Flinders UNIVERSITY
College of Science & Engineering

INSPIRING ACHIEVEMENT

IGS Innovative Groundwater Solutions

Iron-clogging in the Limestone coast aquifers of South Australia - Geochemistry and Microbiology of groundwaters



Iron-clogging in the Limestone coast aquifers of South Australia - Geochemistry and Microbiology of groundwaters

A report prepared for Wine Australia

by

Harriet Whiley, Flinders University

Ilka Wallis, Flinders University

Nikki Harrington, Innovative Groundwater Solutions

August, 2020

Acknowledgements

Funding for this project was provided by Wine Australia through its Incubator Initiative. In-kind support was provided by Flinders University. Thanks to Kerry De Garis (LCGWC), Birte Moser (University of Oldenburg, Germany), and Rachel Roberts, Mira Beknazarova and Thilini Keerthirathne (Flinders University) for providing input to the current work and assistance during field work. Our thanks go to all of the irrigators who took the time to meet and provide information on clogging issues, as well as allowing us to sample a subset of bores on their properties.

Abstract

This study investigated the cause of iron related clogging of bores across the Limestone Coast region. A survey of 26 clogging-affected and unaffected bores found no apparent correlation between the presence of iron related bacteria (IRB) and the clogging status of the bore, with IRB detected in the majority of wells sampled. There was a clear correlation between affected bores and the redox potential of the groundwater, with strongly reducing waters associated with clogged bores. The most severely affected bores were also found to be severely oversaturated in respect to iron oxide minerals indicating a strong potential for iron precipitation. The highest Fe^{2+} concentrations were found in the deepest bores. This relationship needs to be further explored and will inform future mitigation strategies.

Executive summary

Iron clogging of bores, pumps and drippers is a persistent and growing problem for many irrigators in the Limestone Coast region, who rely on groundwater for irrigation. This study examined 26 bores used for viticulture irrigation in the region. Bores were either unaffected by clogging, affected to a minor/moderate degree or strongly affected by clogging. The results found no apparent correlation between the presence of iron related bacteria (IRB) and the clogging status of the well, with IRB detected in the majority of wells. The results suggest that IRB are widespread throughout the aquifer and it appears unlikely, that the perceived increase in iron clogging in the region is a result of the widespread introduction of IRB through drilling equipment. The survey of bores did find a clear correlation between affected bores and the redox potential of the groundwater, with strongly reducing waters associated with clogged bores. The most severely affected bores were also severely oversaturated in respect to iron oxide minerals, indicating a strong potential for iron precipitation. Under these conditions the ingress of oxygen, through well usage, would cause iron to precipitate rapidly. Interestingly, the higher concentrations of Fe^{2+} tended to be in the deeper bores, which were also often the most recently drilled. Further research is needed to explore this relationship in more detail. A sample of clogging material was also examined using the scanning electron microscope. This found evidence of both crystalline material and biological growth. This supports the findings that the clogging is most likely an inorganic problem which may be enhanced through the presence of bacteria and biofilm formation. Future research is needed to expand the dataset and to verify the conclusions drawn on the basis of the currently limited number of examined bores. Funding for this project was provided by Wine Australia through its Incubator Initiative. In-kind support was provided by Flinders University. This project could not have been completed without the input and support from Kerry De Garis (Limestone Coast Grape and Wine Council Inc.) and the irrigators who provided information and allowed us to sample a subset of bores on their properties.

1 Introduction

The Limestone Coast has some of the most productive land in South Australia, with groundwater being the main source of water for agricultural production. The irrigation industry is the most significant user of groundwater with about 1500 irrigators in the region relying on groundwater, many of them wine producers. The main aquifer used for groundwater supply is the unconfined Tertiary Limestone Aquifer, which is generally able to supply good quality irrigation water (Harrington & Harrington, 2017). However, iron clogging of bores, pumps and drippers is a persistent problem in the region (Harrington & Harrington, 2017) and puts serious financial strains on irrigators, as affected bores show a reduction in well yield over time, while pump equipment and/or drippers show a reduction in flow rates up to total blockage and cessation of flow.

Iron clogging within aquifers and of irrigation infrastructure is a well-known and costly problem world-wide. While the issue 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.

Inorganic precipitation of iron oxides occurs in groundwaters with sufficient concentration of dissolved ferric iron (Fe(II)) if these come into contact with oxidants such as nitrate or oxygen (Grenthe, Stumm, Laaksuharju, Nilsson, & Wikberg, 1992; McLaughlan, 1989; Tong et al., 2014). Elevated Fe(II) concentrations are common in reducing groundwaters. These become more common with depth below ground surface, as most aquifers display a distinct redox zonation with the redox potential becoming sequentially more reducing with depth (Figure 1). A borehole exposes these reducing groundwaters to atmospheric conditions allowing dissolved iron to oxidise, leading to oxide precipitation. 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. In addition to the above, iron oxides are known to have a catalytic effect on the oxidation of Fe(II), i.e. the rate of precipitation increases the more iron oxides are present in a well (Teutsch et al, 2005).

Iron related biological clogging is due to the metabolic activity of Iron Related Bacteria (IRB) such as *Gallionella* spp., *Leptothrix* spp*., Thiobacillus* spp. and *Sphaerotilus* spp. (Gino et al., 2010; Sun et al., 2014). These bacteria are commonly found in groundwater and grow well in water with a pH range of 5.4 to 7.2 (Cullimore & McCann, 1977). These bacteria can facilitate the transformation of soluble ferrous ions (Fe^{2+}) to ferric (Fe^{3+}) compounds with low solubility (Gong et al. 2019). 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) (Donlan, 2002). IRB within biofilms are able to multiply profusely in boreholes due to the constant supply of nutrients that occurs through pumping (Houben & Sander, 2020). Their adhesive properties facilitate the colonization of bore casings and screens, enabling them to persist despite high flow velocities (Baveye et al. 2010). This results in the build-up of solids (iron sulphites, iron hydroxides), gas (dinitrogen, methane) and/or slime deposits *(*EPS) which may cause clogging (Vandevivere & Baveye, 1992).

The importance of microbial versus chemical iron oxidation is still a matter of debate. Under neutral pH conditions, as present in the limestone aquifers of the Limestone coast, the oxidation of ferrous iron is a rapid process if mixing with oxygen or nitrate occurs (Straub, Benz, & Schink, 2001). It is speculated that IRB have very little effect under these conditions due to the speed of the inorganic iron precipitation reaction (Davison & Seed, 1983; Singh, Singh, Singh, & Kumar, 2018). However, with decreasing oxygen concentration in the groundwater, IRB become increasingly able to compete with abiotic iron oxidation (Neubauer, Emerson, & Megonigal, 2002; Rentz, Kraiya, Luther, & Emerson, 2007). Below a pH of 4, ferric iron becomes the stable form of iron in groundwater and precipitation of iron oxides even under aerated conditions ceases (Sung & Morgan, 1980). As such it is likely that in natural groundwater systems, the clogging problem can be attributed to a combination of microbiological and inorganic factors (Wang et al., 2014).

This study examines the IRB microbiology and inorganic water chemistry of bore water across the Limestone coast in order to explore the cause of the widespread clogging problem. 26 samples were taken from a range of bores, which were either unaffected, affected to a minor/moderate degree or strongly affected by clogging. Correlations between the severity of clogging and the age and depth of bores, the microbiology and the inorganic chemistry including redox conditions in theses groundwater were examined.

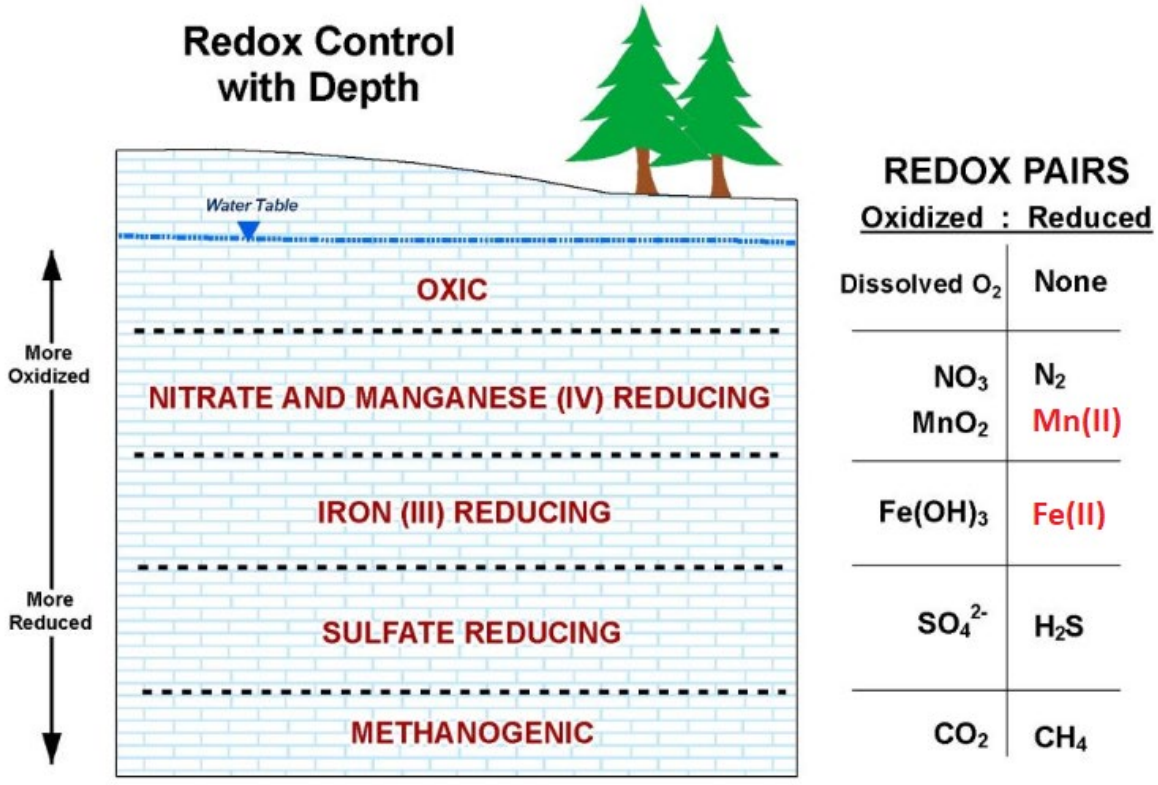


Figure 1: Redox sequence with depth in aquifers. Elevated Mn(II) and Fe(II) concentrations occur naturally under manganese- and iron oxide reducing conditions at depth (after OhioEPA, 2014)

2 Area of investigation

Sampling was conducted from October 2019 to February 2020 in the Limestone Coast region in the south east of South Australia (Fig. 2).

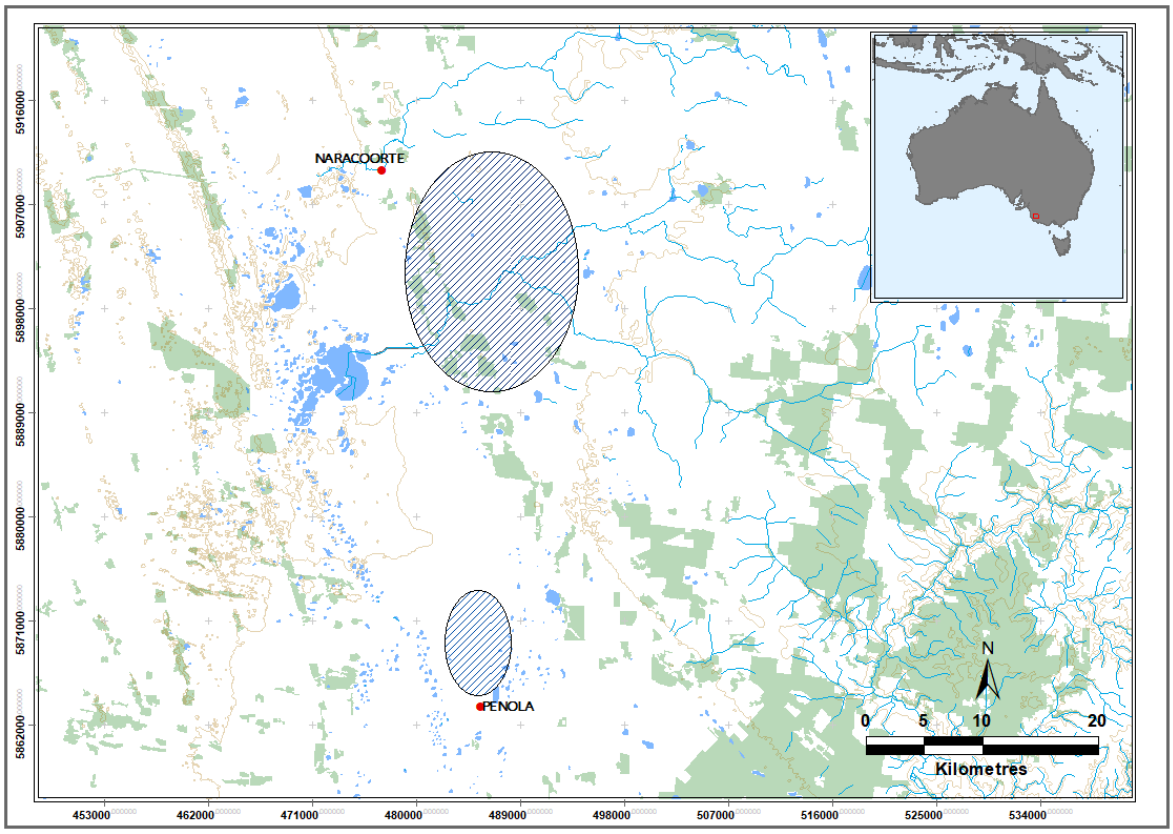


Figure 2: The area of investigation within the Limestone Coast of South Australia. Circles depict the study areas.

The Limestone Coast region is adjacent the border with Victoria and is an important agricultural region for South Australia, contributing an estimated $790 million in agricultural production to the State's Gross Domestic Product (PIRSA, 2010). Irrigated agriculture forms a large portion of this, with the most significant irrigated crops being wine grapes, pasture seed, pasture and potatoes.

The Limestone Coast has a Mediterranean climate and an annual rainfall between 450 mm and 800 mm, with the heaviest rainfall in the south. Surface water occurs as minor creeks, wetlands and an extensive network of interconnected man-made and natural drains that drain the landscape making it suitable for agricultural production. However, irrigation water is sourced almost exclusively from underground aquifers, with the most heavily used being an unconfined Tertiary Limestone Aquifer (TLA). The TLA is karstic, with transmissivities ranging between 200 m^2/day and 10,000 m^2/day. The karstic nature of the aquifer has resulted in a landscape that is dotted by sinkholes ranging in size from centimetres to tens of metres. Irrigation water sourced from the TLA is generally of good quality (salinity less than 1,200 mg/L as Total Dissolved Solids).

Depth to groundwater in the unconfined TLA ranges between approximately 2 m and 20 m and aquifer thickness increases from north to south reaching approximately 300 m around

Mount Gambier (Love et al., 1993). Groundwater levels have declined in some areas over the last 30-40 years as a result of drier environmental conditions and increased extraction. In other parts of the region, rising groundwater levels have been observed due to land clearing.

The TLA was deposited in the lower Miocene and Oligocene (Neogene, Paleogene). The aquifer contains high proportions of dolomite ($CaMg(CO_3)$) and dolostone, as well as calcite ($CaCO_3$, limestone). Besides Ca and Mg, significant concentrations of Fe, Mn, Na and Sr are also found in the dolomite crystals. Siderite ($FeCO_3$) is very rarely present (Kyser et al., 2002).

3 Methodology

A total of 26 water samples were collected in October 2019 and February 2020 from wells, which, based on anecdotal evidence provided by their owners, 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 on the same properties as affected wells. Each well, bar one, 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) indicated a stable composition, representative of the aquifer chemistry. One bore was not equipped with a pump and consequently, a sample was obtained using a bailer. Another bore location lacked a water outlet, from which suitable samples could be obtained. The bore was purged, but water was free-flowing onto the ground, and could only be sampled from a depression on the ground. Two aliquots were collected from each bore for analysis: i) elemental compositions (ICP-MS, ICP-OES, filtered and acidified) and ii) major anions (IC), DOC (Shimadzu TOC analyser) and field alkalinity titration (filtered, untreated sample). Ethics approval for this project was granted by Social and Behavioural Research Ethics Committee (SBREC) at Flinders University (project number 8398).

The results of the inorganic samples are summarised in Table 1, while graphical displays are provided in Figures 3 to 7. Graphical presentations were based on 22 groundwater analyses with the bore obtained through a bailer and another 3 samples with elevated electrical balance errors omitted, as the data was not regarded as reliable (Appendix B). However, the entire data set is provided in Appendix A-C. Microbiological analyses are summarised in Table 2.

3.1 Microbiological analysis

25 out of the 26 collected groundwater samples were analysed for the presence of active bacterial communities including Iron-related Bacteria (IRB), total coliforms and *Escherichia coli*. At one sample location, microbiological samples could not be taken, as there was no dedicated water outlet and subsequently, the pumped water free-flowed onto the ground. Hence it was decided to omit microbiological analysis of this bore, as any analysis would have been impacted by soil/dirt particles.

IRB-BART test

The testing for IRB was conducted according to the manufacturer's instructions (Droycon Bioconcepts Inc., Regina, Canada). When IRB are present, a series of reactions occur as they use the nutrients and ferric iron in the tube to grow. This results in a brown ring, gel and/or clouding in the test tube. The concentration of the IRB in the water sample was estimated based on the speed of the reaction. IRB were recorded as aggressive (higher concentration when the reaction occurred within 3 days) and moderate (lower concentration when the

reaction occurred between 4-8 days). The limit of detection for this test is one organism in 15mL which equates to 700 organisms per L.

Colilert-18 Quantitray test

The manufacturer's instructions (IDEXX Laboratories, Inc., Westbrook, Maine 04092 USA) were followed throughout the analysis. Undiluted bore water samples (100 mL) were mixed with the nutrient indicator provided with the Colilert test kit in a sterile transparent container. The mixture was added to the Quantitray and was sealed followed by incubation at 37^0C overnight. The wells which turned yellow were counted and the Most Probable Number (MPN) was calculated using the provided MPN table. The same procedure was conducted to calculate the MPN of *E. coli*. The number of wells positive for *E. coli* were detected by placing the Quantitray under an Ultraviolet (UV, 365 nm) light at least 5 inches above the Quantitray.

Whole Genome Sequencing

The diversity of bacterial populations was examined in a subset of samples using Whole Genome Sequencing (WGS) (Appendix C). This was conducted by the Australian Water Quality Centre. Briefly, DNA was extracted using the Qiagen DNeasy PowerSoil ® kit as per manufacturer's instructions. Bacterial diversity profiling performed using the Ion 16S™ Metagenomics Kit as per manufacturer's instructions. The metagenomics workflow in the Ion Reporter™ Software enabled microbes to be identified to genus level by using both curated GreenGenes and curated MicroSEQ® 16S reference databases.

3.2 Scanning electron microscopy

A 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 and was visualised using the scanning electron microscope (SEM) (FEI Inspect F50). This work was conducted by Flinders Microscopy and Microanalysis.

4 Results and Discussion

4.1 Inorganic water chemistry

The groundwater in the target limestone aquifer in the study area was found to be generally fresh to slightly brackish (EC range between 1400 and 1800 µS/cm) and characterised by elevated calcium and bicarbonate concentrations, indicating mineral equilibrium with the limestone (Table 1; Appendix A). Accordingly, the saturation index in respect to calcite is close to or above mineral equilibrium (SI => 0) for all samples (Figure 3; Appendix B) and the groundwaters are well buffered and lie within a pH range of 6.7 to 8.0. Water samples of raised salinity are characterised by elevated concentrations of chloride and sodium. Noteworthy are elevated concentrations of arsenic above the drinking water guideline (10 µg/L) in five samples and elevated nitrate concentrations above 50 mg/L in two samples (Figure 4 and 5). The latter may indicate agricultural influence, while arsenic concentrations are correlated to the redox potential of groundwaters with the most reducing groundwaters exhibiting elevated trace metal concentrations (Figure 5).

Redox conditions range from fully oxygenated to strongly reducing waters (Figure 4). Aerated water samples are characterised by elevated dissolved oxygen and nitrate concentrations while reduced species (Fe(II), Mn(II)) are absent or occur at very low concentrations. Reducing

groundwaters, however, exhibit relatively high Fe(II) and Mn(II) concentrations, while appreciable concentrations of oxygen and nitrate are absent.

Table 1: Statistics of groundwater chemistry for the 26 samples collected as part of this study.

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
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

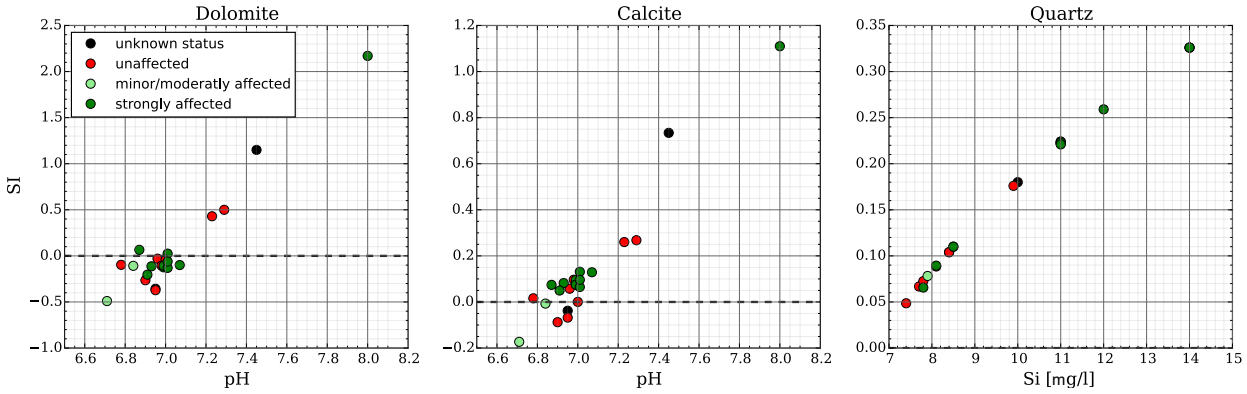


Figure 3: Correlation between the saturation index (SI) for dolomite ($CaMg(CO_3)_2$), calcite ($CaCO_3$) and quartz (SiO_2) with pH and Si concentrations. The dashed line indicates mineral equilibrium (SI=0) with minerals likely to precipitate at SI>0 and dissolve at SI<0. Green symbols relate to clogging-affected 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.

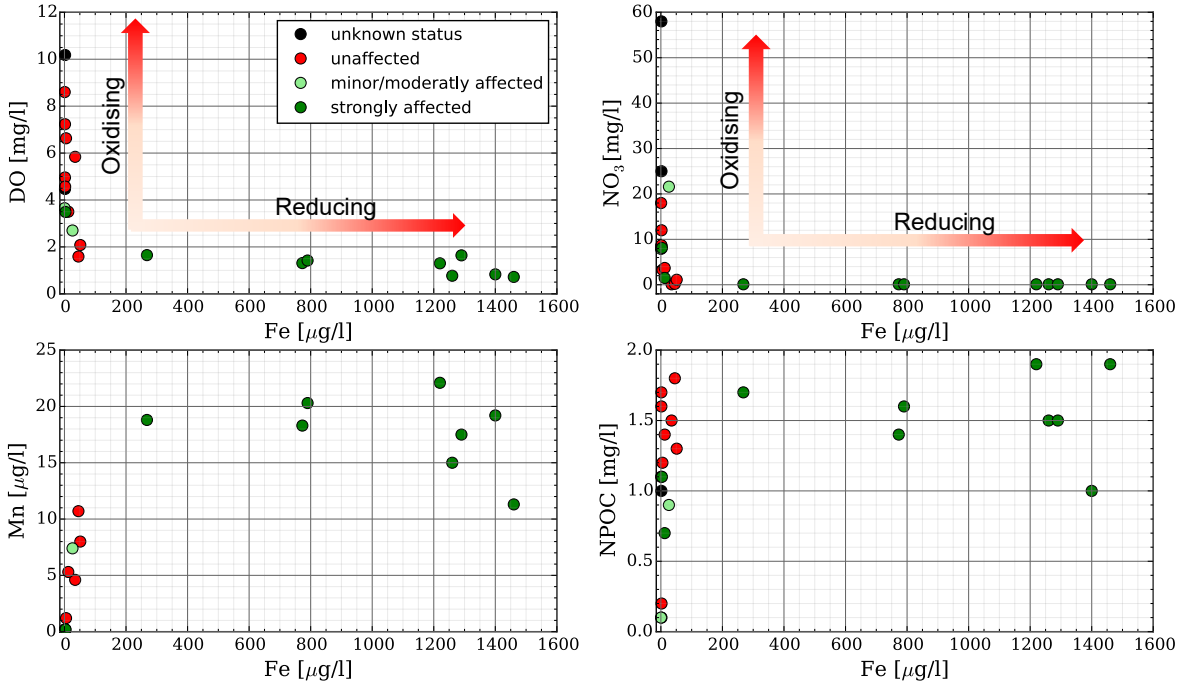


Figure 4: Correlations of Fe with redox sensitive species: dissolved oxygen (DO), nitrate (NO_3^-), manganese (Mn) and non-purgeable organic carbon (NPOC). Green symbols relate to clogging-affected bores, red symbols represent unaffected bores, light green symbols represent mildly/moderately affected bores, while black symbols relate to bores, where the severity of clogging was unknown.

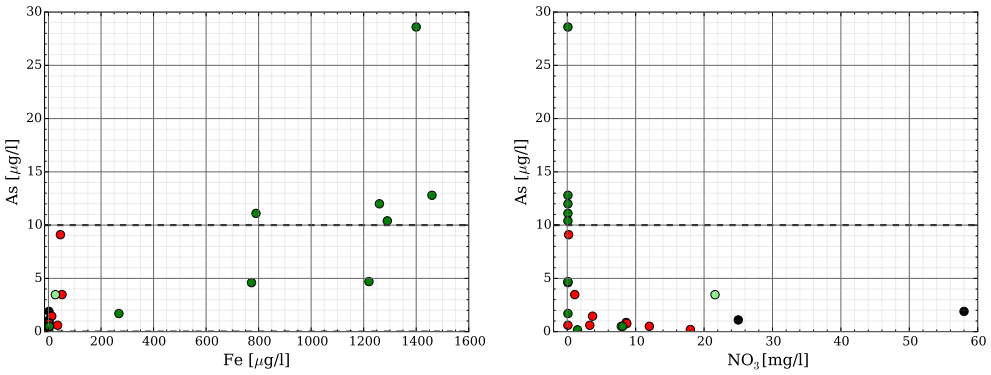


Figure 5: Elevated arsenic and nitrate (NO_3^-) concentrations are correlated to the redox status of the groundwater, with elevated As correlating with higher Fe in reducing waters and inversely correlating with nitrate, which is present in oxidising waters. The dashed line depicts drinking water guideline value for As [10 µg/l]. 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.

4.2 Inorganic chemistry and clogging potential

The results of the sampling program revealed a clear correlation between affected bores and the redox potential of the groundwater. Strongly reducing waters were thereby the most likely to correlate with clogged bores (Figure 4). These bores were found to be the most severely oversaturated in respect to iron oxide minerals (Figure 6), indicating a strong potential for iron precipitation if the water is exposed to oxygen. It is noteworthy that the highest iron concentrations were thereby found in the deepest bores and these were tendentially more recently drilled bores (Figure 7). However, shallow bores turned out not to be a guarantee for low iron concentrations with some bores as shallow as 10m reporting elevated Mn and Fe concentrations also (Figure 7).

Bores reported as unaffected by clogging were all reporting appreciable dissolved oxygen concentrations and elevated redox potentials. If, in addition, the iron concentration was low, the groundwaters were undersaturated in respect to amorphous iron oxides ($Fe(OH)_3$), indicating a low inorganic clogging potential.

All samples from bores strongly affected by clogging had elevated NPOC (> 1 mg/L, Figure 4). Microbial growth is related to the amount of labile carbon and available nutrients in groundwater. However, numerous samples from unaffected bores also had elevated NPOC. Therefore, whilst the presence of organic carbon may facilitate microbial growth and hence microbiological clogging, it is not necessarily a key deciding factor in the onset of clogging.

There were two outlier bores that did not follow the same trends. Bore 24, identified as strongly affected by clogging had a low dissolved iron of 12.3 µg/L compared with other affected bores. However, the sample from this bore was obtained using a bailer (as there was no pump installed) and is therefore unlikely to be representative of aquifer conditions. Bore 4, also identified as strongly affected, had a low dissolved iron concentration, a dissolved oxygen concentration > 2 mg/L and a positive redox potential. The reason for the chemical differences observed in bore 4 compared with the other strongly affected bores is unknown.

It is possible that characteristics of the bore have caused the native groundwater around it to become oxygenated. It is recommended that both these bores be further investigated during future field studies.

The actual clogging material, obtained from an affected screen retrieved from a bore during the first sampling round, was visualised through scanning electron microscope (SEM) (Figure 8). The precipitate was found to be very diverse and generally of low crystallinity. Amorphous iron oxides appear to have formed, however, evidence of bacterial growth was also found, suggesting the co-occurrence of inorganic as well as microbiological clogging.

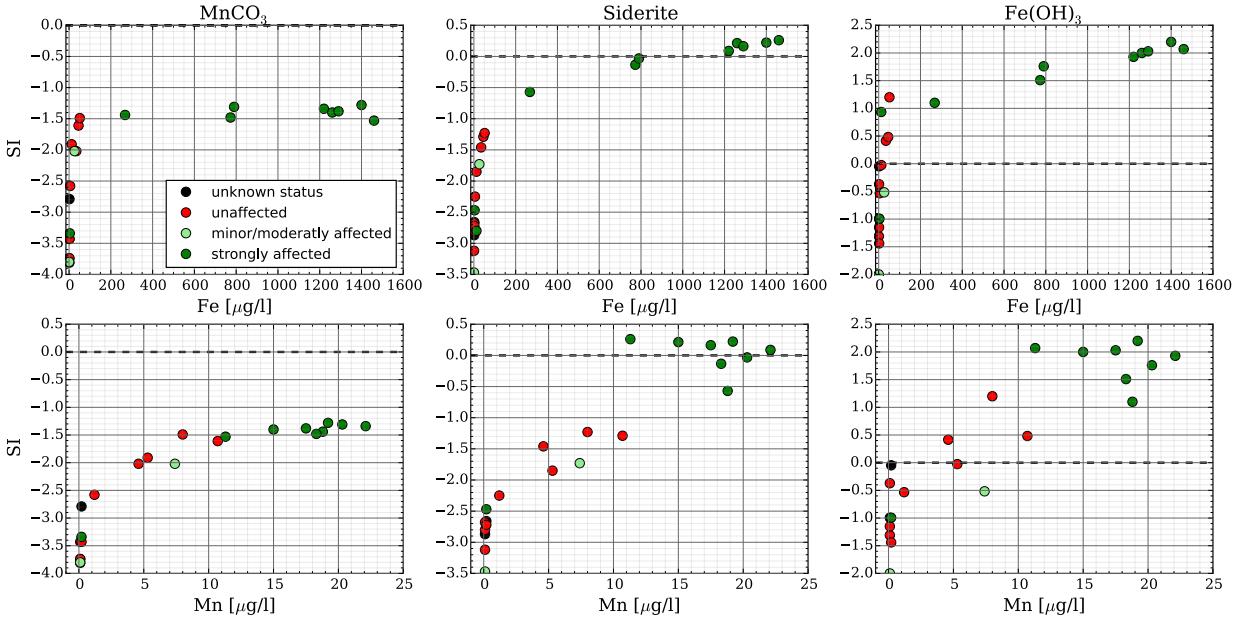


Figure 6: Correlation between Fe(II) and Mn(II) and the saturation of groundwaters in respect to the common aquifer minerals rhodochrosite ($MnCO_3$), siderite ($FeCO_3$) and iron hydroxide ($Fe(OH)_3$). The dashed line indicates mineral equilibrium (SI=0) with minerals likely to precipitate at SI>0 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.

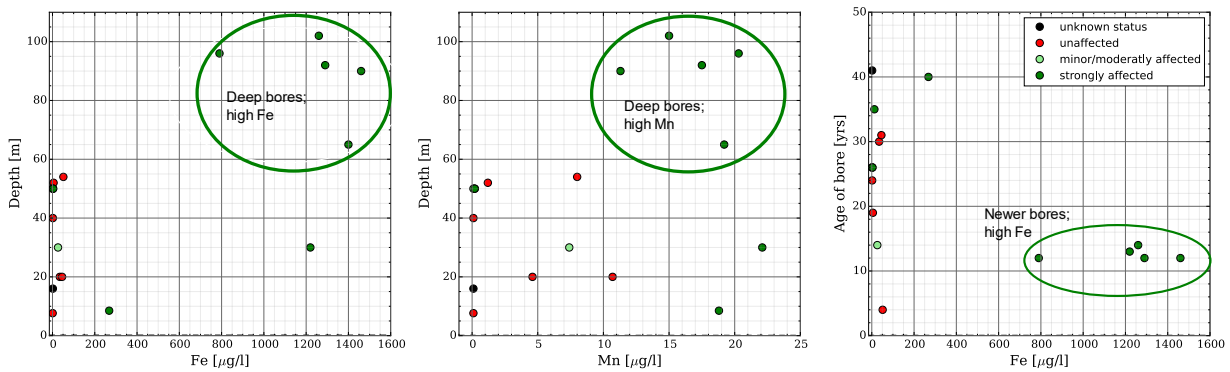


Figure 7: Correlation between the depth of bores and Fe(II) and Mn(II) concentrations as well as borehole age. Information on depth and/or age was not available for all sampled bores.

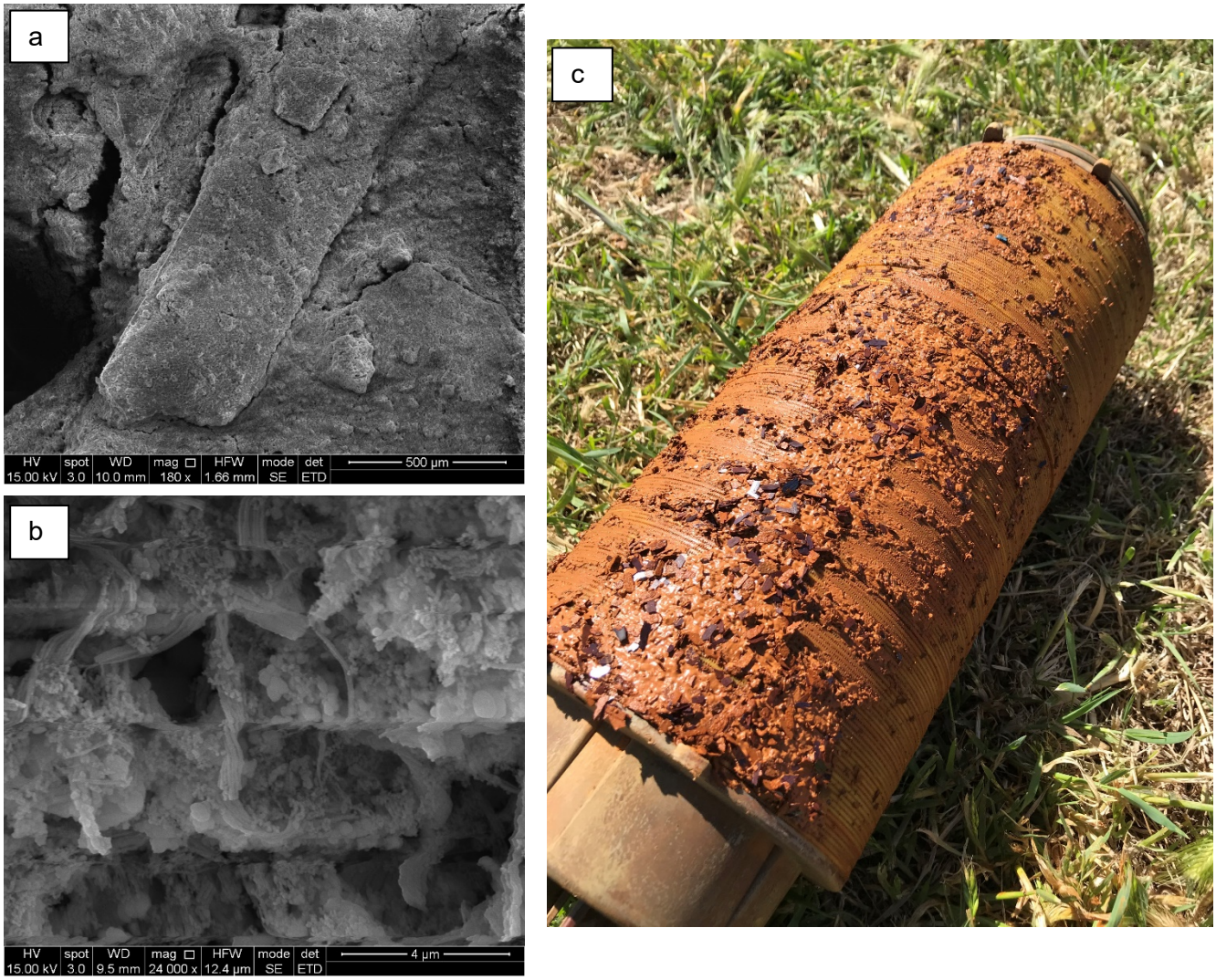


Figure 8: SEM of clogging material: a: amorpheous iron oxides; b: evidence of biological growth; c: incrustations of clogged screen, which were sampled for SEM analysis.

4.3 Microbiological analysis

The IRB and Colilert tests indicated the presence of viable bacterial populations in the bore water samples (Table 1). 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. The whole genome sequences of bacterial populations present in a subset of samples demonstrated that the primary IRB were from the *Gallionella* genus (Appendix C) followed by the *Crenothrix* genus (not shown in Appendix C as it was not in the 15 most prevalent genus). These are considered common iron-oxidising bacteria (Cullimore & McCann, 1977). However, there was no correlation between the presence of IRB and the

clogging status of a bore (Figure 9). There was also no correlation with the presence of IRB and the iron content, pH, location, 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.

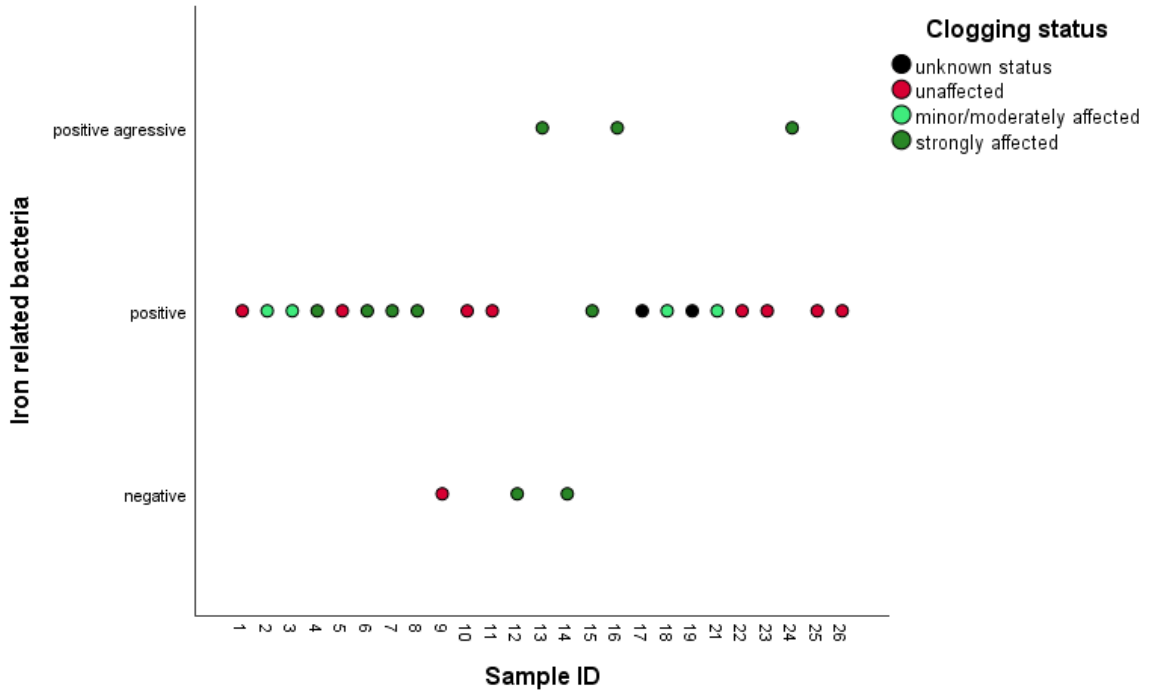


Figure 9: Iron related bacteria (IRB) presence determined using the IRB Bart test and clogging status of bores

Coliforms are commonly used as an indicator of water quality. They are found in large numbers in the faeces of humans and other warm-blooded animals but may also occur in the environment. *E. coli* is a specific indicator of recent faecal contamination because generally it is not capable of growth in the environment and is principally associated with faecal contamination and should not be present in drinking water (National Health and Medical Research Council (NHMRC), 2016). In this study, 22 (out of 25) samples (88%) and 7/25 samples (28%) were positive for coliform bacteria and *E. coli* respectively. 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 whole genome sequencing of bacterial populations also revealed *Acinetobacter, Pseuodmonas, Sphingobium, Lysobacter, Sphingomonas, Mycobacterium, Sulfuricurvum, Flavobacterium, Rickettsiella, Sideroxydans* to be predominant communities in the sampled bore water. These are widespread soil inhabitants and common in well and aquifer samples (Korbel, Chariton, Stephenson, Greenfield, & Hose, 2017). *Pseudomonas* is known to contain crop pathogens and is commonly found in the wells. Its presence might however indicate potential groundwater contamination (Nevondo & Cloete, 1999). No bacterial populations were detected in 2 samples among the 25 either because there were no active bacterial

populations or they were present at concentrations below the limit of detection. No correlation was observed between microbial water quality and the clogging status of a bore.

Table 2: The presence of different microbial populations in the collected bore water samples

Sample number	IRB*	Coliform bacteria	*E. coli* (MPN/100mL)	Clogging status
1	+	+	0	unaffected
2	+	+	0	affected to a minor/moderate degree
3	+	+	0	affected to a minor/ moderate degree
4	+	+	0	strongly affected
5	+	+	0	unaffected
6	+	+	51.2	strongly affected
7	+	+	9.7	strongly affected
8	++	+	0	strongly affected
9	-	+	0	unaffected
10	+	+	0	unaffected
11	+	+	0	unaffected
12	-	-	0	strongly affected
13	++	+	0	strongly affected
14	-	-	0	strongly affected
15	+	+	0	strongly affected
16	++	+	24.6	strongly affected
17	+	+	0	Unknown
18	+	+	0	affected to a minor/moderate degree
19	+	+	0	Unknown
20*	na	na	na	no sample
21	++	+	7	affected to a minor/moderate degree
22	+	+	2	Unaffected
23	+	+	0	Unaffected
24	++		2	strongly affected
25	+	+	0	Unaffected
26	+	+	6	Unaffected

+ = Moderate concentrations of IRB *++ = Aggressive concentrations of IRB

* not sampled

4.4 Limitations

One of the challenges with this study was determining the clogging status of a bore. The reliance on self-reporting would have introduced significant bias. The common preconception that iron-related clogging was due solely to IRB meant that if a bore had been previously tested and found to contain IRB, this bore was then considered affected. Also, it became apparent, that different vineyard owners/managers would have their own scale in regards to the severity of clogging, based on their experience. Thus, what may be reported as a considerable clogging problem in one area may be regarded as less problematic in another

area. The inability to scale the clogging problem impacted on the ability to correlate between the severity of clogging and operational/chemical/biological indicators.

Also, some sampled bores are being treated periodically e.g. through acid application. As such, microbiological analysis may have been impacted if treatment would have occurred sometime prior to sampling with the analysed IRB load lower than under native conditions.

5. Conclusion

The results of the reconnaissance sampling of 26 bores experiencing different levels of iron clogging revealed a clear correlation between affected bores and the redox potential of the groundwater. Specifically, the following groundwater chemical characteristics appear to be associated with iron clogging:

- Strongly reducing waters were the most likely to be associated with clogged bores. All affected bores had dissolved oxygen concentrations less than 4 mg/L (strongly affected < 2 mg/L) and dissolved iron and manganese concentrations greater than 200 µg/L (0.2 mg/L) and 10 µg/L (0.01 mg/L), respectively.
- 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.
- Conversely to the above, samples from bores reported as being unaffected by clogging all had appreciable dissolved oxygen concentrations and elevated (positive) 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.
- All samples from bores strongly affected by clogging had elevated NPOC (> 1 mg/L). Generally, microbial growth is correlated to the amount of labile carbon and available nutrients in groundwater. However, numerous samples from unaffected bores also had elevated NPOC. Therefore, whilst the presence of organic carbon may facilitate microbial growth and hence microbiological clogging, it is not necessarily a key deciding factor in the onset of clogging.
- Severely affected bores were tendentially found to be deeper bores, while bores without clogging problems were generally shallower (<60m).

IRB were detected in the majority of wells sampled; however, there was no apparent correlation between the presence of IRB and the clogging status of the well. The results suggest that IRB are widespread throughout the aquifer.

The clogging material examined using the scanning electron microscope found evidence of both crystalline material and biological growth. This supports the findings from the microbiological and inorganic water analysis that clogging is most likely an inorganic problem which is enhanced through the presence of bacteria.

On the basis of these findings, it appears unlikely, that the perceived increase in iron clogging in the region is a result of the widespread introduction of IRB through drilling equipment. In fact, in bore 23 which was drilled in 2016, IRB were found to be present in moderate numbers despite the fact that the land owner had required the driller of the bore to disinfect their equipment prior to drilling.

The above conclusions are preliminary as they are based on observations from a limited dataset obtained from 26 bores. Expansion of the dataset is required to further explore these observations and the degree to which groundwater redox characteristics can be used to determine the likelihood that a bore will experience clogging.

It can be speculated that a combination of longer term processes may have enhanced the clogging problem in the region over time. The tendency to drill deeper bores and deepen older bores in more recent times may have enhanced the probability of bores/irrigation systems to be affected by clogging, as more reducing conditions favouring higher dissolved iron concentrations are likely to exist in deeper bores (Figure 9). In fact, one irrigator, who was part of this study, stated that the clogging problem increased in the area following the millennium drought and that in general, newer bores were more affected than older bores. The latter were hardly or not affected at all in his experience. Additionally, irrigation practices have changed over the last decades and this could have aided clogging of irrigation infrastructure with drip irrigation systems, with their very narrow emitters more prone to clogging through organic growths and/or iron precipitation.

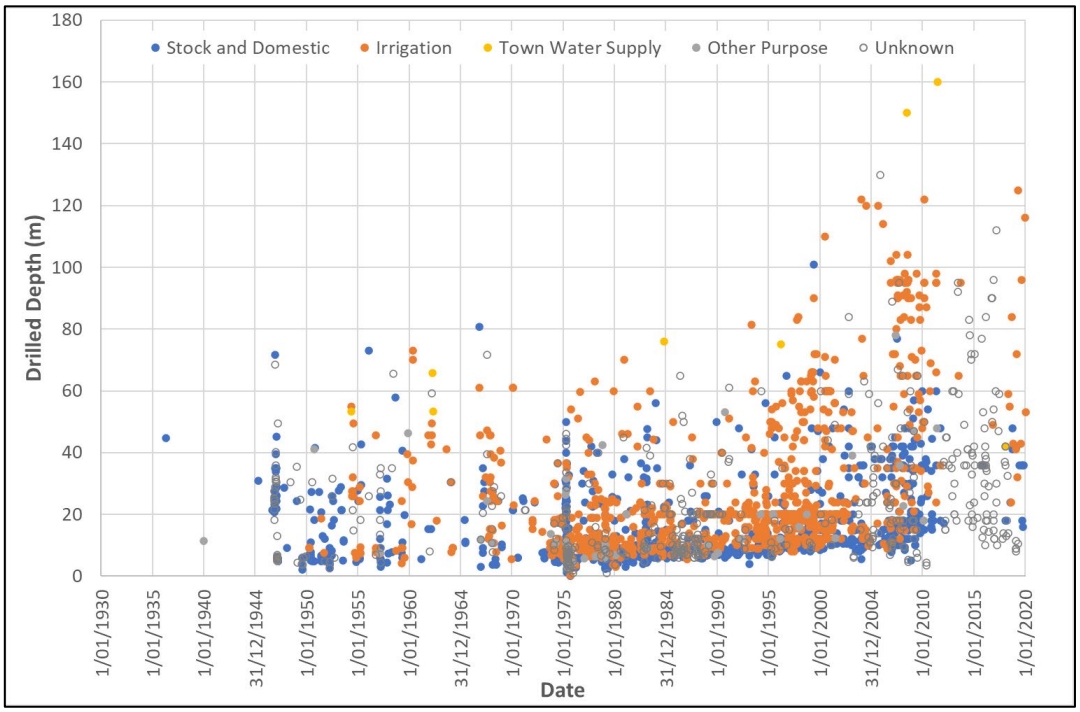


Figure 9: Graph of bore drilled depth versus drilled date for the Coonawarra/Wrattonbully area showing that more bores have been drilled to greater than 60 m depth since 1990.

6. Recommendations for Future Work

Future data collection on the iron clogging problem would benefit from developing a standard and systematic set of questions to more accurately determine the clogging status of a well, as well as the characteristics, timing and locations (pump, screen, dripper lines, etc) of the clogging. It would also be of great value if property owners / managers were to maintain formal records of clogging-related information to improve the accuracy of the anecdotal information available.

The dataset obtained through this study should be expanded to verify the conclusions drawn on the basis of the currently limited number of examined bores. A larger scale analysis would also allow to evaluate the clogging potential in the context of regional-scale information, i.e. aquifer properties, groundwater level trends and trends in drilling depths and groundwater use, which may help to identify larger-scale driving processes and may assist with identification of 'high risk' areas for iron clogging.

Other areas of future research would also include identifying the best operations protocols to reduce oxygen ingress and iron precipitation as well as examining the efficacy of disinfection strategies to control biofilm.

6 References

Baveye, P., Vandevivere, P., Hoyle, B. L., DeLeo, P. C., & de Lozada, D. S. (2010). Environmental Impact and Mechanisms of the Biological Clogging of Saturated Soils and Aquifer Materials. *Critical Reviews in Environmental Science and Technology, 28*(2), 123-191. doi:10.1080/10643389891254197

Cullimore, D., & McCann, A. E. (1977). The identification, cultivation and control of iron bacteria in ground water. *J Aquatic microbiology*, 219-261.

Davison, W., & Seed, G. (1983). The kinetics of the oxidation of ferrous iron in synthetic and natural waters. *J Geochimica et Cosmochimica Acta, 47*(1), 67-79.

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerg Infect Dis, 8*(9), 881-890.

Gino, E., Starosvetsky, J., Kurzbaum, E., & Armon, R. (2010). Combined chemical-biological treatment for prevention/rehabilitation of clogged wells by an iron-oxidizing bacterium. *Environmental Science & Technology, 44*(8), 3123-3129.

Gong, J., Erkelens, M., Lambert, M. F., & Forward, P. (2019). Experimental Study of Dynamic Effects of Iron Bacteria–Formed Biofilms on Pipeline Head Loss and Roughness. *J Journal of Water Resources Planning Management, 145*(9), 04019038.

Grenthe, I., Stumm, W., Laaksuharju, M., Nilsson, A., & Wikberg, P. (1992). Redox potentials and redox reactions in deep groundwater systems. *J Chemical Geology, 98*(1-2), 131-150.

Harrington, N., & Harrington, G. (2017). *Best management practices for irrigation water quality issues in the limestone coast region of South Australia*. Retrieved from Australia,:

Houben, G., & Sander, J. (2020). Preventing the growth of iron bacteria in water wells by copper and silver coating. *J Water Supply, 20*, 1195–1206.

Korbel, K., Chariton, A., Stephenson, S., Greenfield, P., & Hose, G. C. (2017). Wells provide a distorted view of life in the aquifer: implications for sampling, monitoring and assessment of groundwater ecosystems. *Scientific Reports, 7*, 40702.

Kyser, T.K., James, N.P., Bone, Y. (2002) Shallow Burial Dolomitization and Dedolomitization of Cenozoic Cool-Water Limestones, Southern Australia: Geochemistry and Origin Journal of Sedimentary Research 72(1):146-157

Love, A.J., Herczeg, A.L., Armstrong, D., Stadter, F. and Mazor, E. (1993). Groundwater flow regime within the Gambier Embayment of the Otway Basin, Australia: evidence from hydraulics and hydrochemistry., J. Hydrol., 143, pp 297-338.

McLaughlan, R. G. (1989). *Corrosion and incrustation in groundwater bores: a critical review*: Centre for Groundwater Management and Hydrogeology.

National Health and Medical Research Council (NHMRC). (2016). Australian Drinking Water Guidelines 6, 2011 https://www.clearwater.asn.au/user-data/resource-files/Aust_drinking_water_guidelines.pdf

Neubauer, S. C., Emerson, D., & Megonigal, J. P. (2002). Life at the energetic edge: kinetics of circumneutral iron oxidation by lithotrophic iron-oxidizing bacteria isolated from the wetland-plant rhizosphere. *J Applied Environmental Microbiology, 68*(8), 3988-3995.

Nevondo, T., & Cloete, T. E. (1999). Bacterial and chemical quality of water supply in the Dertig village settlement. *Water SA, 25*.

OhioEPA (2014) Reduction-Oxidation (Redox) Control in Ohio's Ground Water Quality. Technical Series on Ground Water Quality 2014

Primary Industries and Resources SA (PIRSA) 2010, South East of South Australia Regional Profile October 2010.

Rentz, J. A., Kraiya, C., Luther, G. W., & Emerson, D. (2007). Control of ferrous iron oxidation within circumneutral microbial iron mats by cellular activity and autocatalysis. *J Environmental science technology, 41*(17), 6084-6089.

Singh, V. K., Singh, A. L., Singh, R., & Kumar, A. (2018). Iron oxidizing bacteria: insights on diversity, mechanism of iron oxidation and role in management of metal pollution. *J Environmental Sustainability, 1*(3), 221-231.

Straub, K. L., Benz, M., & Schink, B. (2001). Iron metabolism in anoxic environments at near neutral pH. *J FEMS microbiology ecology, 34*(3), 181-186.

Sun, H., Shi, B., Lytle, D. A., Bai, Y., & Wang, D. (2014). Formation and release behavior of iron corrosion products under the influence of bacterial communities in a simulated water distribution system. *Environmental Science: Processes Impacts, 16*(3), 576-585.

Sung, W., & Morgan, J. J. (1980). Kinetics and product of ferrous iron oxygenation in aqueous systems. *J Environmental science technology, 14*(5), 561-568.

Teutsch, N., von Gunten, U., Porcelli, D., Cirpka, O. A., & Halliday, A. N. (2005). Adsorption as a cause for iron isotope fractionation in reduced groundwater. *Geochimica et Cosmochimica Acta, 69*(17), 4175-4185. doi:https://doi.org/10.1016/j.gca.2005.04.007

Tong, M., Yuan, S., Zhang, P., Liao, P., Alshawabkeh, A. N., Xie, X., & Wang, Y. (2014). Electrochemically induced oxidative precipitation of Fe (II) for As (III) oxidation and removal in synthetic groundwater. *J Environmental science technology, 48*(9), 5145-5153.

Vandevivere, P., & Baveye, P. (1992). Effect of bacterial extracellular polymers on the saturated hydraulic conductivity of sand columns. *J Appl. Environ. Microbiol., 58*(5), 1690-1698.

Wang, J., Sickinger, M., Ciobota, V., Herrmann, M., Rasch, H., Rösch, P., . . . Küsel, K. (2014). Revealing the microbial community structure of clogging materials in dewatering wells differing in physico-chemical parameters in an open-cast mining area. *J Water research, 63*, 222-233.

Appendix A

Inorganic chemistry

Inorganic chemistry

					|----Ion Chromatography---|				ICP (major ions)							ICP (minor ions)				
	pH	E.C.	Total Alkalinity	NPOC	F^-	Cl^-	Br^-	NO_3^-	SO_4^-	Ca	K	Mg	Na	S	B	Cu	Fe	P	Si	Sr
		dS/m	**meq/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**
1	8.0	1.8	6.7	1.1	0.3	437	0.9	18	50	120	4.5	31	205	14	0.18	<0.05	<0.1	<0.2	7.7	1.2
2	7.6	2.8	8.6	0.1	0.3	771	2.0	7.9	100	154	7.7	55	353	27	0.36	<0.05	<0.1	<0.2	11	2.1
3	7.7	1.8	7.1	1.2	0.5	470	1.2	3.3	45	126	5.8	40	209	12	0.31	<0.05	<0.1	<0.2	11	1.5
4	7.5	2.6	7.5	1.1	0.5	725	2.0	8.1	79	153	8.3	56	308	21	0.27	<0.05	<0.1	<0.2	11	2.3
5	7.5	2.6	7.9	0.2	0.3	722	1.9	12	86	158	7.5	52	322	23	0.32	<0.05	<0.1	<0.2	11	2.0
6	7.6	2.0	6.6	1.7	0.2	488	0.9	<0.2	118	153	5.0	36	226	32	0.07	<0.05	0.3	<0.2	8.1	1.3
7	7.7	2.0	6.7	1.4	0.2	460	0.9	<0.2	133	153	5.2	34	219	36	0.07	<0.05	0.8	<0.2	8.5	1.3
8	7.7	1.9	6.7	1.9	0.2	450	0.9	<0.2	124	153	4.9	33	220	34	0.07	<0.05	1.2	<0.2	8.5	1.3
9	7.6	1.9	6.8	1.5	0.2	458	0.9	<0.2	106	144	6.1	35	211	28	0.09	<0.05	<0.1	<0.2	7.8	1.1
10	7.7	1.6	6.5	1.8	0.2	407	1.0	0.2	34	130	4.3	29	184	9.3	0.07	<0.05	<0.1	<0.2	9.9	1.4
11	7.7	1.8	6.0	1.0	0.2	425	0.9	34	69	138	4.7	30	193	18	0.08	<0.05	<0.1	<0.2	9.8	1.2
12	7.7	1.5	6.9	1.5	0.3	347	0.8	<0.2	24	117	4.9	30	169	6.8	0.09	<0.05	1.3	<0.2	14	1.3
13	7.7	1.5	6.7	1.6	0.2	349	0.8	<0.2	22	118	4.8	29	163	6.2	0.07	<0.05	0.8	<0.2	12	1.3
14	7.8	1.5	6.8	1.5	0.2	359	0.8	<0.2	20	119	4.8	29	165	5.7	0.07	<0.05	1.3	<0.2	14	1.3
15	7.8	1.4	6.6	1.9	0.3	319	0.7	<0.2	17	113	4.7	28	149	4.9	0.07	<0.05	1.5	<0.2	14	1.3
16	7.7	1.4	5.7	1.0	0.2	337	0.8	<0.2	20	118	3.7	23	147	5.7	0.07	<0.05	1.4	<0.2	11	1.1
17	7.7	1.8	5.4	1.0	0.3	508	1.1	58	31	155	4.2	36	174	8.1	0.07	<0.05	<0.1	<0.2	11	1.3
18	7.7	1.5	5.8	0.9	0.2	326	0.7	93	38	121	3.6	29	160	10	0.07	<0.05	<0.1	<0.2	10	0.87
19	8.0	2.0	6.5	0.1	0.2	514	1.0	25	53	165	4.6	35	199	14	0.09	<0.05	<0.1	<0.2	10	1.2
20	7.7	1.5	6.2	1.5	0.2	319	0.6	64	52	129	4.3	28	147	13	0.07	<0.05	<0.1	<0.2	10	0.79
21	7.4	1.8	8.5	0.9	0.4	339.3	0.7	21.6	62.7	130	5.23	41.6	174	20.7	0.24	<0.05	<0.1	<0.2	7.9	1.1
22	7.6	1.7	6.9	1.4	0.6	364.2	1.3	3.7	53.6	103	5.39	41.6	168	17.6	0.20	<0.05	<0.1	<0.2	8.1	1.3
23	7.6	1.8	6.6	1.3	0.7	417.9	1.6	1.1	66.8	105	5.59	43	188	19.7	0.20	<0.05	<0.1	<0.2	8.4	1.3
24	7.7	1.7	7.7	0.7	0.6	343.6	1.4	1.5	51.2	111	5.66	44.2	167	17.4	0.23	<0.05	<0.1	<0.2	7.8	1.3
25	7.5	1.6	6.9	1.7	0.6	327.4	1.1	8.6	30.8	115	5.01	41.6	158	11.6	0.18	<0.05	<0.1	<0.2	7.4	1.3
26	7.6	1.7	6.9	1.6	0.4	355.6	1.3	8.7	47.1	113	4.47	41	166	17.1	0.23	<0.05	<0.1	<0.2	8.4	1.2

Inorganic chemistry (continued)

	ICP												ICP-MS										
	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	As	Se	Mo	Ag	Cd	Sn	Sb	La	Tl	Pb	Bi	U
	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
1	<0.1	<0.8	0.89	<0.5	<0.2	0.8	<0.05	0.7	0.4	18.2	<0.05	<0.4	2.5	<0.2	<0.1	1.48	<0.4	<0.2	<0.05	<0.2	1.9	<0.2	0.7
2	<0.1	<0.8	0.67	<0.5	<0.2	<0.8	<0.05	0.6	0.2	2.8	<0.05	0.5	2.7	0.4	<0.1	0.88	<0.4	<0.2	<0.05	<0.2	<0.1	<0.2	<0.1
3	<0.1	<0.8	0.65	<0.5	1.2	5.1	<0.05	1.3	1.4	13.0	<0.05	0.6	2.8	<0.2	<0.1	1.60	<0.4	<0.2	<0.05	<0.2	2.1	<0.2	0.9
4	<0.1	<0.8	0.95	<0.5	0.2	3.4	<0.05	1.1	1.4	15.8	<0.05	0.5	2.0	<0.2	<0.1	1.26	<0.4	<0.2	<0.05	<0.2	1.6	<0.2	0.7
5	<0.1	<0.8	0.79	<0.5	0.2	2.2	<0.05	0.5	3.6	85.4	<0.05	0.5	2.3	<0.2	<0.1	1.22	<0.4	<0.2	<0.05	<0.2	1.6	<0.2	0.6
6	<0.1	<0.8	0.10	<0.5	18.8		0.15	0.3	0.2	1.9	<0.05	1.7	0.7	0.3	<0.1	1.55	<0.4	<0.2	<0.05	<0.2	1.8	<0.2	<0.1
7	<0.1	1.2	0.33	<0.5	18.3		0.06	0.6	0.1	12.7	<0.05	4.6	0.7	0.7	<0.1	1.45	<0.4	<0.2	<0.05	<0.2	1.7	<0.2	<0.1
8	<0.1	<0.8	0.28	<0.5	22.1		<0.05	2.4	<0.1	73.0	<0.05	4.7	0.8	0.5	<0.1	1.60	<0.4	<0.2	<0.05	<0.2	1.9	<0.2	<0.1
9	<0.1	<0.8	4.47	<0.5	4.6	34.7	0.09	0.7	0.5	1.0	<0.05	0.6	0.9	0.4	<0.1	1.52	<0.4	<0.2	<0.05	<0.2	1.9	<0.2	0.2
10	<0.1	<0.8	0.20	<0.5	10.7	45.4	0.06	0.4	0.1	9.1	<0.05	9.1	1.0	<0.2	<0.1	1.61	<0.4	<0.2	<0.05	<0.2	2.0	<0.2	0.1
11	<0.1	<0.8	2.33	<0.5	0.5	3.6	<0.05	0.3	3.9	9.9	<0.05	1.4	1.2	<0.2	<0.1	1.32	<0.4	<0.2	<0.05	<0.2	1.5	<0.2	<0.1
12	<0.1	<0.8	<0.05	<0.5	15.0		<0.05	0.4	<0.1	<0.5	<0.05	12.0	0.5	0.4	<0.1	1.27	<0.4	<0.2	<0.05	<0.2	1.4	<0.2	<0.1
13	<0.1	<0.8	<0.05	<0.5	20.3		<0.05	0.4	<0.1	1.2	<0.05	11.1	0.3	0.3	<0.1	0.96	<0.4	<0.2	<0.05	<0.2	0.9	<0.2	<0.1
14	<0.1	<0.8	<0.05	<0.5	17.5		<0.05	0.3	0.1	1.5	<0.05	10.4	0.3	0.2	<0.1	1.10	<0.4	<0.2	<0.05	<0.2	1.1	<0.2	<0.1
15	<0.1	<0.8	<0.05	<0.5	11.3		<0.05	0.4	<0.1	4.5	<0.05	12.8	0.4	0.3	<0.1	1.61	<0.4	<0.2	<0.05	<0.2	2.5	<0.2	<0.1
16	<0.1	0.9	<0.05	<0.5	19.2		0.07	0.5	0.1	8.1	<0.05	28.6	0.6	0.2	<0.1	1.59	<0.4	<0.2	<0.05	<0.2	1.9	<0.2	<0.1
17	<0.1	<0.8	0.61	<0.5	<0.2	1.7	<0.05	0.2	0.8	9.8	<0.05	1.9	2.7	<0.2	<0.1	1.74	<0.4	<0.2	<0.05	<0.2	2.4	<0.2	0.3
18	<0.1	<0.8	3.50	<0.5	0.3	3.4	<0.05	0.3	1.6	6.3	<0.05	0.5	2.2	<0.2	<0.1	1.81	<0.4	<0.2	<0.05	<0.2	2.5	<0.2	0.5
19	<0.1	<0.8	3.15	<0.5	0.2	2.0	<0.05	0.3	0.2	1.3	<0.05	1.1	5.2	<0.2	<0.1	1.52	<0.4	<0.2	<0.05	<0.2	1.8	<0.2	0.2
20	<0.1	<0.8	1.65	<0.5	<0.2	<0.8	<0.05	0.2	<0.1	<0.5	<0.05	1.6	1.3	0.4	<0.1	<0.05	<0.4	<0.2	<0.05	<0.2	<0.1	<0.2	0.7
21	<0.1	<0.5	2.1	0.4	7.4	26.1	0.94	1.5	1.7	2.1	<0.1	3.47	3.27	<0.3	<0.3	0.9	<0.4	<0.2	<0.05	<0.1	1.7	<0.1	1.3
22	<0.1	<0.5	0.2	0.5	5.3	12.4	<0.05	1.9	8.7	24.6	<0.1	1.45	1.70	<0.3	<0.3	0.9	<0.4	<0.2	<0.05	<0.1	3.0	<0.1	0.7
23	<0.1	<0.5	0.1	0.3	8.0	51.4	0.11	2.0	0.7	4.9	<0.1	3.48	1.17	<0.3	<0.3	0.9	<0.4	<0.2	<0.05	<0.1	1.7	<0.1	0.4
24	<0.1	1.7	<0.1	<0.2	117.7	12.3	0.15	0.8	0.9	11.2	<0.1	0.17	0.89	<0.3	<0.3	0.7	<0.4	<0.2	<0.05	<0.1	1.1	<0.1	0.6
25	<0.1	<0.5	1.3	0.7	<0.2	1.7	<0.05	3.1	0.8	12.7	<0.1	0.86	3.03	<0.3	<0.3	0.8	<0.4	<0.2	<0.05	<0.1	1.6	<0.1	2.1
26	<0.1	<0.5	1.6	0.8	<0.2	1.7	<0.05	0.4	1.9	10.1	<0.1	0.76	3.83	<0.3	<0.3	0.8	<0.4	<0.2	<0.05	<0.1	1.5	<0.1	1.3

Appendix B

Well-head measurements and Saturation Indices

Well-head measurements and Saturation Indices

	Well head measurements						Electr.	Saturation Indices					
	Alkalinity	EC [uS/cm]	pH	Temp (ºC)	DO [mg/L]	Redox (mV)	balance error [%]	Dolomite	Calcite	Fe(OH)3	Quartz	Rhodocrosite	Siderite
1	251, 246	1726	6.95	17.98	8.6	99.5	-5.745	-0.372	-0.068	-1.313	0.067	-3.743	-3.117
2	337	2765	6.84	17.14	3.65	-9.1	-5.903	-0.108	-0.007	-1.998	0.224	-3.804	-3.467
3	319	1824	6.96	19.23	6.63	24.2	-5.195	-0.030	0.058	-0.533	0.222	-2.576	-2.254
4		2539	6.87	17.88	3.49	95.5	-7.989	0.066	0.074	-0.994	0.223	-3.336	-2.474
5	403, 385	2602	6.78	19	4.57	114	-8.184	-0.097	0.016	-1.437	0.224	-3.425	-2.716
6	309, 325	1985	6.93	16.17	1.65	-84.3	-4.752	-0.113	0.082	1.099	0.089	-1.436	-0.570
7	302, 311	1933	6.91	16.61	1.31	-169.6	-4.373	-0.204	0.050	1.508	0.110	-1.476	-0.134
8	280	1905	6.99	16.77	1.3	-115.7	-2.040	-0.124	0.096	1.929	0.110	-1.345	0.089
9	275, 262	1843	7	16.74	5.84	-5.4	-2.957	-0.121	0.071	0.415	0.073	-2.023	-1.460
10	318, 308	1627	6.98	16.43	1.59	-187	-4.208	-0.106	0.096	0.482	0.176	-1.610	-1.285
11		1727	6.98	21.44	4.69	130	-14.085	0.062	0.186	-0.650	0.172	-2.884	-2.331
12		1494	7.01	17.92	0.77	-79.2	-4.208	0.024	0.131	1.997	0.326	-1.398	0.214
13	312, 308	1475	6.99	17.58	1.42	-25	-3.187	-0.107	0.075	1.756	0.259	-1.312	-0.032
14	286	1488	7.01	17.93	1.64	-58	-1.942	-0.130	0.065	2.028	0.326	-1.385	0.164
15	330, 309	1348	7.01	17.99	0.72	-106.8	-3.955	-0.061	0.096	2.074	0.326	-1.532	0.261
16			7.07	17.88	0.83	-93.5	-4.590	-0.099	0.129	2.203	0.221	-1.282	0.222
17	211, 215	1803	6.95	16.61	4.47	97	-12.204	-0.357	-0.038	-0.995	0.222	-3.810	-2.875
18	272, 255	1482	7.07	16.32	4.6	-26.5	-17.361	-0.119	0.074	-0.409	0.180	-3.070	-2.436
19		1917	7.45	16.94	10.18	95.8	-12.468	1.149	0.734	-0.047	0.180	-2.789	-2.660
20							-16.081	1.858	1.083	-0.555	0.174	-2.858	-4.381
21	345; 298; 343; 329	1910.5	6.71	17.17	2.7	115	-3.453	-0.490	-0.173	-0.517	0.078	-2.023	-1.735
22	297; 300	1738	7	18.86	3.5	56	-5.099	-0.042	0.000	-0.026	0.088	-1.905	-1.849
23	287	1939	7.29	18.54	2.08	-38.2	-5.501	0.500	0.268	1.196	0.104	-1.490	-1.229
24							-5.976	2.173	1.108	0.934	0.066	0.252	-2.797
25	284	1784	7.23	18.16	4.96	50.3	-0.110	0.430	0.260	-0.371	0.049	-3.435	-2.680
26	278; 283; 266	1749	6.9	17.92	7.23	110	-2.753	-0.264	-0.088	-1.151	0.104	-3.745	-2.804

Appendix C

Information on wells and microbiological analysis

Well information and microbiological analysis

	Comments	*Drill Date*	*Purpose*	*Aquifer*	*Depth To (m)*	*Well Yield (L/sec)*	*SWL (m)*	*Date SWL meas.*	*IRB**	*Coliform bacteria*	*E. coli (MPN/100mL)*	*Iron clogging*
1	not affected; bore was running a few hours prior to sampling		Dom	Limest.	7.65			1982	+	+	0	unaffected
2	Moderate problems. Bore affected, but no clogging at dripper lines. Sample analysed in 2010 by AWQC showed low number of iron bacteria and presence of sulphate reducing bacteria	1994	IRR	Limest.	50	37.5	5.9	1994	+	+	0	affected to a minor/moderate degree
3	Cavity encountered - bore issues not related to clogging. Sample analysed in 2010 by AWQC showed moderate number of iron bacteria and presence of sulphate reducing bacteria.	2001	IRR	Limest.	52	30	11.5	2001	+	+	0	affected to a minor/ moderate degree
4	Severely affected. Sample analysed in 2010 by AWQC showed high numbers of iron bacteria and presence of sulphate reducing bacteria	1994	IRR	Limest.	50	15	5	1994	+	+	0	strongly affected
5	Some problems encountered with bore, however high usage is probably the cause of issues rather than clogging. Sample analysed in 2010 by AWQC showed low number of iron bacteria and presence of sulphate reducing bacteria	1994	IRR	Limest.	50	43.5	3	1995	+	+	0	unaffected
6	Affected. Well not recorded on WaterConnect. Noticeable iron on filter paper.	1980	Dom	Limest.	8.5	0.05		1980	+	+	51.2	strongly affected
7	This bore is not on WaterConnect. Bore was not in used since last winter prior to sampling.		IRR	Limest.					+	+	9.7	strongly affected
8	Pump has been on ca. 12 times since September for frost irrigation	2007	IRR	Limest.	30	106.2	5.6	2007	++	+	0	strongly affected

9	Not affected. Bore had been on 12 times since September for frost irrigation. Bore was not running prior to sampling.	1990	IRR	Limest.	20	125	4	1990	-	+	0	unaffected
10		1989	IRR	Limest.	20	125	4	1989	+	+	0	unaffected
11	Not affected - a shallow bore into the sand aquifer. Domestic well - not on WaterConnect		Domestic	Sand	shallow				+	+	0	unaffected
12	Affected. Sampling only possible at dripper, not directly from bore. Pump was running prior to sampling. Magnet installed at pump outlet	2006	IRR	Limest.	102	2	5	2006	-	-	0	strongly affected
13	severely affected; acid treatment	1993 to 18m then deepened to 96m in 2008	IRR	Limest.	96	1.25	7.3	2008	++	+	0	strongly affected
14	severely affected; acid treatment. Only used in growing season (Oct to May)	2008	IRR	Limest.	92	1.25	7.3	2008	-	-	0	strongly affected
15	severely affected; acid treatment and magnet installed. Only used in growing season (Oct to May)	1971 to 21.3m then deepened in 2008 to 90m	IRR	Limest.	90	1.25 (2008); 76 (1987)	7.3	2008	+	+	0	strongly affected
16	No information on well-nr or permit nr. Bore is known to be affected. Bore was not running prior to sampling. Biological samples taken prior to purging.			Limest.	65	40	6.9	2008	++	+	24.6	strongly affected
17	Never tested and no information whether this bore has clogging issues. The bore was running prior to sampling, i.e. biological and inorganic samples taken post purging	1979	IRR	Limest.	16		2	1979	+	+	0	unknown
18	No information on well-nr or permit nr. Bore impacted by sand, but clogging problem is not severe. Bore was running prior to sampling. Could be two different wells on WaterConnect as per the GPS coordinates.		IRR						+	+	0	affected to a minor/moderate degree

19	No possibility to connect flow-through cell, well head measurements done in bucket.		IRR	Limest.					+	+	0	unknown
20	Blockage in pipes due to calcite and iron. Some pipes replaced after 10 yrs because of blockage, even though normal lifespan is 25 yrs. Bore ca 40 yrs old. Deepened ca 10-12 yrs ago. Bore was able to be purged, but no sample outlet - water could only free-flow onto ground, i.e. no well-head measurements could be taken while microbiology samples would have been contaminated, so none taken	1981	IRR	Limest.	20	38		1987	na	na	na	no sample
21	Sand filter. Bore next to a sink hole. Two bores match location on WaterConnect.	2006		Limest.	30	10	4.2	2006	++	+	7	affected to a minor/moderate degree
22	No bore registered in WaterConnect, at the location of sampling. No clogging problems recorded so far.		IRR	Limest.					+	+	2	unaffected
23	No clogging problems recorded so far. Owner made driller disinfect drilling equipment before drilling. Relatively new bore.	2016	IRR	Limest.	54	50	5.2	2016	+	+	0	unaffected
24	Affected and had to be abandoned. No pump on bore. No possibility to purge bore - only able to take a bailer sample. Two bores match location on WaterConnect.	1985		Limest.	was deepend in 2006 from 24m to 60m	40	4.2	1985	++		2	strongly affected
25	No bore registered in WaterConnect, at the location of sampling. Not affected.		IRR	Limest.					+	+	0	unaffected
26	Bore next to a sink hole in limestone. Sample taken after sand filtration. Not affected	1996	IRR	Limest.	40	62		1996	+	+	6	unaffected

The top fifteen most dominant bacterial genera (%) in the subset of samples analysed using whole genome sequencing.

Genus (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Acinetobacter	1	40	2	59	13	4	3	46	65	4	1	52	18	63	39	0	8	0	0
Pseudomonas	0	12	2	32	68	16	2	2	3	0	7	29	12	14	7	0	7	0	2
Sphingomonas	1	1	3	1	0	7	1	0	14	0	1	0	0	0	0	0	65	0	0
Mycobacterium	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	81	0
Sphingobium	0	1	55	0	0	0	0	0	2	0	8	0	0	0	0	0	0	2	0
Sulfuricurvum	0	0	0	0	0	0	9	2	0	4	0	0	9	0	2	39	0	0	0
Flavobacterium	0	1	0	0	0	2	0	1	0	0	0	3	2	0	0	0	0	0	46
Gallionella	0	0	0	0	0	4	2	2	4	15	0	0	0	1	3	6	0	0	0
Lysobacter	32	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Acidovorax	0	0	0	2	0	1	6	2	0	0	18	0	0	0	2	0	0	0	1
Aeromonas	0	1	0	0	3	0	12	2	0	0	0	0	0	0	0	10	0	0	1
Sideroxydans	0	0	0	0	0	3	0	0	0	18	0	0	0	0	1	4	0	0	0
Thiobacillus	0	0	0	0	0	0	6	3	1	1	0	0	6	1	0	5	0	0	0
Hydrogenophaga	0	0	0	0	0	0	1	3	0	0	1	0	0	0	1	1	0	0	16
Rickettsiella	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0