Available online at https://ijmras.com/

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES

ISSN: 2640 7272

Volume:06; Issue:08 (2023)

Doi: 10.33826/ijmras/v06i08.5

Spatial and seasonal distribution, driving forces and producers of 2-MIB in reservoir and treated waters, Sri Lanka

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Abstract

2- Methylisoborneol (2-MIB) is known to cause a musty, moldy taste and odour (T&O) in water. While primarily known for its undesirable effect in the drinking water industry, this compound also causes significant harm in the freshwater fish farming industry. The presence of T&O in potable water supplies is a frequent problem all over the world and Sri Lanka as well. In some parts of Sri Lanka, the highest consumer complaints are related to the T&O issue even in treated water. Most of the source waters in Sri Lanka consist of diverse ranges of algae and cyanobacteria that produce odorous chemicals; Geosmin and 2-MIB. In the present study, a simple and sensitive modified method to determine 2-MIB in water was optimized by headspace solid-phase micro extraction coupled with gas chromatography–mass spectrometry. Quantification of 2- MIB concentrations in 30 raw drinking water sources and treated water in both dry and wet seasons was carried out. Results of the study revealed that 69% of both raw and treated water samples exceed the human threshold level of 2-MIB (5 ng L^{-1}) given by the WHO for drinking water. In wet season, the concentration of 2-MIB in raw water sources ranged from 3.2±1.5 to 57.6±3.1 ng L^{-1} whereas in dry season, from 5.8±1.8 to 96.3±4.2 ngL^{-1}. Moreover, in wet season, 2-MIB concentrations in treated water ranged from 7.6±1.6 to 69.1±1.3 ngL^{-1} while in dry season it ranged from 3.5±1.9 to 98.5±2.8 ngL^{-1}. 2-MIB concentrations in treated water were significantly higher ($p<0.05$) than in the same respective raw water. It was found that the concentrations of 2-MIB was greater in dry season than the wet season ($p<0.01$). In the present study, *Anabaena* sp., *Cylindrospermopsis* sp., *Oscillatoria* sp., *Microcystis* sp., and algae; *Scenedesmus* sp., *Stauastrum* sp., *Dictyosphaerium* sp., *Fragilaria* sp. were identified as T&O producing cyanobacteria in water bodies where 2- MIB was detected. Total T&O forming cyanobacteria and algae count has shown a significantly positive correlation with 2-MIB concentration ($p < 0.05$). Significant positive correlation was found between 2-MIB and total phosphorous ($p<0.05$) and pH ($p<0.05$), which are the factors that normally govern cyanobacteria growth and population density in turn will increase the 2-MIB production.

Keywords: 2- MIB, Taste-Odor, Cyanobacteria, Algae, Headspace Solid-Phase Micro Extraction, Water Quality

1. INTRODUCTION

Surface waters are an essential source for drinking all over the world. Some of the major cities in the world depend on their drinking water requirement from reservoir, lakes and rivers (Tian, 2013). In general, surface water is treated by conventional water treatment processes and some advanced water treatment facilities have also been established to treat water for drinking; however, such technologies are not adequately accessible all part of the world due to high installment and maintenance cost (UNESCO, 2015). Despite this extensive water treatment, many water utilities are confronted with T&O complaints leading to end consumer rejection of treated drinking water, as conventional water treatment facilities are not capable of removing T&O compounds (Suffet et al., 1996; Ganegoda et al., 2019). Hence, water utilities are anxious to mitigate T&O problems quickly and efficiently (Peter et al., 2009). T&O problems in drinking water derogate consumer confidence, consumer satisfaction and water consumption (Awwarf, 2000). Tap water with detectable T&O may be perceived by the consumer as unsafe to drink although it adapts to the

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guideline for regulated constituents (Jiang et al., 2007, Tian, 2013). 2-MIB is one of the most common T&O with a musty (moldy) odour, a semi volatile secondary metabolite, tertiary alcohol with a stable chemical structure which is mainly produced by two groups of aquatic microorganisms; cyanobacteria and actinomycetes (Ding et al., 2014a). The human sensory threshold level of 2-MIB is low as 5ng/L, therefore even an insignificant amount of 2-MIB presence in drinking water, could be sensed by the end consumer, directly turning down of supplied treated water, again give rise to a loss of profit by the water supplier (Ju¨ttner and Watson, 2007). Hence, the ability to reliably predict, confirm, and counteract the occurrence of 2-MIB would be of immense value to not only to water utilities, but also other branches of industry, such as aquaculture farms where T&O can spoil the entire harvests (Jade and Emilia 2013). According to WHO, the provision of drinking-water that is not only safe but also acceptable in appearance in terms of taste and odour (T&O) is of high priority (WHO, 2011). When people are provided with aesthetically bad treated drinking water, that could lead to use of water from sources that are less safe (WHO, 2011). More importantly worldwide water consumers have been looking into the quality of their drinking water with the focus of aesthetic value. A recent study of over 800 utilities in the United States and Canada found that 16% of the utilities experienced serious earthy and musty problems and those utilities spent over 4.5% of their total budget on taste and odour control (Khiari & Watson, 2007). According to McDowall (2008), a survey conducted by the Australian Bureau of Statistics in 2007, found that 18.5% of Australians were dissatisfied with the quality of their drinking water. The problem was most notable in South Australia, with 25.8% of those surveyed being unhappy with their water to the extent that 8.7% would not drink tap water at all.

Intracellular and extracellular metabolites, such as T&O compounds and biotoxins are being produced by many cyanobacteria (Planktonic and periphytic) and actinomycetes (Suffet et al., 1995; Zamyadi et al., 2010). Cyanobacteria: *Anabaena circinalis, Anabaena scheremetievi, Phormidium tenue, Pseudanabaena (planktonic), Oscillatoria f. granulata, Oscillatoria simplicissima, Oscillatoria curviceps,* etc. have been identified as potential T&O forming organisms in reservoirs, rivers, canals, where raw source water taken for drinking purposes (Ju¨ttner and Watson 2007; WHO, 2011). The biological function of these algal metabolites is yet unknown, although they may be intermediates or byproducts of pigment production (Bafford et al., 1993; Zimba et al., 1999). A significant number of USA water treatment plants (WTP) that report T&O associated problems coupled with using surface source water are

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related to algal metabolites (Suffet et al., 1996). Despite both United States Environmental Protection Agency (USEPA) or the World Health Organization (WHO) has not declared 2-MIB as a health hazard, it has been recorded that this odorant can lead to acute health effects such as heat exhaustion and sunstroke, or chronic health effects such as kidney problems (Tian, 2013; Simpson, 2008).

In most parts of Sri Lanka, consumers receive treated and untreated water mainly from lakes and reservoirs and almost all the water sources which are used for drinking purposes are having high diversity of algae and cyanobacteria (Sethunga and Manage 2010, JICA, 2012). Consumer complaints regarding bad T&O has been recorded and this issue is serious among water consumers in north Central, Eastern, Northeast, North, Uva and Southern part of the country (NWSDB, 2011; Ganegoda et al., 2019). Therefore, it's important to consider the level of T&O compounds in raw and treated waters, to improve water treatment facilities and associated technology to safeguard the consumer. Thus, the present study screened 2-MIB level in both raw and treated waters for the first time in Sri Lanka, with the special attention on 2-MIB producers as well as water quality.

2. MATERIALS AND METHODS

2.1. Study area and sampling site

Srilanka is an island, situated in the Indian ocean occupying the tropical climate. The country has main two seasons, dry season and wet/rainy season. In this study, sampling was performed from June 2016 to June 2018, bimonthly, covering both dry and wet seasons. Nineteen (19) raw water reservoirs and fourteen (14) WTPs were sampled where taste and odour problems are prevailing all around the country. Totally thirty-three raw and treated water samples were collected covering North Central province (Anuradhapura and Pollonnaruwa districts), Eastern province (Ampara, Trincomalee, Batticaloa districts) and Southern province (Hambanthota district) in Sri Lanka, given in Figure 1.

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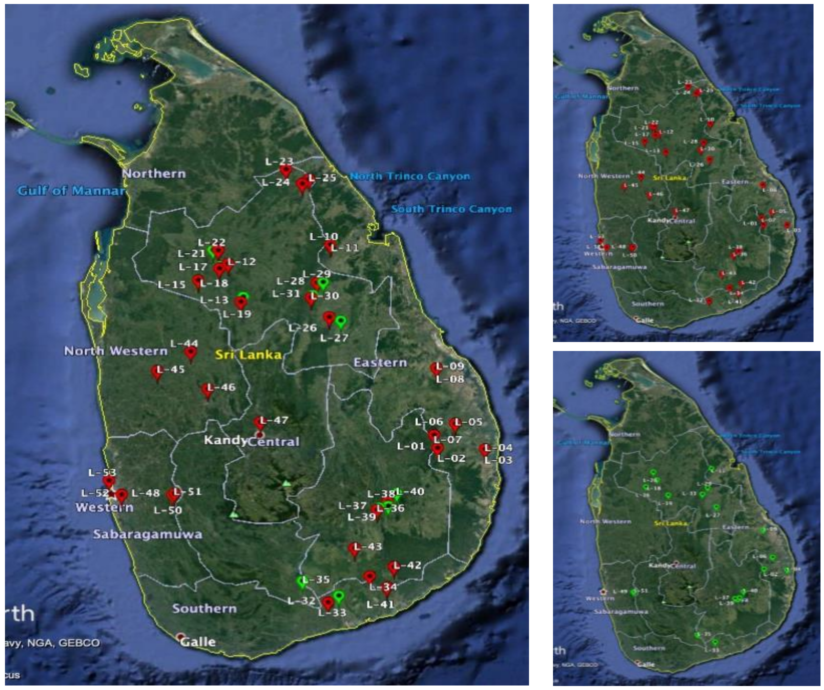

Figure 1: Reservoirs and Water Treatment Plants (WTP) selected to collect water for the present study

2.2. Sample collection and preparation

Samples were collected for 2- MIB analysis following the method given by Ganegoda et al., 2020. The collected samples were placed in an ice box (4 °C) and transported to the laboratory within 12 hours. The samples were stored in the dark at 4 ºC and analysis was performed within 7 days.

2.3. Analysis of 2-MIB

2-MIB in the water samples were determined by HS-SPME/GC–MS analysis according to Ganegoda et al., 2020. GC–MS analysis was carried out with Agilent Model 7890A Gas Chromatograph and Agilent Model 5890 C Mass Spectrometer. A fused-silica capillary column with cross-linked 5% phenyl methyl siloxane of HP-5MS (30 m × 250 µm × 0.25 µm film thickness) was used. The GC operating conditions were as follows: injection and detector temperatures, 270 ºC; inlet helium carrier gas flow rate, 1.1 ml/min is maintained by an electronic pressure controller. Injection port was operated in pulsed split less mode and was fitted with 0.7mm id SPME injection liner. Head pressure was set to 9.35 psi of helium for 1.30 minutes, then changed

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to a constant flow of 1.1 ml/min to give a velocity of 38.4 cm/s. Oven was initially held at 60 °C for 1 minute, then increased by 10 °C/min to 300 °C and held for 4 minutes. The electron impact (EI-MS) conditions were as follows: MS source temperature, 230 ºC, MS quadropole temperature, 150 ºC; ionizing voltage, 70 eV. The full scan mass spectra were obtained at an m/z range of 33–550 D. Fragment ions used for selected ion monitoring (SIM) mode detections for 2- MIB is m/z = 95 which was the most prominent peak. This was monitored alternatively at dwell times of 1001.ts each. At a retention time of 7.85 minute, 2- MIB peak was appeared and mass spectra of 2- MIB peak was matched with NIST library spectra for identification of 2-MIB. 2- MIB produced excellent response to GC–MS-SIM detection and the Minimum Detectable Level (MDL) of 2-MIB by HS-SPME/GC–MS under optimized conditions was found to be 1.3 ng L^{-1}. Minimum Level of Quantification (MQL) was 3.0 ng L^{-1}. Both values are below the minimum threshold levels where human olfactory system detects 2- MIB on or above 5 ng L^{-1}. An excellent linear correlation of peak area and level of 2- MIB was obtained (R^2 = 0.998) for over the concentration range from 5 to 100 ng L^{-1}.

2.4. Identification and enumeration of phytoplankton

100ml of water sample was fixed with acidified Lugol's solution at the final concentration of 1% following natural sedimentation at dark for overnight. Concentrated Lugol's treated samples (3-5ml) were used to identify and enumerate cyanobacteria and algae using a Sedgewick rafter counting chamber under the light microscope (x 40) with identification keys (Prescott, 1978, Manage, 2013).

2.5. Measurement of water quality parameters

Water pH, temperature, Dissolved Oxygen (DO) and Electric Conductivity (EC) were measured at the site itself using standard digital meters [using a pH meter (330 I/ Set, WTW Co., Weilheim, Germany), a thermometer, an oxygen meter (Oxi 320/ Set, WTW Co., Weilheim, Germany) and a conductivity meter (340A-Set 1. WTWCo., Weilheim, Germany) respectively]. Nitrate-N, nitrite-N, ammonia-N, Total Phosphorous (TP) and hardness were measured at the laboratory using standard titrimetric and spectrometric methods (APHA 1999).

2.6. Statistical analysis

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Principal component analysis (PCA) and Pearson's correlation test were performed using MINITAB 17 software to analyze the relationship between 2-MIB level, cyanobacteria cell density and physico chemical parameters.

3. RESULTS

3.1 Detection of 2-MIB in raw and treated water samples

Table 1 shows 2-MIB level in raw and treated water samples collected at various water sources. 2-MIB levels in wet season were much less, when compared to the dry season for raw and treated waters. 2-MIB level in raw water ranged from not detected levels (N.D.) to 57.6±3.1 ng L^{-1} in wet season whereas in dry season it varied from 5.8±1.8 to 96.3±4.2 ng L^{-1}. In treated water, 2-MIB levels ranged from N.D. to 69.1±1.3 ng L^{-1} in wet season and from N.D. to 98.5± 2.8 ng L^{-1} during dry season. Kanthale reservoir, which is situated in the dry zone in Sri Lanka, recorded the highest 2-MIB level in both raw and treated waters, in both seasons. In Sagama reservoir, although 2-MIB was not present in raw water, it was observed in treated water samples (7.6±1.6 ng/L) in wet season. Moreover, in several instances, although 2-MIB presented in raw water, it was not detected in treated water (Table 4). Another highlighted feature was, at several conventional water treatment processes occupied water treatment plants (WTPs), the treated water 2-MIB level was greater than the respective 2-MIB level in raw water. Kawdulla reservoir and Tissa reservoir are two examples.

Table 1: 2-MIB levels (ng/L) in raw and treated water collected at various water resources

Water Source	**Utility**	**Wet season**		**Dry Season**	
		Raw water ng/L	**Treated water ng/L**	**Raw water ng/L**	**Treated water ng/L**
Jayanthi reservoir	D, F, I	11.8±1.3	12.2±2.2	73.8±1.1	22.7±4.5
Sagama reservoir	D, I	N.D.	7.6±1.6	5.8±1.8	8.5±2.4
Kondawatuwana reservoir	D, F, I	3.2±1.5	N.D.	14±1.4	N.D.
Unnichchi reservoir	D, F, I	N.D.	N.D.	6.4±5.1	N.D.
Kanthale reservoir	D, F, I	5.2±1.5	N.D.	80.5±1.6	3.5±1.9
Ridiyagama reservoir	D, F, I	3.6±1.9	N.D.	10.3±3.8	N.D.

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Nachchadoowa reservoir	D, F, I	10.3± 3.5	N.A.	14.5±2.1	N.A.
Kala reservoir	D, F, I	6.5±2.5	8.5±1.6	49.5±1.1	51.2±6.1
Nallachchiya reservoir	D, F, I	8.9±3.3	9.5±2.1	31.7±2.5	41.9±2.2
Thuruwila reservoir	D, F, I	9.2±1.2	9.9±2.0	58.2±1.9	60.3±1.5
Tissa reservoir	D, F, I	10.2±1.6	11.5±1.8	36.4±1.8	40.4±1.7
Nuwara reservoir	D, F, I	15.4±1.7	21.4±1.8	60.2±2.5	62.6±1.9
Parakrama Samudraya reservoir	D, F, I	8.4± 0.5	16.7± 1.8	10.5± 0.5	34.1± 3.3
Kawdulla reservoir	D, F, I	57.6± 3.1	69.1± 1.3	96.3± 4.2	98.5± 2.8
Minneriya tank	D, F, I	6.2± 0.8	8.3± 0.8	7.6± 0.6	10.2± 1.8

ND – Not detected, NA - Water treatment facility not available, D- Drinking, F- fisheries, I- Irrigation.

3.2 Identification and enumeration of phytoplankton

Species composition and abundance of algae, cyanobacteria, diatoms and flagellates in both dry and wet season's raw water reservoirs are given in Table 5, 6, 7, 8, 9 and 10. Some taste and odour producing cyanobacteria; *Anabaena* sp., *Cylindrospermum* spp., and *Oscillatoria* sp. were identified as common organisms in almost all water bodies during the study.

Table 5: Composition and abundance of cyanobacteria and algae in dry and wet seasons in Ampara district water reservoirs

Water Source	Wet season			Dry Season	
	Cyanobacteria/algae species composition	Abundance Cells ml^{-1}		Cyanobacteria/algae species composition	Abundance cells ml^{-1}
Jayanthi reservoir	*Microcystis* spp.	5320±7.34	Taste and Odour forming	*Microcystis* sp.	16,830±2.47
	Cylindrospermopsis sp.	758±2.15		*Cylindrospermopsis* sp.	1280±3.14
	Anabaena sp.	280±1.21		*Anabaena* sp.	850±1.26

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	Oscillatoria sp.	123±0.62		*Oscillatoria* sp.	336±0.47
	Cyclotella sp.	130±0.73		*Cyclotella* sp.	150±1.25
	Volvox sp.	120±0.87		*Volvox* sp.	138±0.88
	Gloeocystis sp.	300±1.12		*Gloeocystis* sp.	127±4.11
	Uroglenopsis sp.	80±0.35		*Uroglenopsis* sp.	91±1.17
	Melosira sp.	1280±3.48	Other	*Chroococcus* sp.	115±2.3
	Chroococcus sp.	80±0.68		*Stephanodisc* sp.	347±1.62
	Stephanodisc sp.	320±1.42		*Gomphosphaeria* sp.	8971±4.27
	Gomphosphaeria sp.	8240±8.24		*Merismopedia* sp.	854±1.42
	Merismopedia sp.	800±0.76		*Pediastrum simplex*	78±1.26
				Melosira sp.	2112±3.17
	Total	**17831±18.21**		**Total**	**32279±175.31**
Sagama reservoir	*Microcystis* sp.	4334±1.14	Taste and Odour forming	*Microcystis* sp.	8752±1.76
	Anabaena sp.	340±0.79		*Anabaena* sp.	1324±5.88
	Synedra ulna	280±0.84		*Synedra ulna*	312±4.31
	Melosira sp.	1325±2.38	Other	*Melosira* sp.	1411±7.05
	Ulothrix sp.	338±0.48		*Ulothrix* sp.	254±1.34
	Stephanodisc sp.	35±0.74		*Stephanodisc* sp.	72±1.26
	Total	**6652±92.43**		**Total**	**12125±151.27**
Kondawa tuwana reservoir	*Cylindrospermopsis* sp.	3250±4.89	Taste and Odour forming	*Microcystis* sp.	14248±10.35
	Microcystis sp.	1500±3.18		*Cylindrospermopsis* sp.	9889±8.41
	Oscillatoria sp.	129±1.16		*Anabaena* sp.	1025±2.32

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	Anabaena sp.	94±2.17		*Oscillatoria* sp.	383±1.22
	Staurastrum sp.	25±0.69		*Pediastrum simplex*	38±1.26
	Gloeocystis sp.	100±0.93		*Scenedesmus arvernensis*	84±1.45
	Melosira sp.	350±0.83	Other	*Melosira* sp.	422±1.51
	Stephanodisc sp.	200±0.99		*Chroococcus* sp.	258±2.32
	Chroococcus sp.	130±0.82		*Navicula graciloides*	39±2.51
	Ulothrix sp.	80±0.96			
	Total	**5858±15.66**		**Total**	**26386±215.34**

Three water reservoirs from Ampara district were sampled where T&O issues were the highest, and the identified cyanobacteria and algae are listed in Table 5. Jayanthi reservoir recorded the highest cyanobacteria and algae cell density in both dry (32279±175.31 Cells ml^{-1}) and wet (17831±18.21 Cells ml^{-1}) seasons. Sagama reservoir recorded the lowest in the dry season (12125±151.27 Cells ml^{-1}), whereas Kondawatuwana reservoir recorded the lowest in the wet season (5858±15.66 Cells ml^{-1}). In majority of the reservoirs, *Microcystis* sp. and *Cylindrospermopsis* sp. were the dominant and co-dominant cyanobacteria genera. However, *Anabaena* sp. was identified as dominant cyanobacteria in Sagama reservoir.

Unnichchi reservoir was selected from the Batticaloa district with prevalent of T&O issues and observed cyanobacteria and algae cell densities are listed in Table 6. Unnichchi reservoir recorded low number of cyanobacteria and algae when compared to all the other reservoirs in both dry and wet seasons, whereas *Cylindrospermopsis* sp. and *Microcystis* sp. were reported as the dominant and co-dominant cyanobacteria genera.

Table 6: Composition and abundance of cyanobacteria and algae in dry and wet seasons in Batticaloa district water reservoirs.

		Wet season		**Dry Season**

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Water Source		Cyanobacteria/algae species composition	Abundance Cells ml^{-1}		Cyanobacteria/algae species composition	Abundance Cells ml^{-1}
Unnichchi reservoir	Taste and Odour forming	*Cylindrospermopsis* sp.	1120±0.93	Taste and Odour forming	*Microcystis* sp.	4592±4.13
		Microcystis sp.	2610±1.73		*Cylindrospermopsis* sp.	2809±2.88
		Oscillatoria sp.	280±0.89		*Anabaena* sp.	1057±1.85
		Anabaena sp.	980±0.82		*Oscillatoria* sp.	388±2.65
		Cyclotella sp.	155±2.32		*Pediastrum simplex*	84±1.52
	Other	*Melosira* sp.	235±1.38		*Synedra ulna*	88±3.12
		Chroococcus sp.	410±1.88	Other	*Melosira* sp.	1133±2.69
		Dichotomosiphon sp.	65±0.58			
		Total	**5855±69.71**		**Total**	**10151±85.42**

Kanthale reservoir in Trincomalee district showed *Microcystis* sp. as the dominant cyanobacteria in both seasons (Table 7). As similar to all the other reservoirs in Eastern province, both *Microcystis* sp. and *Cylindrospermopsis* sp. were the major two cyanobacteria genera presented in the Kanthale reservoir.

Table 7: Composition and abundance of cyanobacteria and algae in dry and wet seasons in Trincomalee district water reservoirs

Water Source		Wet season			Dry Season	
		Cyanobacteria/algae species composition	Abundance Cells ml^{-1}		Cyanobacteria/algae species composition	Abundance Cells ml^{-1}

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Kanthale reservoir	Taste and Odour forming	*Microcystis* sp.	3850±3.37	Taste and Odour forming	*Microcystis* sp.	10214±4.51
		Cylindrospermopsis sp.	2524±1.87		*Cylindrospermopsis* sp.	4118±7.62
		Anabaena sp.	1038±3.81		*Anabaena* sp.	3042±8
	Other	*Melosira* sp.	125±2.55	Other	*Melosira* sp.	312±1.14
		Chroococcus sp.	18±1.23		*Chroococcus* sp.	52±1.38
		Total	**7555±32.72**		**Total**	**17738±98.33**

Ridiyagama reservoir in Hambanthota district showed *Microcystis* sp. being the dominant and *Cylindrospermopsis* sp. was the co-dominant in both seasons (Table 8).

Table 8: Species composition and abundance of cyanobacteria and algae in dry and wet seasons in the Ridiyagama reservoir in Hambanthota district .

Water Source		**Wet season**			**Dry Season**	
		Cyanobacteria/algae species composition	**Abundance Cells ml^{-1}**		**Cyanobacteria/algae species composition**	**Abundance Cells ml^{-1}**
Ridiyagama reservoir	Taste and Odour forming	*Microcystis* sp.	2215±8.54	Taste and Odour forming	*Microcystis* sp.	12425±28.33
		Cylindrospermopsis sp.	1240±5.32		*Cylindrospermopsis* sp.	1596±1.65
		Oscillatoria sp.	95±3.36		*Oscillatoria* sp.	495±2.22
		Anabaena sp.	64±1.54		*Anabaena* sp.	427±0.98
	Other	*Melosira* sp.	325±2.55	Other	*Spirogyra porticalis*	57±2.65

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		Navicula graciloides	18±3.21		*Ulothrix* sp.	58±2.55
		Ulothrix sp.	13±1.40		*Navicula graciloides*	61±3.15
					Melosira sp.	1231±1.63
		Total	**3970±81.22**		**Total**	**16350±140.42**

Six water reservoirs from Anuradhapura district were sampled, where T&O issues are the highest. Table 9 shows the composition and abundance of cyanobacteria and algae in those reservoirs.

Table 9: Composition and abundance of cyanobacteria and algae in dry and wet seasons in four water reservoirs in Anuradhapura district.

Water Source		**Wet season**			**Dry Season**	
		Cyanobacteria/algae species composition	**Abundance Cells ml^{-1}**		**Cyanobacteria/algae species composition**	**Abundance Cells ml^{-1}**
Kala reservoir	Taste and Odour forming	*Microcystis* sp.	2920±2.45	Taste and Odour forming	*Microcystis* sp.	18152±15.27
		Cylindrospermopsis sp.	412±3.41		*Cylindrospermopsis* sp.	14986±3.83
		Oscillatoria sp.	156±2.03		*Anabaena* sp.	8839±0.93
		Anabaena sp.	79±1.52		*Oscillatoria* sp.	1135±3.27
Nallachchiya	Other	*Melosira* sp.	52±3.11	Other	*Merismopedia* sp.	1398±2.18
					Melosira sp.	1330±1.78
		Total	**3619±98.21**		**Total**	**45840±321.58**

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reservoir	Taste and Odour forming	*Microcystis* sp.	1548±2.52	Taste and Odour forming	*Cylindrospermopsis* sp.	6012±2.53
		Anabaena sp.	1184±3.81		*Microcystis* sp.	8221±3.94
		Cylindrospermopsis sp.	1251±0.68		*Anabaena* sp.	7869±4.47
		Oscillatoria sp.	77±1.37		*Oscillatoria* sp.	864±2.58
	Other	*Lyngbya limnetica*	26±4.11	Other	*Lyngbya limnetica*	197±2.18
		Total	**4080±154.3**		**Total**	**23163±163.5**
Nachchadoowa reservoir	Taste and Odour forming	*Microcystis* sp.	2818±3.58	Taste and Odour forming	*Microcystis* sp.	*9300*±8.92
		Cylindrospermopsis sp.	293±1.85		*Cylindrospermopsis* sp.	*4854±5.18*
		Oscillatoria sp.	130±1.72		*Anabaena* sp.	*10468±11.79*
		Anabaena sp.	115±2.16		*Oscillatoria* sp.	*1163±4.38*
		Scenedesmus acuminatus	11±1.21		*Scenedesmus acuminatus*	98±2.31
	Other	*Melosira sp.*	82±3.52	Other	*Lyngbya limnetica*	235±0.38
					Melosira sp	1792±2.39
					Closterium acutum	85±1.39
					Navicula graciloides	87±2.45
		Total	**3449±18.33**		**Total**	**28082±113.72**

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Thuruwila reservoir	Taste and Odour forming	*Microcystis* sp.	4856±6.28	Taste and Odour forming	*Microcystis* sp.	61662±3.35
		Cylindrospermopsis sp.	568±3.47		*Cylindrospermopsis* sp.	12465±3.26
		Oscillatoria sp	322±7.22		*Anabaena* sp.	21500±7.18
		Anabaena sp.	188±2.88		*Oscillatoria* sp.	2467±1.96
		Scenedesmus arvernensis	14±1.07		*Scenedesmus arvernensis*	85±0.26
		Dictyosphaerium sp.	28±1.42		*Dictyosphaerium* sp.	164±0.27
	Other	*Merismopedia* sp.	108±2.53	Other	*Navicula graciloides*	67±3.51
					Merismopedia sp.	1468±3.28
		Total	**6084±128.3**		**Total**	**99878±931.5**
Tissa reservoir	Taste and Odour forming	*Microcystis* sp.	1598±2.15	Taste and Odour forming	*Microcystis* sp.	7665±4.97
		Anabaena sp.	1316±2.87		*Anabaena sp.*	28770±9.41
		Cylindrospermopsis sp.	1292±1.52		*Cylindrospermopsis* sp.	8697±5.42
		Oscillatoria sp.	512±3.71		*Oscillatoria* sp.	2896±1.53
	Other	*Lyngbya limnetica*	51±1.23		*Staurastrum paradoxum*	58±1.52
		Chroococcus sp.	108±4.81		*Scenedesmus acuminatus*	69±2.32
				Other	*Lyngbya limnetica*	168±0.77
					Chroococcus sp.	1305±2.73

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		Total	**4877±15.22**		**Total**	**49628±192.14**
Nuwara reservoir	Taste and Odour forming	*Anabaena* sp.	6281±3.28	Taste and Odour forming	*Anabaena* sp.	35580±42.43
		Cylindrospermum spp.	2135±7.15		*Cylindrospermum* spp.	9850±4.73
		Microcystis sp.	4528±4.22		*Microcystis* sp.	21550±27.64
		Oscillatoria sp.	982±1.08		*Oscillatoria* sp.	2115±3.80
		Lyngbya limnetica	92±1.54		**Total T&O forming**	**71895±583.42**
		Melosira sp.	185±3.72	Other	*Melosira* sp.	1558±8.42
					Lyngbya limnetica	220±1.43
		Total	**14203±195.36**		**Total**	**73673±614.2**

In Anuradhapura district, *Microcystis* sp. and *Cylindrospermopsis* sp. were found to be the dominant and co- dominant genera of cyanobacteria present in most reservoirs. However, *Anabaena* sp. became dominant and co-dominant in few water reservoirs namely Nuwara reservoir, Tissa reservoir and Thuruwila reservoir. These reservoirs showed significantly high ($p<0.05$) levels of 2-MIB when compared with the other tested water bodies. In the dry season, Thuruwila reservoir recorded the highest phytoplankton cell density (99878±931.5 Cells ml^{-1}) whereas Nallachchiya reservoir recorded the lowest (23163±163.5 Cells ml^{-1}). In the wet season, Nuwara reservoir recorded the highest phytoplankton cell density (14203±195.36 Cells ml^{-1}) whereas Nachchadoowa reservoir recorded the lowest (3449±18.33 Cells ml^{-1}).

Table 10 depicts the composition and abundance of cyanobacteria and algae in Parakrama Samudraya reservoir, Kawdulla reservoir and Minneriya reservoir in Pollonnaruwa district. As similar to all other dry zone water bodies, *Microcystis* sp., *Cylindrospermopsis* sp. and *Anabaena* sp. were the key players of dominant and co-dominant 2-MIB producers.

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Table 10: Composition and abundance of cyanobacteria and algae in dry and wet seasons in Parakrama Samudraya reservoir, Kawdulla reservoir and Minneriya reservoir in Pollonnaruwa district

Water Source		**Wet season**			**Dry Season**	
		Cyanobacteria/algae species composition	**Abundance Cells ml^{-1}**		**Cyanobacteria/algae species composition**	**Abundance Cells ml^{-1}**
Parakrama Samudraya reservoir	Taste and Odour forming	*Cylindrospermopsis* sp.	1322±2.22	Taste and Odour forming	*Microcystis* sp.	5894±2.37
		Microcystis sp.	1521±4.32		*Cylindrospermopsis* sp.	825±0.84
		Oscillatoria sp.	116±1.32		*Anabaena* sp.	983±0.97
		Anabaena sp.	105±1.38		*Oscillatoria* sp.	264±1.53
		Scenedesmus acuminatus	15±2.33		*Pediastrum simplex*	92±1.48
	Other	*Melosira* sp.	58±2.37		*Scenedesmus acuminatus*	78±2.37
				Other	*Stephanodisc* sp.	1558±8.42
					Melosira sp.	220±1.43
		Total	**9914±110.45**		**Total**	**3137±29.32**
	Taste and	*Microcystis* sp.	10137±25.8		*Microcystis* sp.	58227±124.6

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Kawdulla reservoir	Odour forming	*Oscillatoria* sp.	522±2.3		*Oscillatoria* sp.	3497±12.1
		Anabaena sp.	3240±4.2		*Anabaena* sp.	25310±72.8
		Cylindrospermopsis sp.	4582±18.7		*Cylindrospermopsis* sp.	8314±7.1
	Other	*Melosira* sp.	432±15.2	Other	*Melosira* sp.	1021±32.7
		Lyngbya limnetica	83±8.5		*Lyngbya limnetica*	412±13.2
		Total	**18996±214.8**		**Total**	**96781±772.4**
Minneriya reservoir	Taste and Odour forming	*Microcystis* sp.	10031±312.5	Taste and Odour forming	*Microcystis* sp.	32567±411.8
		Oscillatoria sp.	1007±32.1		*Oscillatoria* sp.	4312±12.1
		Anabaena sp.	1528±18.4		*Anabaena* sp.	10143±151.2
		Cylindrospermopsis sp.	987±12.1		*Cylindrospermopsis* sp.	15053±8.3
	Other	*Melosira* sp.	27±1.6	Other	*Melosira* sp.	113±4.1
					Stephanodisc sp.	532±11.2
		Total	**13647±315.7**		**Total**	**62818±637.1**

Kawdulla reservoir recorded a significantly high level of cyanobacteria and algae cell density when compared to all other reservoirs in Pollonnaruwa district occupying the highest cell density in both dry (96781±772.4 Cells ml^{-1}) and wet (18996±214.8 Cells ml^{-1}) seasons.

3.4 Measurement of water quality parameters

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Raw water was analyzed for various physico chemical parameters as given in the Table 11. Almost all the raw water bodies tested had acceptable pH, conductivity, Dissolved Oxygen (DO), hardness, Total Phosphorous (TP) and Total Nitrogen (TN) for drinking purpose according to the drinking water standards given by the SLSI except few exceptions. Nachchadoowa reservoir had a significantly high conductivity value ($p<0.05$) compared to other reservoirs. Tissa reservoir had a significantly high ($p<0.05$) TN level in dry season which exceeds the safe range for drinking value according to SLSI drinking water standards.

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Table 11: Physico chemical water quality parameters of raw water reservoirs in dry and wet seasons, Sri Lanka

Water body	**Temperature/(°C)**		**pH**		**DO/(mg/L)**		**EC/(μscm^{-1})**		**TN/ (mg/L)**		**TP/ (mg/L)**		**Hardness/ (mg/L)**	
	Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Jayanthi rese.	32.3 ±0.6	26.3 ±0.7	5.8±1.7	6.14±2.08	6.23±0.12	6.78±0.22	168.0±5.1	147.2 ±7.3	0.08±0.12	0.07±0.22	0.32±5.11	0.02±1.53	78.0±3.2	56.0±2.1
Sagama rese.	29.0 ±1.2	29.0 ±1.2	7.8±1.2	7.57±0.04	6.11±0.04	7.42±0.23	138.0±2.3	129.1 ±1.2	0.02±0.01	< 0.01	0.04±0.01	< 0.01	110.0 ±3.1	58.1±3.1
Kondawatuwana rese.	32.0 ±1.1	28.5 ±0.4	8.8±0.3	8.0±0.8	7.42±0.61	8.97±0.55	185.0±5.2	154.4 ±1.9	0.15±0.25	0.24±0.03	0.18±0.05	0.04±0.01	92.0±3.3	64.0±2.3
Unnichchi rese.	32.1 ±0.2	30.1 ±0.2	8.9±0.8	8.21±1.32	7.31±2.22	8.56±1.54	121.3±2.5	110.7 ±1.8	0.34±0.05	0.31±0.11	0.14±0.01	0.02±0.01	87.0±3.4	60.0±1.2
Kanthale rese.	28.5 ±0.8	28.5 ±0.7	7.6±1.5	7.33±1.25	6.22±1.91	7.81±0.71	198.1±1.8	182.8 ±3.3	0.6±0.5	0.58±0.15	0.08±0.02	< 0.01	120.0 ±3.8	70.0±6.8
Nachchadoowa rese.	28.9 ±0.5	28.8 ±0.4	8.27±0.55	7.1±1.3	7.85±0.85	8.33±1.81	719.0±53.0	234.0 ±3.2	0.74±0.62	0.02±0.01	0.26±2.44	0.015	128.0 ±0.1	78.0±3.2
Kala rese.	31.8 ±0.2	28.2 ±0.2	8.25±1.52	8.1±1.2	8.24±1.12	9.1±0.9	279.1±2.3	180.5 ±8.3	0.74±0.03	< 0.01	8.28±0.09	4.03±0.26	110.0 ±0.2	80.2±2.8

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Nallachchiya rese.	30.2 ±0.6	28.2 ±0.1	8.23±1.27	8.11±1.15	8.07±2.64	8.81±0.16	172.3±2.2	154.7 ±3.6	0.97±0.05	< 0.01	0.14±0.01	< 0.01	72.0±1.4	58.0±1.1
Thuruwila rese.	29.2 ±0.2	28.7 ±3.5	8.41±3.43	6.9±2.2	8.50±1.66	8.9±0.5	478.0±2.1	250.0 ±9.4	1.13±0.03	0.02±0.01	0.29±0.08	0.015	144.0 ±1.5	87.0±2.1
Tissa rese.	30.1 ±0.5	29.8 ±1.1	8.62±1.87	7.1±1.4	8.06±2.33	8.51±0.08	440.0±5.2	325.0 ±4.3	16.78±3.77	2.38±0.53	0.18±0.26	0.02	112.0 ±0.1	64.0±3.2
Nuwara rese.	28.4 ±0.2	28.1 ±2.2	8.51±2.57	7.25±2.51	7.85±1.92	8.2±2.1	382.0±3.4	289.8 ±7.2	2.09±0.21	0.02±0.21	0.13±0.04	< 0.01	112.0 ±0.7	89.1±3.4
Parakrama Samudraya rese.	32.2 ±0.5	30.5 ±0.3	8.7±3.2	8.48±0.11	6.3±2.3	7.5±0.5	198.0±5.2	172.2 ±4.9	0.05	0.161	0.03	0.02±0.01	70.0±3.1	54.0±1.2
Kawdulla rese.	30.7 ±1.5	28.7 ±0.2	6.55±2.28	6.8±0.2	5.88±3.66	6.8±0.3	214.0±2.2	182.0 ±3.1	1.02±0.81	0.56±0.88	0.12±0.66	< 0.04	138.0 ±4.2	111.0±1.1
Minneriya rese.	31.5 ±0.9	30.8 ±0.5	6.5±2.1	7.1±0.3	5.94±2.11	6.39±0.68	380.0±3.1	252.0 ±2.4	0.84±0.11	0.62±0.01	0.24±0.72	< 0.09	185.0 ±2.7	115.55 ±3.29
Ridiyagama rese.	30.3 ±0.3	29.8 ±0.2	8.8±0.9	7.3±1.3	8.1±1.2	8.6±1.5	382.0±2.0	256.0 ±2.5	0.04±0.01	0.03±0.01	0.02±0.01	0.02±0.01	85.0±1.4	56.0±3.1

(Wet-Wet season, Dry-Dry season, DO- Dissolved Oxygen, EC-Electric Conductivity, TN – Total Nitrogen, TP- Total Phosphorous .

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3.5 Principal Component Analysis (PCA)

The PCA was conducted for the results (Figure 4) and the principal components, PC_1 and PC_2 contributed about 71% of the total variance in the data (Table 12). The first principal component, has variance of 4.54 and accounts for 50.5% of the total variance.

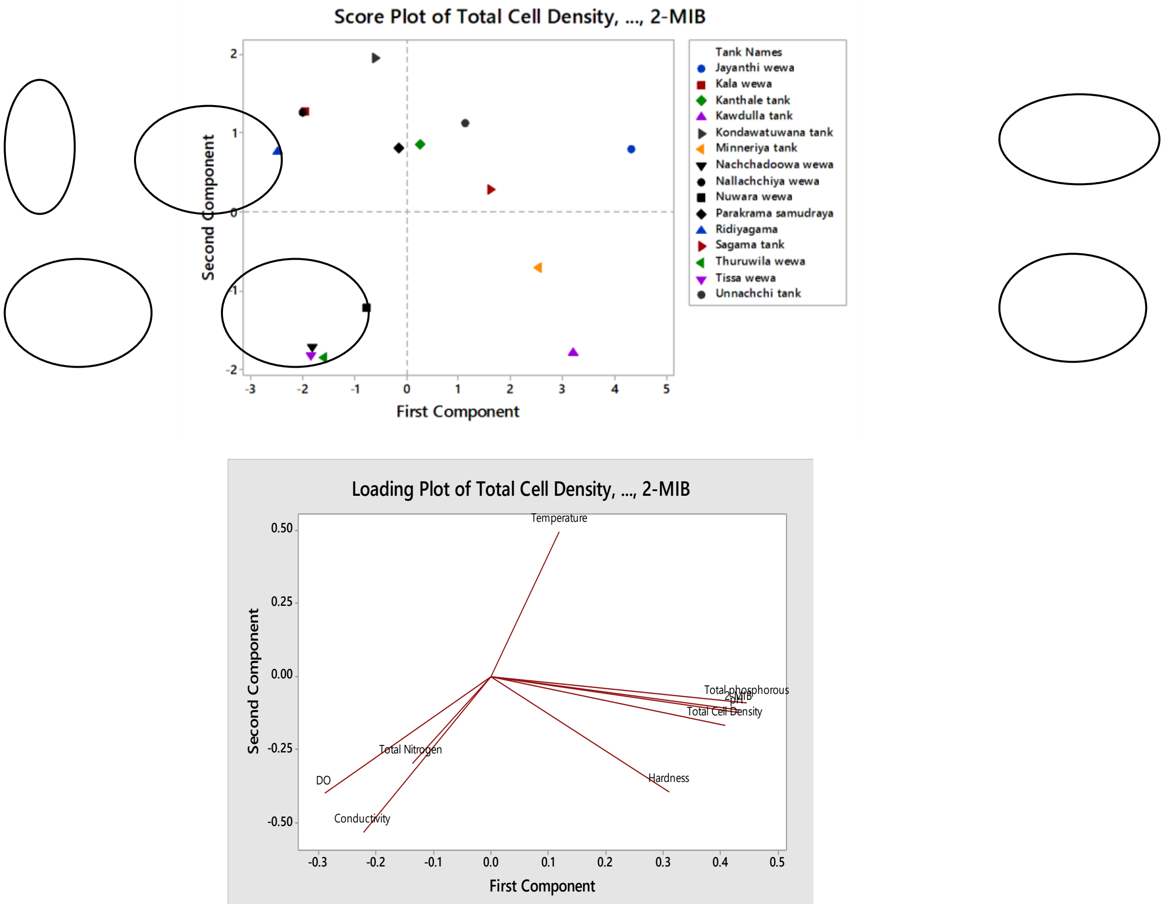

Figure 4: Principal component analysis (PCA): 04 (a): Score plot, 04 (a): Loading plot

Table 12: Summary of the Eigen values & correlation matrix of principal component analysis

Eigen analysis of the Correlation Matrix

Eigenvalue	4.5474	1.8399	0.9087	0.6445	0.4443	0.3381	0.1558	0.1055	0.0159
Proportion	0.505	0.204	0.101	0.072	0.049	0.038	0.017	0.012	0.002
Cumulative	0.505	0.710	0.811	0.882	0.932	0.969	0.987	0.998	1.000

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Correlation matrix of Principal component analysis

Variable	PC1	PC2	PC3
Total Cell Density	**0.408**	-0.167	-0.073
pH	**0.429**	-0.124	0.121
DO	-0.291	**-0.401**	0.115
Conductivity	-0.223	**-0.534**	-0.062
Total Nitrogen	-0.138	-0.298	0.834
Total phosphorous	**0.446**	-0.093	0.172
Hardness	0.310	-0.396	-0.174
Temperature	0.119	**0.494**	0.448
2-MIB	**0.433**	-0.116	0.084

The coefficient listed under PC1 in Table 2 shows the way to calculate the principal component scores: PC_1 = 0.408 Total cell densities + 0.429 pH - 0.291 DO – 0.223 Cond. – 0.138 Total Nitrogen +0.446 Total Phosphorous + 0.310 hardness + 0.119 Temperature + 0.433 2-MIB

PC_1 is contributed by 2-MIB, total phosphorous, Total Cyanobacteria Cell density and pH even though there is no parameter value obtained more than 0.5. The second principal component has variance of 1.83 and accounts for 20.4% of the data variability. It was calculated from the original data using the coefficients listed under PC_2. PC_2 is strongly contributed by DO, EC and temperature. Together, the first two and first three components represent 71% and 81% of the data variability respectively, of the total variability. Thus, most of the data structure can be captured in two or three underlying dimensions. The remaining principal components account for a very small proportion of the variability and are probably unimportant. Eigen values greater than 1.0 was used for PCA scoring and three scores were selected (Table 12). First principal component (PC_1) explains 50.5% of total variance of the data with 50.5% cumulative variation. And the second principal component (PC_2) explains 20.4% of total variance of the data with 71.0% cumulative variation. In the scores plot of PC_2 versus PC_1, four clusters can be identified along PC_1 axis. The groups I, II, III and IV correspond to samples with different water quality parameters. pH, 2-MIB,

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total phosphorous, total cell density, conductivity, hardness, DO and total nitrogen exhibited a strong relationship among them and also influenced the separation of the four groups along PC_1. Ridiyagama reservoir, Nallachchiya reservoir and Kala reservoir were clustered together to create the first cluster whereas Parakrama Samudraya reservoir from North Central province, Unnichchai reservoir, Kanthale reservoir and Sagama reservoir from Eastern province were clustered together with similar temperature values. The third cluster consisted of tanks of Anuradhapura district as Nachchadoowa reservoir, Tissa reservoir, Thuruwila reservoir and Nuwara reservoir with similar DO, Total nitrogen and Conductivity values. Minneriya reservoir and Kawdulla reservoir from Pollonnaruwa district were belonged into the final cluster with high hardness, total cyanobacterial cell density, total phosphorous and pH values. The PCA analysis revealed that 2-MIB level was correlated with water quality parameters and interestingly water reservoirs from Anuradhapura and Pollonnaruwa districts and Eastern province were separately clustered together due to some similarity in geography which may had displayed in the water quality.

4.0 DISCUSSION

2-Methylisoborneol (2-MIB) is known to cause musty taste and odour (T&O) in water making the drinking water aesthetic quality adverse and unpleasant (Guttman & van Rijn, 2012). Hence, presence of 2-MIB has caused major issues in drinking water industry all over the world by deteriorating consumer confidence on treated drinking water leading to consumer rejection, in turn resulting large scale increase of treatment cost (Bristow et al., 2018). Therefore, mitigating the T&O sources into source waters and reducing T&O levels in final treated waters have been a priority for years in the water industry. Several studies have been recorded all over the world related to 2-MIB, however present study is the first study carried out in Sri Lanka. The present results revealed that 75% of the collected raw water covering 06 districts (Anuradhapura, Pollonnaruwa, Ampara, Batticcaloe, Trincomalee and Hambanthota) exceeded the human threshold level for 2-MIB (5 ng L^{-1}) whereas 63% collected treated water samples exceeded the human threshold level.

In the present study, a 2-MB detection method was successfully optimized using Solid Phase Micro Extraction (SPME) coupled with GC/MS, which is a sensitive solvent free extraction method with detection level of technique with the capability of detecting 2-MIB in 'ppt' levels.

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Although there are several studies available in the literature reporting 2-MIB contamination levels in various reservoirs, studies related to seasonal variation of 2-MIB are limited. Majority of the studies suggested that 2-MIB levels were greater in the dry season when compared to the wet season. According to Bertone & O'Halloran (2016), 2-MIB is released more at warm stratified water bodies during the dry period compared to the rainy period. Henatsch & Jüttner (1990) found that increased summer epilimnetic 2-MIB levels in eutrophic Lake Schleinsee (southwest Germany). Sugiura and Nakano (2000) concluded that elevated 2-MIB levels in Lake Kasumigaura (Japan) in summer. Jensen & coworkers (1994) traced the annual spring outbreaks of musty odor in tap water. Results from the present study agree with these findings where elevated levels of 2-MIB was observed in dry season when compared to the wet season (Table 4). Although there is no exact standard level for taste and odour in drinking water by WHO or by Sri Lanka Standard Institute (SLSI), it is clearly mentioned that taste and odour should be at 'unobjectionable' level which again means taste and odour in drinking water should be kept below the human threshold levels or 5 ng/L (BOI, 1999).

Reduction of T&O compounds from treated water is a priority for water utilities, to produce high organoleptic quality treated water, which enhances the confidence and reliance of the consumers towards the drinking water supply system (Bertone & Halloran, 2016). Most of the times, conventional water treatment processes do not remove 2-MIB below the human sensory threshold level (5 ng/L) (WHO, 2017a; Sorial & Srinivasan, 2011; Jung et al., 2004). Therefore, alternative methods to remove 2-MIB from treated water are practiced all over the world. 2-MIB is a secondary metabolite of a range of cyanobacteria and algae in raw water and present of both in solution and in suspended forms mostly associated with the host cyanobacteria (Juttner & Watson, 2007). Moreover, according to Ashitani et al., (1988), when raw water enters specific processing steps of the WTPs, such as pre chlorination, coagulation, etc. cyanobacteria cells in the raw source water disintegrate and cell lysis occurs, followed releasing out 2-MIB into the treated final water. That might cause the increment of 2-MIB levels in treated water than the raw water. This was observed in almost all the treated water samples in the [resent study, where the respective WTP followed only conventional water treatment. During the sampling, it was observed that different water treatment methods have been practiced; Dissolved Air Floatation (DAF), Membrane Filtration (MF), Activated Carbon Beds (ACB) etc. Most of the plants had conventional water

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treatment methods (aeration, pre sedimentation, filtration, sedimentation, flocculation, coagulation, disinfection) where WTPs used modern technologies of Dissolved Air Flotation (DAF), (Kondawatuwana WTP, Unnichchai (Wawnativ) WTP), Membrane Filtration (MF) (Sagama WTP) and Activated Carbon Bed (ACB) or Powder Activated Carbon (PAC) (Unnichchai (Wawnativ) WTP, Kondawatuwana WTP). As mentioned above, most of the times, when only conventional treatments were applied prioritizing sand filtration, an increase in 2-MIB content after treatment was recorded. In the present study it was found that, treated water 2-MIB levels were higher than that of the raw water at the WTPs where conventional water treatment processes (Table 04) are being practiced. As an example, most of the WTPs; Tissa reservoir, Nuwara reservoir, Thuruwila reservoir, Kawdulla reservoir and Madirigiriya, treated water, 2-MIB concentrations were greater than respective raw water, in both dry and wet seasons. According to NWSDB unpublished data, back in 1987, a 5 cm thick black layer was found just underneath the sand filtration layer at the Tissa reservoir WTP. This layer had loads of actinomycetes which could aggravate the 2-MIB content during filtration (Ganegoda et al., 2019; Klausen et al., 2004). Thus, presence of actinomycetes associated with raw source water as well as attached to the treatment steps also might cause the elevation of 2-MIB levels in treated water (Park et al., 2014; Lee et al., 2011; Jüttner & Watson, 2007; Klausen et al., 2005; Klausen et al., 2004). PAC and GAC are one of the key compounds used over the world to solve taste and odour issue in water (Kim et al., 2014, Drikas et al., 2009). It was noted that 2-MIB levels were not detected in treated water which had PAC or GAC beds in the treatment plants (Kondawatuwana and Unnnichchi- Wavnativ). Moreover, it was observed during field visits that GAC or PAC safe doses added into the water as a practice when taste and odour episodes appear in water, time to time even at the treatment plants where they don’t have GAC beds (Ganegoda et al., 2019).

Cyanobacteria and Actinomycetes are the known major producers of 2-MIB (Juttner and Watson, 2007; Lanciotti et al., 2003; Spiteller et al., 2002; Kenefick et al., 1992). Many reservoirs and running waters investigated to date have contained 2-MIB (Ju¨ttner 1999; Hosaka et al., 1995; Ju¨ttner, 1992; Burlingame et al., 1986) with the availability of cyanobacteria and algae. Not all the cyanobacteria and algae are recorded produce 2-MIB, some specific genera are very significant in T&O production. For example, contamination of 2-MIB has a strong correlation along with cell density of *Anabaena* sp. have been reported in several studies (Watson et al., 2007; Sklenar and

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Horne, 1999; Jones and Korth, 1995; Sugiura et al., 1994; Utkilen and Frøshaug, 1992). *Oscillatoria* sp. (Zimba et al., 1999; Izaguirre and Taylor, 1998), *Phormidium* sp. (Smith et al., 2008; Watson, 2003) *Synedra* sp. (Chong et al., 2018), *Planktothrix* sp. (Juttner & Watson, 2007), *Cylindrospermopsis* sp. (Watson et al., 2016*)* and *Pseudanabaena* sp. (Chong et al., 2018) were reported in number of studies in different part of the world. The present study agrees with the above, revealing several cyanobacteria and algae in Sri Lankan reservoirs in both dry and wet seasons. It has been reported that cyanobacteria can maintain a higher growth rate at low water level in dry season (Kulasooriya, 2011) and the present study has recorded comparatively high cyanobacterial cell densities in dry season compared to wet season. This is in agreement with Silva & Reddy, (2005) stating that the density of planktonic algae and cyanobacteria becomes diluted or the water becomes mesotrophic in irrigation tanks with the onset of northeast rainfall resulting in comparatively low measurements for water quality parameters and low planktonic density. *Anabaena* sp., *Oscillatoria* sp., *Cylindrospermopsis* sp., *Microcystis* sp., *Pediastrum* sp. and *Scenedesmus* sp. were frequently identified as T&O producers throughout the study (Sethunga and Manage, 2010). Moreover, Lee et al., (2017) reported production of 2-MIB associated with *Scenedesmus* sp which was recorded in water reservoirs sampled in the present study. In almost all the reservoirs Cyanophyta was found to be the dominant phylum of phytoplankton where Chlorophyta (*Ankistrodesmus* sp., *Staurastrum* sp., *Scenedesmus* sp., *Pediastrum* sp., *Cosmarium* sp.) and Bacillariophyta (*Synedra* sp., *Navicula* sp.) were present in considerable densities too. *Microcystis* sp. and *Cylindrospermopsis* sp. were the dominant and co-dominant in most of the reservoirs during the study. However, at the reservoirs where higher 2-MIB level was detected, *Anabaena* sp. showed a significant abundance ($p<0.05$). As an example, during dry season, Thuruwila reservoir recorded a significantly ($p<0.05$) high concentration of 2-MIB, when *Anabaena* sp. was co dominant species with the cell density of 21500 ± 7 cells/mL. In Tissa reservoir during dry season, *Anabaena* sp., was the dominant cyanobacteria and greater levels of 2-MIB (36.4 ± 1.8 ng L^{-1}) was recorded. However, high levels of 2-MIB were recorded in the reservoirs where *Microcystis* sp. was dominant. As an example, Kawdulla reservoir recorded the highest 2-MIB level (96.3 ± 4.2 ng L^{-1}) in dry season when *Microcystis* sp. and *Anabaena* sp. cell densities were reached to 58227 ± 124 cells/mL and 25310 ± 72 cells/mL respectively. According to Chong et al., (2018), and several other researchers (Smith et al., 2008; Watson, 2003)

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Microcystis sp. which is one of the most frequently discovered cyanobacteria blooms, produces no 2-MIB. Therefore, high abundance of 2-MIB might be due to production of 2-MIB via actinomycetes, *Anabaena* sp. and some other cyanobacteria species. However, production of 2-MIB by another specific strain of *Microcystis* sp. could be another reason. Previous researchers confirm the above fact that different species in the same genera can show different production abilities of 2-MIB (Juttner & Watson, 2007; Hinda´k, 2000).

Water consumers sense even the presence of tiny amount of 2-MIB, subsequently giving rise to all the above-mentioned related issues. Therefore, physico chemical parameters of the source water bodies were studied to identify any association between these odorants and the environmental factors. Water quality variables are closely related to 2-MIB production (Davies et al., 2004; Sugiura et al., 1994) and could be used to predict the likelihood of unacceptable taste and odor events. Several previous studies have recorded that the high pH levels in water making reservoir water alkaline, promotes the higher 2-MIB production. Schrader & Blevins (2001) recorded that the specified pH value more than 7.5 is significantly and positively correlated with high 2-MIB levels. Moreover, Hsieh et al., (2011) reported that 2-MIB occupying a pH-dependent behavior by conducting a series of experiments with different pH values and concluded that 2-MIB production in acidic pH was less than that of alkaline pH. They attributed this observation to dehydration of the tertiary alcohols; 2-MIB under acidic conditions. Further, those studies have been suggested that the dehydration of 2-MIB is reversible, and the analysis can be mitigated by adjusting the water pH back to a neutral condition (Hsieh et al., 2011). In the present study, reservoir water pH values varied from 5.8 to 8.9 and majority of water bodies showed pH greater than 7.5 (Table 11) with considerable level of 2-MIB. Water bodies prevalent with known T&O issues in Sri Lanka were mostly selected in the study, hence this observation agrees with Schrader & Blevins (2001) and Hsieh et al., (2011) showing that alkaline pH promotes 2-MIB production. Moreover, statistical analysis of the present study showed that the 2-MIB having a significant positive correlation with reservoir water pH (2-MIB – p 0.004). As an example, Kala reservoir in the dry zone has been recorded an alkaline water, which is being utilized for drinking, fishing and irrigation purposes (Hettiarachchi, 2017; Piyathilaka, 2015; Sethunge & Manage, 2010; Silva, 1996; Amarasinghe et al., 1983; Gunawardhena and Adikari, 1981) and has been recorded several T&O incidences (Unpublished data, NWSDB). Thus, the results of the present study confirmed

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that the occurrence of the 2-MIB in Kala reservoir exceeding the human threshold level in both dry (2-MIB - 49.5 ± 1.1 ng L^{-1}) and wet seasons (2-MIB- 6.5 ± 2.5 ng L^{-1}) (Table 4).

Linkage of 2-MIB in association with reservoir nutrients has been discussed by several authors. According to Harris et al., (2016), in Midwestern reservoirs, the production of secondary metabolites; 2-MIB is likely to occur when the growth of potential producers was favored by low TN:TP < 30:1 (by mass) and low NO_3^- : NH_3 ratios (Harris et al., 2016). Clercin and Druschel, (2019) agreed with their research findings of low TN:TP ratios promotes 2-MIB. Results of the present study are in concordance with above findings. 2-MIB (p=0.000) showed significant positive correlations with TP concentrations in the reservoir waters. As an example, Kala reservoir recorded the highest TP level in dry season (Table 11) and reported considerable 2-MIB levels as explained previously.

In recent years PCA has been used in many environmental studies to interpret the relationships among physical, chemical and biological parameters (Ganegoda et al., 2018; Hettiarachchi and Manage, 2017). PCA of log-transformed data were used to group the water bodies on the basis of the contribution of the relative dominance of each of the associations (cyanobacterial cell densities, Geosmin and 2-MIB levels and water quality parameters) in each water body. The high correlation between the parameter and the axis of the PCA, the higher is the contribution of the parameter to axis formation was found supporting the results recorded be previous studies (Giannuzzi et al. 2012; Gardner et al. 2000). Therefore, parameters with correlation values of >0.5 (absolute value) were taken as the major contributors to axis formation (Vardaka et al. 2005). The overall results of the present study demonstrated that the PCA analysis grouped the water bodies into four major clusters for each region (Figure 4) based on the correlation of cyanobacterial cell densities, 2-MIB levels and water quality parameters. It was found that the cluster 4 was clearly separated from all other with several indicative parameters of higher pollution with high 2-MIB contamination with high pH, TP, cell density and hardness levels (Figure 4). Loading plot clearly demonstrated this separation showing that comparatively high DO into the cluster 3 direction. The results from PCA analysis reconfirmed that the results from Pearson's correlation analysis where 2-MIB recorded a significant ($p < 0.05$) positive correlation with TP, Cyanobacteria cell density and water pH levels.

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4. CONCLUSION

The current study concludes that presence of 2-MIB in drinking water is one of the responsible factors for T&O issues in some parts of Sri Lanka. When compared the two seasons, 2- MIB detection in reservoirs during dry season was significantly greater (<0.05) than that of the wet season. Total of 69% of the sampling locations covering 6 districts exceeded the human threshold level of 2-MIB (75% of raw water and 63% of treated water). Interestingly, 2-MIB level in treated water from water treatment plants were greater than the respective 2-MIB level in raw water, might be due to cell lysis of cyanobacterial, other bacteria cells and actinomycetes in some water processing steps in the water treatment plants. 2-MIB had significant correlations with total cyanobacteria cell density ($p<0.05$), and with other water quality parameters like total phosphorous ($p<0.05$) and pH ($p<0.05$). PCA analysis pooled four different clusters of water bodies. Ridiyagama reservoir, Nallachchiya reservoir and Kala reservoir were clustered together to create the first cluster whereas Parakrama Samudraya reservoir from North Central province, Unnichchai reservoir, Kanthale reservoir and Sagama reservoir from Eastern province were clustered together forming the second cluster. The third cluster consisted of reservoirs of Anuradhapura district as Nachchadoowa reservoir, Tissa reservoir, Thuruwila reservoir and Nuwara reservoir. Minneriya reservoir and Kawdulla reservoir from Pollonnaruwa district were belonged into the final cluster.

5. ACKNOWLEDGEMENT

National Science Foundation (NSF) is acknowledged for the financial support provided. (Grant number RG/2016/EB04). Center for Water Quality and Algae Research, Department of Zoology, Central Instrumentation Facility, Faculty of Applied Sciences, University of Sri Jayewardenepura, National Water Supply and Drainage Board (NWSDB) and Agilent Technology (PVT) Ltd. are acknowledged for the instrument facilities, technical advises and the other logistic support provided.

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