). As a consequence, the beginning of the first peak was not monitored. The highest concentrations measured were probably not the maximum values of the peak but were, nevertheless, the maxima observed (E = 4681.6 μg L^{-1}; N = 28 μg L^{-1}). It is assumed that the first appearance of E and N occurred very rapidly after the injections. The beginning of the second peak takes place on D + 23 and coincides with the first precipitation event (10 mm) after an 18-day period without rain. This rainfall event intensified on February 16th 2006 (40 mm on D + 28) and concurred with the maxima of the second peak, which is very pronounced for E (E = 4375.8 μg L^{-1}; N = 15.6 μg L^{-1}). The frequency and intensity of rainfall events seem to contribute to the tracer breakthrough in groundwater (PZ30).

3.2.2. UC and NC discharge pipes

Both breakthrough curves display a similar trend (Fig. 4).

A progressive breakthrough of the tracers is observed from the injection to D + 13 in UC and to D + 17 in NC followed by a plateau phase (around 300 and 2500 μg L^{-1} for N and around 100 and 220 μg L^{-1} for E in UC and NC, respectively) and a sharp decrease (D + 25/30 for UC and D + 27/28 for NC). It must be noted that N first appeared several days after the injection (D + 4 in UC and D + 5 in NC), i.e. 1 day after the first rainfall (10 mm) in UC and 2 days after in NC. After a second peak on D + 42/43, a decrease is observed down to minimum values for E, probably corresponding to the fluorescence background levels whereas the N curve is irregular until the end of the experiment. A third increase is observed for N after the third rain period in NC.

The first rain event of the experimental period (January 23rd 2006: 10 mm) induced a sharp increase in the breakthrough of N whereas the increase in E was negligible on the same day. The second rain event (February 11th 2006: 10 mm) had no instantaneous incidence on the curves. An abrupt increase in E was however observed in NC a few days later. The rain event on February 16th 2006 (40 mm) seemed to induce instantaneously a strong decrease of both tracers in UC and NC. Although rain seemed to dilute rapidly the dye tracers, flow measurements at the end of the NC discharge pipe indicated that the pipe flow became more important 1–2 days after rainfall. After each rain period (Fig. 4), an increase in the breakthrough of N is clearly observed. The same trend was noticed for E, except that the increase was not identified after the third rain period. We visually observed the breakthrough of E and N in the discharged water from the SWTS pipe the day the tracers were injected. As a matter of fact, this indicates that the SWTS discharge pipe collects water which comes from the platform.

3.2.3. Maximum breakthroughs

The NC pipe discharges the highest concentrations of N and important concentrations of E (Table 2). The maximum breakthrough rate for E is observed in PZ30 while N concentrations were low. In UC, both tracers are present at significant amounts.

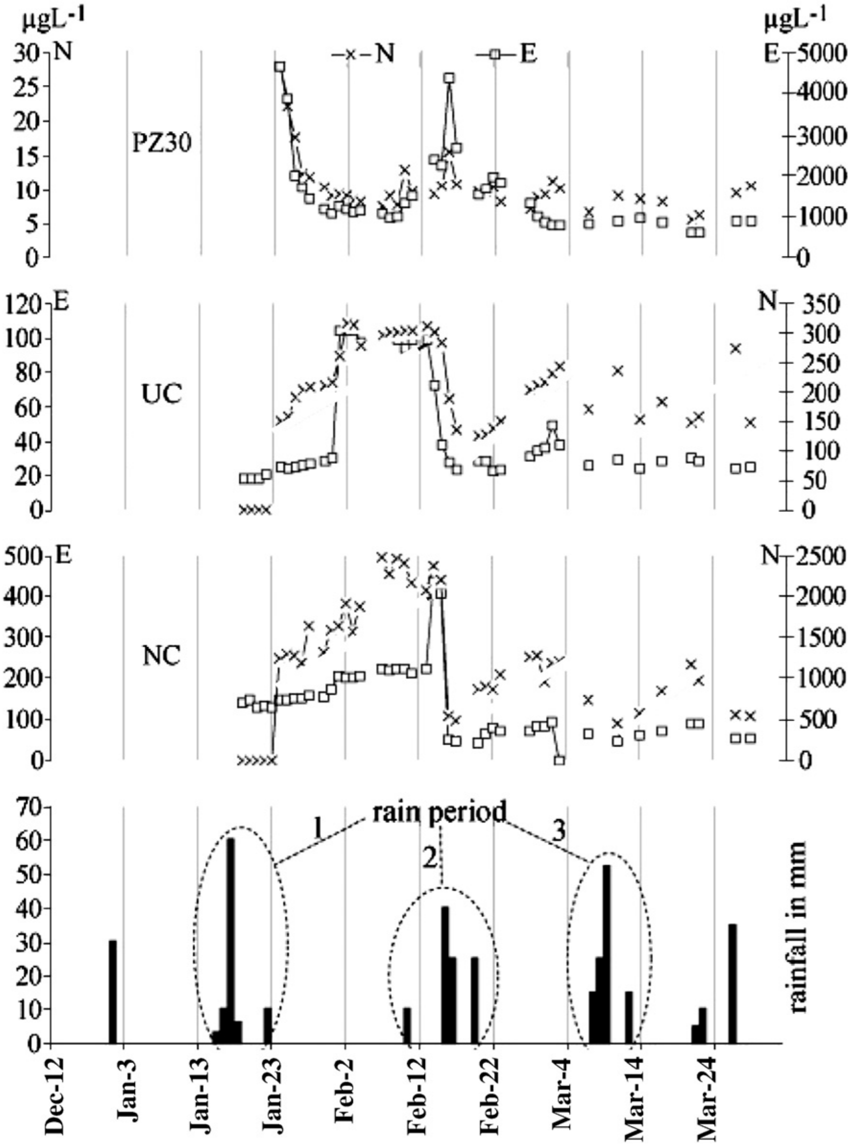

Fig. 4. Breakthrough of tracers N (naphthionate) and E (eosin) in PZ30, the former landfill (FL) pipe and the new cell (NC) pipe vs. time (January 19th 2006–March 27th 2006), with daily rainfall.

4. Discussion

4.1. Origin of bacteria in leachates

The maximum values measured for total coliforms in the leachates were 3000 CFU 100 mL^{-1} and for *Enterococci* 349 CFU 100 mL^{-1}. The only comparable values from the literature ($4.9 \cdot 10^4$ and 10^5 CFU 100 mL^{-1} respectively) are reported by the Washington State Department of Health [29] for a landfill also receiving hospital waste. The Etueffont landfill leachates contain low levels of total coliforms and concentrations of other faecal bacteria are even less significant (Table 1). Faecal coliform survival in natural environments is around 100 days [30] and this value is slightly larger for *Enterococci* [5,9–11]. As waste disposal stopped 4 years prior to the beginning of this study, the presence of faecal bacteria could not be attributed to the landfill. Animal dejecta could not either constitute the main source of faecal bacteria because the bird colony present on the site disappeared 15 days after site closure in July 2002 and very few animals were since seen in the surroundings of the landfill. Their presence in leachate may thus be explained by external inputs, highlighted by the dye tracer tests. The dye experiment showed that the SWTS discharge pipe was the main drain of the SWTS platform. However, the appearance of high amounts of N in the NC pipe seemed to indicate the existence of important leaks originated from the SWTS platform (Fig. 5). As the upper part of the ground was mainly composed by altered and fractured schists, leaching wastewater could easily flow through preferential pathways within the schists and reach the NC. Moreover, according to the significant concentrations of N found in the samples from the UC pipe, which collects water circulating under the NC, leaks from the geomembrane bottom liner of

Table 2
Maximum breakthroughs of eosin (E) and naphthionate (N) in groundwater and leachate (in μg L^{-1}).

	E	N
PZ30	4681.8	28
NC	436.8	2473.5
UC	104.2	316.6

PZ30: piezometer 30.
NC: new cell discharge pipe.
UC: discharge pipe which collects water under the new cell.

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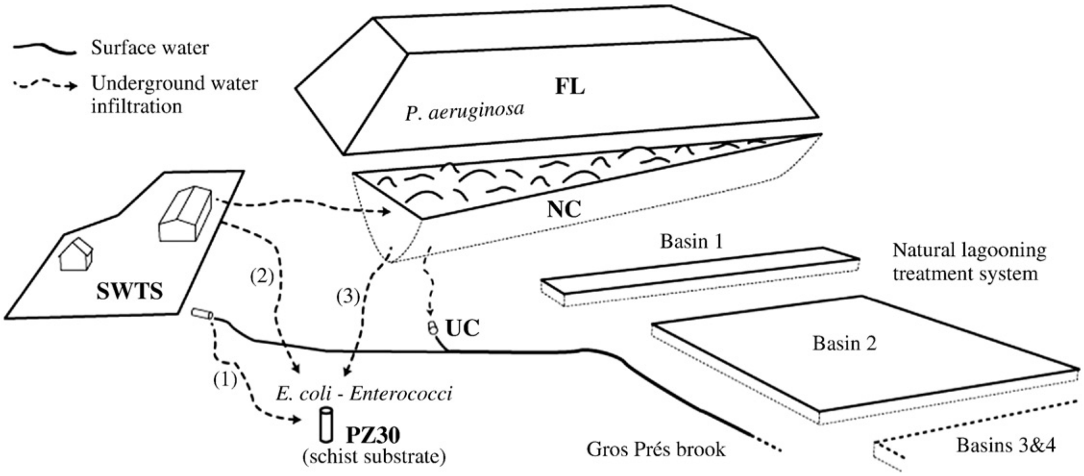

Fig. 5. Schematic map of infiltration pathways in the landfill area. PZ30 is contaminated by the infiltration of SWTS effluents (1), leaks from the SWTS platform (2) and leachate infiltration originated from the NC (3).

the NC should not be dismissed. Consequently, inflows of landfill leachate into the natural environment exist.

The flow measurements at the end of the NC pipe highlighted a shift between discharge and concentrations of tracers after each rain period. An instantaneous dilution effect due to the influx of infiltrating rainwater occurred during heavy rainfall periods but the discharge was more important a few days later. This indicates that rain water and leaching wastewater from the SWTS platform were trapped within the pore spaces of waste and constituted a potential "water storage reservoir".

Small amounts of E were also found in NC and UC, which suggests that leaks occurred in the downgradient side of the platform too. The tracer experiment highlighted the existence of leaks from the SWTS platform, implying leaks from the septic tank system. These results provide some explanations of the presence of faecal bacteria in the NC where waste disposal activity ceased 8 years ago.

P. aeruginosa was present at low levels in leachate, and its abundance increased with rainfall. As *P. aeruginosa* is saprophyte and is widely distributed in moist environments [14], its survival and growth in moisture pockets of the unsaturated zone of the FL is expected, as well as increases in its concentration with precipitation. The increase in *P. aeruginosa* concentrations may also be due to the leaching of bacteria living in wet areas in the unsaturated zone of the landfill. The maximum concentration measured (320 CFU 100 mL^{-1}) was however low compared to the 3500 CFU 100 mL^{-1} recorded in a slightly polluted aquifer (with only 60 CFU 100 mL^{-1} of faecal coliforms) [31].

4.2. Origin of bacteria in groundwater

In contaminated groundwater of non-karstic aquifers, coliforms are always present at concentrations below a few tenth to a few thousand CFU 100 mL^{-1} [32–35]. In the present study, they reached 2.10^4 CFU 100 mL^{-1} in PZ30. Sinton [16] showed that coliform concentrations are generally low in groundwater but may reach values up to 10^8 CFU 100 mL^{-1} in areas with a high density of septic tanks [36]. For septic tank effluents, values as high as 10^7 [37] to 10^{10} CFU 100 mL^{-1} have been recorded [38,39]. However, it should be noted that these values mostly depend on the effectiveness of the septic tank.

Maximum concentrations reported for *E. coli* in aquifers are of the order of magnitude of a few hundred CFU 100 mL^{-1} [40,41]. In this study, concentrations reached 15,000 CFU 100 mL^{-1} in PZ30. Sandhu et al. [36] showed that *E. coli* concentrations may reach 10^7 CFU 100 mL^{-1} near septic tanks, and septic tank effluents may have values as high as 10^5 CFU 100 mL^{-1} [42], 10^6 CFU 100 mL^{-1} [37] or even superior, depending on their efficiency.

Reported concentrations of *Enterococci* in non-karstic aquifers range from a few hundred [41] to a few thousand CFU 100 mL^{-1} [32,43]. In our study, the maximum density of *Enterococci* was 3290 CFU 100 mL^{-1} in PZ30. Sinton [16] found faecal *Streptococci* concentrations close to 10^7 CFU 100 mL^{-1} in septic tank effluents. Globally, most of faecal bacteria concentrations in PZ30 were superior to values generally measured in groundwater. Furthermore, they were much higher than the concentrations found in leachate. Considering *E. coli* and *Enterococci* are unable to proliferate in natural temperate environments [13,44], the faecal bacteria found in PZ30 could not originate from the waste deposited 4 years prior to the study. The dye tracer tests showed that the maximum breakthrough of E occurred in PZ30, located 10 m downstream from the SWTS discharge pipe. Be it reminded that wastewater was discharged into a drainage trench dug through altered schists. In accordance with the high concentrations of E measured in PZ30, it can be concluded that wastewater from the SWTS platform infiltrated into the ground and reached rapidly PZ30 (Fig. 5). The presence in PZ30 of high concentrations of *E. coli* and *Enterococci*, which are indicators of recent faecal contamination and the appearance of the N tracer, indicated that leaks from the septic tank occurred.

P. aeruginosa is present in the septic tank effluents at concentrations around 10^4 CFU 100 mL^{-1} [45]. Their density was very low in groundwater compared to the NC leachate. The tracer tests showed slight concentrations of N in PZ30 and highligted leaks from the bottom liner. The low concentrations of both N and *P. aeruginosa* found in PZ30 can be explained by the infiltration of small quantities of NC leachate into the ground, which migrates in groundwater. Therefore, the seepage of leachate through the geomembrane has a minor impact on groundwater quality.

5. Conclusions

This study presents new data on the concentrations of six bacterial groups in leachate from a municipal solid waste landfill and from groundwater of a schist aquifer, both contaminated by a septic tank system (Table 1). The sources of differences in the bacterial concentrations between the present study and literature (Table 2) are diverse environmental conditions but also the degree of contamination at the study sites. The faecal bacteria survival in municipal solid waste may be favoured by constant moisture as well as high levels of organic matter that induce bacterial growth. Because the presence of bacteria in high numbers in the aquifer is mainly due to a

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higher contamination, it is impossible to compare survival conditions provided in these habitats.

The use of bacterial density monitoring coupled to artificial tracer experiments proves to be useful in locating the exact source of these bacteria. Our study also demonstrates the effectiveness of this method to detect short-term pollution by faecal inputs. Moreover, our data provide further evidence that *E. coli* and *Enterococci*, known markers of recent faecal contamination are as reliable as fluorescent dye tracers (E and N). Finally, this study indicates that the landfill, which activity stopped in 2002, is not a significant source of faecal bacteria.

Acknowledgements

The authors wish to thank the SICTOM (Solid Waste Management Service) of Etueffont (Territoire de Belfort, France) and the ADEME (French Environment and Energy Management Agency) for their technical and financial support.

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Please cite this article as: E. Grisey, et al., Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests, Desalination (2010), doi:10.1016/j.desal.2010.05.007