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TESTING RAINWATER COLLECTED IN TANKS USING THE H_2S METHOD

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ABSTRACT

Rainwater harvesting is common practice in many places throughout the world. It is often the only source of drinking water for many remote areas of the developed and developing countries. Rainwater thus collected was found to be susceptible to bacterial contamination and maintaining the quality of rainwater is the interest of the householder. Only an on-site method that is affordable to the householders enables regular checking of this drinking water source.

In order to test the efficiency of the H_2S method, an affordable and convenient on-site method, rainwater samples were tested from a total of 221 tanks from the households around the city of Perth, capital of, and Collie, a mining town in Western Australia. The general condition and the maintenance pattern of the tanks were assessed through a questionnaire. The efficiency of the H_2S method was tested against the presence of total coliform and faecal coliform bacteria, determined by the standard membrane filtration method. The agreement of the results from the H_2S method and the standard methods was greater at a coliform count of >10CFU/100mL, which indicated that the H_2S method is a suitable method for remote communities and countries where coliform counts greater than 10CFU/100ml is considered as standard for microbial contamination.

KEYWORDS: bacteriological tests, coliform bacteria, on-site test, rain water, the H_2S method

INTRODUCTION

Safe drinking water is a necessity to maintain the health of the public. Many households in remote communities and developing countries do not have access to reticulated water supplies, and therefore must rely on other sources for water. In remote and rural communities of Australia as a whole, 30-85 percent of households depends on alternative supplies to reticulated water (Australian Bureau of Statistics, 1994). Untreated water may contain bacteria from both faecal and non faecal sources such as soil, plant and animal matter. Rainwater is usually considered as a pure source of drinking water. But there are many chances of contamination, which might affect the physical, chemical and microbial quality of the water stored in tanks. Microbial contamination is a major factor affecting the quality of rainwater stored in tanks.

Unfortunately households cannot assess the quality of the rainwater to determine whether it is safe for drinking, due to many reasons. Research has shown that rainwater catchments are susceptible to bacterial contamination, including pathogenic organisms. Sources of contamination include faecal material from animals such as birds, rodents and possums, organic debris from overhanging trees, breakdown from roofing materials, fallout from air pollutants, including chimneys as well as local industries (Fujioka and Chinn, 1987; Yaziz, *et al*., 1989; Thurman, 1995; Thomas and Greene, 1992).

According to the World Health Organisation (WHO, 1993) water intended for drinking should be routinely monitored for total coliforms and *E. coli* and a 100ml drinking water sample should not contain total coliforms and *E. coli*. WHO (1993) recognises that it is often impossible to have water free of microbial contamination especially in remote communities and in many developing countries. Therefore it is recommended that samples with no more than 10 coliforms per 100ml is acceptable, as long as the sample is free of faecal coliforms. It is recommended that targets should be developed so that countries or small communities can progressively improve the quality of their drinking water.

The standard methods used for testing coliform bacteria are expensive and required technical skills to conduct the test. Unfortunately implementing routine tests in remote communities and developing countries is impractical due to the lack of facilities. Therefore there is a need for a simple, inexpensive, on-site test for routine testing of water in these situations. The H_2S method developed by Manja *et al*. (1982) if found reliable, would be a suitable method for routine testing of water quality.

The H_2S method has been adopted as a test for drinking water quality. The major advantages are that it is inexpensive and does not require technical experts or laboratory conditions to test the water. The method, developed by Manja *et al*. (1982), is based on the detection of sulphate reducing bacteria

(SRB) in the water sample. SRB is often associated with faecal contamination. This method if found to be reliable, could be used for routine testing of water. Since the water sample might contain SRB other than of faecal origin the occurrence of false results compared to the presence of faecal coliforms in the H_2S method cannot be overruled. Therefore it is worthwhile to study whether the positive results shown by the H_2S method are true positive results and what percentage of false positive and false negative results could be expected while using this test for routine analysis of water samples from different sources. This paper describes testing of rainwater in tanks using the H_2S method.

MATERIALS AND METHODS

Sampling areas

Collie: Rainwater samples were collected from Collie, a mining town in south west of Western Australia, which relies on alternative water supplies because not all households are connected to reticulated supplies. Rainwater tanks are one of the more desirable sources of potable water for drinking and other household purposes in the area. Houses were selected through the local High School students. A total of 101 households within the Shire of Collie were approached for testing the rainwater quality.

Perth: Samples were collected from households in and around the city of Perth, Western Australia. The households were selected from staff and students working at Murdoch University, Western Australia. A total of 121 samples were tested.

The participants were asked to complete a questionnaire that included information on the location of the tank relative to local industry, a general description and background of the rainwater harvesting system, possible contaminants that the home-owner could identify, and any cleaning or treatment options that had been adopted. Samples were collected in sterile sample bottles. Water samples were stored at $4^{o}C$ until testing. Testing was carried out within 24 hours of collecting the sample.

The samples were tested for total coliform bacteria and faecal coliform bacteria. The membrane filtration technique for the detection of total coliforms and faecal coliforms was conducted as per

Australian Standards (1995) using membrane enriched lauryl sulphate (MELS) agar. Each sample was simultaneously tested using the H_2S method.

The H_2S method

The H_2S medium was prepared as described by Pillai *et al*. (1999). A 100ml-rainwater sample was added to the sample bottle containing the prepared H_2S medium. Samples were incubated at 37^{o}C for 24 and 48 hours for a positive result. A change in the colour of the sample to black was recorded as a positive result. The bottles that did not turn positive after the 48 hours period were considered to be negative.

A true result was recorded when both the H_2S method and the standard method for total coliform bacteria gave the same result. A false positive result was obtained when there were no coliform bacteria, but the H_2S method indicated a positive result. In the case where the H_2S method failed to give a positive result in the presence of coliform bacteria, the result was identified as false negative.

The sensitivity, specificity, the positive predictive value (PPV) and negative predictive value (NPV) of the H_2S method were calculated as described by Mack and Hewison (1988). This comparison with the coliform method was based on the assumption that the coliform test provides 'true' indication of contamination, which may include the presence of environmental derived coliforms in the rainwater sample. The PPV is the ability of a positive test to predict the presence of coliforms, whereas the NPV is the ability of a negative test to predict the absence of coliforms. They are calculated as shown below

H_2S method	Laboratory result (standard method)	
	Positive	Negative
Positive	*a*	*b*
Negative	*c*	*d*
	a+c	b+d

Sensitivity = a/(a+c) X 100

Specificity = d/(b+d) X 100

PPV = a/(a+b) 100

NPV = d/(c+d) X 100

RESULTS

Water from the 101 tanks in the Collie region was used for drinking. All households were unaware of the quality of the rainwater and the majority of tanks were poorly maintained. In total 47 rainwater harvesting systems had a leaf trap over the gutter or tank opening, to prevent larger organic materials from entering the rainwater tank. Only 29 systems had a design in place to prevent the first flush of rain after summer from entering the tank. Animals were known to inhabit the area close to 42 rainwater tanks. Vegetation overhanging the tank and the rainwater harvesting systems occurred at 18 sites sampled. Only 17 householders flushed and cleaned their tank. This occurred once a year or once every two years. Only 20 households filtered/boiled the water before drinking.

In Perth City area, water from 100 tanks was used for drinking purpose and 21 entirely for gardening. Only 20 householders boiled or filtered the water before consumption. None of the tanks was ever tested for microbial contamination. Overhanging trees were present over 41 collecting systems.

Total coliforms (TC) were present in 25 samples out of a total of 101 samples, 3 of which tested positive for faecal coliforms (FC) in Collie region. The 13 tanks that tested positive to total coliforms, were recorded as being frequented by animals. Overhanging trees were present in 4 of the harvesting systems that tested positive for total coliforms. Only 4 samples that tested positive for total coliforms had ever been emptied or cleaned, while 6 systems had a first rains flush mechanism. Out of these 25 only 5 households boiled/filtered the water before consumption.

Coliform counts greater than 10CFU/100ml were observed in 4 samples. In all cases, the rainwater tanks were cleaned, but they were located on farming property, in close proximity to overhanging vegetation and animals. Three of these samples tested positive for faecal coliform colonies.

The H_2S method indicated more true results after 48 hours, than after 24 hours of incubation. Positive results were obtained for 7 samples after 24 hours of incubation while after 48 hours, 37 samples indicated positive results. False negative results were obtained in 22 samples after 24 hours of incubation. After 48 hours of incubation 7 false negatives results (7.1%) were obtained (Table 2). These 7 samples had 1 CFU/100mL of coliform bacteria.

From Perth area total coliforms were identified in 64 samples out of the total of 121 samples and faecal coliforms in 3 samples. High contamination with total coliforms (>10CFU/100ml) was noticed in 35 samples. The percentage of false positive results was higher after 48 hours of incubation whereas false negative results were more after 24 hours of incubation. False negative results were obtained in 7 samples, out of which 5 samples contained low level of coliform bacteria (<5 CFU/100mL), and no *E.coli* were found in those samples.

In addition to the coliforms, *Enterobacter cloacae, E.amnigenous, Proteus, Citrobacter diversus, C. freundii, Serratia sp., Erwinia nigrifens, Xantho maltophilia, Chromobacterium violaceum* were isolated from some of the positive bottles. *Salmonella arizona* was present in two samples. Four samples that were false negative contained *E.coli*. Proteus was a common bacteria that was isolated from the H_2S positive samples even in the absence of coliforms.

Table 1. Percentage of true and false results for the H_2S test in comparison to the standard method for testing coliforms at a total coliform count of 1CFU/100mL

Samples	True results(%)		False positive(%)		False negative(%)	
Rain water	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Collie (101 samples)	74.3	71.3	3.9	19.8	21.8	6.9
Perth City (121 samples)	78.5	75.2	5.7	17.3	15.7	7.4

Table 2. Percentage of true and false results in comparison to the standard methods for testing coliforms at a total coliform count of 10CFU/100mL

Samples	True results(%)		False positive(%)		False negative(%)	
Rain water	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Collie	90.1	65.3	5.9	33.7	4.0	1
Perth City	73.5	64.4	19.0	31.4	7.4	4.1

Sensitivity and NPV were found to be greater after 48 hours of incubation. Comparison of PPV values at 24 and 48 hours showed only slightly higher values at 48 hours. The specificity value was found to be less after 48 hours of incubation, when compared to 24 hours of incubation (Table 3).

Table 3. Sensitivity, Specificity, PPV and NPV values of the H_2S method compared to the total coliform counts

Site	Hrs	Sensitivity		Specificity		PPV		NPV	
		<1 CFU	<10 CFU	<1 CFU	<10 CFU	<1 CFU	<10 CFU	<1 CFU	<10 CFU
Collie	24	12	75.0	94.74	6.2	42.86	33.33	76.6	93.8
	48	68	75.0	72.37	49.3	44.74	8.9	87.3	98.4
Perth City	24	70.1	76.9	87.7	71.9	86.5	56.6	72.4	86.7
	48	86.1	91.8	62.5	53.6	72.7	47.2	76.5	89.8

The pH ranged from 5.46 to 8.65, where more neutral levels were found to occur in cement tanks. Conductivity ranged between 0.62 – 139.1 *u*S. The conductivity of the rainwater was higher in cement than in galvanised tanks. The average conductivity was highest for systems that have a cement tile roof. The pH of the rainwater was also highest for systems with cement tile roofing. Rainwater was more acidic when the roofing was galvanised iron.

DISCUSSION

The results of the present study and past research (Edwards, 1994; Nair *et al*. 2000; Thurman, 1995; Thomas and Greene, 1993; Yaziz *et al*., 1989) indicated the potential for rainwater to become contaminated with microorganisms.

It was observed that 21 out of the 25 of the samples that tested positive for total coliforms from Collie region had microbial counts of less than 10CFU/100ml. Sensitivity was also greater after 48 hours of incubation. All the 4 samples that contained >10CFU/100ml coliforms showed positive results with the H_2S method. However in the Perth area, 35 out of the 64 samples tested contained less than 10CFU/100mL.

Therefore it was suggested that sensitivity, the ability of H_2S method to detect coliforms, was more reliable for coliform counts of <10CFU/100ml than <1CFU/100ml as was also shown by Nair *et al*. (2000).

According to Mack and Hewison (1988), for a test to be useful, the sensitivity and specificity should be 80% or better. The PPV and NPV should also be 100% for successful screening of water samples. In the present study this was not achieved. It is possible that the inability of the H_2S test to test positive for contaminated samples could be due to the low concentration of bacteria in the sample as the 7 samples that showed false negative results contained 1CFU/100ml of TC bacteria and no faecal coliforms. The lower values of specificity and PPV (6.2 and 8.9 respectively) obtained for the Collie water samples were due to the high number of false positive results. Mack and Hewison (1988) suggested that lower value for specificity and PPV could be due to the presence of environmental bacteria that release sulphide as a metabolic product. The poor correlation between the two tests could be associated with many possible organic contaminants from plants and animals. Wallis (1991) reported that on testing rainwater tanks in Thailand 20 % false positive and 41 % false negative results were obtained. It was observed that the percentage of true results was higher in treated community water samples (Nair *et al*., 2000) than the rainwater samples. This shows that treatment reduced the false results and therefore correlated better with the standard methods.

Vegetative material and soil may contain bacteria that can contaminate rainwater in tanks. Soil particles and vegetation can build up in the gutters and on the roof, and after the first rains will be washed down into the tank. Nair *et al*. (2000) identified phytopathogens including *Erwinia sp.* and *Chromobacterium* and suggested that the source of the bacteria could be from leaves. They also identified groups of bacteria belonging to the Enterobacter and *Serratia*. It was suggested that they are common in soil and water as well as in the intestinal tract.

It is possible that the quality of rainwater will vary depending on the time, year and the rate at which water is used (Thomas and Greene, 1993; Thurman, 1995). During this study much of the winter rainfall had already fallen and therefore the tanks had been thoroughly flushed, and overflowing. During the dry season the water levels drop, and the temperatures increase and therefore the possibility of bacterial multiplication is very high. Tanks with high turnover rates may be flushed out before the chemical or microbial aspects of the water become altered (Thurman, 1995). Thurman and Gerba (1988) found that disease-causing organisms could survive for long periods of time depending on the temperature of the water, and particulate concentration. It is possible that disease causing organisms, deposited by birds, rodents and other small animals may survive long enough for them to contaminate the water in the tank (Levine and Levine, 1991; Casemore, 1990; Rose, 1989).

On testing the rainwater for pH and conductivity a wide range of values were obtained. Studies have shown that the conductivity and pH will vary depending on roof material involved in the rainwater harvesting and the type of material that the tank is constructed of (Thurman, 1995). Furthermore the size and age of the tank, the surrounding human activities, and the turn over rate of the rainwater in the tank, may also influence the quality of the rainwater (Thurman, 1995; Thomas and Greene, 1993). Thomas and Greene (1993) found that the quality of water was also influenced by the length of dry periods. They found that there was a positive relationship between suspended solids, turbidity, conductivity and lead in rainwater run-off with the dry period between rainfall events.

The condition including age, size and type of tank, used to store the rainwater can also influence the pH and conductivity of rainwater. The conductivity and pH was highest when the roof material consisted of concrete tiles in comparison to galvanised or colour-bond roofing. In the present study the pH of the water was more acidic when stored in galvanised tanks or collected via galvanised roof system, in comparison to cement tanks and roof tiles. According to Thurman and Greene (1993) this could be due to the coarser surface of the roof material or the composition of the roof material. They suggested that the lower pH in industrial areas could be attributed to excess amounts of carbon dioxide present in the atmosphere as a result of industrial activities. In the rural area, pH ranged from 6.8 – 7.0 with the galvanised roof and 7.2 – 8.1 with the concrete roof. The pH was found to be acidic in most cases, but was lowest in industrial areas, with values as low as 6.7 (Thomas and Greene, 1993).

Higher pH values occurred in new concrete tanks. According to Thurman (1995) this could be attributed to the leaching of carbonate ions and complex alumino silaceous oxides from the walls of the tank, which dissipate with age. Furthermore this is likely to be due to a reduction in the available alkaline substances in the tank wall or the build up of an insulating bio-film on the inside of the tank. Thurman (1995) found that the age of a cement tank might also influence the water quality. Thomas and Greene (1993) suggested that the higher pH in urban and industrial could be due to the deposition of alkaline soil particles on the roofs by wind erosion or due to the absence of excess carbon dioxide in the air, in the rural area. The pH values in the present study could have been influenced by the mining and other industrial activities that operate in the Collie region. The industrial pollutants include CO_2, oxides of nitrogen, and particulate matter into the atmosphere (National Pollutants Inventory, 2000).

CONCLUSION

Rainwater tanks tested were not maintained properly. However the low number of bacteria and the chemical quality of the water could be due to the period of monitoring, as the tanks were over-flowing as it was the rainy season. The low pH values were noted in tanks situated near industries. There is a need for further study to be carried out on the quality of rainwater tanks during all seasons of the year.

Although the percentage of true results was better after 24 hours of incubation, the H_2S method was found to indicate contamination better after 48 hours of incubation. But the high false positive results observed for the rainwater samples indicate possible contamination from a wide range of sources thereby could be considered as indicating better risk of contamination. The false negative results were in the presence of coliform bacteria at a very low level of 1CFU/100mL.

The H_2S method could be an effective test to detect contamination for samples with counts higher than 10 coliforms per 100ml. This would be suitable for remote communities and countries where coliform counts greater than 10CFU/100ml are standard. Further testing of the rainwater quality in all seasons of the year is required to assess the change in quality of rainwater collected in tanks throughout the year.

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