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ORIGINAL ARTICLE

Harmful diatoms and dinoflagellates in the Indian Ocean: a study from Southern coast of Sri Lanka

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Studies on Harmful Algae (HA) are rare in the Indian Ocean around Sri Lanka. The current study investigated diatoms and dinoflagellates in five Sri Lankan Southern coast locations, focusing on potentially harmful species. A total of twenty-seven diatom species and ten dinoflagellate species were identified during the study. Among them, eight diatom species (*Asterionellopsis glacialis*, *Chaetoceros curvisetus*, *Chaetoceros lorenzianus*, *Guinardia flaccida*, *Leptocylindrus minimus*, *Nitzschia* sp., *Proboscia alata* and *Pseudonitzschia fraudulenta*) and three dinoflagellate species (*Ceratium fusus*, *Ceratium furca,* and *Dinophysis caudata*) were identified as potentially harmful species. Specifically, *P. fraudulenta* related to producing domoic acid, causing Amnesic Shellfish Poisoning (ASP), was recorded in all sampling locations. Potentially harmful species showed a significant correlation with turbidity and total phosphorus levels ($p < 0.05$). Discerning the occurrence of these species in the region is vital, as the seascape under investigation is in anthropogenic pressure with many sea routes. Even though bloom conditions were not observable during the study period, the risk of transporting microalgae to many different locations and the possibility of bloom formations cannot be ignored. As a country surrounded by the ocean, the results demonstrated the importance of continuous monitoring of potentially HA and regulating maritime and land-based activities, covering a broader area to identify and manage potential threats to the Indian Ocean.

Keywords: Indian Ocean; Sri Lanka; coastal zone; harmful algae; diatoms; dinoflagellates; water quality

Introduction

Microalgae are a significant component of aquatic food webs and a key element in aquatic productivity. Some microalgal species, including diatoms, dinoflagellates, raphidophytes, prymnesiophytes, cyanophytes, and silicoflagellates, can intensively grow under favorable environmental conditions. This can leads to a natural phenomenon called "blooms" (Anderson, 2009; Padmakumar et al., 2012). Among the bloom-forming harmful algae (HA), diatoms and dinoflagellates are the most frequently reported groups (Gelin et al., 1999; Hinder et al., 2012; Draredja et al., 2018).

Occasionally, such algae could be a "nuisance" or even a "disaster" due to the harm they bring in through toxin production or high cell concentrations (Anderson et al., 2000; Landsberg, 2002; Landsberg et al., 2005; Fire and Van Dolah, 2012; Hallegraeff et al., 2017). They can cause numerous adverse impacts to the environment, marine biota, and even humans. HA are capable of affecting the marine environment (Granéli and Turner, 2006; D'Anglada, 2015) by reducing light penetration due to dense growth (Anderson, 2009), producing noxious gases (Fleming et al., 2005), and by generating hypoxia (Burkholder et al., 2006). Some species produce toxins that can harm the marine biota (Fire and Van Dolah, 2012). Deaths of marine mammals and sea birds along the coast of Southern California attributable to domoic acid is a well-known example of the harm caused by Harmful Algal Blooms (HAB) (Scholin et al., 2000; Lefebvre et al., 2002; Smith et al., 2018). Moreover, HABs could cause severe impacts, including the alternation of marine ecology and food webs (Zimba et al., 2004). Similarly, HA contribute to contaminated sea food (Backer and Mcgillicuddy, 2006), affecting commercial fisheries (Yan and Zhou, 2004). Besides, the impacts of HA on marine food security and tourism could have massive influences on the economy and society (Ritzman et al., 2018; Gobler, 2020).

Sri Lanka is an island situated in the Indian Ocean at India's tip (7.8731° N, 80.7718° E). However, very few studies (Senanayake et al., 2010; Jayasiri et al., 2015; Wijethilake and Ranatunga, 2015; Jayawardhane et al., 2018) have focused on HA species and possible threats in the coasts of the country. The present study was carried out to identify potentially harmful species in two significant microalgae groups, diatoms, and dinoflagellates, on the southern coast. The study further investigated the water quality parameters that are associated with these species. According to the authors' knowledge, the present study considered to be the first detailed study conducted on HA on the Southern coast of Sri Lanka. The Southern region of the country plays an important role in South Asian maritime commercial activities (Weerakoon and Perera, 2014; Ekanayake et al., 2016). Nationally, the Southern coast of the island performs a significant role in socio-political, economic, and environmental settings on account of its legendary tourist attraction, and the area is affected by busy sea routes (BOBLME, 2013; NARA, 2016). Furthermore, the oceanic area is active and dynamic with the influence of a wide array of anthropogenic activities as 12 % of the country's population resides there (Department of Census and Statistics, 2012).

The lifestyles of people in the region are interwoven with the coastal waters, and marine phytoplankton is an essential biological component. However, unfortunately, Sri Lanka does not have a regular monitoring system for microscopic biota. Therefore, detecting the presence of harmful species, identifying the factors influencing their development, and foreseeing the potential harm they could bring would benefit the sustainable management of coastal resources and activities.

Materials and methods

Study area:

The five study sites, Ambalangoda (6°14'3.99"N 80°2'55.83"E), Hikkaduwa (6° 8'36.67"N 80° 5'46.98"E), Dodanduwa (6°6'20.22"N 80°7'16.14"E), Mirissa (5°57'0.18"N 80°27'0.96"E) and Tangalle (6° 1'33.32"N 80°47'57.38"E), which are associated with fishery harbors are located in the Southern coast of the island (Figure 1). From the preliminary field visit, five sub-sampling stations were identified from each of the sampling locations. The sample collection was carried out from June to December 2019, parallel to the coastline at 1–2 km distance towards the sea.

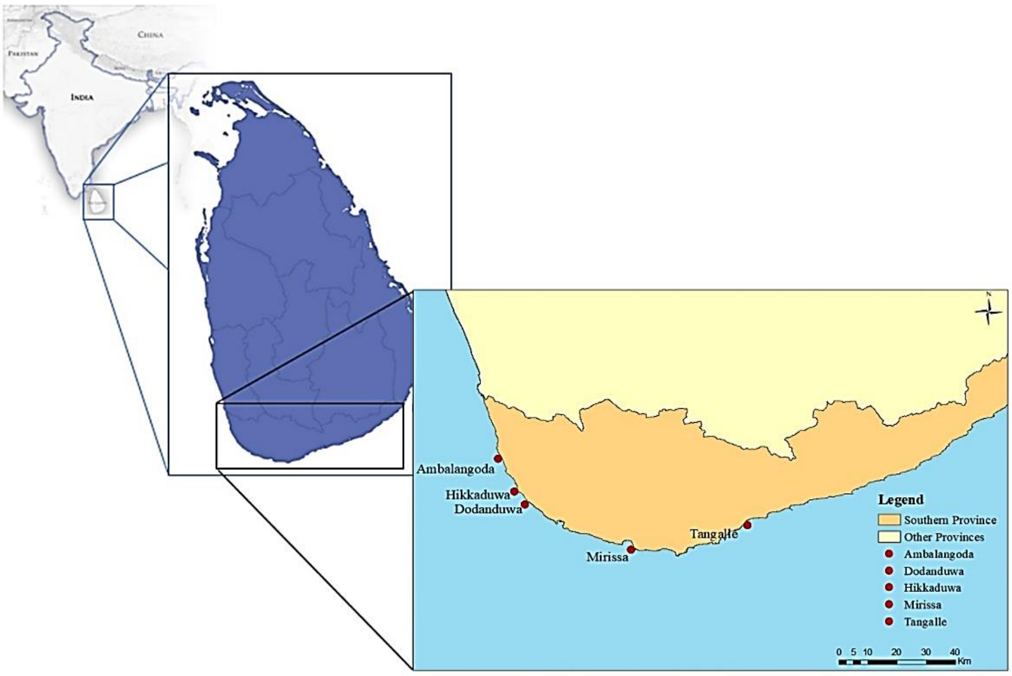

Figure 1. Sampling locations along the Southern coast of Sri Lanka.

Identification and enumeration of planktons

Samples were collected by boat dragging a 55 µm plankton net along the water surface (0-1 m) at a known speed for 2 minutes (a modified method of Manage and Piyasiri (1995). The boat speed was measured using a GPS device (GARMIN GPS 73). A further volume of 1 L water sample was taken from each substation to analyze the species composition. All samples were collected in opaque plastic bottles. The collected water samples were fixed with acidified Lugol's solution at a final concentration of 1% and were kept cool. The samples were stored for natural sedimentation for 24 hours. Later, the sedimented plankton was concentrated to 5 mL, removing supernatant for species identification and enumeration.

A Sedgwick–Rafter counting chamber was used to enumerate the microalgae. Identification and enumeration of microalgae were carried out under 40X magnification of binocular compound light microscope (Olympus CX21, Japan). When appropriate, when the microalgae density was high, 1:10 dilution was carried out using distilled water.

Microalgae were identified using identification keys (Tomas, 1997; Faust and Gulledge, 2002; Botes, 2003; Al-Kandari et al., 2009; Sahu et al., 2013). Also, potentially toxic species were verified according to Phyto'pedia - The report of Phytoplankton Encyclopaedia Project from Department of Earth, Ocean and Atmospheric Sciences of the University of British Columbia (Ivanochko et al., 2012) and Taxonomic Reference List of Harmful Micro Algae from the Intergovernmental Oceanographic Commission of the UNESCO (Moestrup et al., 2009). Moreover, verifications of species identification were obtained from relevant expertise by sending photographs.

Physicochemical parameters of coastal water

In-situ water quality measurements were taken for water temperature, pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), salinity, turbidity, and light intensity above the water surface. The parameters were measured using the respective standard meters, namely, glass thermometer, handheld pH meter (Sper Scientific Large-Display pH pen), conductivity meter (BANET 540, China), dissolved oxygen meter (55/50 FT, YSI, USA), salinity meter (BANET 540, China), Turbidity meter (Thermoscientific EUTECH TN-100, USA) and Light meter (Brannan, England).

The water samples were collected into polypropylene bottles and then transported under chilled conditions and stored in the laboratory under 4 °C. Laboratory analyses were performed for Chemical Oxygen Demand (COD), Chlorophyll a, Nitrate nitrogen (NO_3^- -N), Nitrite nitrogen (NO_2^--N) and Ammoniacal nitrogen (NH_3-N) according to APHA (2012), and total phosphorous (TP) according to USEPA (1978). All samples were analyzed in triplicates, and the mean was taken (Appendix).

Statistical analyses

Microalgae data and water quality parameters were statistically analyzed using Kruskal–Wallis test and Spearman Correlation Test in SPSS Statistics 25 software. Kruskal–Wallis test was performed to determine the variations of water quality parameters among the sampling sites, and correlation analyses were implemented to determine the relationships between water quality parameters and the abundance of harmful diatoms and dinoflagellates.

Results

Species profile

Altogether, twenty-seven species of diatoms and ten species of dinoflagellates were identified from all locations during the study period. Among them, eight species from class Bacillariophyceae (21.62%), eight species from class Coscinodiscophyceae (21.62%), eleven species from class Mediophyceae (29.73%), and ten species from class Dinophyceae (27.03%) were recorded. Diatoms were the most dominant group, which represented nearly 73% of the identified species. Among all the diatoms and dinoflagellates recorded, eight diatoms and three dinoflagellates represented potentially harmful species (Table 1), signifying 29.72 % of the total species recorded.

Table 1. Potentially harmful diatoms and dinoflagellate species were recorded during the study.

Phylum	**Species**	**Potential harm (references)**
Bacillariophyta (Diatoms)	*Asterionellopsis glacialis*	Capable of forming blooms (Mishra et al., 2006)
	Chaetoceros curvisetus	Capable of forming blooms and may cause physical damage to the fish gills (Ivanochko et al., 2012)
	Chaetoceros lorenzianus	Capable of forming blooms (Sunesen et al., 2008)
	Guinardia flaccida	Capable of forming blooms (Yan et al., 2002)
	Leptocylindrus minimus	May cause fish mortalities (Ivanochko et al., 2012)
	Nitzschia sp.	Many species produce domoic acid - Amnesic Shellfish poisoning (Bates et al., 2018; Kotaki et al., 2000)
	Proboscia alata	Capable of forming blooms (Thomas et al., 2014)
	Pseudonitzschia fraudulenta	Produce domoic acid – Amnesic Shellfish poisoning (Almandoz et al., 2017)
Dinophyta/ Miozoa (Dinoflagellates)	*Ceratium fusus*	Capable of forming extensive blooms (Ivanochko et al., 2012)
	Ceratium furca	Capable of forming extensive blooms (Ivanochko et al., 2012)
	Dinophysis caudata	Diarrhetic shellfish poisoning and associated with massive fish kills (Nishitani et al., 2008; Faust and Gulledge, n.d.)

P. fraudulenta were present in all sampling locations, and their abundance was highest compared to other potentially harmful species (Table 2).

Table 2. The occurrence of potentially harmful diatoms and dinoflagellates in sampling locations.

Species	TG	MR	DD	HK	AM
Asterionellopsis glacialis	++	++	-	-	-
Chaetoceros curvisetus	+++	++	-	+++	-
Chaetoceros lorenzianus	+++	+++	++	-	++
Guinardia flaccida	++	++	-	-	-
Leptocylindrus minimus	++	++	-	-	-
Nitzschia sp.	-	++	-	++	++
Proboscia alata	-	++	-	-	-
Pseudonitzschia fraudulenta	++++	++++	+++	++	++++
Ceratium fusus	++	++	-	-	++
Ceratium furca	++	++	-	-	++
Dinophysis caudata	-	-	-	-	++

- denotes absence and + denotes presence (+:<1000 cells L^{-1}; ++: 1000-5000 cells L^{-1}; +++: 5000-10000 cells L^{-1}; ++++: >10000 cells L^{-1}) (TG: Tangalle, MR: Mirissa, DD; Dodanduwa, HK; Hikkaduwa, AM; Ambalangoda)

Physicochemical parameters of water

Physicochemical parameters were checked against quality standards for Sri Lanka's coastal waters (CEA, 2001 cited in BOBLME, 2013) (Table 3). Other than the mean salinity levels in all sampling locations and mean pH in Tangalle and Mirissa, all other parameters tested were laid within the stipulated water quality standards, where available.

Table 3. Variation of physicochemical parameters during the study.

Parameter	Mean ± std. deviation					Proposed coastal water quality standards
	TG	MR	DD	HK	AM	
Water temperature (°C)	28.66 ± 0.47	30.26 ± 0.44	29.93 ± 0.77	29 ± 0.81	29.46 ± 1.08	<32
pH	**6.77 ± 0.67***	**6.92 ± 0.60***	7.32 ± 0.28	7.16 ± 0.37	7.37 ± 0.30	7 – 8.5
Electrical Conductivity (mS/cm)	24.96 ± 19.11	22.26 ± 12.77	26.35 ± 16.81	26.47 ± 16.32	25.77 ± 14.35	-
Dissolved Oxygen (ppm)	8.66 ± 0.58	8.13 ± 0.19	8.18 ± 0.39	8.07 ± 0.49	8.17 ± 0.67	-

Salinity (ppt)	**16.19 ± 15.84***	**14.48 ± 11.28***	**17.70 ± 14.87***	**17.48 ± 14.77***	**16.69 ± 13.63***	29 - 35
Light intensity (klx)	33.18 ± 8.88	58.24 ± 21.96	60.29 ± 7.43	26.19 ± 21.41	36.23 ± 17.73	-
Turbidity (NTU)	2.62 ± 1.94	1.53 ± 1.46	0.79 ± 0.57	1.43 ± 1.11	1.35 ± 0.67	-
Chemical Oxygen Demand (ppm)	61.21 ± 49.49	69.88 ± 54.71	50.28 ± 44.39	44.32 ± 46.27	65.58 ± 52.03	-
Total phosphorus(ppb)	119.52 ± 172.99	144.28 ± 220.76	53.11 ± 65.28	11.92 ± 6.49	10.84 ± 3.16	-
Nitrate nitrogen (ppm)	1.14 ± 1.42	0.95 ± 0.58	0.60 ± 0.49	0.39 ± 0.19	1.06 ± 0.61	-
Nitrite nitrogen (ppb)	0.71 ± 0.15	2.39 ± 2.53	1.65 ± 1.01	1.94 ± 0.84	2.33 ± 2.14	-
Ammoniacal nitrogen (ppb)	0.57 ± 0.21	0.57 ± 0.19	0.56 ± 0.21	0.59 ± 0.18	0.56 ± 0.23	-
Chlorophyll *a* (ppb)	2.20 ± 0.99	8.01 ± 3.81	2.16 ± 2.35	10.55 ± 9.16	10.86 ± 9.23	-

(TG: Tangalle, MR: Mirissa, DD; Dodanduwa, HK; Hikkaduwa, AM; Ambalangoda). *Figures that do not comply with the proposed quality standards for different use classes of coastal water in Sri Lanka (CEA, 2001 cited in BOBLME, 2013).

Kruskal–Wallis test (df = 4, significance level = 0.05) reveals that turbidity (*p* = 0.016), chlorophyll *a* (*p* = 0.000), ammoniacal – nitrogen (*p* = 0.002), light intensity (*p* = 0.000) and water temperature (*p* = 0.000) displayed significant variations among the five sampling locations.

Correlation between physicochemical parameters of water and harmful species identified in the study

Spearman correlation analysis (significance level = 0.05) revealed that most of the potentially harmful species counts show a significant relationship with the turbidity. *A. glacialis* displayed significant positive correlations with turbidity (ρ=0.237, *p* =0.041) and total phosphorus (ρ =0.234, *p* =0.044). *C. curvisetus* showed positive correlation with total phosphorus (ρ =0.274, *p* =0.017). *C. lorenzianus* showed positive correlation with turbidity (ρ =0.283, *p* =0.014) and negative correlation with chlorophyll *a* (ρ = -0.280, *p* =0.015). *G. flaccida* showed negative correlation with nitrite - nitrogen (ρ = -0.228, *p* =0.049) and positive correlation with total phosphorus (ρ = 0.238, *p* =0.047). Turbidity was positively correlated with both *L. minimus* (ρ = 0.257, *p* =0.026) and *C. fusus* (ρ = 0.241, *p* =0.037). *C. furca* showed negative correlation with nitrite – nitrogen (ρ = - 0.290, *p* =0.012).

Discussion

This study reports the first-ever HA species found in the Indian ocean around the Southern coast of Sri Lanka. Eight diatom species (*A. glacialis, C. curvisetus, C. lorenzianus, G. flaccida, L. minimus, Nitzschia* sp., *P. alata* and *P. fraudulenta*) and three dinoflagellate species (*C. fusus, C. furca* and *D. caudata*) were identified as potentially harmful species. Taking the capability to produce domoic acid in the genus, even though not all *Nitzschia* sp. are deemed harmful, it is considered to retain potential harm during this study. Out of these, three species are known to produce health impacts on humans directly or indirectly, and the rest are capable of forming blooms that could be a threat to coastal health. More profoundly, *P. fraudulenta* related to producing domoic acid, resulting in Amnesic shellfish poisoning, is recorded in all five sampling locations.

Most of the potentially harmful diatoms and dinoflagellates recorded during the study period were present in Mirissa, and in contrast, the lowest was recorded from Dodanduwa. However, no evidence of "bloom forming" was recorded during the study period at any of the sampling sites.

There are only a handful of studies carried out on HA in Sri Lanka, and most of them were focused on Colombo harbor, which is one of the key harbors in Sri Lanka, located in the Western province of the country. Senanayake et al. (2010) have mentioned *C. furca* and *C. fusus* in Colombo harbor in an investigation of the marine organisms in ship ballast water, indicating the possibility of transporting harmful algal species from elsewhere to the coasts of the island. Jayasiri et al. (2015) reported 13 toxic dinoflagellates out of the 42 species reported in the Colombo harbor. A study conducted by Wijethilake and Ranatunga (2015) reported cysts belonging to *Scrippsiella* spp. that cause fish mortalities. Further, Jayawardhane et al. (2018) reported two species (*C. fusus* and *D. caudata*) identified in the present study from the Western coast of Sri Lanka. As Sri Lanka and India are placed in the same ocean and given the chances of transporting microalgae from one location to another, studies on harmful algae are significant to the Indian Ocean. A review of algal bloom occurrences in Indian waters by D'Silva et al. (2012), from 1908 to 2009, showed a total of 101 bloom incidents, where blooms of *A. glacialis* and *P. alata* were also documented, revealing the capability of these species to develop into blooms.

Studies on risk assessment of HA and the potential threats they present in Sri Lankan coasts are mostly inadequate. For instance, *Pseudonitzschia* sp. was first logged on the US west coast in the 1920s and went unnoticed for many years until extremely high contents of domoic acid were found, which resulted in adverse impacts on fish kills (Lewitus et al., 2012). The risk of HA upon communities who consume fish and engaged in seafood harvesting, shipping, and processing occupationally and environmental workers (Backer et al., 2003) have not gained scientists' and policy makers' attention.

In the Sri Lankan context, compared to freshwater phytoplankton, studies on marine plankton remain unnoticed, and even the existing marine studies are mainly focused on the diversity and abundance of plankton (Chandrasekera and Fernando, 2009; Jayasiri et al., 2016; Wickramasingha and Jayasiri, 2016; Weerakoon et al., 2017). Furthermore, as Sri Lanka is situated in the middle of the trans-oceanic route connecting east and west, there is a considerable possibility of alien plankton being introduced into the Sri Lankan coastal zone via ballast water (Chandrasekera and Fernando, 2009).

With the global trend of expanding the distribution of HA, Sri Lanka could also be at risk. If so, tourism and recreation can also be imperiled by HA, as tourism has focused primarily on scenic sandy beaches and coastal lagoons. Also, coastal tourism accounts for 70 % of the total tourism infrastructure in Sri Lanka and a significant portion of the national economy (BOBLME, 2013).

Monitoring the water quality is crucial in understanding coasts' ecological health and any link between physicochemical parameters with occurrence and density of microalgae (Parmar et al., 2016). Inland activities could modify coastal water quality. During the study period, the mean salinity level at each sampling site was lower than the quality standards for Sri Lanka's coastal waters. According to Rajaram et al. (2005), large amounts of freshwater discharges, land runoff, heavy rains in the monsoons, and proximity to the land can cause lower salinity levels. Similarly, the pH of water, an essential parameter for biota, can also be influenced by river discharges. A decrease in pH values can be caused by riverine influx (Saraswat et al., 2011; Vajravelu et al., 2018). For instance, mean pH values in Tangalle and Mirissa were lower than the quality standards for coastal waters in Sri Lanka, and Raven et al. (2020) have reported that some HA favored in acidified aquatic environments. Turbidity of coastal waters can be linked to the inputs from inland, while some studies report that turbidity affects the growth of HA (Park et al., 2017). In the present study, many of the potentially harmful species showed a significant correlation with turbidity, including *A. glacialis.*

Moreover, another study relates turbidity to bloom-forming species such as *A. glacialis* (Pannard et al., 2008). Elevated nutrient levels can influence the general algal growth (Muñiz et al., 2018). For instance, phosphorus availability affects the algal growth and metabolic activities, whereas nitrogen is a contributory factor for the somatic growth of cells (Granéli and Flynn, 2006). The present study also provides indications on the correlation between nutrient levels and HA abundances. All stations investigated under this study are under significant influence of anthropogenic activities from possible influxes of various physicochemical and biological materials from the inland through many small-scale waterways. Besides, fishery harbors located close could affect the water quality due to increased human activities. Some potentially harmful algae recorded from the present study were correlated positively with turbidity and total phosphorus concentration which could be due to anthropogenic activities.

However, a variety of research gaps exist that include inadequacy of information on profiling microalgae species, distribution in time and space, and assessment of toxicity, which hinder the risk identification and assessment of HA in the coastal region. This study provides the foundation for the studies on HA in the southern region, leading to more informative studies in the future.

Conclusions

The present study reports eleven harmful diatoms and dinoflagellates from the Southern coast of Sri Lanka. To the authors' knowledge, this is the first record of harmful algae in this region of the Indian Ocean. Three species were recorded (*Nitzschia* sp., *P. fraudulenta, D. caudata*) that can be directly related to shellfish poisoning in humans, and eight species were recorded that are capable of forming blooms. Further, as Sri Lanka is located in a strategic location of the Indian Ocean, the risk of spreading HA to other parts of the ocean could not be ignored. To make the situation worse, the vulnerability of oceans to increased nutrient pollution and impacts of climate change which results in elevated ocean temperature and acidity could affect the occurrence and frequency of HA. Thus, proper long-term monitoring, risk assessment, and management are becoming increasingly important.

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Contributions

Sajini Dissanayake performed field and lab investigations and contributed to writing. Deepthi Wickramasinghe designed the study and contributed to writing. Pathmalal Manage designed the study and guided the chemical analysis.

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