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RESEARCH ARTICLE

Geosmin contamination status of raw and treated waters in Sri Lanka

Sathya Ganegoda[1], S.D.M. Chinthaka[2] and Pathmalal M. Manage[1*]
[1] *Centre for Water Quality and Algae Research, Department of Zoology, University of Sri Jayewardenepura, Nugegoda.*
[2] *Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda.*

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Abstract: Off taste and odour in drinking water cause a serious issue in some parts of Sri Lanka and higher number of customer complaints is related to off taste and odour in treated water. Water sources are having diverse range of algae and cyanobacteria community and most of them produce chemical compounds like geosmin. A simple and sensitive modified method for the determination of earthy odorant geosmin in water was developed by headspace solid-phase micro extraction coupled with gas chromatography-mass spectrometry. Quantification of the geosmin contamination level in 22 drinking water sources (raw water bodies and water treatment plants) was carried out for the first time in Sri Lanka. Results revealed that 68% of the sampling locations exceed the human threshold level of geosmin (5 ng L^{-1}). The concentration of geosmin in raw water bodies ranged from 7.825 to 10.929 ng L^{-1} whereas in treated water it ranged from 8.113 to 11.196 ng L^{-1}. Geosmin concentrations in treated water were higher than in the respective raw water. Some taste and odour producing cyanobacteria and algae were identified and quantified in the same water bodies where geosmin was detected. *Anabaena* sp., *Cylindrospermopsis* sp., *Microcystis* sp., *Oscillatoria* sp. and *Volvox* sp. are the common cyanobacteria and algae in water bodies. Total taste and odour forming cyanobacteria and algae count have shown a significantly positive correlation with geosmin concentration ($p < 0.05$). A significant positive correlation was found between geosmin and total phosphorus ($p < 0.05$), electrical conductivity ($p < 0.05$) and pH ($p < 0.05$), factors that normally favour cyanobacterial growth.

Keywords: Cyanobacteria, gas chromatography-mass spectrometry, geosmin, headspace solid-phase micro extraction, taste and odour.

INTRODUCTION

The provision of adequate volumes of safe, clean drinking water to the world's growing population is a continual and increasing challenge for water authorities around the world. Although health aspects of water are the primary focus, consumers generally judge the quality of water by its aesthetic value. A common and recurrent problem in drinking water is the occurrence of taste and odour (T&O) producing compounds (Jiang *et al*., 2007). T&O compounds are the cause of most consumer complaints and rejections specially related to potable water where flavour and smell of the water is the only measure of water quality for the end-user (AwwaRF, 2000). Geosmin (*trans*-1, 10-dimethyl-*trans* -9-decalol) is one of the most common chemicals which produces T&O in water, which produces an earthy odour (Jiang *et al*., 2007). Secondary metabolites of two groups of aquatic microorganisms; cyanobacteria and actinomycetes have been considered to be the main cause of earthy taints in drinking water (Zuo *et al*., 2009; Ding *et al*., 2014a). Geosmin is a tertiary alcohol first isolated in 1965 (Mcdowall, 2008). Often lakes and reservoirs are contaminated with geosmin and other odourants (Ding *et al*., 2014b) where people can smell the odour in water samples. Human threshold level for geosmin is found to be in the range of 5 to 40 ng L^{-1} and although there are very low levels of geosmin present in water, consumers still can taste an unpleasant earthy flavour in water.

* Corresponding author (pathmalal@sjp.ac.lk; https://orcid.org/0000-0002-2014-2060)

Geosmin is a semi volatile compound and is produced by algae, cyanobacteria and actinomycetes and has a stable chemical structure (Sorial & Srinivasan, 2011). Therefore, conventional water treatment processes such as aeration, coagulation, flocculation, boiling, chlorination and filtration have failed to reduce the levels below human threshold (Sorial & Srinivasan, 2011). Hence, this has become a challenge to conventional water treatment processes. Odour may develop downstream of water treatment as a result of heterotrophic biological activity in distribution pipes and sand bed filtration post treatments. Production of geosmin has been documented in several studies (Ju¨ttner & Watson, 2007). For example, heterotrophic eukaryotes such as fungi, which colonise biofilms in activated filters and distribution pipes can generate potent musty-smelling metabolites such as trichloroanisol (Ju¨ttner & Watson, 2007). According to Ju¨ttner and Watson (2007), geosmin in treated drinking water was traced to the disturbance of thick biofilms that had developed on the pipe surfaces of a distribution system from a groundwater-supplied treatment plant. After a change in water treatment process in the plant to remove iron from the iron-rich source water, the biofilms degraded and sloughed off, releasing high levels of geosmin, leading to consumer complaints. In another case, biological activity in poorly maintained filtration media was considered to be the most likely cause of high geosmin levels downstream of filter beds in a small rural treatment plant (Ju¨ttner & Watson, 2007). Geosmin contamination in either raw or treated water certainly raises the issue of water rejection and complaints by consumers. A survey conducted in more than 800 utilities in the United States and Canada had found that 16 % of the utilities experienced serious earthy odour problems, and that utilities spend an average of about 4.5 % of their total treatment budget on earthy smell control (Tian, 2013). Therefore, water utilities around the world struggle to deal with the irregular pattern of appearance of these compounds in their source waters, which increases the treatment cost. The ability to reliably predict, confirm and counteract their occurrence would be important to water utilities and other branches of industry, such as aquaculture farms where these compounds can spoil entire harvests (Zuo *et al*., 2009, Jade & Emilia 2013). According to WHO, the provision of drinking-water that is not only safe but also acceptable in appearance, taste and odour (T&O) is of high priority (WHO, 2011). Tap water with detectable T&O may be perceived by the consumer as unsafe to drink although it adapts to the guideline for regulated constituents (Tian, 2013). More importantly this could lead to the use of water from sources that are less safe (WHO, 2011). Although neither the United States Environmental Protection Agency (USEPA) nor the World Health Organization (WHO) has declared geosmin as a health hazard, geosmin can lead to acute health effects such as heat exhaustion and sunstroke, or chronic health effects such as kidney problems (Tian, 2013). Moreover, it is reported that toxins and geosmin frequently co-occurred indicating odour may serve as a warning that cyanotoxins are likely to be present (Chen *et al*., 2010). Many studies have been conducted in this field worldwide, but so far no studies have been published related to geosmin in Sri Lanka.

Climatic conditions around the world are changing such that more extreme events of flooding and drought, and a general increase in ambient temperatures are favouring both the occurrence and intensity of blue-green algae (cyanobacteria) blooms (Sethunga & Manage, 2010; Cayelan *et al*., 2012). The increasing frequency and intensity of harmful cyanobacterial proliferation in water sources is a growing global issue (Preecea *et al*., 2017; Yatigammana & Perera, 2017). Cyanobacteria blooms have been recognised as a nuisance in drinking water and aquaculture industries because some species produce potent odour compounds. When large numbers of cyanobacteria and bacteria flourish in a water body, T&O compound concentrations increase to levels above the human threshold and cause T&O problems (Sethunga & Manage, 2010; Idroos, 2015; Idroos *et al*., 2015). Earthy T&O producing geosmin is one of the most frequently identified T&O compounds associated with cyanobacterial blooms (Sethunga & Manage 2010; Zamyadi *et al*., 2010). Several cyanobacterial species are potent producers of T&O compounds and toxins and Watson *et al*. (2008) have documented the presence of geosmin producing cyanobacteria in surface water bodies. *Anabaena circinalis, A. scheremetievi, Phormidium tenue, Pseudanabaena (planktonic), Oscillatoria f. granulata, O. simplicissima, O. curviceps, O. tenuis* are found to be toxic and produce geosmin in source water (Izaguirre & Taylor, 2007; Ju¨ttner & Watson, 2007; Ho *et al*., 2009; Sethunga & Manage, 2010; Zamyadi *et al*., 2010; WHO, 2011).

In most parts of Sri Lanka, consumers receive treated and untreated water mainly from lakes, reservoirs and ponds, which are contaminated with algae and cyanobacteria. According to available literature on algae and cyanobacteria, in Sri Lanka almost all water sources used for drinking purposes have a high diversity of algae and cyanobacteria (Sethunga & Manage 2010; Hettiarachchi *et al*., 2013; Hettiarachchi & Manage 2014; Idroos *et al*., 2015) which are responsible for the production of chemicals such as geosmin (JICA, 2012). Further, seasonal cyanobacteria and algal blooms have been recorded in raw water bodies where water is used

for drinking by the National Water Supply and Drainage Board (NWSDB) of Sri Lanka. Consumer complaints regarding earthy taste and odour show an increasing trend and this issue is serious among water consumers in North Central, Eastern, North East, North, Uva and southern part of the country (NWSDB, 2011).

Therefore, determination of contamination status by such compounds must be given priority to improve water treatment facilities and technology to safeguard drinking water. The present study was carried out to detect and quantify geosmin contamination levels in both raw water and treated water, covering 5 districts in Sri Lanka (Anuradhapura, Polonnaruwa, Ampara, Batticaloa and Trincomalee districts). These sampling locations were selected on the basis of available information from the NWSDB (*unpublished data*) where T&O problems were prevalent. Further, all raw water bodies selected are used by the NWSDB to provide drinking water after treatment.

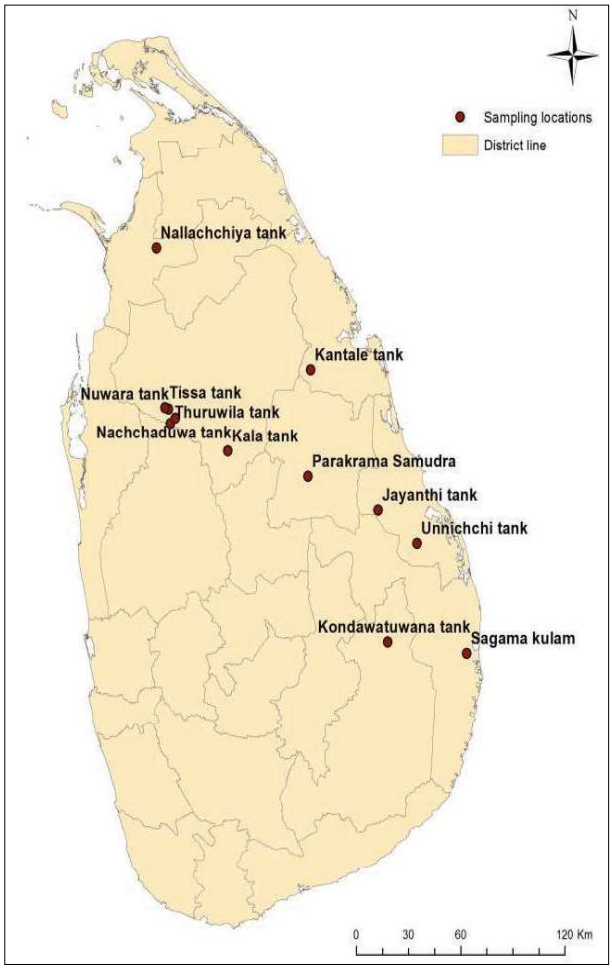

Figure 1: Sampling locations of the study (WTP – water treatment plant)

METHODOLOGY

Materials

Geosmin was purchased from Sigma Aldrich, USA and dissolved in deionised water to make a stock solution at a concentration of 200 μgL^{-1}. The solution was stored at 4 ºC and used after dilution with deionised water. Molecular grade sodium chloride was obtained from Sigma Aldrich, USA.

Manual fiber assembly of solid-phase micro extraction (SPME) with an extraction fiber coated with stable flex divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) microfiber with film thickness 50 µm/30 µm was purchased from Supelco (Tokyo, Japan).

Sample collection and preparation to detect geosmin

A volume of 10 mL of surface water from raw water bodies and 10 mL of treated water from water treatment plants (WTPs) (Jayanthi wewa, Sagama tank, Kondawatuwana tank, Unnichchai tank, Kantale tank, Nachchadoowa tank, Kala tank, Nallachchiya tank, Thuruwila tank, Tissa tank, Nuwara tank and Parakrama Samudraya)

Collection source	District	Province
Jayanthi tank		
Jayanthi WTP		
Sagama tank		
Sagama WTP	Ampara	Eastern
Kondawatuwana tank		
Kondawatuwana WTP		
Unnichchai tank	Trincomalee	
Unnichchai (Wavnativ) WTP		
Kantale tank	Batticaloa	
Kantale WTP		
Nachchadoowa tank		
Kala tank		
Kala WTP		
Nallachchiya WTP		
Thuruwila tank		
Thuruwila WTP	Anuradhapura	North Central
Tissa tank		
Tissa WTP		
Nuwara tank		
Nuwara WTP		
Parakrama Samudraya	Polonnaruwa	

were collected directly into 20 mL headspace SPME vials. The vials were sealed with a twist cap (Saito *et al*., 2008). Samples were placed in an ice box (4 ºC), transported to the laboratory and stored in the dark at 4 ºC until analysis. Sampling was performed from June 2016 to June 2017 from the raw water bodies where T&O problems were prevailing in Sri Lanka. Twenty two raw and treated water samples were obtained covering the North Central province (Anuradhapura and Polonnaruwa districts) and Eastern province (Ampara, Trincomalee, Batticaloa districts) (Figure 1). Analysis was performed within 7 days. The water samples were saturated with molecular grade solid sodium chloride and subjected to HS-SPME/GC-MS analysis.

Gas chromatography-mass spectrometry (GC/MS)

GC-MS analysis was carried out with Agilent Model 7890A gas chromatograph-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 maintained by an electronic pressure controller. Injection port was operated in pulsed splitless mode and was fitted with 0.7 mm id SPME injection liner. Head pressure was set to 9.35 psi of helium for 1.30 min, then changed to a constant flow of 1.1 mL/min to give a velocity of 38.41 cm/s. Oven was initially held at 60 ºC for 1 min, then increased by 10 ºC/min to 300 ºC and held for 4 min. The electron impact (EI-MS) conditions were as follows: MS source temperature, 230 ºC, MS quadropole temperature, 150 ºC; ionising voltage, 70 eV. The full scan mass spectra were obtained at an m/z range of 33–550 D. Selected ion monitoring (SIM) mode detections for geosmin were selected at m/z = 112 (GSM) and m/z = 125. These were monitored alternatively at dwell times of 1001.ts each. Optimised conditions of GC-MS are: geosmin retention time 11.15 min, precursor ion 112, product ion 97, dwell 20 s and collision energy 10. The correlation area was measured to construct a calibration curve and to determine the concentration of geosmin in samples.

Headspace solid-phase micro extraction (HS-SPME)

A 10 mL volume of sample was transferred to a 20 mL headspace vial along with 3.0 g of molecular grade sodium chloride. The vial was sealed with a twist cap prior to placement on the vortex machine (1000 rpm) for 1 min for agitation. The SPME needle pierced the septum of sample vial and the fiber was exposed in the headspace above the sample for 15 min at 40 ºC. After extraction, the fiber was retracted into the needle; the needle was removed from the septum and then inserted directly into the GC-injection port of the GC-MS instrument. Immediately after exposition of fiber, GC-MS temperature programming was started and the fiber was held in the GC-injection port for 5 min. Then, the fiber was retracted into the needle. The needle was removed from the GC-injection port and used for the HS-SPME of the next sample after 5 min.

Optimisation of headspace solid-phase micro extraction (SPME)

In order to optimise the extraction of geosmin by HS-SPME, several extraction conditions including the sample volume, extraction time, temperature and agitation time were studied. The best sample size was found to be 10 mL, and optimum extraction time was 15 min while the best extraction temperature was 40 ºC. Agitation rate became constant by stirring at more than 1000 rpm, but faster agitation tends to be uncontrollable and might cause a poor measurement precision. Therefore, the stirring rate was maintained at 1000 rpm (Saito *et al*., 2008). Use of 3.0 g of sodium chloride (NaCl) was found to be the best salting-out agent. Various SPME fiber types have been tested for geosmin extraction efficiency from water. Medium polar SPME fiber (divinylbenzene/ carboxen/ polydimethylsiloxane) (DVB/ CAR/ PDMS) microfiber with film thickness 50/30 µm proved to be the most efficient for geosmin extraction as geosmin is a semi volatile odorous compound. Extraction yield also increased by 1.2 times by a salting-out effect (Saito *et al*., 2008). Geosmin extracted on the fiber was completely desorbed within 5 min by heating in the GC-injection port at 270 ºC and carry over was not observed because the fiber was washed during exposition. The geosmin peak was not detected by re-exposition of the fiber after heating (Saito *et al*., 2008). The absolute amount of geosmin extracted by HS-SPME method was calculated by comparing the correlation area of the peak.

Application of optimised method for the detection of geosmin in raw and treated water samples

The optimised method was applied to several raw and treated water samples collected from source water and water treatment plants. Geosmin peak in the water samples was identified by mass spectrum analysis.

Identification and enumeration of phytoplankton

Phytoplankton samples were collected by filtering 100 L of water through 55 µm plankton net for identification purposes. For enumeration of algae and cyanobacteria,

100 mL of water was fixed with acidified Lugols' solution to a final concentration of 1 % followed by natural sedimentation. Identification of cyanobacteria and other algae was carried out under light microscopy using standard keys (Prescott, 1978, Manage, 2013). Enumeration was done using a Sedgewick rafter counting chamber under the light microscope (× 40) in order to get species composition and abundance of cyanobacteria and algae in water samples.

Measurement of water quality parameters

During sampling, pH, temperature, dissolved oxygen (DO) and electric conductivity of water were measured at the site using standard digital meters [pH meter (330 I/ Set, WTW Co., Weilheim, Germany), thermometer, oxygen meter (Oxi 320/ Set, WTW Co., Weilheim, Germany) and conductivity meter (340A-Set 1. WTWCo., Weilheim, Germany, respectively]. Nitrate-N, nitrite-N, ammonia-N, total phosphorous (TP) and hardness were measured in the laboratory using standard titrimetric and spectrometric methods (APHA, 1999).

Evaluation of the preference of treated water for drinking

The NWSDB-treated water samples having T&O problems were collected from the Tissa tank, Nuwara tank and Thuruwila tank WTPs and given to a selected group of people aged 20–60 who do not consume geosmin contaminated water in daily life. A questionnaire was prepared to evaluate the preference of water for drinking purposes. A total of 100 students, age ranging 20–24 years, both male and female and 100 adults, age ranging 25–70 years, both male and female were randomly recruited to the survey.

Statistical analysis

Principal component analysis (PCA) test and Pearson's correlation test were carried out using MINITAB 17 software.

RESULTS AND DISCUSSION

Detection of geosmin and quantification

Geosmin 200 μgL^{-1} extracted by HS-SPME method was used to detect and identify geosmin *via* scan mode in GC/MS as per Figure 2, which demonstrates the total ion chromatogram (TIC). Geosmin peak appeared at a retention time of 11.15 min and mass spectra of geosmin peak was matched with NIST library spectra. Since human sensory threshold is very low as ngL^{-1} level SIM mode was occupied for further detection. The selected

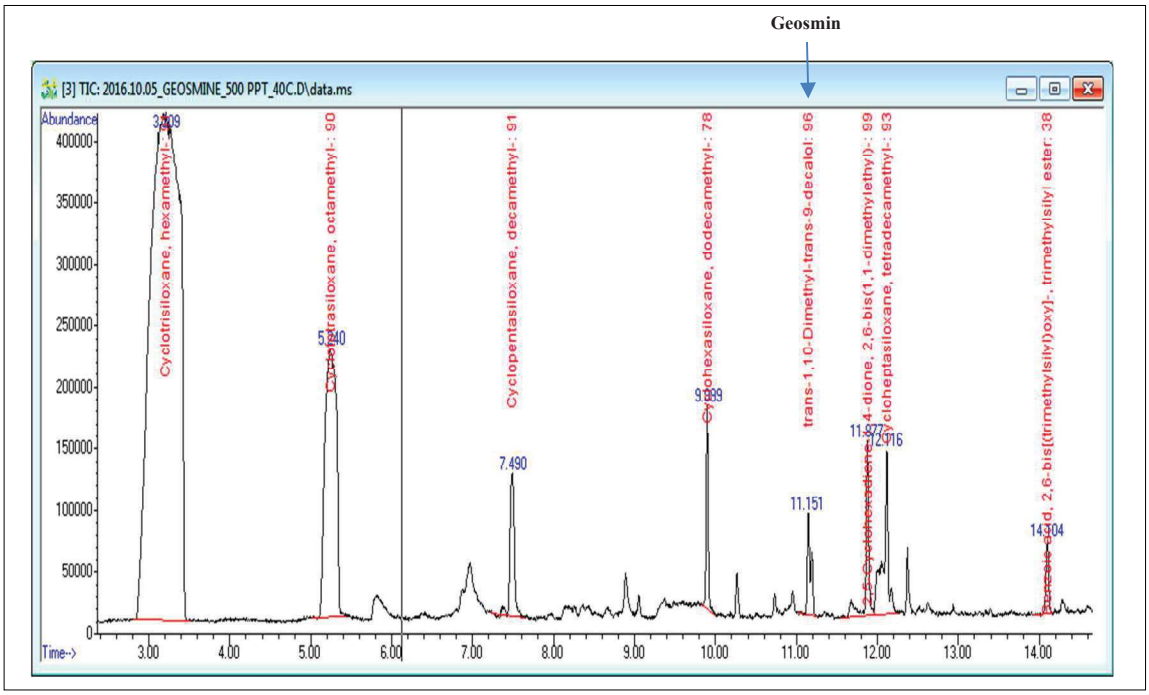

Figure 2: Total ion chromatogram of a water sample
Geosmin (*trans*-1, 10-dimethyl-*trans*-9-decalol) retention time is at 11.15min

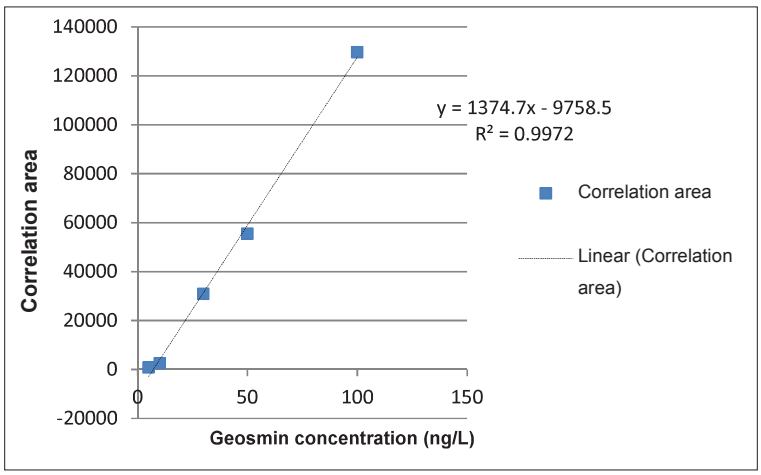

Figure 3: Calibration curve for geosmin quantification

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

Water source	Raw water geosmin (ng/L)	Treated water geosmin (ng/L)	Type of treatment	District	Province
Jayanthi tank	ND	ND	Sand filter system and alum are used at coagulation step	Ampara	Eastern
Sagama tank	ND	8.1	Membrane filter system and alum and poly aluminium chloride are used at coagulation step		
Kondawatuwana tank	ND	ND	Dissolved air floatation system with sand filter system used. Alum, poly aluminium chloride and powdered activated carbon are used at coagulation step		
Unnichchai tank	ND	ND	Dissolved air floatation system with sand filter system used. Alum, poly aluminium chloride and powdered activated carbon are used at coagulation step	Batticaloa	
Kantale tank	ND	ND	Sand filter system and alum are used at coagulation step	Trincomalee	
Nachchadoowa tank	8.7	NC	-	Anuradhapura	North Central
Kala tank	8.2	10.3	Sand filter system and alum are used at coagulation step		
Nallachchiya tank	7.8	NC	-		
Thuruwila tank	8.1	8.5	Sand filter system and poly aluminium chloride are used at coagulation step		
Tissa tank	8.8	11.1	Sand filter system and alum are used at coagulation step		
Nuwara tank	10.9	11.2	Sand filter system and alum are used at coagulation step		
Parakrama Samudra	8.1	NC	-	Polonnaruwa	

ND - Not detected; NC - water was not collected from the treatment plant

ions for quantification of geosmin are of m/z 112 and 125. Geosmin eluted as a single and symmetrical peak at 11.15 min. It gave excellent response to GC-MS-SIM detection and the minimum detectable level (MDL) of geosmin by HS-SPME/GC-MS under optimised conditions was 1.5 ng L^{-1}. Minimum level of quantification (MQL) was 3.5 ng L^{-1}. Both values are below the minimum threshold levels where human olfactory system detects geosmin at 5 ng L^{-1}. As shown in Figure 3, an excellent linear correlation of peak area and level of geosmin was obtained (R^2 = 0.998) for the concentration range from 5 to 100 ng L^{-1}. Solid-phase micro extraction integrates sampling, extraction, concentration and sample introduction into a single solvent-free step and analytes in the sample are directly extracted and concentrated to the extraction fiber. The optimised method is cost effective and saves sample preparation time.

Application of optimised method for the detection of geosmin in raw and treated water samples

Table 1 shows geosmin levels in raw and treated water samples collected at various water resources. The sampling was done in October (dry season) in the North Central province and in February (wet season) in the Eastern province. The levels of geosmin in raw water bodies ranged from 7.8 to 10.9 ng L^{-1} whereas in treated water it ranged from 8.1 to 11.2 ng L^{-1}.

Among the selected raw water bodies, the highest level of geosmin was recorded in Nuwara tank (10.9 ng L^{-1}) while the lowest was detected in Nallachchiya tank (7.8 ng L^{-1}). Detectable levels of geosmin was not present in Sagama tank, Kantale tank, Kondawatuwana tank, Jayanthi tank and Unnichchai tank during sampling. This may be due to the samples being collected during the rainy season in these raw water bodies causing dilution of the available geosmin level. Further, according to literature, geosmin is released more at warm stratified season of the dry period compared to the rainy period (Bertone & O'Halloran, 2016). Therefore, geosmin levels not being detected during the wet season in the above water bodies can be explained. Further, treated water from Nuwara tank WTP recorded the highest geosmin concentration (11.2 ngL^{-1}) while treated water from Sagama tank WTP recorded the lowest (8.1 ngL^{-1}). Treatment plants of Kondawatuwana tank, Jayanthi tank, Unnichchai tank and Kantale tank did not record geosmin at detectable levels during the wet season. Not detecting geosmin in treated water may be due to the lack of presence of geosmin in respective raw water bodies used for treatment processes. Water treatment plants at Kondawatuwana and Unnichchai tanks have activated carbon beds, which are capable of removing geosmin (Drikas *et al*., 2009; Kim *et al*., 2014). Kanthale WTP uses both reservoir water and Mahaweli river water as a mixture for treatment processes. It has been recorded that cyanobacteria cell growth in rivers is significantly lower than in reservoirs (Okogwu & Ugwumba, 2009). This could be another reason for not detecting geosmin in treated water from Kanthale tank. Although there is no recommended standard level for T&O in drinking water by WHO or by the Sri Lanka Standards Institute (SLSI), it is clearly mentioned that T&O should be at 'unobjectionable' levels, which again means below the human threshold levels or 5 ngL^{-1} (BOI, 1999). The present results revealed that in 68 % of the sampling locations covering 5 districts (Anuradhapura, Polonnaruwa, Ampara, Batticaloa and Trincomalee), the human threshold level for geosmin was exceeded. Moreover, geosmin levels in treated water from WTPs were higher than the geosmin levels in the respective raw water bodies and some water purification steps may have caused that elevated level of geosmin, which again lead to consumer rejection of treated water. Moreover, at Sagama tank WTP, treated water recorded a geosmin level of 8.1 ngL^{-1} when reservoir water did not detect geosmin. At Sagama tank WTP, there are no activated carbon beds although they use powdered activated carbon occasionally when T&O episodes occur. The treatment facility in Sagama tank is a membrane filter system with alum and poly aluminium chloride at coagulation step. These facilities do not seem to be very effective in removing geosmin when compared to activated carbon (Drikas *et al*., 2009; Kim *et al*., 2014). Geosmin is a secondary metabolite of a range of cyanobacteria and algae in raw water, and it is present both in solution and suspended form mostly associated with the host cyanobacteria (Ashitani *et al*., 1988). According to Ashitani *et al*. (1988), geosmin in suspended form were removed well by coagulation and sedimentation alone. Geosmin present in solution can be removed almost to an undetectable level in the rapid sand filter where no pre-chlorination is practiced. However, when raw water enters specific processing steps of the WTPs such as pre-chlorination, cyanobacteria cells disintegrate and cell lysis occurs (Ashitani *et al*., 1988). At that point, geosmin leaks out to the water and leads to an elevated level of geosmin in treated water than the geosmin level in the respective raw water. During sampling, a noted feature was the different water treatment methods used in various water treatment steps such as dissolved air floatation systems, membrane filtration systems and activated carbon beds. Most of the plants had conventional water treatment methods (aeration, pre-sedimentation, filtration, sedimentation,

Table 2: Species composition of algae and cyanobacteria *vs* geosmin concentration in water bodies

Raw water body	Odour and taste forming algae/cyanobacteria/ diatoms/flagellates	Cell density cells/ml	Other algae/ cyanobacteria/diatoms/ flagellates	Cell density cells/mL	Total cell density cells/ mL	Geosmin concentration (ng/L)
Jayanthi wewa	*Anabaena* sp.	280 ± 1.21	*Melosira* sp.	1280 ± 3.48		
(February 2017)	*Microcystis* sp.	5320 ± 7.34	*Chroococcus* sp.	80 ± 0.68		
	Oscillatoria sp.	123 ± 0.62	*Stephanodisc* sp.	320 ± 1.42		
	Cylindrospermopsis sp.	758 ± 2.15	*Gomphosphaeria* sp.	8240 ± 8.24		
	Cyclotella sp.	130 ± 0.73	*Merismopedia* sp.	800 ± 0.76	17831 ± 212.38	N.D.
	Volvox sp.	120 ± 0.87				
	Gloeocystis sp.	300 ± 1.12				
	Uroglenopsis sp.	80 ± 0.35				
	Total	7111 ± 128.37				
Sagama tank	*Microcystis* sp.	4334 ± 1.14	*Melosira* sp.	1325 ± 2.38		
(February 2017)	*Anabaena* sp.	340 ± 0.79	*Ulothrix* sp.	338 ± 0.48	6652 ± 158.14	N.D.
	Synedra ulna	280 ± 0.84	*Stephanodisc* sp.	35 ± 0.74		
	Total	4954 ± 131.85				
Kondawatuwana	*Cylindrospermopsis* sp.	7250 ± 4.89	*Melosira* sp.	350 ± 0.83		N.D.
tank	*Anabaena* sp.	925 ± 2.17	*Stephanodisc* sp.	200 ± 0.99		
(February 2017)	*Oscillatoria* sp.	129 ± 1.16	*Chroococcus* sp.	130 ± 0.82	11689 ± 261.43	
	Microcystis sp.	2500 ± 3.18	*Ulothrix* sp.	80 ± 0.96		
	Staurastrum sp.	25 ± 0.69				
	Gloeocystis sp.	100 ± 0.93				
	Total	10929 ± 92.43				
Unnichchai tank	*Anabaena* sp.	980 ± 0.82	*Melosira* sp.	235 ± 1.38		
(February 2017)	*Cylindrospermopsis* sp.	1120 ± 0.93	*Chroococcus* sp.	410 ± 1.88		
	Cyclotella sp.	155 ± 2.32	*Dichotomosiphon* sp.	65 ± 0.58	5855 ± 274.15	N.D.
	Microcystis sp.	2610 ± 1.73				
	Oscillatoria sp.	280 ± 0.89				
	Total	5145 ± 151.62				
Kanthale tank	*Microcystis* sp.	3850 ± 3.37	*Melosira* sp.	125 ± 0.55	6499 ± 99.23	N.D.
(February 2017)	*Cylindrospermopsis* sp.	2524 ± 1.87				
	Total	6374 ± 148.49				
Nachchadoowa	*Anabaena* sp.	10468 ± 11.79	*Lyngbya limnetica*	235 ± 0.38		
wewa	*Cylindrospermopsis* sp.	4854 ± 5.18	*Melosira* sp	1792 ± 2.39	27812 ± 215.18	8.7
(October 2016)	*Microcystis* sp.	9300 ± 8.92				
	Oscillatoria sp.	1163 ± 4.38				
	Total	25785 ± 218.47				
Kala wewa	*Cylindrospermopsis* sp.	14986 ± 3.83	*Merismopedia* sp.	1398 ± 2.18		
(October 2016)	*Anabaena* sp.	8839 ± 0.93	*Melosira* sp.	1330 ± 1.78	45840 ± 261.42	8.2
	Microcystis sp.	18152 ± 15.27				
	Oscillatoria sp.	1135 ± 3.27				
	Total	43112 ± 272.41				

continued -

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Nallachchiya	*Cylindrospermopsis* sp.	6012 ± 2.53				
wewa	*Microcystis* sp.	8221 ± 3.94	*Lyngbya limnetica*	197 ± 2.18	23163 ± 180.43	7.8
(October 2016)	*Anabaena* sp.	7869 ± 4.47				
	Oscillatoria sp.	864 ± 2.58				
	Total	22966 ± 183.48				
Thuruwila wewa	*Anabaena* sp.	21500 ± 7.18				
(October 2016)	*Cylindrospermopsis* sp	12465 ± 3.26	*Merismopedia* sp.	1468 ± 3.28	99562 ± 657.84	8.1
	Oscillatoria sp.	2467 ± 1.96				
	Microcystis sp.	61662 ± 3.35				
	Total	98094 ± 756.39				
Tissa wewa	*Anabaena* sp.	28770 ± 9.41				
(October 2016)	*Cylindrospermopsis* sp.	8697 ± 5.42	*Lyngbya limnetica*	168 ± 0.77	48196 ± 364.36	8.8
	Microcystis sp.	7665 ± 4.97				
	Oscillatoria sp.	2896 ± 1.53				
	Total	48028 ± 250.75				
Nuwara wewa	*Anabaena* sp.	35580 ± 42.43	*Melosira* sp.	1558 ± 8.42		
(October 2016)	*Cylindrospermum* spp.	9850 ± 4.73	*Lyngbya limnetica*	220 ± 1.43		
	Microcystis sp.	21550 ± 27.64			73673 ± 276.48	10.9
	Volvox sp.	2800 ± 9.39				
	Oscillatoria sp.	2115 ± 3.80				
	Total	71895 ± 284.42				
Parakrama	*Microcystis* sp.	5894 ± 2.37	*Stephanodisc* sp.	39 ± 1.46		
Samudraya	*Cylindrospermopsis* sp.	825 ± 0.84	*Melosira* sp.	1772 ± 2.87	9777 ± 128.18	8
(November	*Anabaena* sp.	983 ± 0.97				
2016)	*Oscillatoria* sp.	264 ± 1.53				
	Total	7966 ± 112.37				

flocculation, coagulation, disinfection) where some plants used modern technological methods such as dissolved air floatation systems (DAF) (Kondawatuwana WTP, Unnichchai (Wawnativ) WTP), membrane filtration systems (Sagama WTP) and activated carbon beds (Kondawatuwana WTP, Unnichchai WTP). Generally, when sand filtration is applied as a step in the treatment of raw water, there is an increase in geosmin content after treatment. The highest increase in geosmin content (Table 1) after treatment was from the Tissa tank in Anuradhapura. According to NWSDB (*unpublished data*), back in 1987, a 5 cm thick black layer was found just underneath the sand filtration layer of the Tissa WTP. This layer had loads of actinomycetes, which could aggravate the geosmin content during filtration (Klausen *et al*., 2005). Moreover, previous studies have concluded that geosmin is produced by actinomycetes (Gerber & Lechevalier, 1965; Klausen *et al*., 2004; Klausen *et al*., 2005; Jüttner & Watson, 2007; Lee *et al*., 2011; Park *et al*., 2014). The special water treatment facilities in each plant except for the conventional processes are stated in Table 1. Powder activated carbon (PAC) and granular activated carbon (GAC) are one of the key compounds used over the world to solve the issue T&O in water (Drikas *et al*., 2009; Kim *et al*., 2014). It was noted that geosmin levels were not detected in treated water, which had PAC or GAC beds in the treatment plants (Kondawatuwana and Unnichchi) (Table 2). It was observed during field visits that GAC or PAC safe doses added into the water has been found to be a good solution when T&O problem appears in water occasionally even with the treatment plants where PAC or GAC beds are unavailable (Kim *et al*., 2014, Drikas *et al.*, 2009).

Identification and enumeration of phytoplankton

Species composition and the number of algae/ cyanobacteria/ diatoms and flagellates in each raw water body, geosmin concentration *vs* T&O forming species composition and number are stated in Table 2. Some T&O producing cyanobacteria and algae species such as *Anabaena* sp., *Cylindrospermum* spp., *Microcystis* sp., and *Oscillatoria* sp. were identified and quantified in the selected raw water bodies during the study.

T&O producing algae and cyanobacteria cell density ranged between 4954 to 98094 cells mL^{-1} and Sagama tank had the lowest and Thuruwila tank had the highest cell density during February 2017 and October 2016, respectively. Considering the geosmin concentration, in Sagama tank where the lowest cell density was recorded, geosmin was not detected; in Thuruwila tank where the highest cell density was recorded, a high level of geosmin was detected. Nuwara wewa, where the highest geosmin level was detected (10.9 ng/L) also recorded high algae and cyanobacteria cell density (71895 ± 2 84 cells mL^{-1}). A strong positive correlation between total T&O forming algae and cyanobacteria cell count and geosmin levels was detected and the results were statistically significant with a Pearson correlation coefficient of 0.691 according to Cohen *et al*. (1998) ($p < 0.05$). The present study shows that when the number of T&O forming cyanobacteria and algae are higher, geosmin level also increased significantly ($p < 0.05$). During the sampling

Table 3: Physico chemical parameters of collected water

Raw water body	Time of collection	Tem. °C	pH	DO/ mg/L	Cond./ µscm^{-1}	Nitrate-Nmg/L	Nitrite-Nmg/L	Ammonia-Nmg/L	Total Phosphorous (TP) mg/L	Total Hardness mg/L
Recommended level for drinking			6.5 - 8.5		3500	50	3	0.06	2	250
Jayanthi wewa (February 2017)	7.53 AM	25.3	7.14	6.78	147.2	0.07	< 0.01	< 0.01	< 0.01	56.0
Sagama tank (February 2017)	10.51 AM	29.0	7.57	7.42	129.1	< 0.01	< 0.01	< 0.01	< 0.01	56.0
Kondawatuwana tank (February 2017)	1.00 PM	32.0	8.00	8.97	154.4	0.24	< 0.01	< 0.01	< 0.01	64.0
Unnichchai tank (February 2017)	5.00 PM	30.1	8.21	8.56	168.1	0.31	< 0.01	< 0.01	< 0.01	60.0
Kanthale tank (February 2017)	9.00 AM	28.5	7.33	7.81	182.8	0.577	< 0.01	< 0.01	< 0.01	120.0
Nachchadoowa tank (October 2016)	10.00 AM	28.5	8.27	7.85	719.0	0.27	0.47	< 0.01	0.28 ± 2.4	128.0 ± 0.01
Kala tank (October 2016)	11.37 AM	29.1	8.11	7.24	279.1	0.29 ± 0.01	0.45	< 0.01	0.26 ± 0.09	36.0 ± 0.05
Nallachchiya tank (October 2016)	2.22 PM	30.2	8.23	8.07	172.3	0.45	0.52	< 0.01	0.14	72.0 ± 0.02
Thuruwila tank (October 2016)	4.12 PM	28.2	8.41	8.50	478	0.58	0.55 ± 0.01	< 0.01	0.29 ± 0.08	144.0 ± 0.03
Tissa tank (October 2016)	5.02 PM	29.1	8.62	8.06	440	0.24	16.54	< 0.01	0.18	112.0 ± 0.12
Nuwara tank (October 2016)	5.58PM	28.4	8.51	7.85	462	0.24	1.85	0.01	0.13	112.0 ± 0.01
Parakrama Samudraya (November 2016)	3.00 PM	30.5	8.48	7.38	320.5	0.03	0.02 ± 1.76	< 0.01	0.16 ± 0.04	70.0.0

period *Anabaena* sp. was recorded as the dominant species in Tissa tank (28770 ± 9.41 cells mL^{-1}), Nuwara tank (35580 ± 42.43 cells mL^{-1}), and Nachchadoowa tank (10468 ± 12 cells mL^{-1}). *Cylindrospermum* spp. (7250 ± 5 cells mL^{-1}), was recorded as the dominant species in Kondawatuwana tank. *Microcystis* sp. was dominant in majority of the tanks such as Parakrama Samudra, Thuruwila tank, Nallachchiya tank, Kala tank, Kantale tank, Unnichchai tank, Sagama tank and Jayanthi tank. Further, *Oscillatoria* sp., *Anabaena* sp. and *Cylindrospermopsis* sp. had significant positive correlations ($p < 0.05$) with the geosmin level with Pearson correlation coefficients of 0.765, 0.750 and 0.620, respectively. However, any significant correlation was not observed between *Microcystis* sp. cell density and geosmin level. According to Shang *et al*. (2018) the concentration of geosmin had no relationship with *Microcystis* sp. count. Chen *et al*. (2010) also supports this evidence with their study. Interestingly, highest levels of geosmin was recorded in the tanks where *Anabaena* sp. was dominant such as Nuwara tank (10.9 ngL^{-1}), Tissa tank, (8.8 ngL^{-1}) and Nachchadoowa tank, (8.7 ngL^{-1}). *Anabaena* sp. cell densities in these tanks were recorded as 35580, 28770 and 10468 cells mL^{-1}, accordingly. This clearly shows that geosmin level and *Anabaena* sp. cell density has a strong correlation. This result resembles Oh *et al*. (2017) where *Anabaena* sp. was found to be a high geosmin producing genus. Moreover, the reason for Thuruwila tank to not record the highest level of geosmin despite it having the highest level of T&O forming cell density can also be explained using this correlation. In Thuruwila tank, *Microcystis* sp. is the dominant species and *Anabaena* sp. is the co-dominant species. It was observed that at Parakrama Samudra, the total T&O forming cyanobacteria cell density was lower compared to other raw water bodies (7966 ± 112.37 cells mL^{-1}), but the recorded geosmin level was comparatively high as 8 ng/L. This result was observed during the rainy season and the dominant species was *Microcystis* sp. during that time period as well. Although algal blooms, and thus T&O events typically occurred in warm stratified seasons, some studies have shown that high geosmin levels can be detected during lake circulation periods and how low, instead of high, temperatures can stimulate the production of geosmin (Bertone & Halloran, 2016). Moreover, the sources of geosmin are not only cyanobacteria but could be due to the presence of geosmin producing actinomycetes, other vegetation and some standing timber. Therefore, these sources might have caused the high geosmin level in Parakrama Samudra at that time period despite the low cyanobacteria availability.

Measurement of water quality parameters

Raw water was analysed for various physico-chemical parameters as given in Table 3. According to the results, all the raw water bodies tested had safe pH, conductivity, hardness, total phosphorous (TP), N-nitrate and N-ammonia for drinking purposes according to SLSI drinking water standards. Tissa tank had a higher N-nirite level, which exceeds the safe range for drinking according to SLSI drinking water standards. All other raw water bodies had a safe N-nirite level for drinking.

Phosphorous availability is generally believed to be the limiting nutrient determining the rate of production of cyanobacteria and is likely the limiting factor for algal growth in most freshwater systems (Saadoun *et al*., 2001; Ji *et al*., 2017). A significant positive correlation between total phosphrous and geosmin level with a Pearson correlation coefficient of 0.850 ($p < 0.05$) was found indicating that total phosphorous may be the limiting factor for algae and cyanobacteria growth which enhances the production of geosmin (Saadoun *et al*., 2001; Christinesen *et al*., 2003). This evidence corresponds with Oh *et al*. (2017), which concluded that the growth of *Anabaena* sp., one of the main geosmin producing genera, is dependent on phosphorous concentration. Moreover, there was a significant positive correlation between electrical conductivity and geosmin level with a Pearson correlation coefficient of 0.796 ($p < 0.05$). A significant strong correlation was found between pH and geosmin level with a Pearson correlation coefficient of 0.788 ($p < 0.05$). This indicates that a high concentration of dissolved ions, high alkalinity and algae and cyanobacteria support the production of geosmin in water. According to Ji *et al*. (2017), alkalinity is a known optimum condition for cyanobacteria and a high cyanobacteria concentration will produce higher geosmin content (Okogwu & Ugwumba, 2009; Ji *et al*., 2017). Significant positive correlations were observed between total phosphorous and total T&O forming cell density with Pearson correlation coefficient of 0.689 ($p < 0.05$), and between electrical conductivity and total T&O forming cell density with a Pearson correlation coefficient of 0.612 ($p < 0.05$). This suggests total T&O forming cells thrive more in environments where high concentrations of total phosphorous and dissolved ions are available and in return produces more geosmin (Saadoun *et al*., 2001; Oh *et al*., 2017). This provides evidence to the fact that cyanobacteria cells produce geosmin. Moreover, from statistical analyses it was found that there is a direct positive correlation among geosmin, cyanobacteria and total phosphorous with a Pearson correlation coefficient

of 0.691 ($p < 0.05$) (Oh *et al*., 2017). This points to the fact that high phosphorous levels increase the growth of cyanobacteria and high cyanobacteria levels produce higher geosmin levels in water (Saadoun *et al*., 2001; Christinesen *et al*., 2003). Nutrients, particularly nitrogen and phosphorous have been identified as major reasons why much of the Nation's surface water is considered degraded with respect to water quality (USEPA, 2000). Nutrients are also important in T&O occurrences. According to TN:TP ratio calculation (Abell *et al*., 2010), Jayanthi tank, Sagama tank, Nachchadoowa tank, Kala tank, Nallachchiya tank, Thuruwila tank and Parakrama Samudra were found to have a nitrogen limiting situation (N:P < 10), while Kondawatuwanana tank, Unnichchai tank, Kanthale tank and Tissa tank have a phosphorous limiting situation (N:P > 17). Nuwara tank showed a N:P ratio indicating that either nitrogen or phosphorous can be limited (N: P = 10–17). Nitrogen and phosphorous can affect the trophic conditions or the degree of biological activity. Nutrient enrichment (eutrophication) can lead to excessive growth of algae, particularly cyanobacteria, with subsequent geosmin production and T&O problems (Davis & Shaw, 2009). Principal component analysis was conducted for the results (Figure 4) and the principal components, PC1 and PC2 contributed about 69.7 % of the total variance in the data (Table 4). PC1 as given in Table 4, has variance 4.67 and accounts for 52 % of the total variance.

Table 4: Summary of eigenvalues and correlation matrix of principal component analysis

Eigen analysis of the correlation matrix

Eigenvalue	4.6783	1.5964	1.0739	0.7534	0.4129	0.2903	0.1395	0.0359	0.0193
Proportion	0.520	0.177	0.119	0.084	0.046	0.032	0.016	0.004	0.002
Cumulative	0.520	0.697	0.817	0.900	0.946	0.978	0.994	0.998	1.000

Correlation matrix of principal component analysis

Variable	PC1	PC2	PC3
Total Cell Density	0.388	-0.093	-0.117
pH	0.365	0.092	0.438
DO	0.125	-0.588	0.499
Conductivity	0.405	0.179	-0.108
Nitrate-N	0.191	-0.636	-0.238
Nitrite-N	0.193	0.166	0.598
Total phosphorous	0.402	0.171	-0.235
Hardness	0.369	-0.248	-0.251
Geosmin	0.407	0.285	-0.038

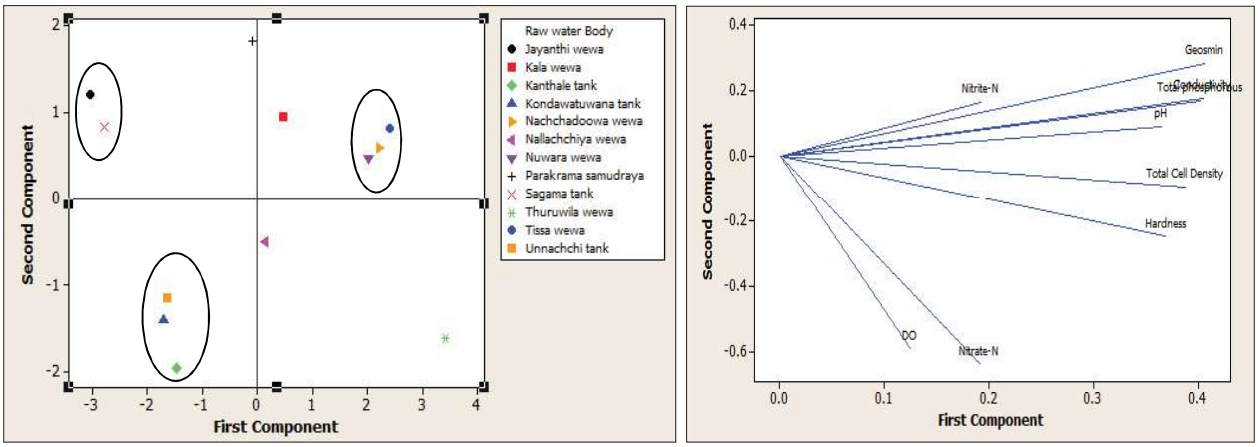

Figure 4: Principal component analysis (PCA): (a) score plot; (b) loading plot

The coefficients listed under PC1 in Table 4 shows the calculation of principal component scores: PC1 = 0.388 total cell density + 0.365 pH + 0.125 DO + 0.405 cond. + 0.191 nitrate-N +0.193 nitrite-N + 0.402 total phosphorous + 0.369 hardness + 0.407 geosmin.

PC1 is contributed by geosmin, total phosphorous and conductivity, although there is no parameter value obtained above 0.5. The second principal component has a variance of 1.59 and accounts for 17.7 % of the data variability. It is calculated from the original data using the coefficients listed under PC2. PC2 is strongly contributed by nitrate-N and dissolved oxygen. Together, the first two and three components represent 69.7 % and 81.7 % 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 were used for PCA scoring and three scores were selected (Table 2). PC1 explains 52 % of total variance of the data with 52 % cumulative variation and PC2 explains 17.7 % of total variance of the data with 69.7 % cumulative variation. In the score plot of PC2 versus PC1, three clusters can be identified along the PC1 axis. The groups I, II and III correspond to samples with different water quality parameters. pH, conductivity, geosmin, total phosphorous, total cell density, hardness, DO, nitrite-N and nitrate-N exhibited a strong relationship among them and also influence the separation of the three groups along PC1. Nuwara wewa, Tissa wewa and Nachchadoowa wewa were clustered together with high geosmin, pH, conductivity, total phosphorous and total odour and taste forming cell density values, whereas Unnichchai tank, Kondawatuwana tank and Kanthale tank were clustered together with similar nitrate-N and DO values. The third cluster consisted of Jayanthi wewa and Sagama tank with similar nitrite-N values.

In the questionnaire survey, 95 % of students rejected geosmin contaminated water, whereas only 5 % accepted geosmin contaminated water for drinking purposes (n = 100). In the non-student category, 98 % adults rejected and only 2 % adults accepted geosmin contaminated water for drinking purposes (n = 100).

CONCLUSION

The present study concludes that geosmin is one of the causes for T&O problems in drinking water in some parts of Sri Lanka. More than 95 % consumers rejected drinking water contaminated with geosmin. A total of 68 % of the water sampling locations covering 5 districts exceeded the human threshold level of geosmin. Geosmin concentration in treated water from water treatment plants were higher than the geosmin concentration in relevant raw water bodies. PCA analysis pooled three different clusters of water bodies. The solvent-free HS-SPME/GC-MS method developed in this study is simple and sensitive for analysis of geosmin. It can be directly applied to the analysis of a small volume of environmental water samples without any pretreatment. Therefore, this method provides a useful tool for the screening and determination of geosmin in water analysis.

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