Communication

Investigation into the Cause of Iron-Related Clogging of Groundwater Bores Used for Viticulture in the Limestone Coast, South Australia

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Abstract: Iron-related clogging of boreholes, pumps and dripper lines is a significant and costly problem for irrigators worldwide. The primary cause of iron-related clogging is still debated. Previous studies have described complex interactions between biological clogging and inorganic iron/manganese oxide precipitation. This case study examined groundwater bores used for viticulture irrigation in the Limestone Coast region, a highly productive wine growing area in the SE of South Australia. Iron clogging of bore screens, pumps and dripper systems has been a persistent problem in the region and the issue is perceived to be growing, with irrigators suggesting the widespread introduction of iron-related bacteria (IRB) through drilling equipment to be the root cause of the problem. Analysis of the groundwater microbiology and inorganic chemistry found no apparent correlation between the presence of IRB and the clogging status of wells. In fact, IRB proved to be widespread throughout the limestone aquifer. However, a clear correlation could be found between clogging affected bores and the redox potential of the groundwater with the most severely affected bores strongly oversaturated in respect to iron oxide minerals. Elevated dissolved concentrations of Fe(II) thereby tended to be found in deeper bores, which also were generally more recently drilled. Following decades of less than average rainfall, a tendency to deepen bores in response to widespread declines in water levels has been documented for the SE of South Australia. The gradually widening clogging problem in the region is postulated to be related to the changes in climate in the region, with irrigators increasingly driven to rely on deeper, anoxic iron-rich groundwater resources.

Keywords: iron-clogging; iron-related bacteria; microbiological iron clogging; inorganic iron clogging

1. Introduction

Iron clogging within aquifers and of irrigation infrastructure is a well-known and costly problem worldwide [1,2]. The most common problems for water supply bores are the clogging of screens and filter material, thereby reducing the subsurface permeability and the coating of pipes and pumps, which reduces water throughflow rates. If the produced groundwater feeds into an irrigation system, clogging of irrigation pipes is often an added problem [3]. While iron-related clogging is widespread and has been reported from a variety of environments, the cause of the incrustation and the environmental and/or operational factors contributing to the problem are still a matter of debate. This is partly

due to uncertainty in whether microbial activity or inorganic iron precipitation is the root cause of the problem [2].

Inorganic precipitation of iron oxides is a common occurrence in boreholes penetrating reducing groundwaters with sufficient concentration of dissolved ferric iron [4–6]. A borehole facilitates the exposure of these reducing groundwaters to atmospheric conditions allowing dissolved iron to oxidize. Abstraction may enhance the mixing of aerated and reducing waters within the well due to turbulent flow conditions, further increasing the ability for iron to precipitate. Once incrustation has commenced, the catalytic effect of iron oxides on the oxidation of Fe(II) accelerates the rate of precipitation, enhancing the problem further [7].

Iron-related biological clogging, however, is due to the metabolic activity of iron-related bacteria (IRB) such as *Gallionella* spp., *Leptothrix* spp., *Thiobacillus* spp. and *Sphaerotilus* spp. [8,9]. These bacteria can facilitate the transformation of soluble ferrous ions (Fe^{2+}) to ferric (Fe^{3+}) compounds with low solubility [10] and are commonly found in groundwaters [11,12]. IRB can develop and be incorporated into biofilms, which are a community of microorganisms attached to a surface by extracellular polymeric substances ((EPS) composed mainly of polysaccharides, proteins and DNA) [13]. IRB within biofilms are able to multiply profusely in boreholes due to the constant supply of nutrients that occurs through pumping [14]. Their adhesive properties facilitate the colonization of bore casings and screens, enabling them to persist despite high flow velocities [15]. This results in the build-up of solids (iron sulphides and iron hydroxides) and/or slime deposits (EPS) within the bore, pump and/or pipes [16–18].

The importance of microbial versus chemical iron oxidation is still a matter of debate. Under neutral pH conditions, the oxidation of ferrous iron is a rapid process if contact with oxygen or nitrate occurs [19]. It is speculated that IRB have very little effect under these conditions due to the speed of the inorganic iron precipitation reaction [20,21]. However, with decreasing oxygen concentration in the groundwater, IRB become increasingly able to compete with abiotic iron oxidation [22,23]. In natural groundwater systems, the clogging problem is likely to be attributable to a combination of microbiological and inorganic factors [2].

The Limestone Coast region in the south east of South Australia (Figure 1) has some of the most productive land in South Australia, with groundwater being the main source of water for agricultural production. It is also a unique wine growing region with over 250 growers and 15,800 ha of vineyards [24]. The region produces nearly one-third of the value of South Australia's agricultural produce from only about 2% of its land mass. Iron clogging of bores, pumps and drippers has been a persistent problem for many irrigators in the region, however, the issue is perceived to be growing [3]. Many irrigators suspect biological clogging to be the root cause of the problem and a common belief exists among irrigators that the problem is spreading due to the introduction of IRB through contaminated drilling equipment [25].

In order to investigate the cause of the widespread clogging problem, this study examines the IRB microbiology and inorganic water chemistry of bore water across two selected sub-regions of the Limestone Coast. Groundwater was analysed from a range of production bores with no, unknown or known clogging problems. Correlations between the severity of clogging and the age and depth of bores, the microbiology and the inorganic chemistry including redox conditions in these groundwaters was able to deduce the most likely cause of the clogging problem. Hypotheses were able to be drawn as to why the problem appears to be becoming more widespread.
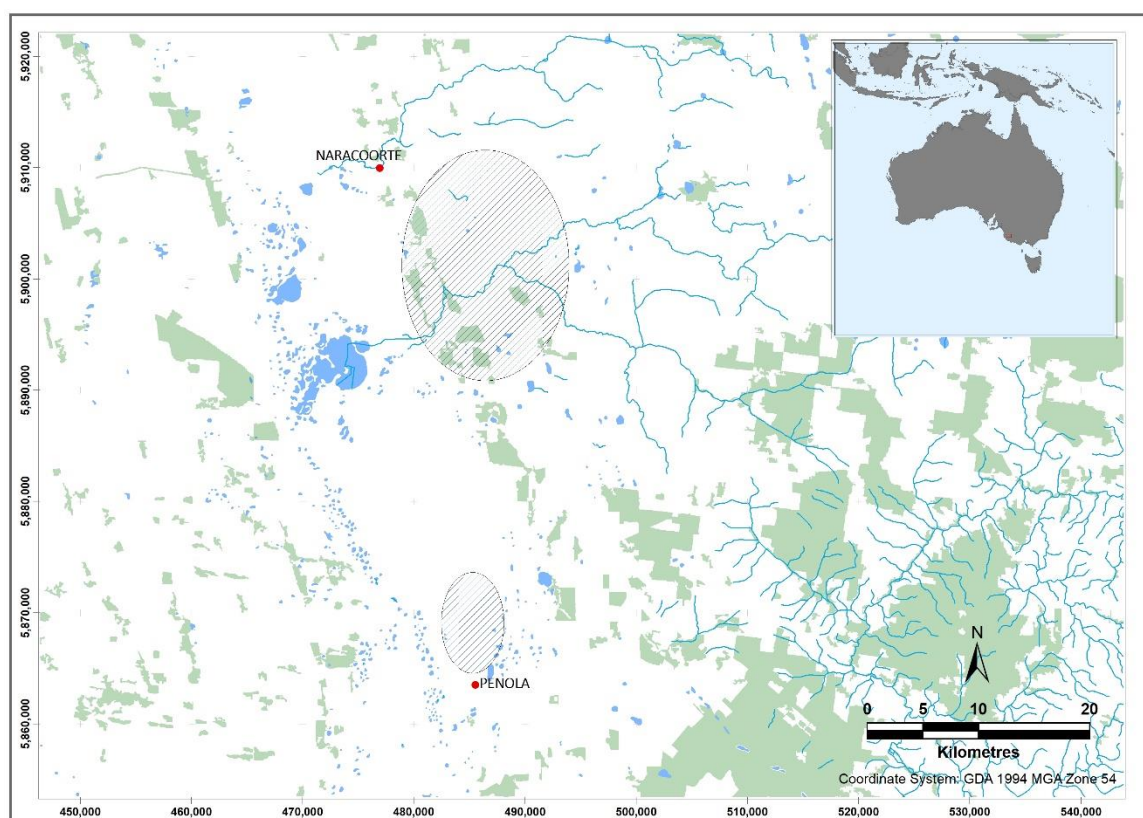
Figure 1. Location of area of investigation. Shaded oval circles indicate bore locations, however, accurate locations had to remain anonymous based on the request of landowners.

2. Materials and Methods

2.1. Field Site and Groundwater Sampling

The Limestone Coast of South Australia is located in the South East of South Australia, approximately 300 km south-east of Adelaide. The region is characterized by low-lying coastal plains, rising to ca. 70 m above sea level within the Naracoorte Ranges in the east and north-east of the region (Figure 1). The annual average rainfall in the region ranges from more than 800 mm in the south to ~500 mm in the north with the climate being considered Mediterranean, with cool wet winters and mild to hot, dry summers. Most irrigation bores access the unconfined Tertiary Limestone Aquifer (TLA) in the area, with a maximum thickness of about 300 m. The aquifer consist to a large degree of Dolomite ($CaMg(CO_3)$) and to a lesser degree of Calcite ($CaCO_3$) and Siderite ($FeCO_3$) [26]. The unconfined aquifer is recharged mainly by diffuse rainfall and the depth to water varies between 2 and ca. 20 m below ground level relative to topography. Water levels have overall declined over the last few decades across the region [27]. These declines have been attributed to a combination of a significant longer-term rainfall decline over southern Australia and below average summer rainfalls over the last decades [28].

Sampling of production bores used in viticulture irrigation was conducted from October 2019 to February 2020. A total of 26 water samples were collected from wells, which, based on self-reporting by landowners, were either unaffected by clogging, affected to a minor/moderate degree or strongly affected by clogging. Two wells had uncertainty about potential clogging problems but were included in the field program as they were located in close proximity to affected wells.

2.2. Inorganic Water Parameters

Each well (bar two, bore number 16 and 24) was purged a minimum of three casing volumes and inorganic water samples were taken after continuous well-head measurements

of pH, temperature, electrical conductivity (EC), dissolved oxygen (DO) and redox potential (Eh), which indicated a stable composition, representative of the aquifer chemistry. Two aliquots were collected from each bore for inorganic analysis: (i) elemental compositions (ICP-MS, ICP-OES, filtered and acidified) and (ii) major anions (IC), DOC (Shimadzu TOC analyser) and alkalinity titration (filtered and untreated sample).

Aqueous speciation and saturation state of groundwaters in respect to common mineral phases were computed using the PHREEQC code, utilizing the PHREEQC-2 thermodynamic database [29].

2.3. Microbiological Analysis

Two samples were obtained from each well for the analysis of the presence of active bacterial communities including iron-related bacteria (IRB), total coliforms and *Escherichia coli*. Water was collected in sterilized bottles and kept refrigerated until analysed. Analysis occurred within 72 h following collection.

2.3.1. IRB-BART Test

The testing was conducted according to the manufacturer's instructions (Droycon Bioconcepts Inc., Regina, SK, Canada). Presence of IRB was indicated by a brown ring, gel and/or clouding in the test tube, indicating the production of ferric iron. The concentration of the IRB in the water sample was estimated based on the speed of the reaction. IRB were recorded for each water sample as aggressive (higher concentration when the reaction occurred within 3 days), moderate (lower concentration when the reaction occurred between 4 and 8 days) or not present.

2.3.2. Colilert-18 Quanti-Tray Test

Total coliforms and the presence of *Escherichia coli* was analysed through a Colilert-18-Quanti-Tray test (IDEXX Laboratories, Inc., Westbrook, ME, USA), where undiluted bore water samples (100 mL) were mixed with a nutrient indicator in a sterile transparent container. The mixture was added to the Quanti-Tray and was sealed followed by overnight incubation at 37 °C. The Most Probable Number (MPN) of *E. coli* and total coliforms was calculated using the provided MPN table. The number of wells positive for *E. coli* were thereby detected by placing the Quanti-Tray under an Ultraviolet (UV, 365 nm) light at least 5 inches above the Quanti-Tray.

2.3.3. Scanning Electron Microscopy

A single sample of the clogging material was obtained from an affected screen retrieved from a bore located on one of the properties included in the study. Samples were fixed in 4% glutaraldehyde and dehydrated by immersion in sequential ethanol solutions for 10 min per rinse starting at $1 \times 70\%$ *v/v*, followed by $1 \times 90\%$ *v/v*, $1 \times 95\%$ *v/v* and $2 \times 100\%$ *v/v*, with a final rinse in hexamethyl-disilazane (HMDS) for 30 min. Samples were then air dried and sputter coated with 3 nm of platinum prior to analysis on Inspect FEI F50 Scanning Electron Microscope. This work was conducted by Flinders Microscopy and Microanalysis.

3. Results

3.1. Inorganic Water Chemistry

The groundwater in the target limestone aquifer was found to be generally fresh to slightly brackish (EC range between 1400 and 2800 μS/cm) and characterized by elevated calcium and bicarbonate concentrations, indicating mineral equilibrium with the limestone (Appendix A Table A1). Accordingly, the saturation index in respect to calcite and dolomite was close to or above mineral equilibrium (SI $\geq$ 0) for all samples (Figure 2). All groundwaters were well buffered in a pH range of 7.4–8.0 with the dissolution of carbonate minerals providing an effective pH buffering mechanism, due to fast dissolution kinetics [30,31]. The groundwater was also oversaturated in respect to quartz, a further major mineral

phase within the target aquifer, but was undersaturated with respect to gypsum, despite some groundwater samples showing elevated SO_4^{2-} concentrations (Figure 2).
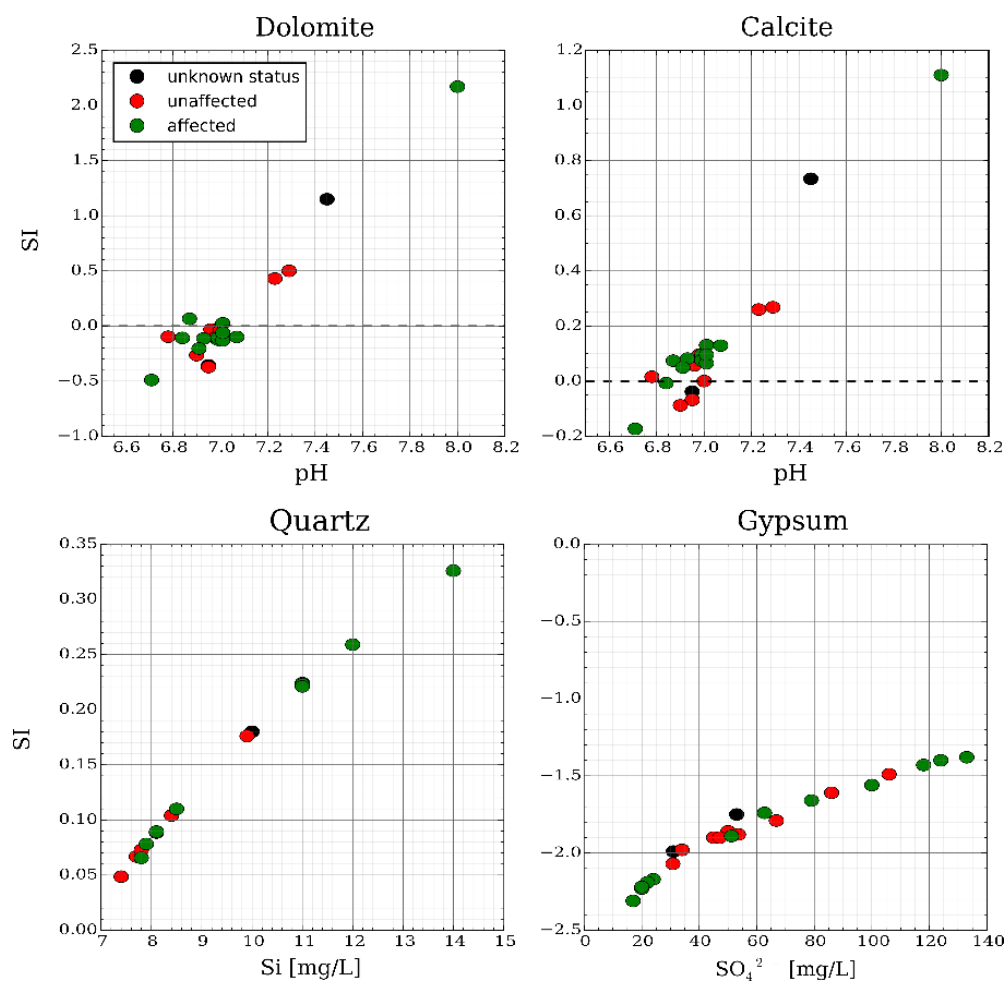

Figure 2. Correlation between the saturation index (SI) for dolomite ($CaMg(CO_3)_2$), calcite ($CaCO_3$), quartz (SiO_2) and gypsum ($CaSO_4$) with dissolved Ca, Si and SO_4^2 concentrations. The dashed line indicates mineral equilibrium (SI = 0) with minerals likely to precipitate at SI > 0 and dissolve at SI < 0. Calculations were performed by PHREEQC using the PHREEQC-2 thermodynamic database.

Redox conditions ranged from fully oxygenated to strongly reducing waters (Figures 3 and 4). Aerated water samples were thereby characterized by elevated dissolved oxygen and nitrate concentration while reduced species (Fe(II), Mn(II)) were absent or occurred at very low concentrations. Reducing groundwaters, however, exhibited relatively high Fe(II) and Mn(II) concentrations, while appreciable concentrations of oxygen and nitrate were absent. In addition, several trace metal and metalloids were found to be strongly correlated to the redox status of the groundwater and indicative of oxygenated or reducing conditions. Elevated arsenic concentrations were found in reducing groundwaters, with some bores exhibiting concentrations above the drinking water guidelines (e.g., As > 10 μg/L). Under reducing conditions, iron hydroxides are unstable. Their dissolution is commonly associated with a release of trace metals such as arsenic due to the corresponding loss of surface binding sites [32,33], which could explain the correlation of elevated As and Fe as shown in Figure 4. Other trace metals showed elevated mobility under aerobic, oxidizing conditions. For instance, SeO_4, the most prevalent selenium species in the presence of dissolved oxygen and/or nitrate and the neutral pH conditions in the Tertiary Limestone Aquifer is known to be a poor sorbent and highly mobile, leading to elevated concentrations under aerobic conditions [34].
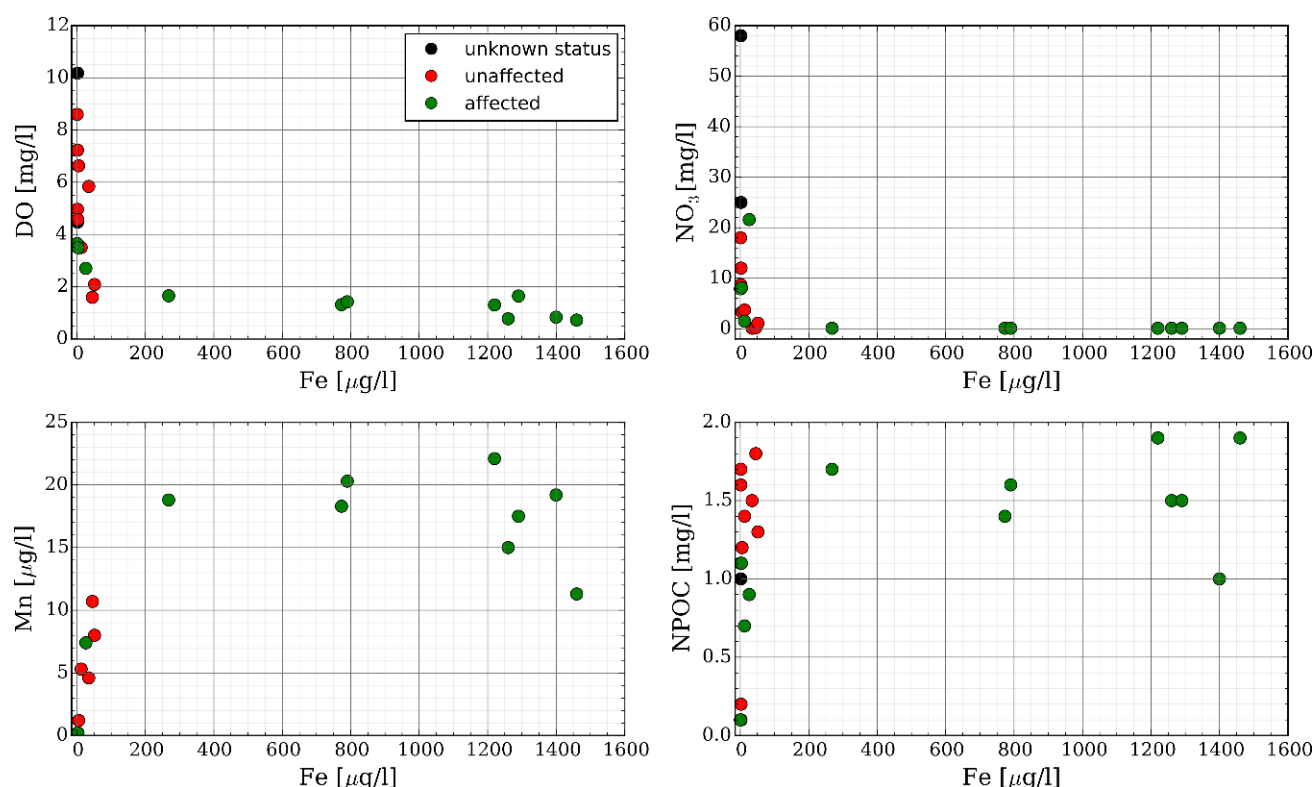
Figure 3. Correlations of Fe with redox sensitive species: dissolve oxygen (DO), nitrate (NO_3^-), and manganese (Mn). Additionally, shown is the correlation of Fe^{2+} and organic matter concentrations. Green symbols relate to clogging-affected bores, red symbols represent unaffected bores, while black symbols relate to bores where the severity of clogging was unknown.

A clear correlation between affected bores and the redox potential of the groundwater was identified. Specifically, strongly reducing waters were the most likely to be associated with clogged bores. Affected bores were found to be the most severely oversaturated in respect to iron oxide minerals, indicating a strong potential for iron precipitation if the water is exposed to oxygen (Figure 5). These ferrous-iron-rich groundwaters were also supersaturated for siderite, indicating slow siderite precipitation kinetics [35]. Besides iron mineralization, oxidation of manganese is often implicated in clogging of bores and irrigation systems [36]. However, despite elevated Mn concentrations, reducing waters remained below saturation of manganese carbonates ($MnCO_3$) as well as manganese oxides (Figure 5). Conversely to the above, samples from bores reported as being unaffected by clogging and all had appreciable dissolved oxygen concentrations and elevated redox potentials. If, in addition, the iron concentration was low, these groundwaters were undersaturated in respect to amorphous iron oxides ($Fe(OH)_3$), indicating a low inorganic clogging potential.

Severely affected bores were tendentially found to be deeper bores (Figure 6), while bores without clogging problems were generally shallower (<60 m). This reflects the natural redox sequence as residence time of groundwater increases with depth and groundwaters evolve from oxygenated to reducing waters. Higher ferrous iron concentrations are, therefore, more likely to be encountered in bores drilled to greater depth.
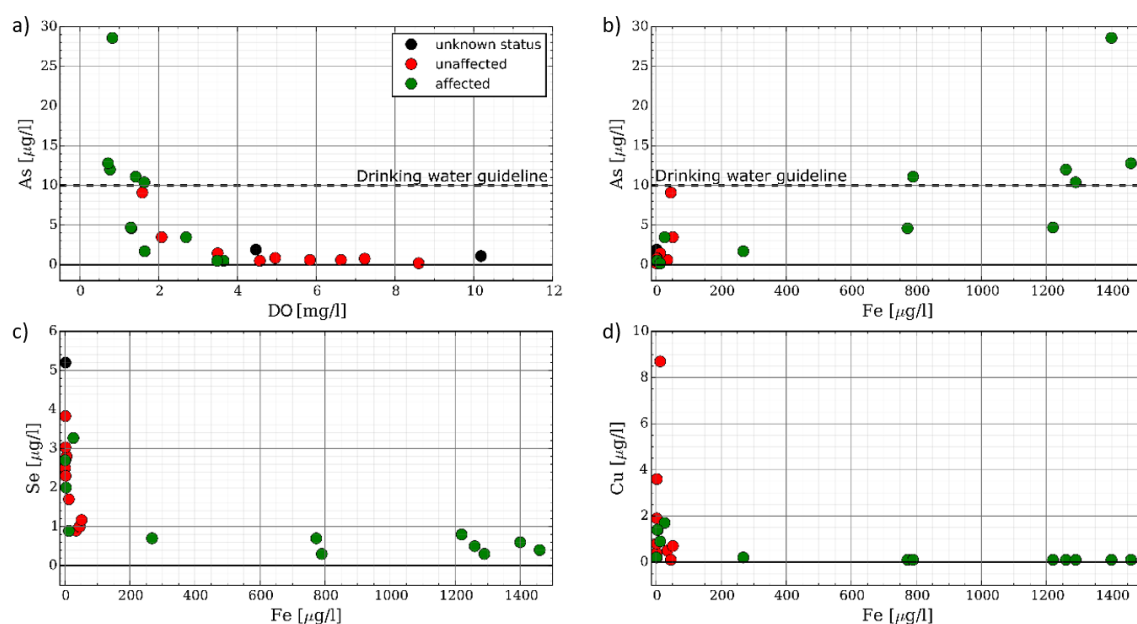
Figure 4. Trace metal and metalloid concentrations are strongly correlated to the redox conditions in groundwaters from the Tertiary Limestone Aquifer. Shown here are arsenic concentrations versus (**a**) dissolved oxygen, (**b**) ferrous iron, (**c**) selenium and (**d**) copper versus ferrous iron concentrations.
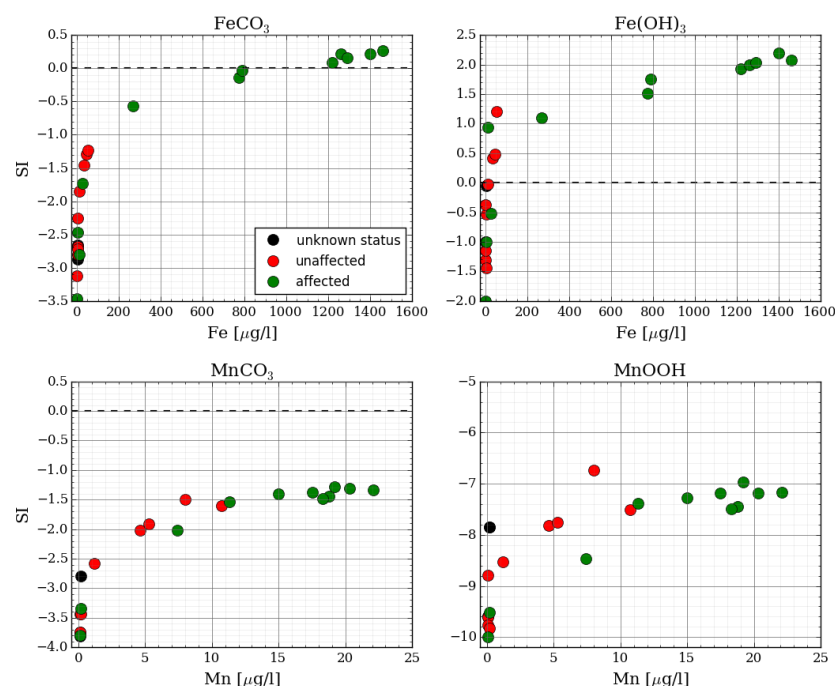

Figure 5. Correlation between Fe(II) and Mn(II) and the saturation of groundwaters in respect to the common aquifer minerals rhodochrosite ($MnCO_3$), siderite ($FeCO_3$) and iron and manganese oxides ($Fe(OH)_3$ and MnOOH). The dashed line indicates mineral equilibrium (SI = 0) with minerals likely to precipitate at SI > 0 and dissolve at SI < 0. Green symbols relate to clogging-affected bores, red symbols represent unaffected bores, light green symbols represent mildly/moderately affected bores, while black symbols relate to bores where the severity of clogging was unknown. Calculations were performed by PHREEQC using the PHREEQC-2 thermodynamic database.
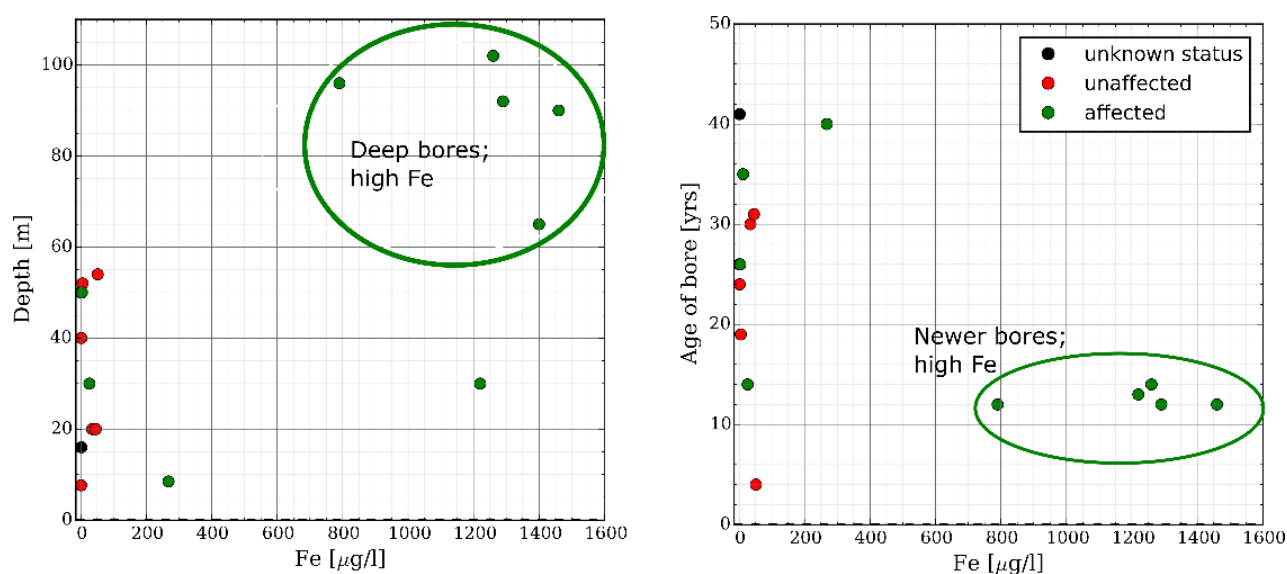
Figure 6. Correlation between the depth of bores and Fe(II) and Mn(II) concentrations as well as borehole age. Information on depth and/or age was not available for all sampled bores.

3.2. Microbiological Analysis

The IRB and Colilert tests indicated the presence of viable bacterial populations in the bore water samples (Appendix A Table A2). IRB was detected in 88% (n = 21/25) of the bore water samples. Among the IRB-positive samples, five were considered to contain aggressive IRB and 17 contained moderate IRB. However, there was no correlation between the presence of IRB and the clogging status of a bore (Figure 7). There was also no correlation with the presence of IRB and the iron content, pH, location, non-purgeable organic carbon. (NPOC), EC, temperature and DO. Two wells identified as being strongly affected by iron-related clogging were negative for IRB; however, it is unknown if these bores underwent disinfection treatments. It is possible that these were false negatives due to prior disinfection reducing the viable microorganisms to below the limit of detection.
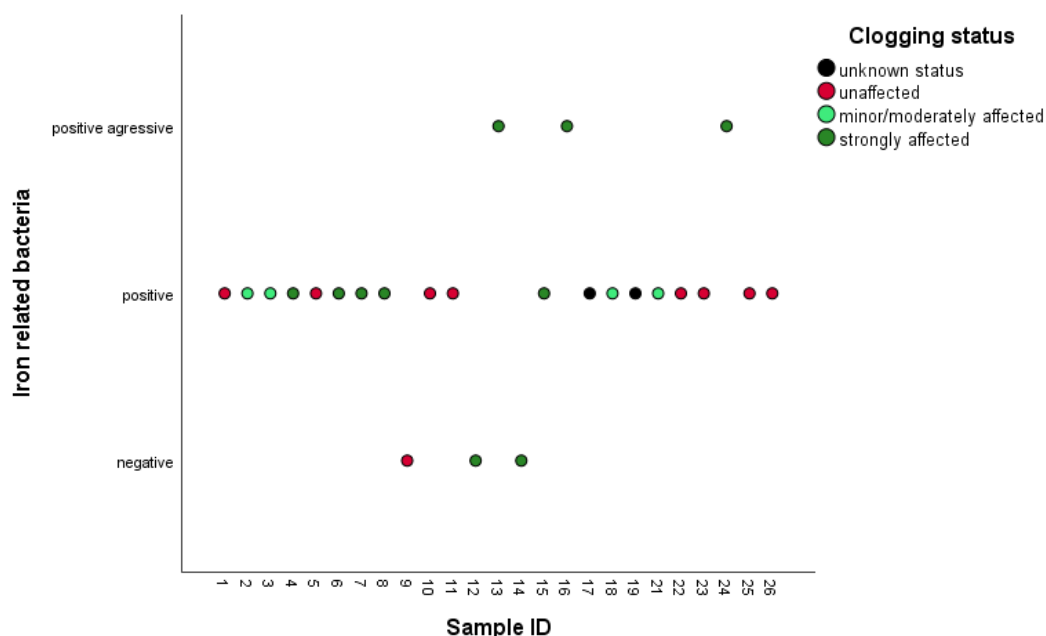

Figure 7. Iron-related bacteria (IRB) presence determined using the IRB Bart test and clogging status of bores.

Twenty-two (out of 25) samples (88%) and 7/25 samples (28%) were positive for coliform bacteria and *E. coli*, respectively. The presence of coliforms and *E. coli* indicate potential faecal contamination and are the most commonly used indicator organisms for microbial water quality [37]. Bores affected by *E. coli* correlate with very shallow bores and/or bores, where samples for bacterial analysis were taken during the first flush, prior to well purging.

The nature of the clogging material, obtained from a screen retrieved from an affected bore, was visualized through scanning electron microscope (SEM) (Figure 8). The precipitate was found to be very diverse with both crystalline structures, suggesting amorphous iron oxides, and biological structures, suggesting bacterial growth. This suggests the co-occurrence of inorganic as well as microbiological clogging.
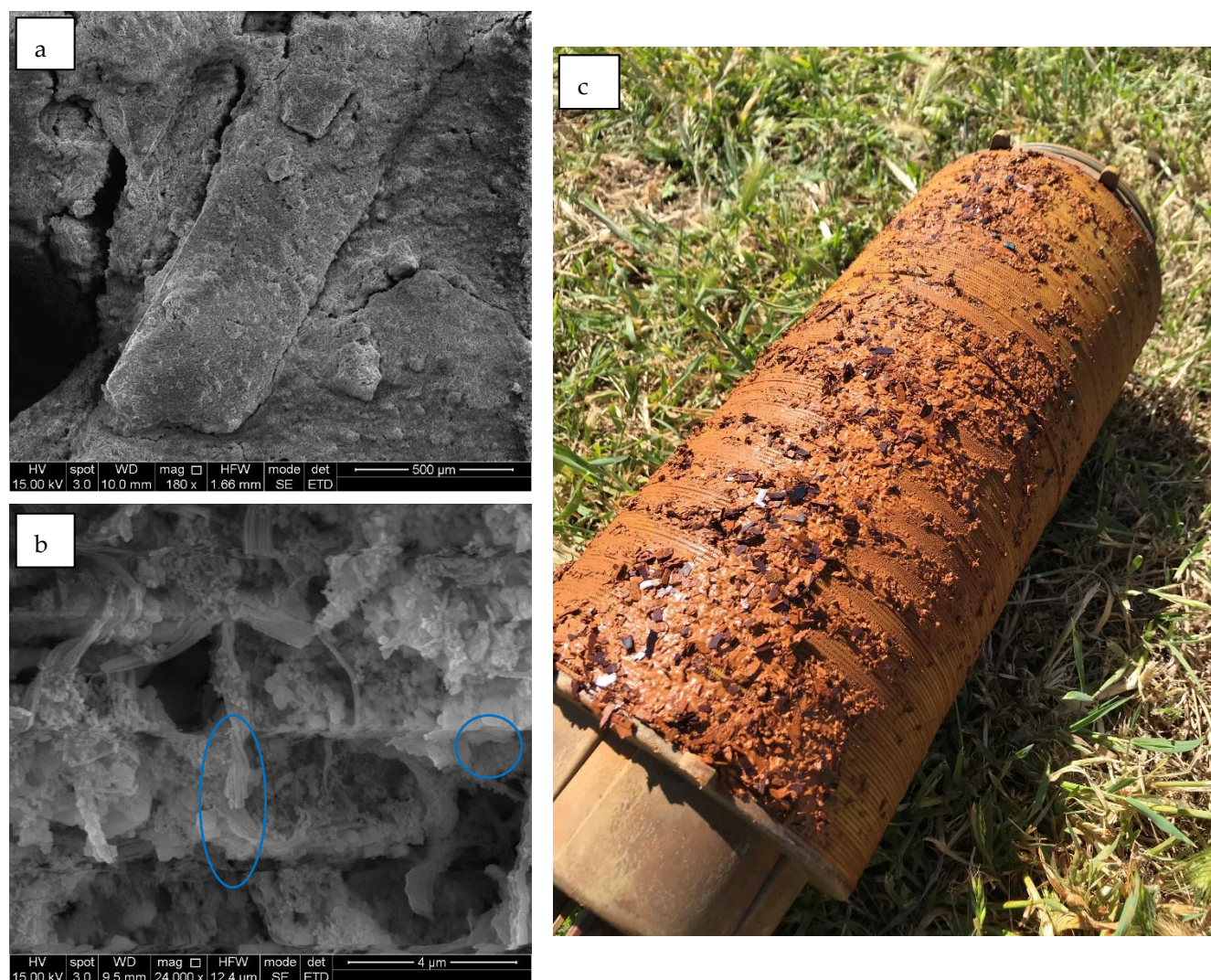

Figure 8. SEM of clogging material: (**a**): crystalline structure suggesting amorpheous iron oxides; (**b**): blue circles show the evidence of biological structure suggesting microbial growth; (**c**): incrustations of a clogged screen, which was sampled for SEM analysis.

4. Discussion

Sampling revealed that strongly reducing waters were the most likely to correlate with clogged bores. These bores were found to be the most severely oversaturated in respect to iron oxide minerals, indicating a strong potential for iron precipitation if the water is exposed to oxygen through pumping. No correlations could be found between the presence of IRB and the clogging status of a bore. There was no apparent correlation

with the presence of IRB and the ferrous iron concentration, the pH, location of bores, the salinity of the water, its temperature or DO concentration. Two wells were identified as being unaffected by clogging had high concentrations of IRB, and one affected well was negative for IRB. Samples with elevated organic carbon (Figure 3), however, did show a tendency to be found in bores affected by clogging. This is consistent with a higher microbiological clogging potential in the presence of dissolved carbon, as in the absence of chemical disinfectants, the degree of microbial growth is related to the amount of labile carbon in groundwater.

The aqueous chemistry and microbiology (which is supported by the SEM analysis of one sample) suggest that the widespread occurrence of reducing groundwaters with elevated iron concentrations is the primary cause of iron clogging, however, biological clogging may contribute to the problem in the area. It, therefore, appears unlikely that the perceived increase in iron clogging in the region is a result of an unintentional introduction of IRB through drilling equipment. Rather, we postulate that the documented change in regional climate, increased viticulture and irrigation demand are more likely drivers for the widening problem [38]. Since several decades, southern Australia has experienced longer-term rainfall declines with the prolonged dry spell experienced from mid 1990s to mid-2010 (the "Millennium Drought") representing the driest period in the last 110 years of reliable climate records [39]. The less than average rainfall has led to an increase in the reliance on groundwater extraction in the region, and, overall to decline in water levels [23]. Subsequently, the region has seen a tendency to drill deeper bores and deepen existing bores since the 1990s to secure irrigation supplies (Figure 9). With a higher likelihood, however, of deeper bores accessing more reducing waters, this tendency would have led to an increase in the probability of iron clogging, as redox conditions would favour higher dissolved iron concentration at depths (Figures 5 and 6).
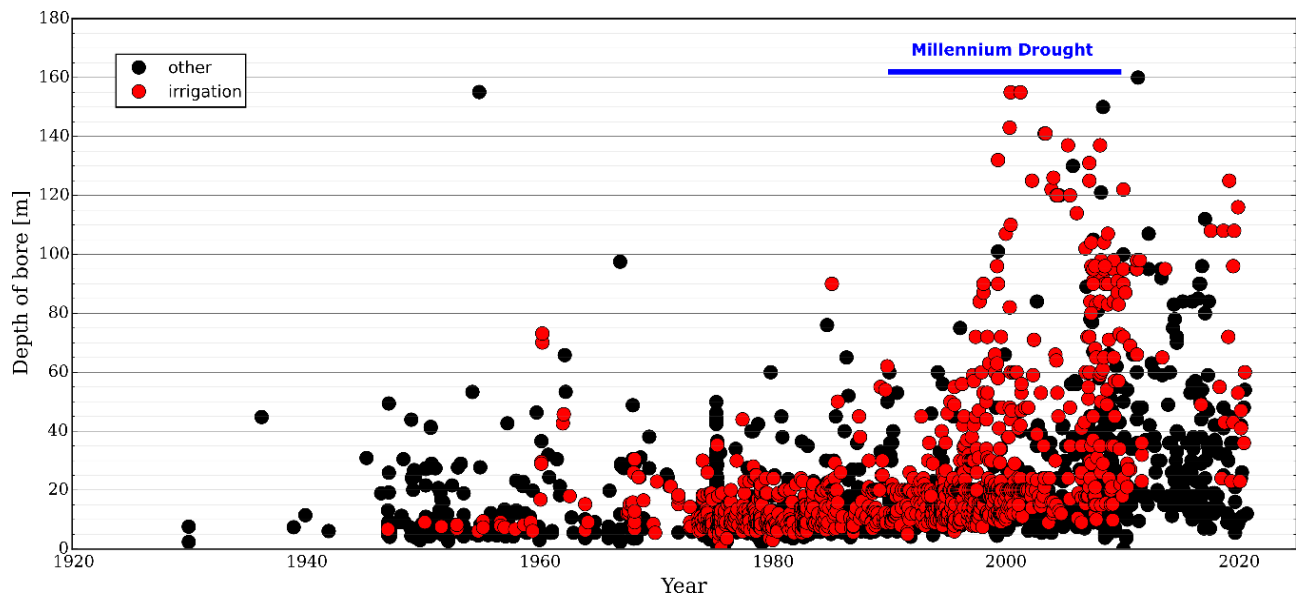

Figure 9. The drilled depth of bores in the study area has increased since the mid-1990s, coinciding with the onset of the "Millennium Drought." Data obtained from the SA Government Groundwater Data: Obswell database [40].

In addition to the above, it can be speculated that the transition from flood/pivot irrigation to drip irrigation systems over the last few decades would have further exacerbated the clogging problem. Drip irrigation systems, with their very narrow emitters may have increased the susceptibility of irrigation systems to clog up.

A correlation between changes in climate and irrigation practices and the widening of the clogging problem has not been postulated to date. Instead, many irrigators view contamination through drilling equipment as the likely route cause of the problem. As

changes in climate and irrigation practices are impacting the study area very gradually, its follow-on impacts on clogging in the region would have not been easily discernible, however, these changes are regarded as the most likely cause of the widening problem to date.

Limitations

A major limitation in the current study has been the reliance on self-reporting on the clogging status of a bore by bore owners in the absence of a quantitative measure of iron clogging. This would have introduced significant bias. The absence of an adoptable scale for the severity of clogging, impacted on the ability to correlate between the severity and operational/chemical/biological indicators. Future work in this area would benefit from the development of a standard and systematic approach in determining the clogging status of a well based on quantitative measures. A larger sample size is also needed to explore the statistical significance of observed correlations and relationships.

Appendix A

Table A1. Overview of the inorganic groundwater chemistry.

Components	Units	Min	Max	Average	Median	*n*
pH		7.4	8.0	7.7	7.7	26
E.C.	dS/m	1.4	2.8	1.8	1.8	26
Total alkalinity as $CaCO_3$	mg/L	270	429	341	336	26
NPOC	mg/L	0.1	1.9	1.2	1.3	26
F	mg/L	0.2	0.7	0.3	0.3	26
Cl	mg/L	319	771	436	413	26
Br	mg/L	0.6	2.0	1.1	0.9	26
NO_3	mg/L	0.2	93.3	21.7	8.7	26
SO_4	mg/L	17.3	133	59.0	51.6	26
Ca	mg/L	103	165	131	128	26
K	mg/L	3.6	8.3	5.2	4.9	26
Mg	mg/L	22.9	55.5	36.5	34.9	26
Na	mg/L	147	353	198	179	26
B	mg/L	0.1	0.4	0.1	0.1	26
P	mg/L	<0.2	<0.2	na	na	26
Si	mg/L	7.4	13.6	9.9	10.0	26

Table A1. *Cont.*

Components	Units	Min	Max	Average	Median	*n*
Sr	mg/L	0.8	2.3	1.3	1.3	26
Be	ug/L	<0.1	<0.1	na	na	26
Al	ug/L	<0.5	1.7	0.1	0.0	26
V	ug/L	<0.05	4.5	1.0	0.6	26
Cr	ug/L	<0.2	0.8	0.1	0.0	26
Mn	ug/L	<0.2	117.7	11.5	5.0	26
Fe	ug/L	<0.8	1460	332	12.4	26
Co	ug/L	<0.05	0.9	0.1	0.0	26
Ni	ug/L	0.2	3.1	0.8	0.5	26
Cu	ug/L	0.1	8.7	1.4	0.8	26
Zn	ug/L	1.0	85.4	14.2	9.5	26
Ga	ug/L	<0.05	<0.1	na	na	26
As	ug/L	<0.4	28.6	4.4	1.5	26
Se	ug/L	0.3	5.2	1.7	1.2	26
Mo	ug/L	<0.2	0.7	0.2	0.0	26
Ag	ug/L	<0.1	<0.1	na	na	26
Cd	ug/L	<0.05	1.8	1.2	1.3	26
Sn	ug/L	<0.4	<0.4	na	na	26
Sb	ug/L	<0.2	<0.2	na	na	26
La	ug/L	<0.05	<0.05	na	na	26
Tl	ug/L	<0.1	<0.1	na	na	26
Pb	ug/L	<0.1	3.0	1.7	1.7	26
Bi	ug/L	<0.1	<0.1	na	na	26
U	ug/L	<0.1	2.1	0.4	0.2	26

Table A2. Microbial analysis of sampled bores and the iron clogging status. All samples (expect bore number 16 and 24 were sampled after purging. Bore number 16 and 24 were sampled without purging).

Bore No.	Drill Date	Depth To (m)	IRB *	Coliform Bacteria	*E. coli* (MPN/100 mL)	Iron Clogging
1		7.65	+	+	0	Unaffected
2	1994	50	+	+	0	Affected to a minor/moderate degree
3	2001	52	+	+	0	Affected to a minor/moderate degree
4	1994	50	+	+	0	Strongly affected
5	1994	50	+	+	0	Unaffected
6	1980	8.5	+	+	51.2	Strongly affected
7			+	+	9.7	Strongly affected
8	2007	30	++	+	0	Strongly affected
9	1990	20	-	+	0	Unaffected
10	1989	20	+	+	0	Unaffected
11		Shallow	+	+	0	Unaffected
12	2006	102	-	-	0	Strongly affected
13	1993 to 18 m then deepened to 96 m in 2008	96	++	+	0	Strongly affected
14	2008	92	-	-	0	Strongly affected
15	1971 to 21.3 m then deepened in 2008 to 90 m	90	+	+	0	Strongly affected
16		65	++	+	24.6	Strongly affected
17	1979	16	+	+	0	Unknown

Table A2. *Cont.*

Bore No.	Drill Date	Depth To (m)	IRB *	Coliform Bacteria	*E. coli* (MPN/100 mL)	Iron Clogging
18			+	+	0	Affected to a minor/moderate degree
19			+	+	0	Unknown
20	1981	20	na	na	na	No sample
21	2006	30	++	+	7	Affected to a minor/moderate degree
22			+	+	2	Unaffected
23	2016	54	+	+	0	Unaffected
24	1985 to 24 m, deepened in 2006 to 60 m	60 m	++	+	2	Strongly affected
25			+	+	0	Unaffected
26	1996	40	+	+	6	Unaffected

* Positive or negative according to the IRB BART test.

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