Report

Assessment of EOXIDE® LQ 75 solution and sodium hypochlorite as a disinfectant for Namibian water samples

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Executive summary

This report discusses the outcomes of laboratory tests conducted on source water samples from three Namibian towns, Oshakati, Gobabis and Karabib. The background bacterial concentration was determined by serial dilution and plate counting and these values corresponded well with data from the NamWater laboratories.

The samples were treated with EOXIDE® LQ 75, a chlorine dioxide product, at increasing dosing concentrations (0.01-5 mg/l) and different contact times (1, 10 and 30 minutes). The disinfection efficiency was determined by performing plate counts after the specified contact time.

For the Oshakati sample all coliform bacteria were eliminated at a dosing of 1 mg/l and all heterotrophic bacteria at 2 mg/l. For the Gobabis sample 1 mg/l ClO_2 was sufficient to eliminate all bacteria. The Karibib sample, despite having the lowest background counts required the highest dosing (5 mg/l) to eliminate both coliform and heterotrophic bacteria. There was no dose dependent reduction of bacterial numbers below 1 mg/l. This showed a different trend to the other two samples and suggested the Karibib sample contained some component which consumed the oxidant before it had a chance to affect the bacteria.

Manganese levels were low in all three samples, but the dissolved organic content, measured by TOC was significant. The Karibib sample had a TOC of 8.2 mg/l, more than double that of the other two samples. This most likely accounted for the higher oxidant requirement. Further evidence for this was provided by the redox potential (ORP) measurements where the Karibib sample did not exceed 600 mV at a dosing of 5 mg/l. In addition the sample developed a light brown colour after dosing, suggesting the formation of tannin. This level of dissolved organic material could lead to trihalomethane (THM) formation during conventional chlorination.

A comparative study, using sodium hypochlorite (NaOCl) rather than ClO_2, showed that the dosing required to achieve complete sterilisation of the water was at least two times higher than that of ClO_2.

The efficacy of EOXIDE® LQ 75 was stable of a pH range of pH 7-9, with a very limited reduction at pH 10. No residual chlorite could be detected in the samples after dosing with EOXIDE® LQ 75.

1. Introduction

This report documents an assessment of two water treatment chemicals, EOXIDE® LQ 75 solution and sodium hypochlorite, in terms of their bacterial disinfection efficiency.

2. Background

AquaSmarter Namibia holds the distribution rights for EOXIDE® LQ 75, a chlorine dioxide generation system, in Namibia. As part of a motivation to trial the product for the disinfection of drinking water in Namibia, samples from three sites were selected to test the efficiency of the product. Sodium hypochlorite, a commonly utilised disinfection chemical, was selected as a basis for comparison.

Preliminary tests to assess the efficiency of the product were conducted in Namibia, but results proved inconclusive and a number of anomalies were reported. Samples were subsequently sent to the University of Cape Town, in South Africa, where a comprehensive set of microbiological analyses were conducted and the results described below.

3. Terms of reference

The terms of reference of this study are as follows:

- Assess the efficacy of EOXIDE® LQ 75 for the disinfection of three Namibian waters samples as a function of dosing and contact time.
- Compare the performance of EOXIDE® LQ 75 to a conventional water treatment chemical (NaOCl ot HTH).

Following the presentation of the preliminary data in Windhoek (14/12/2010) the request was made to report on a number of additional factors. These were:

- The efficacy of the product across a pH range of pH 7-9.
- The presence of residual chlorite.
- The concentration of manganese and dissolved organic carbon in the raw water samples.
- An assessment of redox potential (ORP) as a proxy measurement for disinfection.

4. Sample preparation and test protocol

Water samples (10 l) were received from three sites in Namibia, Oshakati, Gobabis and Karibib. The samples were refrigerated at 4°C upon arrival. Prior to all tests a subsample (250 ml) was aseptically removed from the sample bottles and allowed to equilibrate to room temperature (25°C ± 1°C).

The coliform and heterotrophic bacterial numbers for the individual samples were determined by serial dilution and plate counting. A series of 10-fold dilutions (10^{-1} – 10^{-4}) were performed by pipetting 1 ml of raw water into a McCartney bottle containing 9 ml of sterile (autoclaved) water (10^{-1} dilution). The contents were vigorously mixed using a vortex mixer and 1 ml transferred to the next bottle (10^{-2} dilution) and the process repeated. A subsample (100 µl) of each dilution was spread onto the surface of an agar plate and incubated at 30°C (nutrient agar plates for heterotrophic bacteria) or 37°C (MacConkey agar plates for coliform bacteria). Replicate plates were prepared for each dilution to assess reproducibility. The plates were checked after 24 and 48 hours and visible colonies counted where colony numbers were lower than 300.

Chlorine dioxide was prepared according to the manufacturer's instructions to produce a stock solution at 7 500 ppm ClO_2 (0.75%). A working solution (75 ppm) was prepared by dilution. Commercial sodium hypochlorite (NaOCl – 3.5% solution) was diluted to prepare a similar working solution.

The test protocol involved decanting 20 ml of the water sample into a sterile McCartney bottle and adding the appropriate volume of 75 ppm working solution to achieve the desired dosing concentration (Appendix A). Following dosing the sample was thoroughly mixed and a 100 µl subsample plated onto nutrient and MacConkey agar plates after exposure times of 10 and 30 minutes. The plates were incubated at 30°C and 37°C and visible colonies counted after 24 and 48 hours. An untreated sample was included as a positive control and an autoclaved sample as a negative control.

The test work was conducted in three phases. During the first phase samples were dosed with EOXIDE® LQ 75 at 0.01, 0.05, 0.1 and 0.3 ppm. Dosing concentrations were selected based on recommendations for treating European drinking water. The concentrations were increased to 0.3, 0.5, 0.75 and 1 ppm during the second phase and 1.5, 2 and 5 ppm during the third phase. Parallel samples were treated with NaOCl at 0.5, 1, 2 and 5 ppm. The higher NaOCl concentrations were selected based on the outcome of European trials comparing the two chemicals. The redox potential (ORP) of the treated samples were measured in each case to assess the correlation between extent of disinfection and ORP.

Based on the outcome of the microbiological analysis, two additional analyses were performed. The manganese concentration and total organic carbon (TOC) content of

the raw water samples were determined by atomic absorption spectroscopy (AAS) and using an SGC AnaTOC total organic carbon analyser respectively.

5. Results and discussion

Microbiological assessment

The results of the initial serial dilution tests to determine the concentration of coliform and heterotrophic bacteria in the individual samples are summarised in Table 1.

Table 1: Coliform and heterotrophic bacterial counts in water samples from different locations, determined by serial dilution and plate counting

Location	Coliforms (cfu/ml)	Heterotrophs (cfu/ml)
Oshakati	875	13 000
Gobabis	630	7 300
Karibib	190	4 400

The bacterial concentrations determined are similar to those obtained for the same samples in tests performed by NamWater (Kangootui, pers comm.)

Visual observation of the colony morphologies on the plates suggests two species of coliforms and 7-8 species of heterotrophic bacteria. Seven strains have been isolated and purified. DNA has been extracted from these isolated and is currently being sequenced to allow identification. The pure cultures will be used for later tests to determine the relative susceptibility of each species to chlorine dioxide and sodium hypochlorite.

The combined data, across the three trials, for chlorine dioxide dosing are summarised in Tables 2 to 4. In all cases the coliform bacteria are more susceptible to the chlorine dioxide dosing than the heterotrophic bacteria.

Of the three samples the Gobabis water was completely disinfected (all coliform and heterotrophic bacteria destroyed) at the lowest dosing (1 mg/l). The Karibib sample had the lowest background bacterial concentration, but even at the highest dosing tested (5 mg/l) complete disinfection could not be achieved. This indicates that the oxidant was being consumed by something other than the bacterial. The most likely explanation is that this sample had a higher dissolved organic content. During discussions with NamWater personnel (14 December 2011) the possibility of manganous ions was raised. TOC and manganese levels were subsequently measured.

Table 2: Reduction in coliform and heterotrophic bacterial counts in Oshakati water sample as a function of increasing chlorine dioxide dosing. TMTC = too many to count which is typically more than 300 colonies per plate.

	Coliform bacteria			Heterotrophic bacteria		
Dose (mg/l)	Count	Cfu/ml	% reduction	Count	Cfu/ml	% reduction
0.01	71	710	18.9	TMTC	> 3 000	-
0.05	57	570	34.9	TMTC	> 3 000	-
0.1	23	230	73.7	TMTC	> 3 000	-
0.3	1	10	98.9	TMTC	> 3 000	-
0.3	1	10	98.9	TMTC	> 3 000	-
0.5	0	0	100	TMTC	> 3 000	-
0.75	2	20	97.7	TMTC	> 3 000	-
1.0	0	0	100	8	80	99.4
1.5	0	0	100	1	10	99.9
2.0	0	0	100	0	0	100

Table 3: Reduction in coliform and heterotrophic bacterial counts in Gobabis water sample as a function of increasing chlorine dioxide dosing. TMTC = too many to count which is typically more than 300 colonies per plate.

	Coliform bacteria			Heterotrophic bacteria		
Dose (mg/l)	Count	Cfu/ml	% reduction	Count	Cfu/ml	% reduction
0.01	62	620	1.6	TMTC	> 3 000	-
0.05	25	250	60.3	TMTC	> 3 000	-
0.1	4	40	93.7	TMTC	> 3 000	-
0.3	2	20	96.8	70	700	90.4
0.3	1	10	98.4	55	550	92.5
0.5	1	10	98.4	38	380	94.8
0.75	2	20	96.8	17	170	97.7
1.0	0	0	100	0	0	100

In terms of exposure time, there was no significant difference in the results between 10 and 30 minutes of contact time, with only a marginal decrease in efficiency at a contact time of one minute.

Table 4: Reduction in coliform and heterotrophic bacterial counts in Karibib water sample as a function of increasing chlorine dioxide dosing. TMTC = too many to count which is typically more than 300 colonies per plate.

	Coliform bacteria			Heterotrophic bacteria		
Dose (mg/l)	Count	Cfu/ml	% reduction	Count	Cfu/ml	% reduction
0.01	19	190	0	TMTC	> 3 000	-
0.05	16	160	15.8	TMTC	> 3 000	-
0.1	21	210	0	TMTC	> 3 000	-
0.3	24	240	0	TMTC	> 3 000	-
0.3	17	170	10.5	TMTC	> 3 000	-
0.5	15	150	21.1	TMTC	> 3 000	-
0.75	17	170	10.5	TMTC	> 3 000	-
1.0	15	150	21.1	TMTC	> 3 000	-
2.0	1	10	94.7	23	230	99.5
5.0	0	0	100	0	0	100

In order to compare the data achieved using EOXIDE® LQ 75 with a conventional disinfectant the samples were treated with sodium hypochlorite at dosing concentrations of 0.5, 1.0, 2.0 and 5.0 mg/l (Table 5). The concentrations were selected based on previous tests, conducted in Europe on the relative efficacy of the two chemicals. For all three samples 1.0 mg/l NaOCl was sufficient to eliminate coliform bacteria, which is consistent with the chlorine dioxide data. However, the data differed significantly with respect to the heterotrophic bacteria. Only the Gobabis sample was completely sterilised, at a concentration of 2 mg/l compared to 1 mg/l for chlorine dioxide. The Oshakati and Karibib samples were not sterilised at dosages of 5 mg/l, with the Oshakati sample returning a heterotrophic bacterial count of 450 cfu/ml after 30 minutes of dosing at 5 mg/l.

Table 5: Reduction in heterotrophic bacterial counts across the three water sample as a function of increasing NaOCl dosing.

Dosage	Oshakati		Gobabis		Karibib	
mg/l	Cfu/ml	% reduction	Cfu/ml	% reduction	Cfu/ml	% reduction
0.5	> 3 000	-	130	99.82	> 3 000	-
1.0	> 3 000	-	140	99.81	670	98.48
2.0	2170	98.33	0	100	30	99.93
5.0	450	99.75	0	100	1	99.98

In all cases autoclaved samples resulted in no growth upon subsequent plating, indicating that the trials were run under sterile conditions and no contamination occurred.

Photographs of the relevant plates are detailed in the Appendices.

Assessment of chemical parameters

A number of non-biological parameters were assessed to account for their impact on the results obtained. The dissolved manganese concentration was measured using AAS. The results showed that the Oshakati and Gobabis samples contained less than 1 mg/l dissolved manganese, while the Karibib sample had a manganese concentration of 1.12 mg/l. Manganese could therefore not account for the consumption of the oxidant.

The TOC analysis showed clear relationship between the organic content of the samples and the amount of oxidant required to eliminate all bacteria. The Karibib sample had the highest organic load at 8.2 ± 0.4 mg/l. The Gobabis and Oshakati samples had similar dissolved organic loads at 3.9 ± 0.3 and 3.5 ± 0.6 mg/l respectively. Dissolved organic carbon often occurs in the form of humic and fulvic acids, which are resistant to microbial breakdown so persist in the water. These substances are not acutely toxic and animal studies have shown that concentrations of up to 1 000 mg/l had no effect on rats. Their presence in source waters is undesirable as they react with chlorine to form toxic trihalomethanes (THMs). The TOC of the Karibib sample exceeds Canadian specification for source waters (4 mg/l). Following dosing with EOXIDE® LQ 75 at 5 mg/l the sample developed a light brown colour, while the other two samples remained clear. While chlorine dioxide does not react with fulvic and humic acids to form THMs, the partial oxidation of these phenolic molecules can lead to polymerisation and tannin formation. This would be consistent with the observed colour change.

Anecdotal evidence has suggested that if the redox potential (ORP) is maintained above 600 mV the sample would be effectively sterilised. This was assessed by recording the ORP of each sample, after dosing, using a Metrohm 827 pH lab ORP meter. The data are summarised in Table 6

Table 6: ORP (mV) measurements as a function of chlorine dioxide dosing.

Dosing (mg/l)	Oshakati	Gobabis	Karibib
0	201	193	209
0.01	225	210	245
0.05	211	236	262
0.1	210	233	261
0.3	219	236	265
0.5	247	251	272
0.75	324	302	278
1.0	393	307	285
1.5	512	308	301
2.0	573	482	319
5.0	684	673	424

The ORP data shows the same trends as the microbiological studies and is consistent with the TOC data.

The pH of the raw water samples ranged from 8.19 (Karibib) to 8.49 (Oshakati). The efficacy of the EOXIDE® LQ 75 product was unaffected across a pH range of pH 7-9, although a minor loss of efficacy was noted at pH 10, which is consistent with previous trials conducted in Europe.

No residual chlorite was detected in the treated water samples. This result is consistent with the claims by the manufacturer that their novel ClO_2 generation system does not result in the release of chlorite.

6. Conclusions

Based on the data generated in the laboratory tests described above the following conclusions may be drawn.

- The bacterial counts obtained in this study are similar to those determined by the laboratory in Namibia, indicating that transportation to Cape Town did not significantly affect sample integrity.
- The concentration of EOXIDE® LQ 75 required to achieve complete bacterial destruction exceed the manufacturer's recommendation. The difference in source water quality could account for this.
- Complete elimination of coliform bacteria was achieved at 1 mg/l ClO_2 dosing for the Oshakati and Gobabis samples. The higher organic load resulted in the dosing for the Karibib sample lying just above 2 mg/l.
- The heterotrophic organisms were more resistant, requiring a higher dose for disinfection.
- The concentration of sodium hypochlorite required to achieve equivalent levels of disinfection were 2-5 times higher, confirming the greater efficiency of the chlorine dioxide.
- The levels of dissolved organic carbon are relatively high in the source water samples and could result in THM formation during conventional chlorination.

7. Appendices

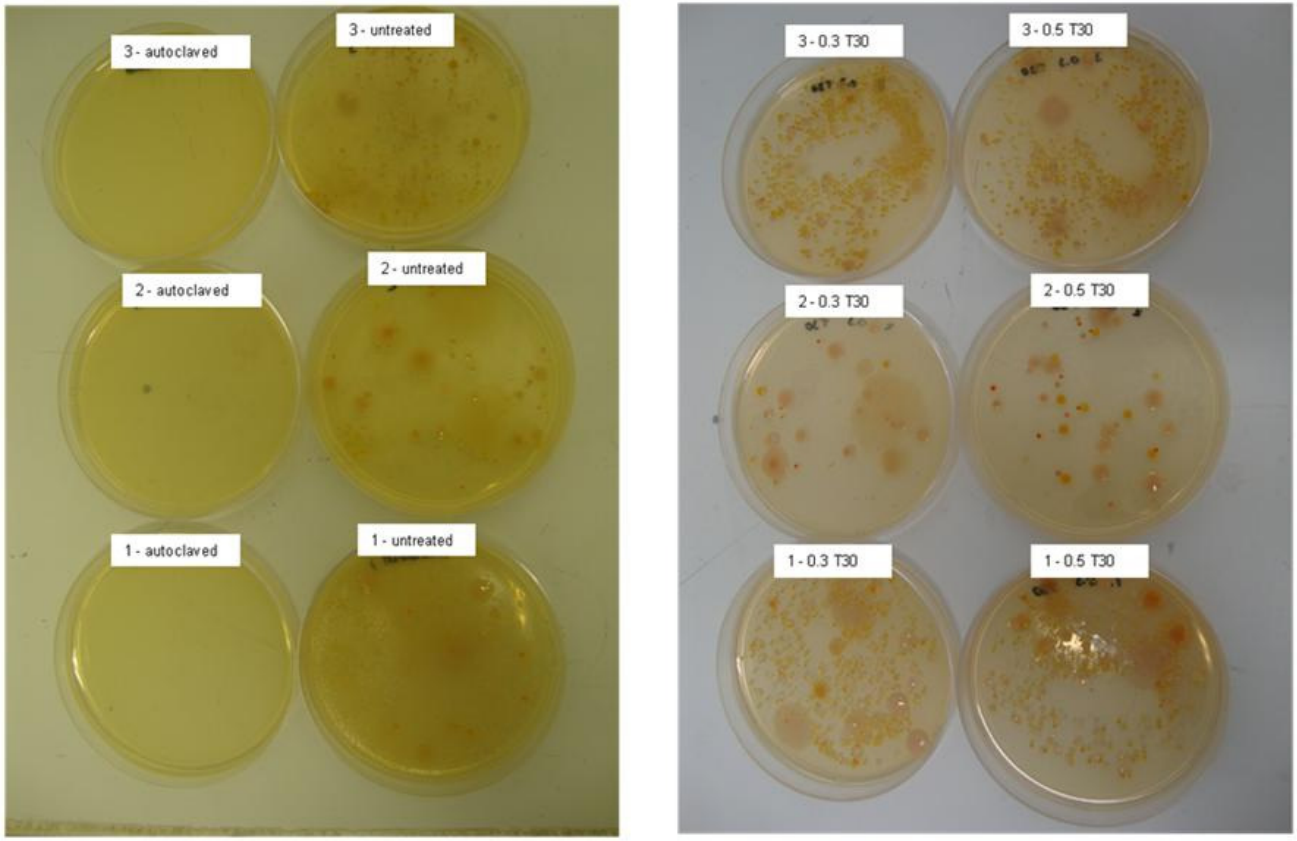

Figure A1: Photograph of plates used to determine heterotrophic bacterial count. 1 = Oshakati, 2 = Gobabis, 3 = Karibib; 0.3 = dosing, T30 = 30 minute exposure time. Autoclaved plates represent the positive control.

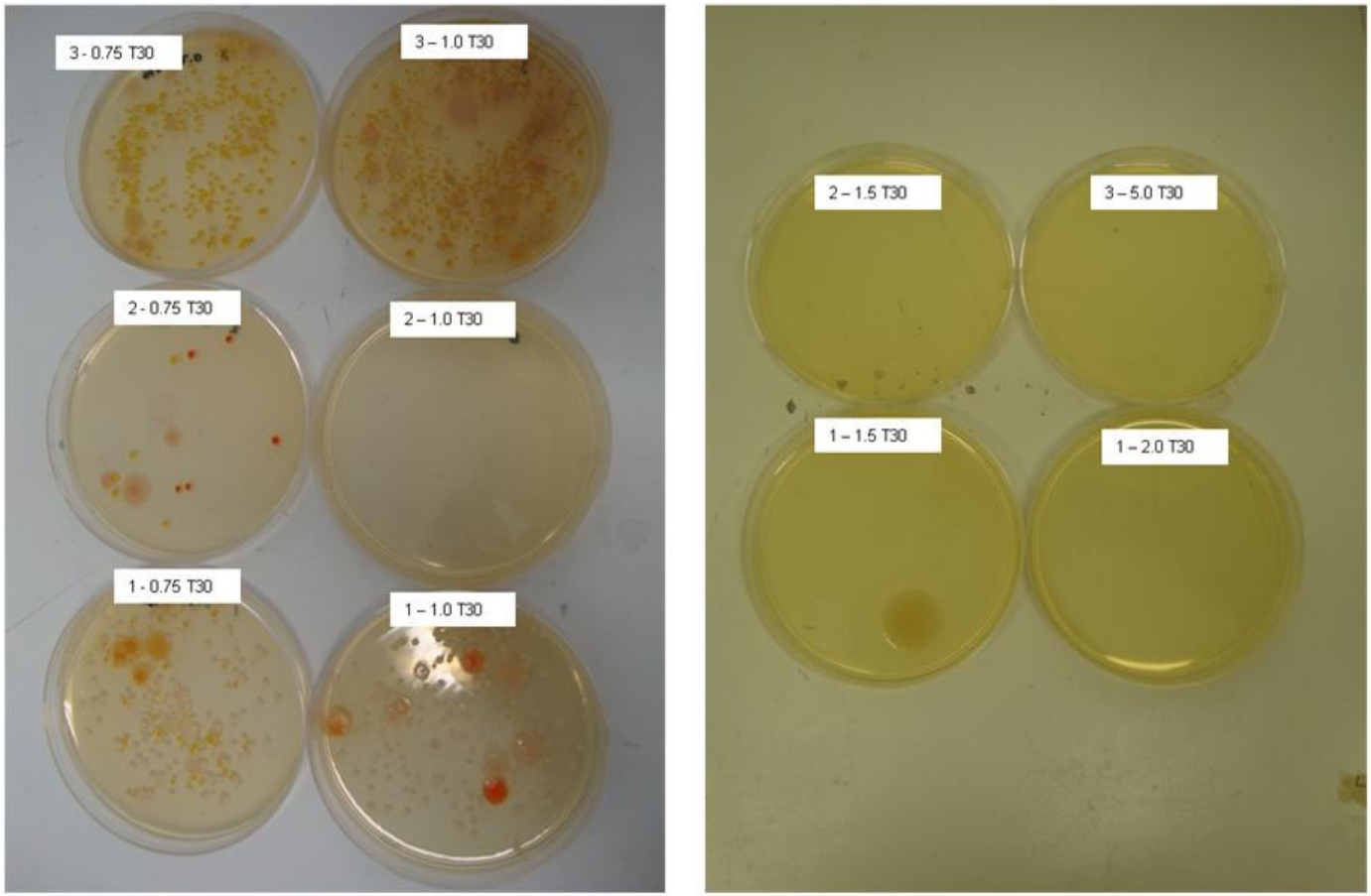

Figure A2: Photograph of plates used to determine heterotrophic bacterial count. 1 = Oshakati, 2 = Gobabis, 3 = Karibib; 0.75 = dosing, T30 = 30 minute exposure time. Diversity of heterotrophic organisms is visible.

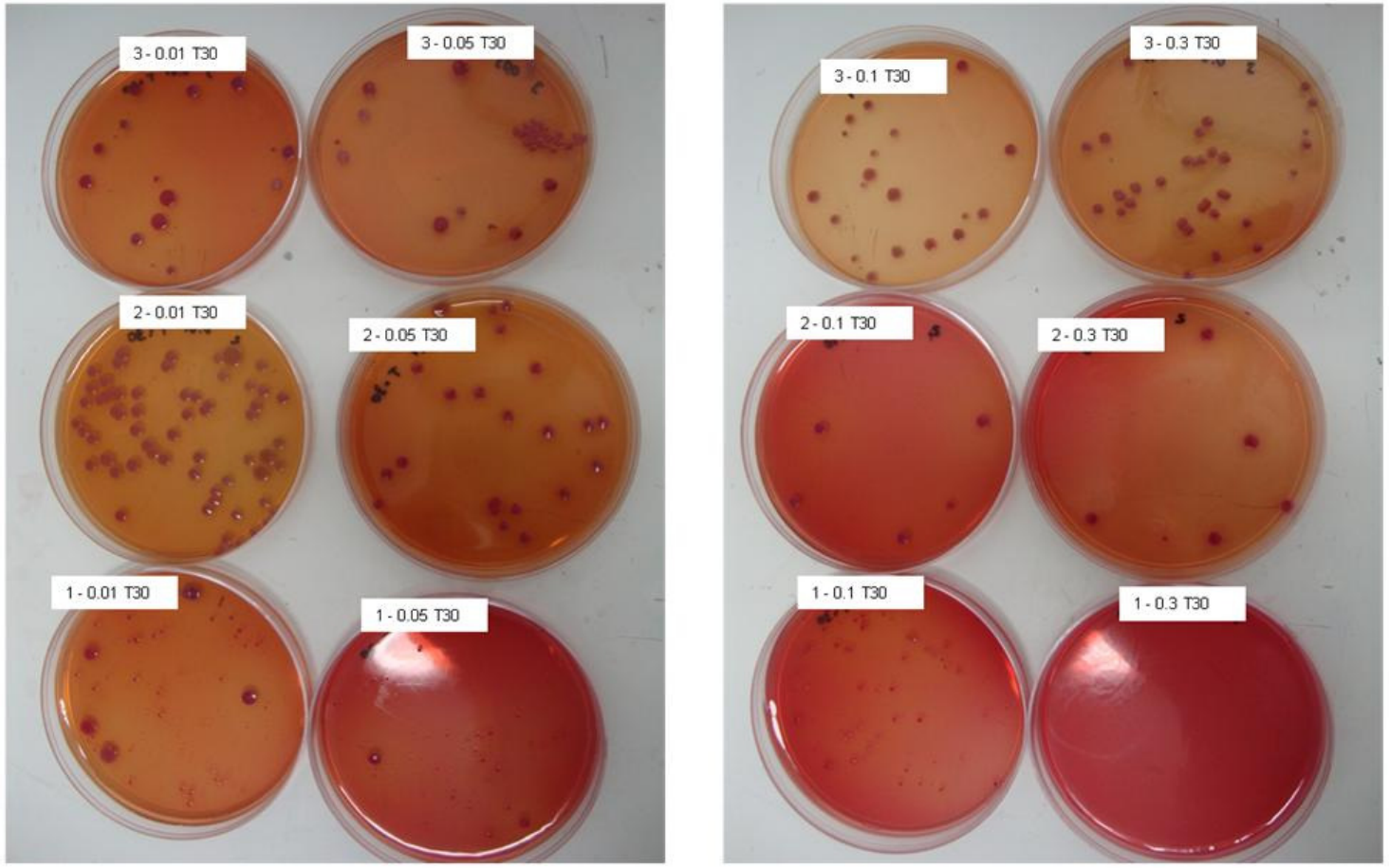

Figure A3: Photograph of plates used to determine coliform bacterial count. 1 = Oshakati, 2 = Gobabis, 3 = Karibib; 0.01 = dosing, T30 = 30 minute exposure time. Diversity of heterotrophic organisms is visible.