Survival of *Legionella* in earthquake-induced soil disturbance (liquefaction) in residential areas, Christchurch, New Zealand: implications for disease

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ABSTRACT

AIM: To investigate a possible link between liquefaction dust exposure and the noticeable increase in legionellosis cases in response to major earthquakes in 2010 and 2011 that resulted in widespread soil disturbance (liquefaction) in parts of Christchurch, New Zealand.

METHOD: We culture tested liquefaction-affected soil for *Legionell*a spp. in the six months following the first earthquake in 2010. Thirty silt samples were collected randomly from locations within Christchurch's metropolitan area that were affected by liquefaction. The samples were tested to determine the presence of *Legionella* using qualitative and quantitative methods. Liquefaction-affected soil samples from three sites were further subjected to particle size distribution analysis and determination of major oxides. A controlled field study was established using six silt samples and one control (commercial compost), seeded with a wild-type strain of *Legionella bozemanae* serogroup (sg) 1 and persistence monitored over a 60-day period by culturing for the presence of *Legionella*. Dry matter determinations were undertaken so that total *Legionella* could be calculated on a dry weight basis.

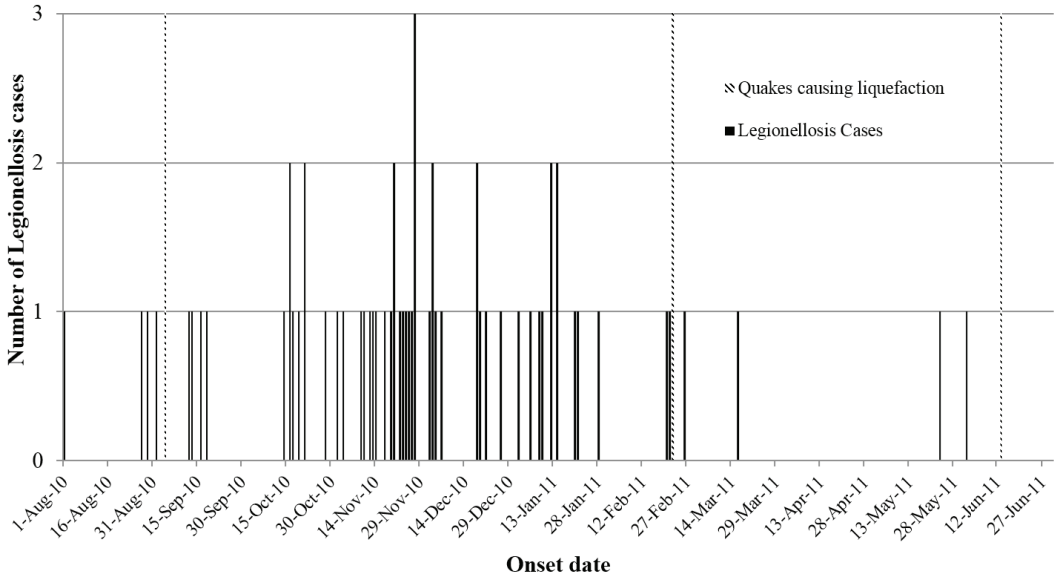
RESULTS: *Legionella* bacteria were undetectable after day one in the silt samples. However, *L. bozemanae* sg1 was detected in the control sample for the entire study period.

CONCLUSION: This study showed that the liquefaction-affected soil could not contribute directly to the observed increase in legionellosis cases after the earthquakes due to its inability to support growth and survival of the *Legionella* bacteria.

Legionellosis is an important notifiable disease often causing sporadic community-acquired pneumonia in New Zealand.[1] The predominant *Legionella* species responsible for disease are *L. pneumophila* and *L. longbeachae*; collectively contributing to greater than 80% of the laboratory-diagnosed cases each year.[1] However, *L. bozemanae* is the second most prevalent *Legionella* species isolated from compost material after *L. longbeachae* in New Zealand, although it is rarely associated with human disease.[1] The Canterbury region (including Christchurch) experiences a high rate of legionellosis relative to the rest of New Zealand.[2]

Active disease surveillance of legionellosis cases is reported annually and showed a step change in the numbers of laboratory-proven cases between 2009 and 2010, with 76 in 2009 and 178 in 2010.[3] Between September 2010 and March 2011, a combination of clinical testing, source tracing and case interviews identified a noticeable increase in compost-associated *Legionella* cases in Christchurch. This was well above the previous 10-year average: in 2009, 13 cases; 2010 52 cases, making a 300% year-on-year increase.[3] Elevated case numbers of legionellosis for the Canterbury region were again observed in 2011 (Figure 1A).

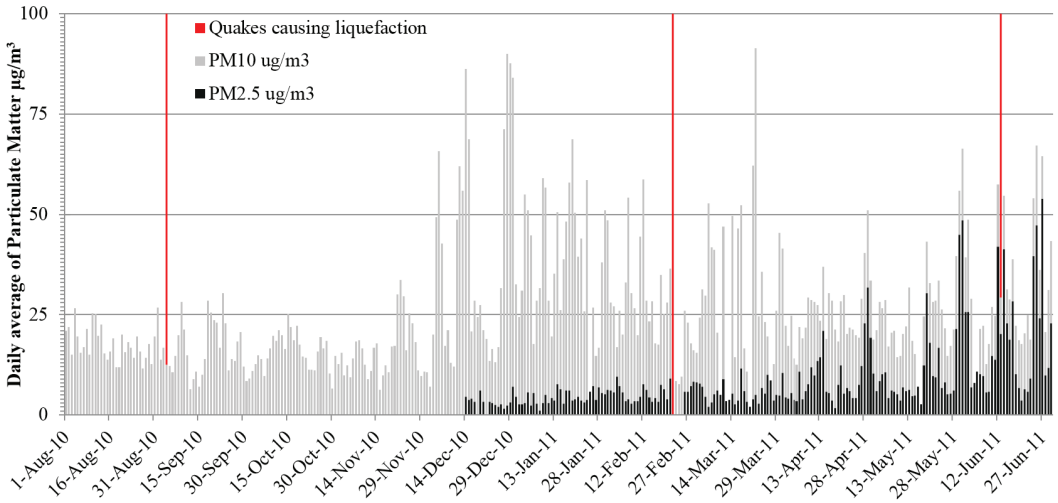
Figure 1A: Number of legionellosis cases in the Canterbury region.



Since legionellosis occurs following environmental exposure and infection, a significant environmental change that may have contributed to the observed increase in legionellosis cases may be the large increase in suspended particulate matter in the ambient air following the earthquakes. Between 4 September 2010 and 13 June 2011, the city of Christchurch was shaken by a series of strong earthquakes, and in particular the devastating 22 February 2011 earthquake, which resulted in 185 fatalities and produced widespread soil disturbance (liquefaction). Several other smaller or more distant earthquakes also produced further liquefaction in parts of the city for the remainder of 2011.[4] One of the most pervasive effects of the earthquakes was the widespread change in the below-ground environment due to liquefaction, with 10 distinct liquefaction episodes occurring between 4 September 2010 and 23 December 2011.[5]

The increased atmospheric $PM_{2.5}$ and PM_{10} (Particulate Matter smaller than 2.5 and 10 micrometres) concentrations was the result of the presence of earthquake-induced soil disturbance (liquefaction) dispersed by the prevailing wind and vehicle movement (Figure 1B). In an attempt to explain the

Figure 1B: Daily concentrations of Particulate Matter (column height) comprising of $PM_{2.5}$ and PM_{10}.



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sudden increase in case numbers and in particular the increase in cases in the Canterbury region following the Canterbury earthquake series, we postulate that dust inhalation may have predisposed a case to an invasive infection/disease like legionellosis when exposed to the organism via contact with compost or soil/silt due to damaged lung tissue causing inflammation. Inflammation could allow opportunistic pathogens such as *Legionella* bacteria, to more successfully infect the human host. The scant research in this area has tended to evaluate the potential relationship between dust inhalation and lung inflammation in occupational settings and seasonal climatic events.[6]

Liquefaction was most severe in residential areas located to the east of Christchurch's Central Business District (CBD) as a result of stronger ground shaking due to the proximity of the causative fault, a high groundwater table approximately 1m from the surface and soils with states of high susceptibility and potential for liquefaction.[7] Figure 2 shows areas of different liquefaction severity, which include: (a) moderate to severe liquefaction including sand ejecta, large cracks and fissures in the ground and significant liquefaction-induced impacts on buildings; (b) low to moderate liquefaction with generally similar features as for the severe liquefaction, but of lesser extent and intensity; and (c) minor liquefaction primarily affecting roads.[7]

The earthquakes in Canterbury provided a unique opportunity to investigate a possible causal effect peculiar to *Legionella* and its pathogenesis that we want to explore further. Overseas studies have shown that in the aftermath of a catastrophic earthquake, the immersion in tsunami waters is a risk factor for infection caused by *Legionella* spp.[8] However, very little is known about the microbiological agents that may reside in liquefaction-affected soil or dust. Trials conducted overseas have shown that sterile soil was colonised by the *Legionella* bacteria from the atmosphere within 25 days (the first sampling), however, to date this has never been replicated outside controlled conditions,[9] so as yet it is unclear whether exposure to *Legionella* species in natural soil can lead to disease.[10] Therefore, the key research questions were: (1) whether *Legionella* bacteria is present in the liquefaction-affected soil and (2) if present, how long can *Legionella* survive in the liquefaction-affected soil, following a major earthquake and in turn be a potential exposure route for this pathogen through the inhalation of aerosolised liquefaction-affected soil. The objective of answering these questions was to establish a possible direct link between exposure to the liquefaction soil and the observed elevation in legionellosis cases in the study area following the earthquakes in 2010 and 2011.

Materials and methods

Legionellosis case data collection

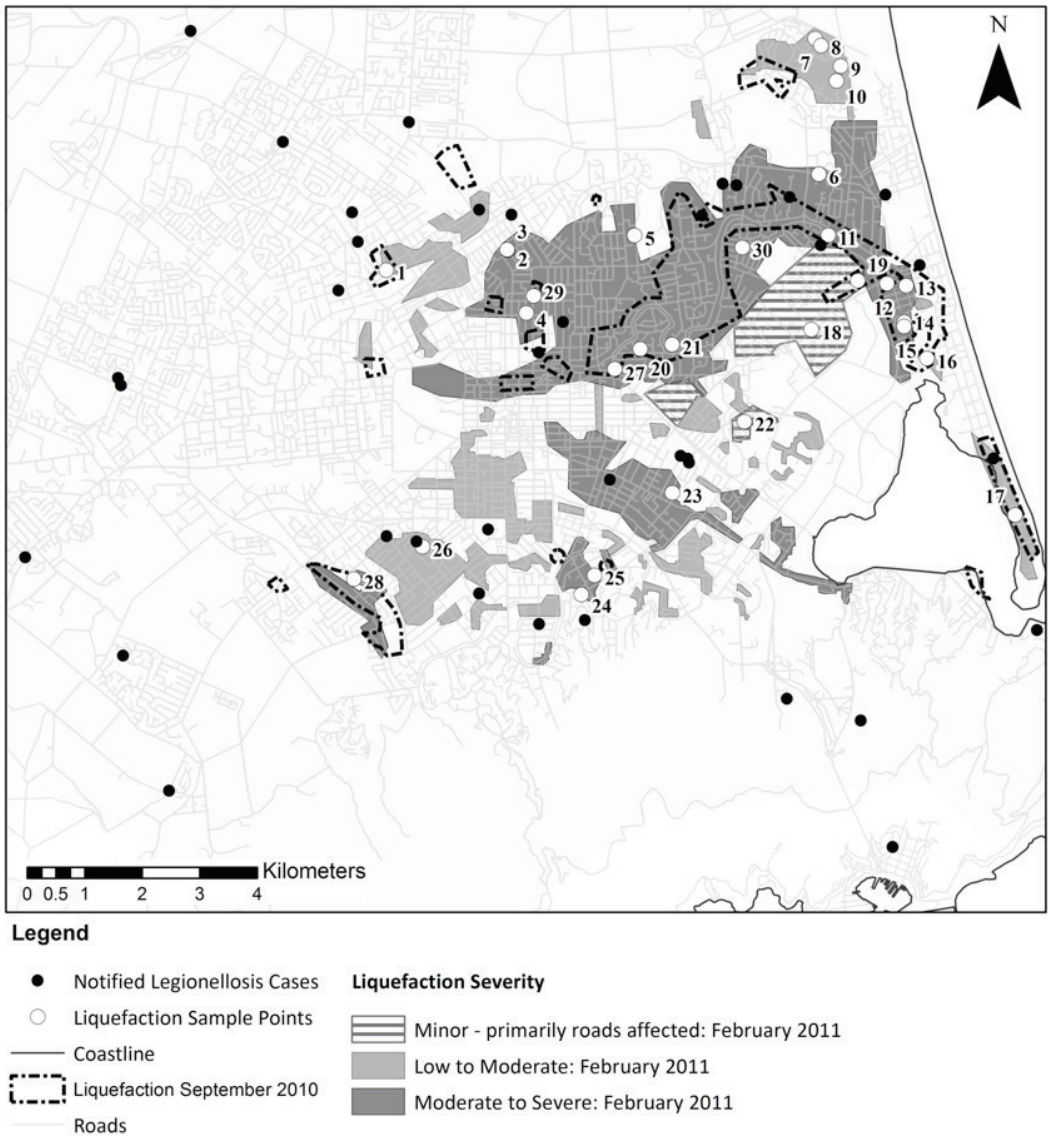
In New Zealand, infections caused by any *Legionella* species are notifiable under the Health Act 1956. All notified laboratory-confirmed cases of legionellosis were obtained from the National Notifiable Disease Surveillance system (EpiSurv), operated and managed since 1996 by the Institute of Environmental Science and Research Ltd (ESR), under contract to the Ministry of Health. The data was accessed to obtain the residential location of all cases between 1 August 2010 and 30 June 2011. Using a geographic information system (GIS), the location of the residential locations were overlaid on the map of the study area (Figure 2).

Sample collection

On 22 November 2011, 30 samples of undisturbed liquefaction-affected soil were collected within the metropolitan area of Christchurch affected by soil disturbance (liquefaction), including areas where there were confirmed cases of legionellosis reported (Figure 2). To ensure objectivity, digitised address points within the liquefaction-affected areas were randomly selected. Using GIS, the location of the sampling sites were overlaid on areas of observed liquefaction, which were based on drive-through reconnaissance that was conducted in the period from 23 February to 1 March 2011.[11]

Liquefaction-affected soil samples of about 250g (dry weight) were obtained from undisturbed sites at a depth greater than 1cm to avoid inclusion of non-silt contaminants using a sterile spoon and placed in plastic bags. Samples were transported in an insulated bin to ESR and stored in sealed plastic

Figure 2: Areas of liquefaction severity and location of notified cases and sampling addresses within the liquefaction area of urban Christchurch.



bags at ambient temperature, in the dark. The material sampled was geographically representative of the liquefaction-affected soil. All 30 samples were cultured on a 90mm diameter plate with Glycine-Vancomycin-Polymyxin-Cycloheximide (GVPC) media by ESR for the presence of *Legionella* in accordance with Steele et al,[12,13] who set out a qualitative test method for testing of potting mixes, composts and other solid matrices for *Legionella* species. A quantitative approach was used measuring the total *Legionella* by plate count. A range of dilutions were plated after acid treatment.

Persistence modelling of *Legionella* in liquefaction soil

To establish if liquefaction soil could sustain the prolonged survival of *Legionella* bacteria, the following experiment was carried out: six liquefaction-affected soil samples (transcending west to east sample—nos. 3, 10, 17, 21, 23 and 26) were randomly selected and seeded with the *Legionella bozemanae* for the persistence modelling experiment. This species is the second most prevalent *Legionella* species isolated from compost material after *L. longbeachae* in New Zealand, and has been

demonstrated to survive in aerosol derived from composted material.[1,14,15] *L. bozemanae* was chosen in the seeding experiment because unlike *L. longbeachae* and *L. pneumophila,* it fluoresces blue under ultra violet (black lamp) illumination. This assists with more accurate and quicker counting of the seeded *Legionella* colonies among mixed bacterial growth on plate culture. The seeded samples and controls were placed outside from 1 November 2012 until 30 March 2013 to mimic ambient climatic conditions. This was because in New Zealand the peak incidence of legionellosis occurs from late spring (November) until autumn (March) and is possibly related to increased gardening activity and use of compost during this period.[1] A compost sample obtained from a commercial supplier in Christchurch that was proven culture-negative for *Legionella* was used as a comparator (control) in the persistence modelling experiment, as compost is known to support the growth and persistence of legionellae.

Due to the limited amount of liquefaction material available for the persistence modelling under field conditions, approximately 200g (wet weight) of sample was placed in free-draining plastic tubs with a diameter of 10cm and a depth of 15cm. The tubs were buried to the top in a gravel bed and placed outdoors at ESR's research facility located at Porirua, Wellington to expose the liquefaction material to normal environmental conditions. At each sampling time point, the whole of the material in each tub was mixed thoroughly and a single 4–6g grab sample was taken for *Legionella* culture testing and dry matter determinations. The 'Day O' samples were collected immediately after the inoculum had been added and tested. Further samples for testing for *Legionella* culture and dry matter determinations were taken on Days 1, 7, 30 and 60. The dry weight measurement of the samples was used when determining the *Legionella* concentration, as this was more accurate than using the wet weight since moisture levels varied significantly between the samples and over the sampling period. Organic matter content (%OM) of the six samples was determined as weight loss on ignition (LOI)[16] by ashing a representative sediment sub-sample at 400°C for four hours.

Silt characterisation: elemental composition and particle size

Particle size distributions in disaggregated samples from the bulk material were determined using a Beckman-Coulter laser diffraction particle size analyser. The samples from the bulk material were dried and sieved to exclude particles >2000µm (2mm) diameter prior to analysis. Gradistat V8,[17] a Microsoft Excel format program, was used to analyse the grain-size data and to determine parameters, such as the percentage of sand, silt and clay, as well as mean and median grain-size, sorting and skewness.

Major oxides were determined using a Panalytical MiniPal-4 X-ray fluorescence (XRF) spectrometer. A fused disk of each sample was made by mixing 1g of sample with 10g of Lithium-Borate flux and melting the powder in an induction furnace at 1100°C. The melt was then poured into a cast and allowed to cool. Each disk was then analysed three times by XRF and an average of the results taken.

Meteorological investigation

For the field experiment, meteorological data for 1 November 2012 to 31 January 2013 was retrospectively accessed from CliFlo, a web-based system that provides access to the National Climate Database, New Zealand's principal repository of meteorological data (http://cliflo.niwa.co.nz/). This system is hosted by the National Institute of Water and Atmospheric Research, a crown research institute. Data (minimum and maximum) temperature, precipitation and relative humidity were sourced from a fixed continuous monitoring station located in metropolitan Wellington.

Microbiological: Preparation of *L. bozemanae* serogroup 1 inoculum

The *L. bozemanae serogroup 1* strain used in this study was a wild-type strain isolated from the same commercial compost brand as the control material. This strain was chosen to avoid using a laboratory-adapted strain. The strain was identified by mip-gene sequence analysis and using commercial DFA reagents. The strain auto-fluoresced bright blue when exposed to black light (UV 660 nm). The *L. bozemanae* strain was sub-cultured onto buffered charcoal yeast extract (BCYE) agar plates (Oxoid)

for three days at 36°C. The plate grown *L. bozemanae* was then used to seed a liquid culture of AYE medium supplemented with BSA (0.5%)[18] and was grown for 48h at 36°C (post-exponential growth phase). The concentration of *L. bozemanae* serogroup 1 in the prepared inoculum was measured by a direct spread plate method: 100µL 10-fold serial dilutions of the final inoculum were cultured on BCYE agar plates and the colonies counted after three days growth at 36°C. The prepared liquid inoculum *of L. bozemanae* serogroup 1 was used to seed each of the liquefaction soil samples and also the 'control compost' material.

Seeding and sampling procedure

All samples used in the study had been previously cultured for Legionellae and were found to be culture-negative. A total of 2.4mL of the *L. bozemanae* inoculum was slowly seeded into each soil sample while undergoing continual stirring to ensure even mixing. The concentration of the *L. bozemanae* sg1 inoculum was determined by 10-fold dilution plating in triplicate from 10^3 to 10^7 and calculated to be $4.08x10^6$ colony forming units (cfu). The concentration of culturable *L. bozemanae* organisms inoculated into each liquefaction sample at time zero and at each subsequent sampling time point was determined by using dilution plating in triplicate at 10^0, 10^1, 10^2 and 10^3. The 'Day 0' samples were collected immediately after the inoculum had been added by taking between 4–6g of the seeded material and cultured for the presence of *Legionella* using the method prescribed in Steele et al.[12] Subsequent samples were collected from each sample on Day 1, Day 7, Day 30 and Day 60, and tested in the same manner. Dry matter determinations were carried out on each sample day so that total *Legionella* concentration could be calculated on a dry weight basis. The concentration of total *Legionella* isolated from each sample at each time point was expressed as the number of *Legionella* colonies growing by plate culture per gram of the original sample using the dry weight determination to correct for the varying moisture content of the sample material.

Results

Microbiological examination of liquefaction silt samples

All 30 liquefaction-affected soil samples were culture-negative for Legionellae. Table 1 shows that a large proportion (80%) of the 30 samples tested had less than 200 microbial (bacterial and fungal) colonies on the plate surface. This was unexpected as the soil samples generally contained high levels of culturable microflora.

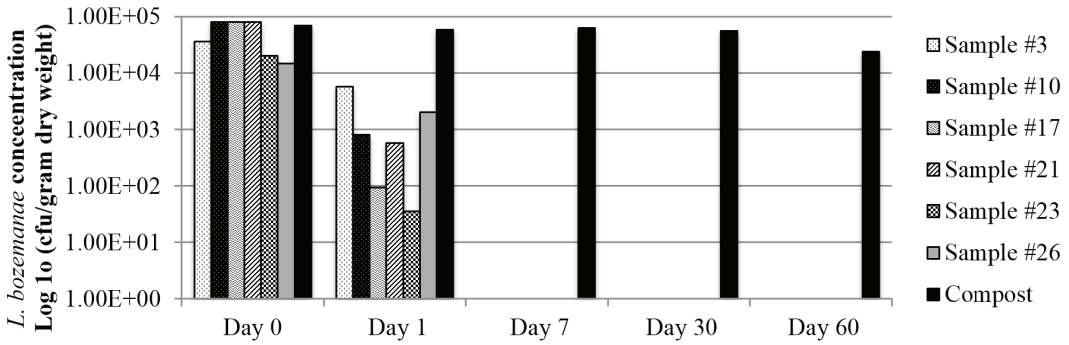
Persistence study using *Legionella*-seeded liquefaction-affected soil

The results for microbial persistence/die-off of *L. bozemanae* in the controlled field experiment are presented in Figures 3A and B. *L. bozemanae* was detectable one day after seeding of liquefaction-affected soil with the bacteria (Figure 3A). For the field samples the moisture content changes depending on precipitation, temperature and wind and varied over the course of the experiment, whereas the dry matter remains constant. The only sample in which the *Legionella* was detected over the full-time course of the field experiment was the seeded commercial compost sample.

Table 1: Microbial growth on GVPC plate medium.

Total (%) number of liquefaction samples (N=30)	Relative microbial growth on GVPC medium (cfu – colony forming unit)	Number of samples from which *Legionella* species isolated
43.3	Low: <50 cfu	None—not detected
36.7	Medium: 51–200 cfu	None—not detected
20	High: ≥201 cfu	None—not detected

Figure 3A: Persistence of *L. bozemanae* in seeded liquefaction-affected soil and compost.



Silt characterisation: elemental composition and particle size

Elemental composition of major oxides present in liquefaction-affected soil is summarised in Appendix 1. Of particular note are the concentrations of silica (SiO_2), which were 72.6%, 76.2% and 76.3% on a mass basis. These results indicate that there is the potential for airborne particulate matter to contain a high proportion of crystalline silica. The analysis also found that 11% of the liquefaction-affected soil was aluminium oxide, which occurs naturally in silicates.[19] The loss-on-ignition results showed low levels of organic carbon in the liquefaction samples, indicating low organic matter.

Figure 4 shows the results of the particle size analysis for sample numbers 10, 21 and 26. The particle size analysis indicated that the liquefaction ejecta were generally very similar to ejected sediment seen elsewhere in Christchurch. Sample No. 26 indicates that the liquefaction ejecta is finer grained than the sand it passes through. Samples Nos 10 and 21 show a well-sorted sand which is very similar to beach sand.

Meteorological investigation

When values for meteorological parameters for the field experiment were compared with a 30-year historical mean, the key finding was that the total rainfall between 1 November 2012 to 31 January 2013 was higher than average (Appendix 2).

Figure 3B: Comparative moisture content over time for the seeded liquefaction-affected soil and compost.

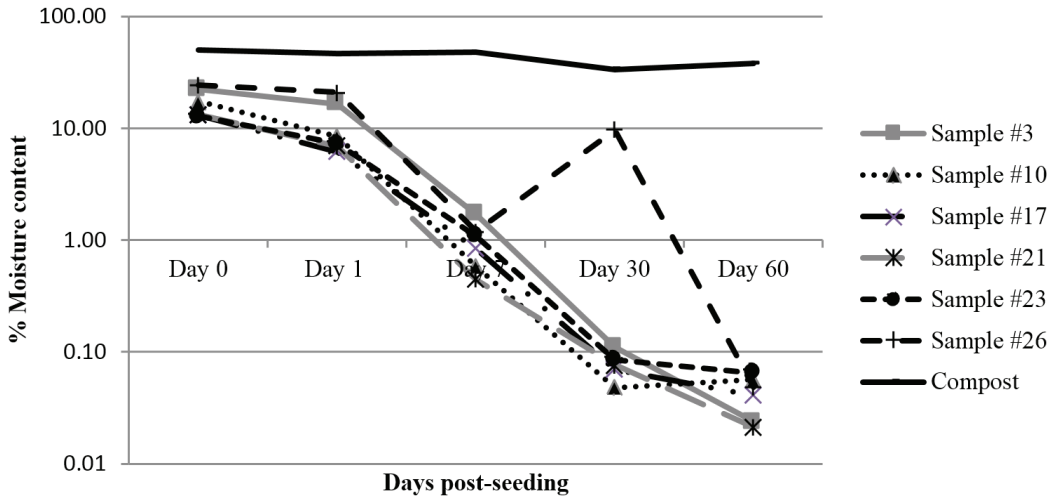


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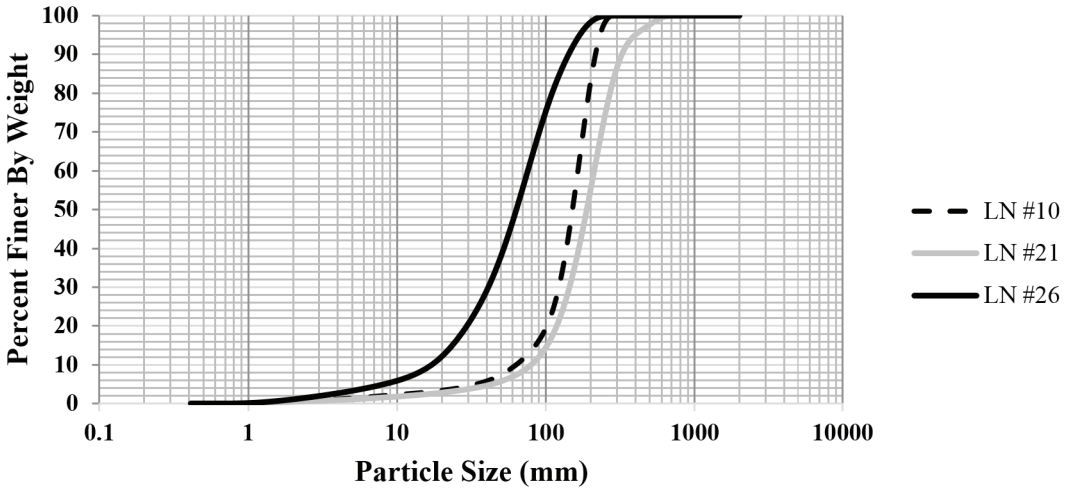
Figure 4: Laser particle size analysis results for three liquefaction samples.



Discussion

Difficulties in isolating legionellae directly from natural organic matter in soil and the focus on manufactured composts and potting mixes[20] may account for the paucity of reported investigations of indigenous soil for *Legionella* spp.[13] A series of damaging earthquakes near Christchurch, New Zealand during 2010 and 2011 provided a unique opportunity to study the survival of *Legionella* bacteria in the associated liquefaction silt generated by these seismic events. The interest in investigating liquefaction silt for the presence and persistence of *Legionella* bacteria was an attempt to elucidate a cause for the observed large and sharp increase in the number of *Legionella* infections in Christchurch following the earthquakes. The observed increase in *Legionella* infections closely aligned with an increase in atmospheric PM_{10} concentrations as a result of the aerosolisation of liquefaction-affected silt, especially following the 22 Feb 2011 quake. This points to environmental dust particles being a likely risk factor for legionellosis.[1,21] The increase in case numbers also coincided with a change in the testing algorithm for *Legionella* infection at Canterbury Health Laboratories (CHL) from August 2010 with the introduction of *Legionella* polymerase chain reaction (PCR) testing.[22] These cases dropped significantly after February 2011 with no cases identified between April and June 2011. However, there has been no change in the testing protocol at CHL since September 2010, suggesting the large spike in cases over the summer was related to seasonal factors, compost use or confounding factors such as weather and source material other than liquefaction-affected silt.

During the liquefaction process, subsoil was ejected vertically onto the existing soil surface. When designing our study, one of our primary goals was to develop a snapshot of the effects of earthquake-induced disturbance on the survival of pathogenic bacteria such as *Legionella*. The methods used in this study, while they were capable of detecting 10^3 cfu of Legionellae per gram of liquefaction-affected soil, did not detect any populations from the 30 random samples. A controlled field investigation to demonstrate multiplication of Legionellae in liquefaction-affected soils also gave negative results after Day 1. The inability of the liquefaction-affected soil to retain moisture compared to the compost under the same weather conditions may have contributed to the rapid die-off in the *Legionella* seed (Figure 3B). Moisture retention ability is proportionately linked to the amount of organic matter in the sample. This influences the presence and make-up of the microflora in the silt, and these may be natural hosts for *Legionella* bacteria. It is possible that small numbers of Legionellae in some source material could multiply to form populations of detectable size given that samples were collected in areas where this disease was recorded. The nutritional requirements of the Legionellae dictate that they live communally with other species or as parasites. Indeed, when cultured,

Legionella bacteria require a medium that supplies them with amino acids as their main carbon source as well as fulfilling a specific need for L-cysteine and iron (Fe).[23] Decaying organic matter may support *Legionella* growth, as suggested by earlier studies of aquatic bacterial utilisation of nutrients released by excretion and decomposition of algae.[24] Our study showed that liquefaction-affected soils had low organic matter content consistent with other findings[25] and was nutrient deficient, having low concentrations of exchangeable bases (Fe, Ca, Mg, Na). A study of agricultural land affected by liquefaction following the 4 September 2010 earthquake found the sandy textured soils to have low water-holding capacity.[25] In addition to the chemical limitations of the sandy sediment as a growing medium for supporting the growth of Legionellae, and the possibility of negatively impacting hydrology, there is speculation that soil aeration was also negatively impacted. Because liquefied soil has been known to compress as it dewaters, fine sand may have reduced aeration.[26] This is significant since as aerobes, *Legionella* also require oxygen, either ambient or supplied by the other organisms with which they are associated, hence their preference for air-water surface bio-films.[27]

The hypothesis was developed that liquefaction-affected soil may contain *Legionella*. After all, the ability of *Legionella* to adapt and withstand stressful changes in their environment is well documented. Also, many studies that have demonstrated *Legionella* bacteria can gain a large measure of environmental protection while living within host cells. For example, amoebae have been found to protect the intracellular Legionellae from temperature extremes, saline conditions, increased external osmopolarity,[28] oxygen deprivation, biocides such as 50ppm chlorine from hypochlorite[29] and dehydration. Indeed, when conditions become too dry, the protozoan host cell will encyst and enter a dormant phase to await rehydration, protecting the Legionellae within host cells.[30] It may explain how *L. longbeachae* or *L. bozemanae* (among other species) survives in the non-liquid medium of compost when it dries out.

Amoebal life consists of cycles of encystment and excystment according to available moisture.[31] When encysted, they can survive from months to years, and at any one time in the soil, a good proportion of the population will be encysted.[31] The drier the soil environment, the higher the percentage in this dormant form. The drying out of liquefaction-affected soils may have allowed any *Legionella* present to survive and when the dried soil becomes airborne, pose an infection risk. A limitation of this paper is the lack of testing for the presence of amoebae in the liquefaction soil, which has resulted in a lack of evidence to support this argument.

The low moisture content and the observed relatively low abundance of other culturable microorganisms detected on plate culture, along with the relatively low organic matter in the liquefaction-affected soil all contribute to it not harbouring *Legionella* bacteria and, it can be assumed, a lack of host organisms. In the field experiment where liquefaction-affected soil was deliberately contaminated (seeded) with a heavy inoculum of *Legionella* bacteria, it did not persist for more than one day, contrary to a seeded commercial compost sample exposed to the same conditions. This adds to the evidence that *Legionella* bacteria do not survive adverse environmental conditions readily without the support of host organisms, such as those naturally present in organic material such as compost.

While the persistence of *Legionella* in some matrices can occur due to protection, the exposure of *Legionella* bacteria to sunlight, high temperature and low humidity is likely to inactivate them. The low to non-existent organic content in the liquefaction-affected soil is likely to decrease the presence of *Legionella* hosts. Collectively, this suggests that liquefaction-affected soil that becomes airborne as dust (liquefaction silt dust) is unlikely to represent a frequent exposure route to infectious organisms such as *Legionella*. This observation was consistent with this study's findings that all of the residential liquefaction-affected soil samples analysed did not show evidence of the *Legionella* bacteria.

Chemical and size analysis of the liquefaction-affected soil shows it consisted of >65% silica and between 2–8% (w/w) is <10µm in diameter, of which 30% of the respirable dust was quartz that was less than 10µm

in size (Figure 4). This contributed to the increased number of high-pollution days.[32,33] It is also known that airborne particulates can cause inflammation and increase the risk of respiratory infections.[34,35] We propose that inhalation of earthquake-associated airborne liquefaction-affected soil can damage lung tissue and cause inflammation. Inflammation and damage could allow opportunistic pathogens, such as *Legionella* bacteria, to more successfully infect the human host. Inflammation of the lung epithelial cells results in an influx of macrophages to the site. This may be a similar mechanism resulting in the seasonal increase in meningococcal meningitis seen in sub-Saharan Africa (particles ranging from 0.55 to 7.9µm in size).[6] The inhalation of silica dust is known to increase the risk of lung infections, including pulmonary tuberculosis.[36] It is thought that the inhalation of crystalline silica in inorganic dust including quartz damages the ability of pulmonary macrophages to kill bacteria. This is because crystalline silica has sharp faces rather than the round edges of sedimentary quartz, so the lungs cannot expel the sharp minute crystals and the silica (quartz) is retained in the lungs where it may eventually cause disease due to lung epithelial cell damage and even death.[37] Other studies found that the inhalation of inorganic dust (including quartz) increased mortality from infectious pneumonias, especially lobar pneumonia and pneumococcal pneumonia among construction workers.[38] These factors combine to suggest that dust inhalation could then predispose a case to an infection/disease like legionellosis when exposed to the organism. The scant research in this area has tended to evaluate the potential relationship in occupational settings and seasonal climatic events. Little is known about the relationship between *Legionella* infection and events that result in elevated levels of airborne dust such as aerosolised liquefaction-affected soil following a major earthquake.

Another plausible explanation for the increased notifications might be physical disturbance of the water systems. This may be caused by two different actions. Firstly, by physically shaken and movement of reticulated water lines. Recent studies have shown the presence of *Legionella* and other opportunists in disinfected water distribution systems.[39] Disturbance of such pipelines might cause release of biofilm, and surface attached microorganisms creating a 'pseudo-bloom' of *Legionella* at points of use. Secondly, the ingress of silica-based material such as that generated during the liquefaction event may have caused scouring of the reticulated water system and building water systems.[27] This would also lead to elevated numbers of bacteria being released at point of use. Either or both of these events combined might explain an increase in the cases of water-associated legionellosis immediately subsequent to earthquake events.

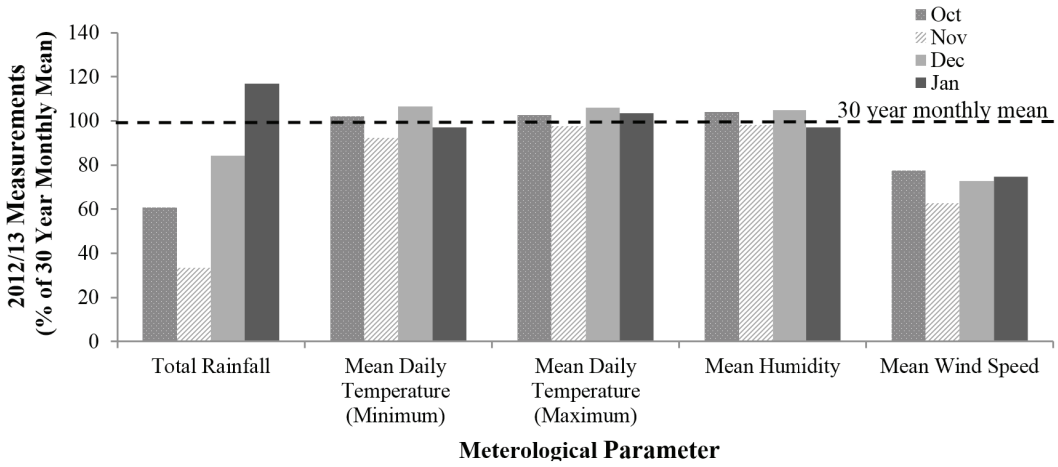
In conclusion, this study set out to expand our understanding of the environmental exposure risks to *Legionella* and whether seemingly unrelated environmental factors, such as aerosolised liquefaction-affected soil resulting from the Christchurch earthquakes had the potential to impact on disease prevalence. No direct causal link between exposure to liquefaction-affected soils/silt and legionellosis was established since no Legionellae were isolated from any samples tested and *Legionella* was shown not to survive in the seeded silt. This does not provide support for the notion that aerosolised liquefaction soil/silt (quartz silica, per se) could be a vector for *Legionella* infection with the methodology followed in this study. Disturbance of and infiltration of liquefaction material into distributions lines cannot be discounted as a possible contributing factor. The contribution of an increased amount of airborne dust on the incidence of legionellosis as a predisposing factor for infection should exposure to the bacteria occur, is still unknown. In addition, the impact of respiratory tract epithelial injury from particulate matter on host susceptibility to opportunistic infections like *Legionella* should be considered. In other words, whether it may reduce immune-system function leading to increased susceptibility to *Legionella*. With careful study design this could add considerably to our understanding of the interactions between environmental events and invasive diseases processes.

Appendix 1: X-ray fluorescence major oxide analyses.

	ChCh 10	Ave of 3	ChCh 21	Ave of 3	ChCh 26	Ave of 3
	Average	2sd%	Average	2sd%	Average	2sd%
SiO_2	76.314	0.239	76.199	0.249	72.554	0.285
TiO_2	0.398	2.527	0.406	1.706	0.482	1.726
Al_2O_3	11.596	0.926	11.716	0.558	13.193	0.523
Fe_2O_3	2.700	0.074	2.553	0.431	3.134	0.327
MnO	0.041	4.878	0.081	1.420	0.044	2.644
MgO	0.778	8.783	0.786	2.701	1.001	15.900
CaO	0.931	2.316	0.954	1.348	1.073	1.456
Na_2O	2.936	2.264	2.876	6.331	3.051	2.503
K_2O	2.394	0.720	2.346	0.323	2.711	0.777
P_2O_5	0.093	7.525	0.098	1.450	0.109	2.794
L.O.I.	1.924	19.545	1.998	17.004	2.745	10.139

LOI = loss on ignition at 1000◦C for one hour.
Results are expressed as weight % on oven dried (110◦C).

Appendix 2: Comparison of meteorological parameters for October–December 2012 and January 2013 with 30 years monthly means (1981–2010), using percentage of the mean.



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