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RESEARCH ARTICLE

DISTRIBUTIONAL CHARACTERISTICS OF DIMETHYL SULPHIDE (DMS) RELATED TO PHYTOPLANKTON BIOMASS AND NUTRIENT DYNAMICS IN THE COCHIN ESTUARY

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ARTICLE INFO	ABSTRACT
Article History: Received 04 th July, 2013 Received in revised form 10 th August, 2013 Accepted 25 th September, 2013 Published online 23 rd October, 2013	Distribution of Dimethyl Sulphide (DMS) was measured in relation with phytoplankton density in the Cochin estuarine system during the year 2010. A total of 120 species of phytoplankton were identified which represents different distinct classes viz: <i>Bacillariophyceae</i> (65), <i>Chlorophyceae</i> (25), <i>Dinophyceae</i> (21), <i>Cyanophyceae</i> (6), <i>Dictyochophyceae</i> (1), <i>Chrysophyceae</i> (1) and <i>Zygnematophyceae</i> (1). The phytoplankton identification reveals that Cochin estuary is a diatom dominated estuary. The maximum concentration of diatom species was high in pre-monscon season
<i>Key words:</i> Dimethyl sulphide, Phytoplankton, Salinity, Chlorophyll <i>a</i> , Nutrients.	(av.57693 cell/m ³) followed by monsoon (av.45073 cell/m ³) and post monsoon (av.40320 cell/m ³) whereas dinoflagellates range av.14413 cells/m ³ (post monsoon), av.7840 cells/m ³ (pre monsoon) and av.4593 cells/m ³ (monsoon). Hydrographical parameters and nutrient distribution were also measured to ascertain a relationship with phytoplankton. Chlorophyll <i>a</i> , salinity and phosphate exhibit a positive correlation with DMS. The DMS concentration varied from non detectable levels to 19.5 nM in post monsoon, while (0.2 to 1.8 nM) in pre monsoon and (0.2 to 1.1 nM) in monsoon. Elevated levels of DMS were observed in saline stations of the estuary. The data represented above is the first baseline study of DMS in the Cochin Estuarine system.
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INTRODUCTION

Dimethyl sulphide (DMS) is considered to be the most abundant form of volatile organic sulfur. DMS contributes about two-third of global natural sulfur emission to the atmosphere and extends its role in the sulfur cycle and climate (Lovelock et al., 1972., Rodhe, 1999). The natural occurrence of DMS was first discovered by Haas in 1935. The production of DMS by several classes of phytoplankton through biological activity in the aquatic realm was extensively studied (Lovelock et al., 1972., Charlson, 1987). The concentration of DMS in water sample depends on the production by phytoplankton, other microorganisms. bacterial and photochemical consumption (Andreae, 1986), zooplankton grazing (Dacey and Wakeham, 1986) in addition to microbial decomposition of DMSP to DMS (Andreae, 1985). Dimethylsulphonio propionate (DMSP) is the major precursor of DMS, which is synthesized by marine phytoplankton as an internal cell component. DMSP is also considered as a compatible solute involved in osmoprotection and cryoprotection in algae (Stefels, 2000).

Cochin estuary is classified as a tropical dynamic estuary. Although several studies accounting this dynamic behavior of

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the estuary, but no database yet published regarding the complex and consumption of biogenic sulfur gas (DMS). This is the first preliminary report on DMS with respect to phytoplankton community. Estuaries are the cradle grounds for flora and fauna, where sundry activities regularly occur and are the most productive of the aquatic ecosystem. These ecosystems are highly vulnerable and easily subjected to stresses induced by environment or human. Several studies have been accomplished in this estuary on various physicochemical (Sankaranarayanan and Qasim, 1969., Shyanamma and Balakrishnan, 1973) and biological characteristics (Rao et al., 1975., Madhupratap and Haridas, 1975., Qasim, 2003., Martin et al. 2008).

The spatial and temporal variability of DMS production is widely studied (Yang et al., 2000a, b., Jiao et al., 2003). But less data base is available from the Indian sector rather than in estuary (Kumar et al. 2009, Shenoy et al. 2002, Shenoy and Patil 2003). The distribution of DMS in the marine water was influenced by various environmental factors. Salinity is one of the responsible factor for DMS production, in which algal cells produce organic solutes such as quaternary ammonium compounds (Keller et al., 1999a, b) and tertiary sulfonium compounds (DMSP) (Blunden and Gordon, 1986., Bisson and Kirst, 1995). Previous work by Sunda and Hardison, in 2007 highlights the effect of nitrogen limitation on cellular DMSP and DMS release in marine phytoplankton.

MATERIALS AND METHODS

Site details

Cochin estuary is a bar-built micro-tidal system connected to the Arabian Sea at two locations one at Cochin (latitude 9°10 N) and at Azhikode (latitude 10°10 N). The estuary is flanked between two parts: the southern arm extending from Cochin to the south and the northern arm extending from Cochin to Azhikode. The Cochin bar mouth is about 450 m wide, whereas the Azhikode inlet is relatively narrow. The Cochin metropolis receives an annual rainfall of 320 cm, of which 60% occurs during the southwest monsoon period, July-Sept (Qasim 2003). The estuary, receives a high volume of fresh water annually $(20 \times 10^9 \text{ m}^3 \text{year}^{-1})$ from the six rivers in the State, Kerala (Srinivas et al., 2003). During the months of December to April, construction of a salinity barrier bund at Thanneermukkam virtually cuts off the tidal propagation further towards south and modifies the circulation patterns in the remaining part of the estuary. The samples were collected during 2010 in three prominent seasons; post monsoon (Jan.), pre monsoon (Apr.) and monsoon (Aug.). Fifteen stations in the estuary were selected for DMS measurements. Sampling was conducted twice in each season and the average values for each parameter is reported. The sampling sites were best ascribed in Figure 1 and the specifications are as follows.

features leading to a hodgepodge of multidimensional behaviors. The remaining stations from 11 to 15 flows closely through industrial region and many small and large scale industries on the river bank discharges effluents directly into water ultimately leading varying amount of nutrients in to the lower river.

Sampling and analysis

Samples have been taken for qualitative and quantitative analysis of physico-chemical parameters. Surface and bottom water samples were collected by using a clean plastic bucket and Niskin water sampler respectively. The temperature was measured by using a thermometer. Salinity was calculated by Mohr-Knudsen titration technique. Water samples were analyzed for nutrients (nitrate, phosphate and silicate) within 6 hours after collection following standard procedures and protocols (Grasshoff *et al.*, 1999).

Chlorophyll a analysis

Chlorophyll *a*, in water samples were determined by filtering the sample through GF/C filter paper and extracting with 90% acetone (Parsons *et al.*, 1984). The mixture is kept for overnight under dark condition. After incubation, the mixture is grinded well and centrifuged at 5000rpm for 15 minutes. The supernatant was used for the pigment analysis using UV visible spectrophotometer (GENESYS 10UV).



Fig.1. Study area in the Cochin estuary showing the station locations

The stations numbered from 1 to 4 are generally fresh water in nature and industrial effluents are relatively less than municipal waste. The stations 5 to 7 lie in the coastline section. Moving together, stations 8 to 10 become estuarine in character and its connection to the Arabian Sea exhibits vivid

Analysis and identification of phytoplankton

For analyzing phytoplankton cell counts and composition, water samples were filtered through a phytoplankton net of 20μ mesh size made of bolting silk. The filtrate was preserved in 3% Lugol's iodine solution. A setting and siphoning

procedure was followed to concentrate samples from 250ml to 20ml (Utermohl, 1958). For counting phytoplankton cells and identification of genera and species, the concentrated samples were thoroughly shaken and from each, 1ml replicates were transferred into a sedge wick-rafter plankton counting chamber and examined by using biological microscope (OLYMPUS; MLX) at 200x magnification. The planktonic micro algae filtered from 100 L of water was made up to a fixed volume concentrate. 1 ml of this sample was transferred to the sedge wick-Rafter counting cell (the volume of this chamber is 1 ml). The number of micro algae present in the cell 1000 grids was calculated. Repeated the counting for three times and took the average. The total number of planktonic algal species present in water sample was calculated using the formula,

$$N = \frac{n * v}{V}$$

N= total number of phytoplankton cell per liter of water filtered; n= average number of phytoplankton cells in 1 ml of plankton sample; v = volume of plankton concentrate (ml); V =volume of total water filtered (L).

DMS analysis

Water samples for DMS were transferred to 60 ml amber colored bottles. Care was taken to avoid atmospheric contact and samples were preserved immediately in the dark at 4° C. Analysis was completed within ten hours. DMS was measured using AGILENT 7890 gas chromatograph equipped with flame photometric detector (FPD). A known volume of sample (10ml) was purged (15min) using nitrogen gas and the stripped sulfur gases were passed through moisture traps (ice bath, glass wool and potassium carbonate). These traps were replaced very frequently. The sulfur gases were cryogenically (liquid nitrogen) trapped in a teflon loop. The loop was then transferred to a water bath, maintained at $>80^{\circ}$ C, for removal of the trapped gases. Separation was done on a DB-5 capillary column. Temperature ramp program was set at initial 800°C for 5 minutes and final 180°C for 25 minutes. DMS calibrations were done using DMS standard (Sigma), ethanol (Merck) and milli-Q water. The retention time of DMS was 2.8min and detection limit 0.05 nM. The linear detection range is from 0.2 nM to 25 nM. The calibration curve with precision of analysis was presented in Figure 2.



Fig. 2. Calibration curve of Dimethyl sulphide (DMS)

RESULTS

Hydrographical parameters and nutrient distribution

Hydrographical parameters serve as nucleus for investigating water quality. Estuaries which are in the brim of oceanic and marine environment undergo rapid changes which are reflected in the quality of water. Sea water intrusion and fresh water mixing pose serious fluctuations in the estuarine ecosystem and as a result hydrodynamic parameters keeps on oscillating. During the study, temperature varied from 32-34.5°C pre monsoon (PRM), 26-34°C post monsoon (POM) and 26-30°C monsoon (MON), where as in bottom it ranges from 31-33°C (PRM), 29-31°C (POM) and 26-27.5°C (MON). The average temperature recorded in the surface was pre monsoon>post monsoon with 33°C, 31°C and 29°C and the bottom also follows the same trend with 32°C, 30°C, 26.6°C respectively (Figure 3).



Fig.3. Distribution pattern of temperature at both surface and bottom waters in three prominant seasons

Levels of nitrate ranges from $3.03-16.74\mu$ mol L⁻¹(MON), $3.7-23.75\mu$ mol L⁻¹(POM) and $1.03-30.99\mu$ mol L⁻¹(PRM) in the surface. The bottom values vary $7.55-38.44\mu$ mol L⁻¹(MON), $2.56-15.72\mu$ mol L⁻¹(POM) and $5.01-26.15\mu$ mol L⁻¹(PRM). The phosphate values showed considerable discrepancies in all seasons and in surface ranges from $0.88-6.56\mu$ mol L⁻¹(MON), $0.83-8.42\mu$ mol L⁻¹(POM) and $1.08-8.12\mu$ mol L⁻¹(PRM). In bottom it fluctuates from $2.15-8.22\mu$ mol L⁻¹(MON), $1.32-7.88\mu$ mol L⁻¹(POM) and $0.54-10.23\mu$ mol L⁻¹(PRM). The surface silicate concentrate varied between $0.06-5.82\mu$ mol L⁻¹(MON), $21.67-92.4\mu$ mol L⁻¹(POM), $8.52-82.16\mu$ mol L⁻¹(PRM) and the bottom between $0.48-4.36\mu$ mol L⁻¹(PRM) (Figure 4).



Fig. 4. Distribution pattern of nutrients (Nitrate, Phosphate and Silicate) at both surface and bottom waters in three prominant seasons

Estuary	Concentration Range (nM)	Average Concentration (nM)	References
Estuary in North America	1-18	-	Iverson et al. 1989
Canal de Mira	0-18	2.9-5.3	Cerqueira & Pio, 1999
Scheldt Estuary	0-2.5	0.4-0.6	Scaire et al. 2002
Zuari Estuary	0.3-15.4	-	Shenoy et al. 2002
Gironde Estuary	0-1.7	0.2-0.7	Min Hu et al. 2005
Elbe Estuary	0-2.5	0.9	Min Hu et al. 2005
Rhine Estuary	0-10	0.2	Min Hu et al. 2005
Loire Estuary	0.5-3.6	1.3	Min Hu et al. 2005
Pearl River Estuary	0.05-56.7	3.0-8.6	Min Hu et al. 2005
Cochin Estuary	0-19.5	-	This Work

Table 1. Comparison of DMS values with other estuaries



Fig. 5. Distribution pattern of DMS, Chl.a and Salinity at both surface and bottom waters in three prominant seasons

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Allocation of DMS, Chlorophyll a and Salinity

The DMS values in the surface estuary ranged from 0.25-1.3nM (MON), 0.2-1.8nM (PRM) and 0-1.81nM (POM) and bottom DMS concentration varied from undetectable levels to 19.5nM (POM), 0.2-3.4nM (PRM) and finally 0.54-3.6nM in (MON). Higher concentration of DMS was observed in the post monsoon season. The elevated concentrations of DMS were observed in the saline regions of the estuary especially station 6, 7, 8 and 9. Comparison of DMS concentration with other estuaries is appended in (Table 1) and the values are in agreement with that of Iverson et al. (1989) and Cerqueira and Pio, 1999. Chlorophyll a, a significant biomarker for assessing phytoplankton biomass was spatially and temporally estimated. The surface concentrations ranges from 0.23-10.44nM (POM), 4.49-33.52nM (PRM) and 0.22-22.24nM (MON) where as in bottom it varies from 0.22-12.14nM (POM), 2.21-15.36nM (PRM) and 0.07-8.24nM (MON). Salinity the foremost key of an estuary in the surface varies in MON (3.48-19.25ppt) where as considerable increase in PRM (2.41-31.26ppt), POM (0.65-26.4ppt) and in bottom (3.49-27.96ppt) in MON, (1.29-31.57ppt) POM, and (1.45-27.45ppt) PRM respectively (Figure 5).

Total phytoplankton biomass

In the present study, a total of 120 species of planktonic microalge were identified within classes 7 viz. bacillariophyceae, chlorophyceae, dinophyceae, chrysophyceae, cyanophyceae, dictyochophyaceae and zygnematophyceae. Qualitative and quantitative analysis of planktonic microalagae reveals bacillariophyceae is the dominant taxa with 65 species and the abundance consist of $(av.57693 \text{ cell/m}^3)$ PRM, $(av.45073 \text{ cell/m}^3)$ MON and (av.40320 cell/m³) POM, followed by *chlorophyceae* with 25 species, dinophyceae with 21 species; dictyochophyceae, cyanophyceae, chrysophyaceae and zygnematophyceae with comparatively low numbers (Table 2 & Figure 6). The dominant diatom species comprised of Skeletonema costatum, Coscinodiscus spp., Thalassiothrix spp., Nitzschia spp., Chaetoceros spp. and Rhizosolenia spp. whereas Ceratium spp., Dinophysis spp., Diplosalis spp., Protoperidinium spp. and Prorocentrum spp. were the dominant dinoflagellates.

Statistical analysis

A statistical analysis were also employed to find the correlation between the different environmental parameters such as temperature, salinity, nitrate, phosphate, silicate, chlorophyll a and DMS using SPSS 13.

DISCUSSION

The phytoplanktons are the major source of DMS production in the marine environment (Kiene *et al.*, 1996). Earlier, Aneeshkumar and Sujatha, 2012 reported that in Cochin estuarine system, fucoxanthin was the most abundant caroteniod pigment which indicates profuse of diatom community. Regional studies in the Coast of Goa (Shenoy *et al.*, 2012), North Sea (Turner *et al.*, 1988) and the East Coast of the U.M.A. (Iverson *et al.*, 1989) have accounted the measurement of regional DMS fluxes and compared these with biological parameters such as phytoplankton biomass and

Table 2. Qualitative identification of phytoplankton records

CHLOROPHYCEAE (25)

Ankistrodesmus falcons Arthodesmus convergens Chlorella sp. Chlorococcum sp. Closterium sp Coelastrum sp. Euastrum sp. Micrasterias foliacea Pediastrum duplex Pediastrum simples Pleodorina sp. Scenedesmus arcuatus Scenedesmus quadicauda Selenastrum gracile Sphaerozosma granulatum Staurastrum asteroideum Staurastrum gracile Staurastrum leptocladium Staurastrum pingue Staurastrum sp. Tetraedron trigonum Tetraspora sp. Ulothrix tenuissima Kuetzing Volvox aureas Ehrenberg Xanthidium antilopaeum **BACILLARIOPHYCEAE (65)** Actinocyclus sp. Achnanthes sp. Amphiprora alata Amphora sp. Asterionella Formosa Asterionella japonica Asteromphalus flabellatus Aulacoseira granulate Bacillaria paradoxa Bacteriastrum varians Biddulphia aurita Biddulphia mobilianis Biddulphia rhombus Biddulphia sp. Cerataulina bergonii Cerataulina pelagic Chaetoceros affinis Chaetoceros coarctatus Chaetoceros decipiens Chaetoceros denticulatum Cheatoceros densus Coscinodiscus asteromphalus Coscinodiscus centralis Coscinodiscus granii Coscinodiscus marginatus Coscinodiscus oculis-iridis Coscinodiscus perforatus Coscinodiscus radiates Coscinodiscus subtilis Cyclotella sp. Cyclotella meneghiana Cyclotella striata Cylindrotheca closteridium Cymbella marina Dityllum brightwelli Dityllum sol Fragilariopsis sp. Gyrosigma sp.

Hemidiscus hardmannianus Hyalodiscus subtilis Leptocylindrus danicus Navicula henneidyi Nitzschia closterium Nitzschia fasiculata Nitzschia longissima Nitzschia marina Nitzschia seriata Nitzschia sigma Pleurosigma directum Pseudonitzschia seriata Rhizosolenia imbricate Rhizosolenia robusta Rhozosolenia styliformis Skeletonema costatum Surirella elegans Surirella sp. Thalassionema nitzschioides Thalassiosira subtilis Thalassiothrix frauenfeldii Thalassiothrix longissima Triceratium affine Triceratium favus Triceratium reticulam Triceratium sp. Tropidoneis sp. DINOPHYCEAE (21) Ceartium breve Ceratium furca Ceratium lineatum Ceratium macroceros Ceratium tripos Dinophysis caudata Dinophysis miles Diplopsalis lenticula Diplosalis acuta Gonyalux sp. Gymnodinium sp. Heterocapsia sp. Noctiluca miliaris Peridinium claudicans Prorocentrum maximum Prorocentrum micans Protoperidinium depressum Protoperidinium oceanic Protoperidinium pellucidum Protoperidinium sp. Scrippsela sp. CYANOPHYCEAE (6) Anabaena sp. Katagnymene spiralis Merismopedia sp. Nostoc colony Tolypothrix sp. Trichodesmium sp. **CHRYSOPHYCEAE** (1) Dinobryon sp. DICTYOCHOPHYCEAE (1) Dictyocha fibula **ZYGNEMATOPHYCEAE (1)** Spirogyra sp.

chlorophyll *a*. In the present study a significant correlations were observed on DMS with chlorophyll *a* (Table 3) in the surface waters of monsoon, post monsoon and pre monsoon season. Similar trends were cited by (Barnard *et al.*, 1982., Tanzer, 1992 and Belviso *et al.*, 1993a). Pingree *et al.*, 1975 reported the high concentration of DMS in surface water and showed a clear association with chlorophyll *a* levels.







Fig. 6. Abundance of phytoplankton species (cells/m³) at three prominant seasons during the study period

The physiological factors such as light intensity and salinity greatly affect the amount of DMS from DMSP produced (Dickson and Krist, 1986., Van Bergeijk et al., 2002). Laboratory studies have shown that intracellular DMSP in marine macroalgae (Dickson et al., 1980., 1982., Reed, 1983) and in phytoplankton (Vairavamurthy et al., 1985) increases with increasing salinity. Similar result was cited by Zhang et al. in (1999). The present study also supports this statement. Salinity favorably affected the DMS production in surface samples of both POM and MON season whereas bottom sample resulted in PRM season. Furthermore, modeling studies also reflects that limited nutrient concentrations favors an increase in DMS concentrations apart from the increase in salinity, chlorophyll a, temperature and light (Laroche et al., 1999). Inadequate positive correlation between DMS and phosphate were observed both in surface (POM) and bottom (PRM) waters during the study period whereas a strong negative correlation were exhibited between DMS and nitrate in (MON) bottom water (Table 3).

DMS concentrations were determined in surface waters at stations 6,7,8,9,11,13,14 and 15 inclusive of riverine, estuarine and coastal system in all the seasons. However, the maximum concentration of DMS (19.5 nM) was found at station 7 during post monsoon season at bottom water. Impact of stratification may be the reason for higher salinity which inturn leads to high DMS values (Ramamirtham et al., 1986) and enrichment of DMS producing phytoplankton species. The major identified DMS producing species include: Skeletonema costatum, Cylindrotheca closterium, Thalassiosira snn Rhizosolenia spp., Heterocapsa spp., Prorocentrum minimum, Prorocentrum micans and Scrippsiella spp. This observation well in support with the results of previous studies conducted by Keller et al. in 1989, and investigated the DMS production in some strains of marine phytoplankton such as dinoflagellates (Ceratium spp., *Heterocapsa* spp., Prorocentrum spp. and Scrippsiella spp.), and diatoms (Skeletonema costatum, Thalassiosira spp., Rhizosolenia spp., Cylindrotheca closterium and Nitzschia spp.). Besides, the

Table 3. Correlation analysis of DMS with other environmental variables at three different seasons in both surface and bottom waters

MON-Surface	Variables	DMS	Chl. a	Salinity	NO ₃	PO_4	SiO ₃	Temp.
	DMS	1	.560(*)	.626(*)	.180	.365	324	324
	Chl. a		1	.401	.101	089	262	617(*)
	Salinity			1	082	.188	126	713(**)
	NO ₃				1	.135	.338	040
	PO_4					1	174	.046
	SiO_3						1	116
	Temp.							1
MON-Bottom	DMS	1	.061	141	682(**)	108	.389	.508
	Chl. a		1	.297	173	.276	.233	.160
	Salinity			1	239	.440	.056	.427
	NO_3				1	112	779 (**)	321
	PO_4					1	038	.192
	SiO_3						1	151
	Temp.							1
POM-Surface	DMS	1	.523(*)	.806(**)	316	.594(*)	.237	.365
	Chl. a		1	.393	402	.128	.098	.120
	Salinity			1	343	.598(*)	.289	.270
	NO ₃				1	.038	.060	.469
	PO_4					1	.187	.250
	SiO ₃						1	.018
	Temp.							1
POM-Bottom	DMS	1	115	.457	149	235	.027	103
	Chl. a		1	454	259	185	.311	119
	Salinity			1	492	.064	360	484
	NO ₃				1	.052	004	.426
	PO_4					1	.102	306
	S1O ₃						1	.078
DDMC	Temp.	1	50 4 (*)	411	222	0.50	121	1
PRM-Surface	DMS	1	.594(*)	.411	232	052	.131	.078
	Chl. a		1	024	393	276	216	044
	Samity			1	3/1	000	.199	.428
	NO ₃				1	.255	.185	479
						1	.459	058
	SIO_3						1	.544
DDM Dottom	DMS	1	447	517(*)	022	504(*)	207	109
PKM-DOII0III	Chla	1	.447	.517(*)	.025	.594 (*)	307	.198
	Cill. a		1	.420	311 576(*)	.112	002	.034
	NO			1	570(*)	.309	192	.118
					1	1/8	1/5	039
	104 SiO					1	010	003
	SIU3 Temp						1	091(***) 1
	remp.							1

species composition of phytoplankton is also a determining factor for the DMS production in an aquatic system (Groene, 1992) and our studies revealed that *Skeletonema coastatum*, an ubiquitous species enriched in all the seasons at saline stations.

Conclusion

The present research work provides baseline information on the distributional characteristics of the DMS related hydrography, nutrients, biomass and taxonomic composition of the phytoplankton. Microscopic observation of phytoplankton cell counts points that in general the diatom community dominated and the abundant groups in terms of species diversity and density rather than other taxonomic groups. Results obtained in this study suggest that the production of DMS was species specific and influenced by different growth stages of algae. Moreover the salinity conditions also displayed the physiological and ecological complexity of the DMS production. There was no methodical drift in the DMS values; yet reviewing of this all pervading gas becomes unique in nature due to its intervention with global climate.

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