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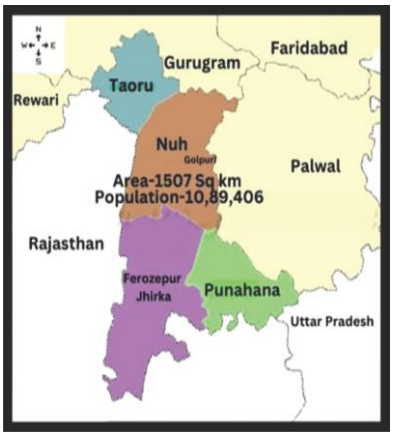

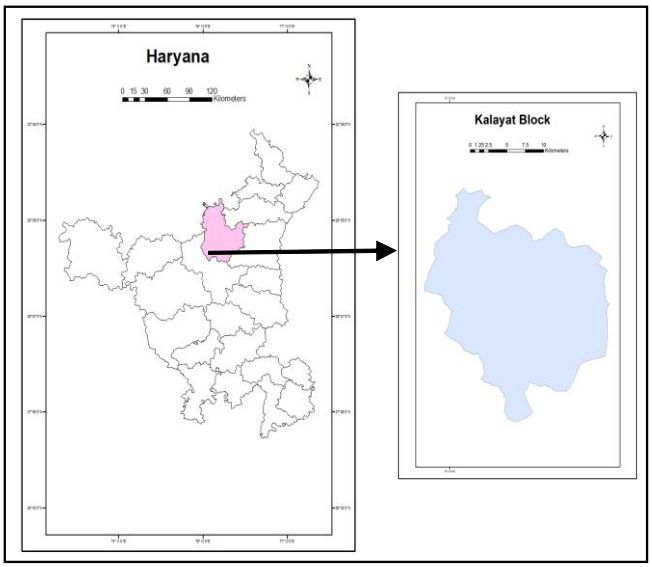

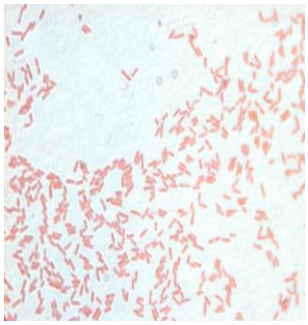

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The IJEHS is an official publication of Save The Environment (STE). It publishes peer reviewed quarterly, original articles (Research paper, Review articles, Short Communication, Case studies, etc.) related to all fields of Environment and Health Sciences. It disseminates the scientific research and recent innovations.

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International Journal of Environment and Health Sciences

From The Editor's Desk…

As we welcome the New Year 2022, the time has come to work together for creating a sustainable and environment-friendly earth around us by making the most of this recovery phase. New policies are being formulated for improving air, soil and water quality which will further improve the health status of public as well as the environment quotient. Undoing the economic losses and health crisis incurred in the past two years, by implementing more responsible actions will be the main pledge.

One important aspect of the 75th year of Indian independence under 'Azaadi ka Amrit Mahotsav' theme has been designated as repurposing natural compounds for therapeutic functions by harnessing the vast knowledge about traditional medical systems available in our ancient texts. Also, another major focus will be necessitating agricultural reforms in order to reduce gaps in crop production, while ensuring benefits of farmers, who are one of the most important pillars of nation-building.

In view of this, all of us have to act more responsibly by 'life management' such that we move a step closer towards achieving the goal of sustainability, as suggested by The United Nations.

Striving to achieve the aforesaid, The International Journal of Environment and Health Sciences (IJEHS) proposes to provide a reliable platform to discuss relevant technologies and strategies. IJEHS will be quintessential to academicians, industry professionals and researchers who are actively engaged in the areas of environmental issues and related health effects. We are pleased to inform that ISSN for IJEHS is available as 2582-5283. IJEHS is referenced in Crossref, the official Digital Object Identifier Agency (doi 10.47062). IJEHS is now also indexed in the International Scientific Indexing (ISI).

We invite original research articles, short communications and critical reviews directed towards an academic, clinical and industrial audience. The first section of the journal focuses on burning environmental issues like pollutants and their fate, waste management, resource conservation, remediation technologies, etc. The second section includes all topics relevant to physiological impact of environmental risk factors and application of alternative medicinal approaches as remedial measures. Detailed scope can be found in the home page of the journal (www.stenvironment.org/journals). Notes on development of any novel and validated strategy or tool to address environmental challenges are welcome. Discussion on proceedings of conferences conducted on environmental themes and related health aspects will also be considered.

All submissions will be meticulously scrutinized by pioneers in the field to ensure publication of only articles of high quality and relevance. Authors are requested to take special precautions to avert plagiarism and redundancy. It is high time that we realize the gravity of circumstances and take potent steps to undo the adversities already triggered. In this pursuit, IJEHS expects to be the ideal platform to discuss sustainable ideas and potential solutions.

We thank all authors who have contributed to the journal and have consistently been with us in the past years. With this, I wish all our readers a Very Happy New Year, 2022 and I hope our audience and patrons shall come together in this effort to promulgate their part in resurrecting our valuable environment.

Dr. Kshipra Misra
Editor-in-Chief, IJEHS

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ASSESSMENT OF GROUNDWATER QUALITY FOR DRINKING PURPOSE IN KALAYAT BLOCK, KAITHAL DISTRICT, HARYANA

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Abstract
Water is important for survival of life. Monitoring of drinking water quality is necessary for checking water related health problems. Anthropogenic activities have deteriorated quality of surface water and groundwater. Excessive use of groundwater for irrigation and industrial uses lead to the declining of groundwater depth and quality deterioration. The present study area Kalayat block is located in Kaithal district, Haryana. Kaithal block in Kaithal district. The geo-coordinates of the study area are latitudes 29.55^0 N to 29.79^0 N and longitudes 76.17^{0}E to 76.39^{0}E and covers an area of 342.68 sq. km. Geologically alluvium and geomorphologically alluvial plain are present. The main objective was to study groundwater quality for drinking purpose in the study area. In the study area nine groundwater samples were collected in 250 ml double capped plastic bottles from tube wells and hand pump. Geo-coordinates of sample locations were noted with the help of mobile GPS. Chemical analysis of nine groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board, Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids, fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine. Results of groundwater samples analysis were compared with BIS (IS 10500:2012) drinking water standards to know groundwater quality for drinking purpose. In the study area pH ranges 6.5 to 9, alkalinity 150 mg/l to 600 mg/l, hardness 140 mg/l to 1120 mg/l, chloride 30 mg/l to 730 mg/l, TDS 396 mg/l to 2904 mg/l, fluoride 1.0 mg/l to 5.0 mg/l, iron nil in all the nine groundwater samples, ammonia nil to 2.0 mg/l, nitrite 0.2 mg/l to 1.0 mg/l, nitrate 45 mg/l to 100 mg/l, phosphate nil in all the nine groundwater samples and residual chlorine nil to 0.2 mg/l. The study is highly useful for planning and monitoring of groundwater quality in the study area.

Keywords
Groundwater, quality assessment, drinking, Kalayat, Kaithal, Haryana.

INTRODUCTION

Water is important for survival of life on the planet Earth. Excessive use of water drinking due to increasing population, industrialization and agriculture practices leads to decline of availability of water per capita. Monitoring of drinking water quality is necessary to check the water related health problems like fluorosis, methemoglobinemia. Aghazadeh et al. (2010), Spanos et al. (2014), Hanumantharao et al. (2019), Kumar et al. (2015), Punia et al. (2015), Choudhary et al. (2016), Vijaya Lalitha et al. (2017), Sinha et al. (2018) studied groundwater quality for drinking purpose in different types of areas.

STUDY AREA

Kalayat block is located in Kaithal district, Haryana (Fig.1). The geo-coordinates of the study area are latitudes 29.550 N to 29.790 N and longitudes 76.170E to 76.390E and covers an area of 342.68 sq. km. Geologically alluvium and geomorphologically alluvial plain are present.

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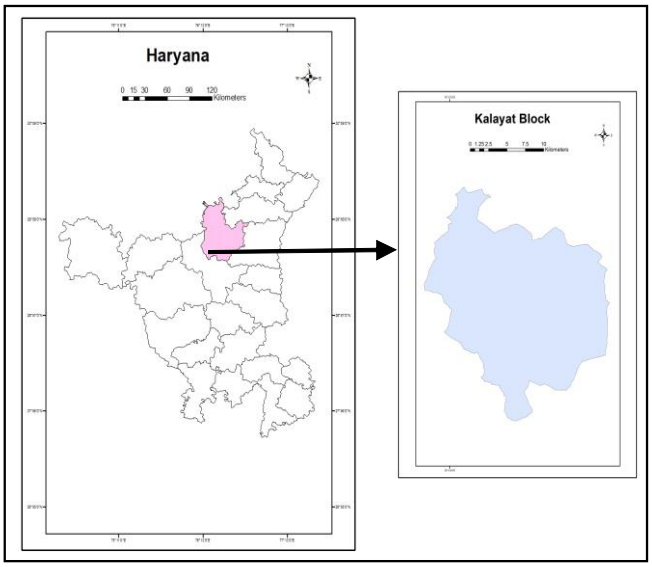

Fig.1: Location map of the study area.

OBJECTIVE

The main objective was to study groundwater quality for drinking purpose in the study area.

MATERIALS AND METHODOLOGY

In the study area nine groundwater samples were collected in 250 ml double capped plastic bottles from tube wells and hand pump. Geo-coordinates of sample locations were noted with the help of mobile GPS. Chemical analysis of nine groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board, Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine (Table 1). Chemical analysis of groundwater samples data were entered in excel software and prepared bar graphs for each chemical parameter. Result of groundwater samples analysis was compared with BIS (IS 10500:2012) drinking water standards (Table 2) to know groundwater quality for drinking purpose

Table1: Results of groundwater samples analysis in the study area

S. No.	Sample Location	Latitude	Longitude	Source	pH	Alkalinity (mg/l)	Hardness (mg/l)	Chloride (mg/l)	TDS (mg/l)	Fluoride (mg/l)	Iron (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Phos-phate (mg/l)	Residual Chlorine (mg/l)
1	Simla	29.64	76.22	TW	7	200	140	50	468	5	0	0	0.5	100	0	0
2	Batta	29.69	76.29	TW	8	390	480	500	1371	5	0	2	1.0	100	0	0
3	Kurar	29.72	76.19	TW	7.5	310	300	100	852	5	0	1	0.2	45	0	0
4	Dubbal	29.74	76.22	TW	7.5	280	200	300	936	3	0	0	0.2	75	0	0.2
5	Kailram	29.71	76.36	TW	8	380	390	180	1140	3	0	0.5	0.5	75	0	0
6	Mator	29.62	76.26	TW	8	600	1120	700	2904	1	0	1	0.5	75	0	0
7	Vajir Nagar	29.68	76.34	HP	6.5	150	150	30	396	2	0	1	0.5	100	0	0
8	Kheri Lamba (I)	29.69	76.23	TW	7	200	970	730	2280	1.5	0	0.5	0.2	45	0	0
9	Kheri Lamba (ii)	29.69	76.23	TW	9	550	300	400	1500	5	0	0	0.5	75	0	0

Table 2: BIS drinking water standards (IS: 10500:2012)

Sl. No.	Parameters	Potable		Non potable
		Desirable	Permissible	
1	pH	6.5-8.5	-	<6.5 and >8.5
2	Alkalinity (mg/l)	200	200-600	>600
3	Hardness (mg/l)	200	200-600	>600
4	Chloride (mg/l)	250	250-1000	>1000
5	Total Dissolved Solids (mg/l)	500	500-2000	>2000
6	Fluoride (mg/l)	<1.0	1.0-1.5	>1.5
7	Iron (mg/l)	<0.3	-	>0.3
8	Ammonia (mg/l)	<0.5	-	>0.5
9	Nitrite (mg/l)	<0.1	-	>1.0
10	Nitrate (mg/l)	<45	-	>45
11	Phosphate (mg/l)	<1.0	-	>1.0
12	Residual Chlorine (mg/l)	<0.2	0.2-1.0	>1.0

RESULTS AND DISCUSSION

i. pH

In the study area pH ranges 6.5 to 9 (Table 1, Fig.2). As per BIS (IS 10500:2012) drinking water standards pH is desirable between 6.5 to 8.5 and non-potable if less than 6.5 and more than 8.5 (Table 2). pH is desirable in eight groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram, Mator,Vajir Nagar, Kheri Lamba (i) and non-potable in one groundwater sample (Kheri Lamba (ii)).

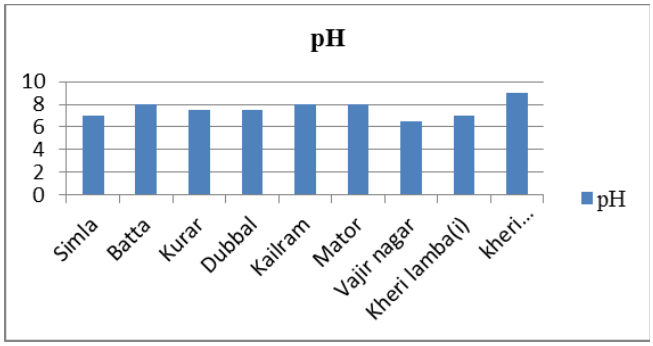

Fig.2: pH in groundwater samples.

ii. Alkalinity

In the study area alkalinity ranges 150 mg/l to 600 mg/l (Table 1, Fig.3). As per BIS (IS 10500:2012) drinking water standards alkalinity is desirable if less than 200 mg/l, permissible between 200 mg/l-600 mg/l and non-potable if more than 600 mg/l (Table 2). Alkalinity is desirable in one groundwater sample (Vajir Nagar) and permissible in eight groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram, Mator, Kheri Lamba (i) and Kheri Lamba (ii)).

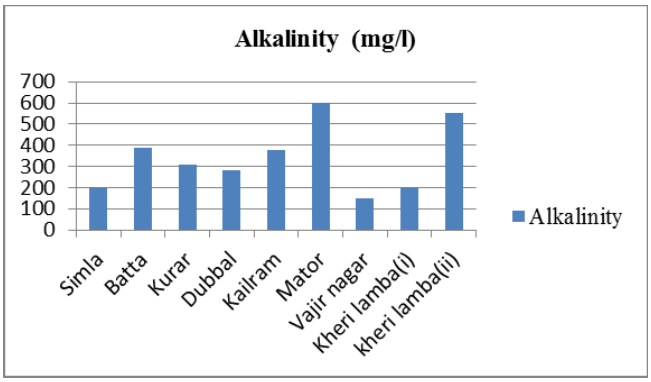

Fig.3: Alkalinity in groundwater samples .

iii. Hardness

In the study area hardness ranges 140 mg/l to 1120 mg/l (Table 1, Fig.4). As per BIS (IS 10500:2012) drinking water standards hardness is desirable if less than 200 mg/l, permissible between 200 mg/l - 600 mg/l and non-potable if more than 600 mg/l (Table 2). Hardness is desirable in two groundwater samples (Simla, Vajir Nagar),permissible in five groundwater samples (Batta, Kurar, Dubbal, Kailram, Kheri Lamba (ii) and non-potable in two groundwater samples (Mator, Kheri Lamba (i)).

iv. Chloride

In the study area chloride ranges 30 mg/l to 730 mg/l (Table 1, Fig.5). As per BIS (IS 10500:2012) drinking water standards chloride is desirable if less than 250 mg/l, permissible between 250 mg/l-1000 mg/l and non-potable if more than 1000 mg/l (Table 2). Chloride is desirable in four groundwater samples (Simla, Kurar, Kailram,Vajir Nagar) and permissible in five groundwater samples (Batta, Dubbal, Mator, Kheri Lamba (i), Kheri Lamba (ii)).

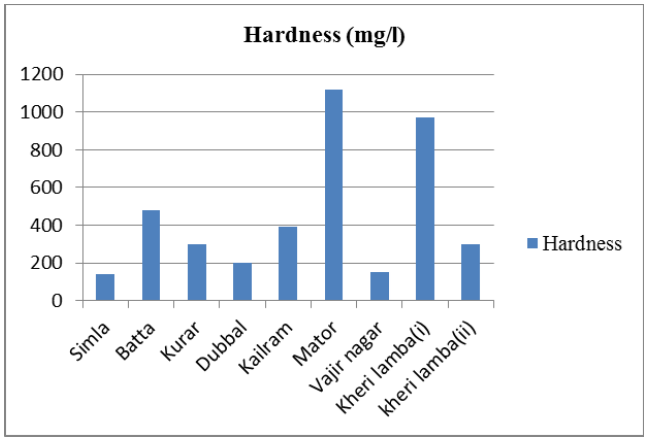

Fig.4: Hardness in groundwater samples.

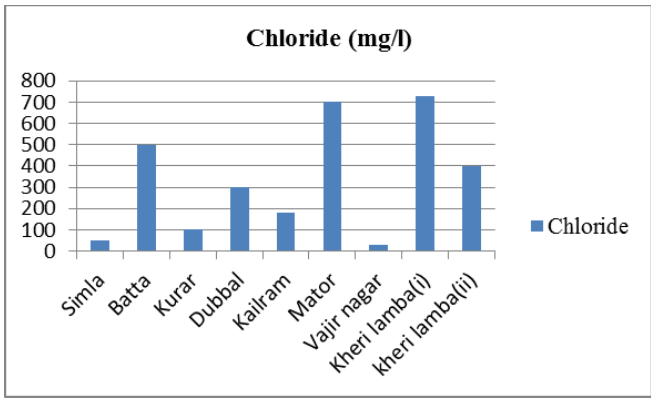

Fig. 5: Chloride in groundwater samples.

v. Total Dissolved Solids

In the study area TDS ranges 396 mg/l to 2904 mg/l (Table 1, Fig.6). As per BIS (IS 10500:2012) drinking water standards TDS is desirable if less than 500 mg/l, permissible between 500 mg/l-2000 mg/l and non-potable if more than 2000 mg/l (Table 2). TDS is desirable in two groundwater samples (Simla, Vajir Nagar), permissible in five groundwater samples (Batta, Kurar, Dubbal, Kailram, Kheri Lamba (ii) and non-potable in two groundwater samples (Mator, Kheri Lamba (I)).

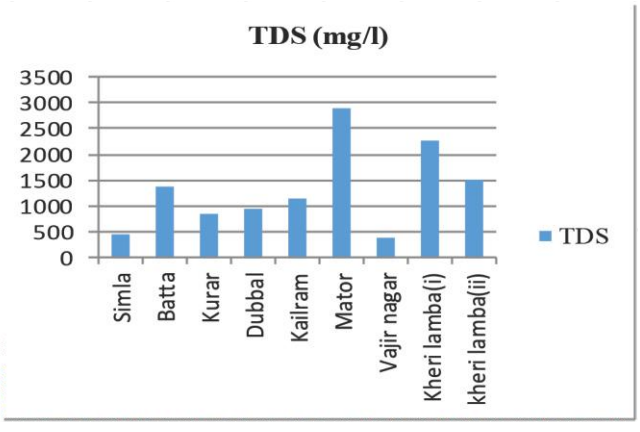

Fig. 6: TDS in groundwater samples.

vi. Fluoride

In the study area fluoride ranges 1.0 mg/l to 5.0 mg/l (Table 1, Fig.7). As per BIS (IS 10500:2012) drinking water standards fluoride is desirable if less than 1.0 mg/l, permissible between 1.0 mg/l-1.5 mg/l and non-potable if more than 1.5 mg/l (Table 2). Fluoride is permissible in two groundwater samples (Mator, Kheri Lamba (i) and non-potable in seven groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram,Vajir Nagar, Kheri Lamba (ii)).

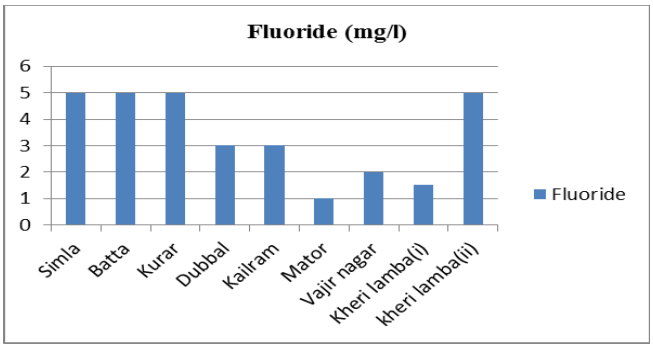

Fig. 7: Fluoride in groundwater samples.

vii. Iron

In the study area iron is nil in all the nine groundwater samples (Table 1, Fig.8). As per BIS (IS 10500:2012) drinking water standards iron is desirable if less than 0.3 mg/l and non-potable if more than 0.3 mg/l (Table 2). Iron is desirable in all the nine groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram, Mator, Vajir Nagar, Kheri Lamba (i), Kheri Lamba (ii)).

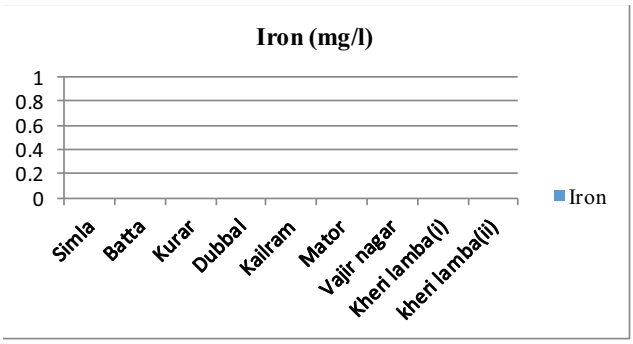

Fig. 8: Iron in groundwater samples.

viii. Ammonia

In the study area ammonia ranges nil to 2.0 mg/l (Table 1, Fig.9). As per BIS (IS 10500:2012) drinking water standards ammonia is desirable if less than 0.5 mg/l and non-potable if more than 0.5 mg/l (Table 2). Ammonia is desirable in five groundwater samples (Simla, Dubbal, Kailram, Kheri Lamba (i), Kheri Lamba (ii) and non-potable in four groundwater samples (Batta, Kurar, Mator, Vajir Nagar).

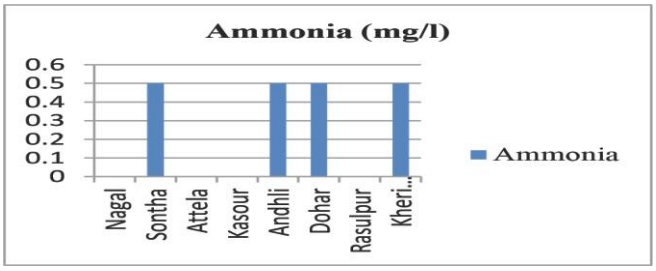

Fig. 9: Ammonia in groundwater samples.

ix. Nitrite

In the study area nitrite ranges 0.2 mg/l to 1.0 mg/l (Table 1, Fig.10). As per BIS (IS10500:2012) drinking water standards nitrite is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Nitrite is desirable in all the nine groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram, Mator,Vajir Nagar, Kheri Lamba (i), Kheri Lamba (ii)).

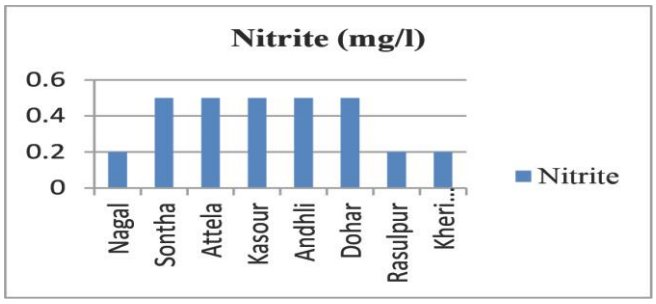

Fig.10: Nitrite in groundwater samples.

x. Nitrate

In the study area nitrate ranges 45 mg/l to 100 mg/l (Table 1, Fig.11). As per BIS (IS 10500:2012) drinking water standards nitrate is desirable if less than 45 mg/l and non-potable if moer than 45 mg/l (Table 2). Nitrate is desirable in two groundwater samples (Kurar, Kheri Lamba (i)) and non-potable in seven groundwater samples (Simla, Batta, Dubbal, Kailram, Mator, Vajir Nagar, Kheri Lamba (ii)).

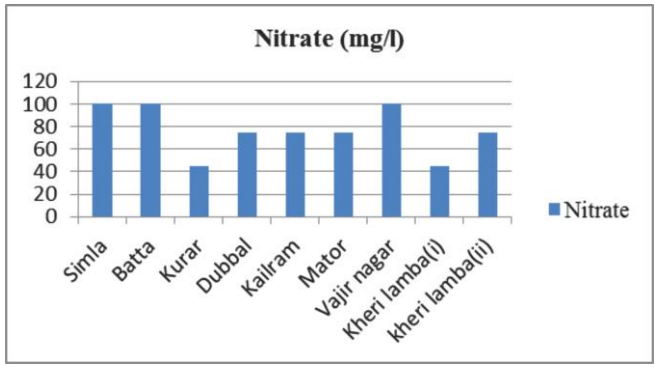

Fig. 11: Nitrate in groundwater samples.

xi. Phosphate

In the study area phosphate is nil in all the nine groundwater samples (Table 1, Fig.12). As per BIS (IS 10500:2012) drinking water standards phosphate is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Phosphate is desirable in all the nine groundwater samples (Simla, Batta, Kurar, Dubbal, Kailram, Mator,Vajir Nagar, Kheri Lamba (i), Kheri Lamba (ii)).

xii. Residual Chlorine

In the study area residual chlorine ranges nil to 0.2 mg/l (Table 1, Fig.13). As per BIS (IS 10500:2012) drinking water standards residual chlorine is desirable if less than 0.2 mg/l, permissible between 0.2 mg/l-1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Residual Chlorine is desirable in eight groundwater samples (Simla, Batta, Kurar, Kailram, Mator, Vajir Nagar, Kheri Lamba (i), Kheri Lamba (ii)) and permissible in one groundwater sample (Dubbal).

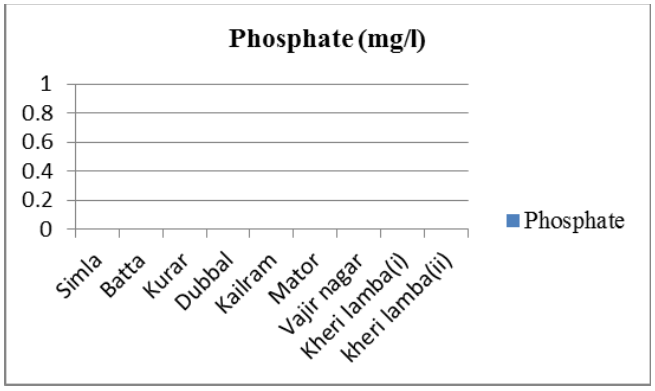

Fig.12: Phosphate in groundwater samples.

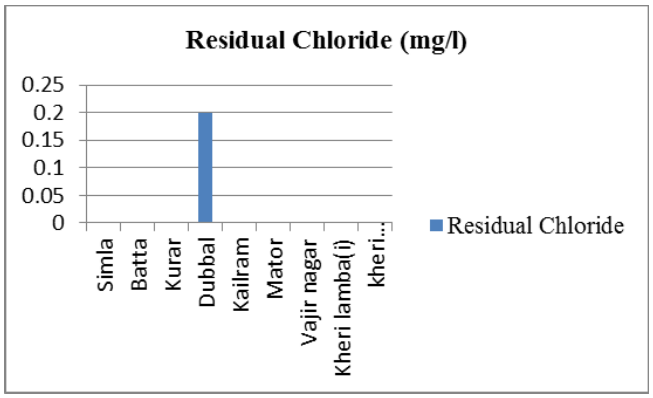

Fig.13: Residual Chlorine in groundwater samples.

CONCLUSIONS

In the study area pH is desirable in eight groundwater samples and non-potable in one groundwater sample. Alkalinity is desirable in one groundwater sample and permissible in eight groundwater samples. Hardness is desirable in two groundwater samples, permissible in five groundwater samples and non-potable in two groundwater samples. Chloride is desirable in four groundwater samples and permissible in five groundwater samples. TDS is desirable in two groundwater samples, permissible in five groundwater samples and non-potable in two groundwater samples. Fluoride is permissible in two groundwater samples and non-potable in seven groundwater samples. Iron, nitrite and phosphate are desirable in all the nine groundwater samples. Ammonia is desirable in five groundwater samples and non-potable in four groundwater samples. Nitrate is desirable in two groundwater samples and non-potable in seven groundwater samples. Residual Chlorine is desirable in eight groundwater samples and permissible in one groundwater sample. This study is highly useful for planning and monitoring of groundwater quality in the study area.

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ASSESSMENT OF GROUNDWATER QUALITY FOR DRINKING PURPOSE IN SIWAN BLOCK, KAITHAL DISTRICT, HARYANA

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Abstract
The study area Siwan block is located in Kaithal district of Haryana state. The geo-coordinates of the study area are latitudes 29.83^0 **N** to 29.99^0**N** and longitudes 76.17^0 E to 76.45^0 E and covers an area of 291.49 sq. km. Geologically alluvium and geomorphologically alluvial plain are present. The main objective was to assess groundwater quality for drinking purpose in the study area. In the study area eight groundwater samples were collected in 250 ml double capped plastic bottles from tube wells. Geo-coordinates of sample locations were noted with the help of mobile GPS. Chemical analysis of eight groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board, Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine. Results of groundwater samples analysis were compared with BIS (IS 10500:2012) drinking water standards to know groundwater quality for drinking purpose. In the study area pH ranges 7 to 8, alkalinity 260 mg/l to 510 mg/l, hardness 70 mg/l to 340 mg/l, chloride 50 mg/l to 70 mg/l, TDS 480 mg/l to 996 mg/l, fluoride 1.0 mg/l to 5.0 mg/l, iron nil in all the eight groundwater samples, ammonia nil to 0.5 mg/l, nitrite 0.2 mg/l to 0.5 mg/l, nitrate 75 mg/l to 100 mg/l, phosphate and residual chlorine nil in all the eight groundwater samples. The study is highly useful for planning and monitoring of groundwater quality in the study area.

Keywords
Groundwater, quality, drinking, Siwan, Kaithal, Haryana.

INTRODUCTION

Water is important for survival of living beings, irrigation and industrial uses. Increasing population, industrailisation and irrigation practices have put pressure on the availability and quality of water. Groundwater is available, hence, more vulnerable to quality deterioration due to anthropogenic activities. In agriculture dominant areas farmers are using manures and herbicides in excess which ultimately deteriorated the groundwater quality. Many workers (Sarala et al. (2012), Singh and Kumar (2014), Spanos et al. (2014), Annapoorna and Janardhana (2015), Punia et al. (2015), Choudhary et al. (2016), Vijaya Lalitha et al. (2016), Lalitha et al. (2017), Madhav et al. (2018)) had done work on groundwater quality assessment for drinking purpose in different areas.

STUDY AREA

The study area Siwan block is located in Kaithal district of Haryana (Fig.1). The geo-coordinates of the study area are latitudes 29.83^0 **N** to 29.99^0 **N** and longitudes 76.17^0 E to 76.45^0 E and covers an area of 291.49 sq. km. In the study area geologically alluvium and geomorphologically alluvial plain are present.

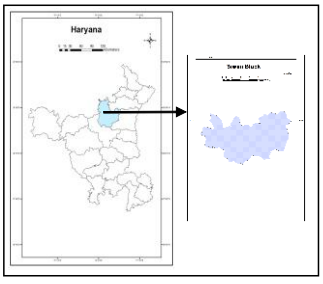

Fig.1: Location map of the study area.

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OBJECTIVE

The main objective of the study was to assess groundwater quality for drinking purpose in the study area.

MATERIALS AND METHODOLOGY

In the study area eight groundwater samples were collected in 250 ml double capped plastic bottles from tube wells (TW). Geo-coordinates of sample locations were noted with the help of mobile GPS. Chemical analysis of eight groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board, Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine (Table 1). Chemical analysis of groundwater samples were entered in excel software and pprepared bar graphs for each chemical parameter. Results of groundwater samples analysis were compared with BIS (IS 10500:2012) drinking water standards (Table 2) to know groundwater quality for drinking purpose.

Table 1: Results of groundwater samples analysis in the study area.

S. No.	Sample Location	Latitude	Longitude	Source	pH	Alkalinity (mg/l)	Hardness (mg/l)	Chloride (mg/l)	TDS (mg/l)	Fluoride (mg/l)	Iron (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Phos-phate (mg/l)	Residual Chlorine (mg/l)
1	Nagal	29.89	76.28	TW	7	330	230	50	732	1.5	0	0	0.2	75	0	0
2	Sontha	29.92	76.34	TW	7.5	290	240	50	696	1	0	0.5	0.5	75	0	0
3	Attela	29.85	76.29	TW	8	370	270	70	852	1	0	0	0.5	100	0	0
4	Kasour	29.96	76.22	TW	7.5	270	270	70	828	1.5	0	0	0.5	75	0	0
5	Andhli	29.91	76.25	TW	7.5	260	200	70	624	1.5	0	0.5	0.5	75	0	0
6	Dohar	29.87	76.43	TW	8	510	140	70	864	5	0	0.5	0.5	75	0	0
7	Rasulpur	29.92	76.42	TW	7	290	70	70	480	1.5	0	0	0.2	75	0	0
8	Kheri Gulamali	29.88	76.30	TW	7.5	420	340	70	996	2	0	0.5	0.2	75	0	0

Table 2: BIS Drinking water standards (IS 10500:2012).

Sl. No.	Parameters	Potable		Non potable
		Desirable	Permissible	
1.	pH	6.5-8.5	-	<6.5 and >8.5
2.	Alkalinity (mg/l)	200	200-600	>600
3.	Hardness (mg/l)	200	200-600	>600
4.	Chloride (mg/l)	250	250-1000	>1000
5.	Total Dissolved Solids (mg/l)	500	500-2000	>2000
6.	Fluoride (mg/l)	<1.0	1.0-1.5	>1.5
7.	Iron (mg/l)	<0.3	-	>0.3
8.	Ammonia (mg/l)	<0.5	-	>0.5
9.	Nitrite (mg/l)	<0.1	-	>1.0
10.	Nitrate (mg/l)	<45	-	>45
11.	Phosphate (mg/l)	<1.0	-	>1.0
12.	Residual Chlorine (mg/l)	<0.2	0.2-1.0	>1.0

RESULTS AND DISCUSSION

i. pH

In the study area pH ranges 7 to 8 (Table 1, Fig.2). As per BIS (IS 10500:2012) drinking water standards pH is desirable between 6.5 to 8.5 and non-potable if less than 6.5 and more than 8.5 (Table 2). pH is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

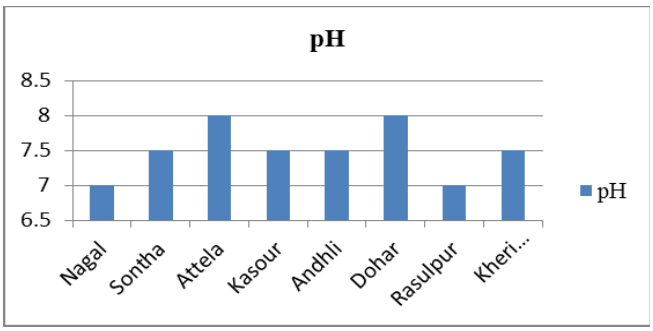

Fig.2: pH in groundwater samples.

ii. Alkalinity

In the study area alkalinity ranges 260 mg/l to 510 mg/l (Table 1, Fig.3). As per BIS (IS 10500:2012) drinking water standards alkalinity is desirable if less than 200 mg/l, permissible between 200 mg/l-600 mg/l and non-potable if more than 600 mg/l (Table 2). Alkalinity is permissible in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

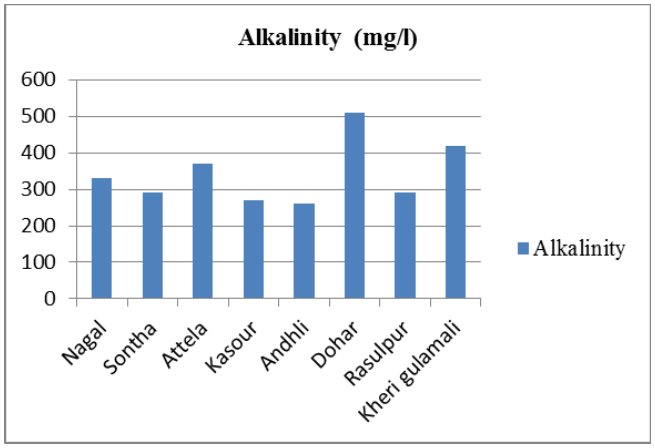

Fig. 3: Alkalinity in groundwater samples.

iii. Hardness

In the study area hardness ranges 70 mg/l to 340 mg/l (Table 1, Fig.4). As per BIS (IS 10500:2012) drinking water standards hardness is desirable if less than 200 mg/l, permissible between 200 mg/l-600 mg/l and non-potable if more than 600 mg/l (Table 2). Hardness is desirable in two groundwater samples (Dohar, Rasulpur) and permissible in six groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Kheri Gulamali).

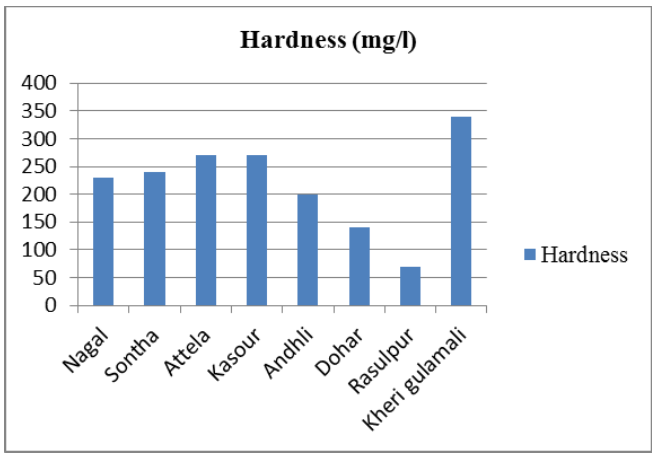

Fig.4: Hardness in groundwater samples.

iv. Chloride

In the study area chloride ranges 50 mg/l to 70 mg/l (Table 1, Fig.5). As per BIS (IS 10500:2012) drinking water standards chloride is desirable if less than 250 mg/l, permissible between 250mg/l-1000 mg/l and non-potable if more than 1000 mg/l (Table 2). Chloride is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

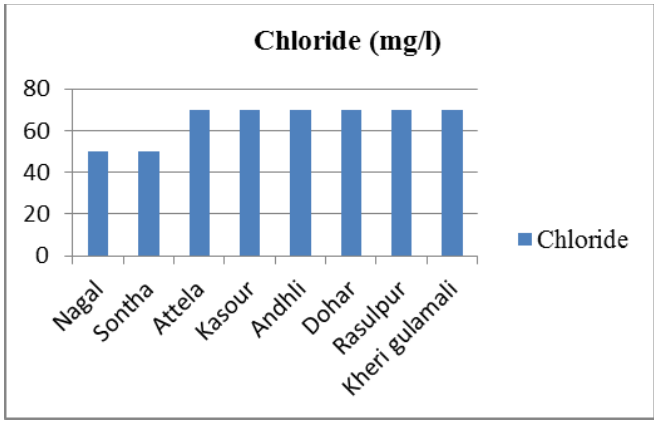

Fig. 5: Chloride in groundwater samples.

v. Total Dissolved Solids (TDS)

In the study area TDS ranges 480 mg/l to 996 mg/l (Table 1, Fig.6). As per BIS (IS 10500:2012) drinking water standards TDS is desirable if less than 500 mg/l, permissible between 500mg/l-2000 mg/l and non-potable if more than 2000 mg/l (Table 2). TDS is desirable in one groundwater sample (rasulpur) and permissible in seven groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Kheri Gulamali).

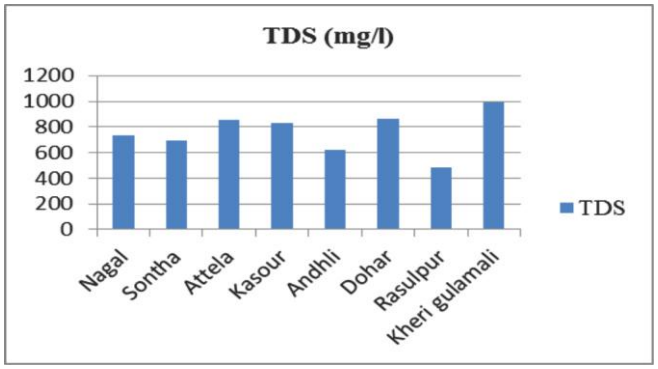

Fig. 6: TDS in groundwater samples.

vi. Fluoride

In the study area fluoride ranges 1.0 mg/l to 5.0 mg/l (Table 1, Fig.7). As per BIS (IS 10500:2012) drinking water standards fluoride is desirable if less than 1.0 mg/l, permissible between 1.0 mg/l -1.5 mg/l and non-potable if more than 1.5 mg/l (Table 2). Fluoride is permissible in six groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Rasulpur) and non-potable in two groundwater samples (Dohar (5 mg/l), Kheri Gulamali (2 mg/l)).

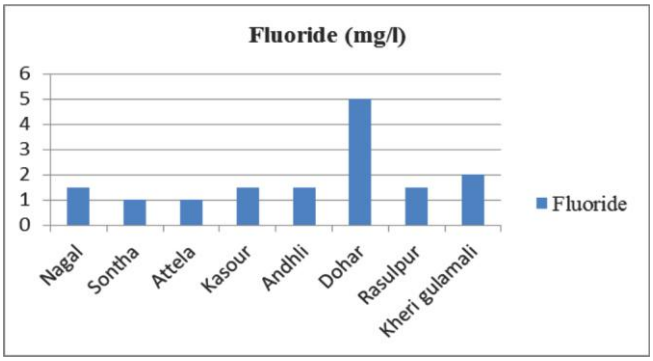

Fig.7: Fluoride in groundwater samples.

vii. Iron

In the study area iron is nil in all the eight groundwater samples (Table 1, Fig.8). As per BIS (IS 10500:2012) drinking water standards iron is desirable if less than 0.3 mg/l and non-potable if more than 0.3 mg/l (Table 2). Iron is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

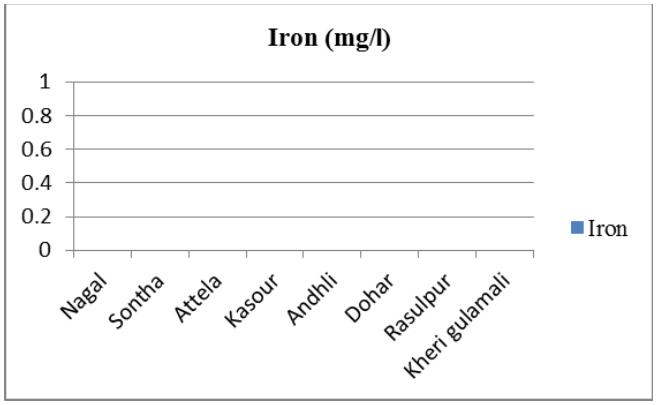

Fig. 8: Iron in groundwater samples.

viii. Ammonia

In the study area ammonia ranges nil to 0.5 mg/l (Table 1, Fig.9). As per BIS (IS 10500:2012) drinking water standards ammonia is desirable if less than 0.5 mg/l and non-potable if more than 0.5 mg/l (Table 2).Ammonia is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

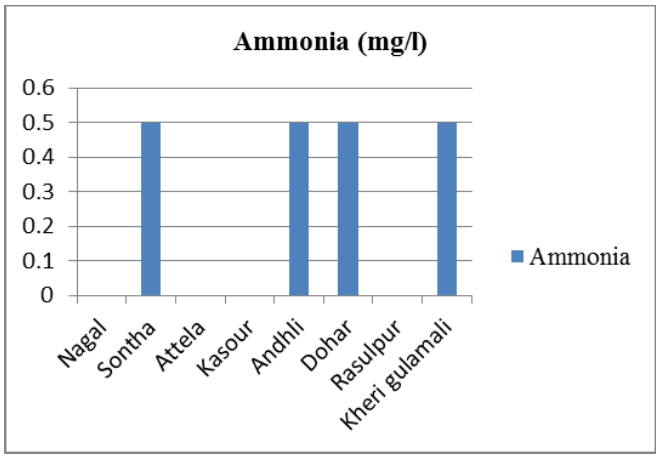

Fig.9: Ammonia in groundwater samples.

ix. Nitrite

In the study area nitrite ranges 0.2 mg/l to 0.5 mg/l (Table 1, Fig.10). As per BIS (IS 10500:2012) drinking water standards nitrite is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Nitrite is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

x. Nitrate

In the study area nitrate ranges 75 mg/l to 100 mg/l (Table 1, Fig.11). As per BIS (IS 10500:2012)drinking water standards nitrate is desirable if less than 45 mg/l and non-potable if more than 45 mg/l (Table 2). Nitrate is non-potable in all eight groundwater samples (Nagal (75 mg/l), Sontha (75 mg/l), Attela (100 mg/l), Kasour(75 mg/l), Andhli (75 mg/l), Dohar (75 mg/l), Rasulpur (75 mg/l), Kheri Gulamali (75 mg/l)).

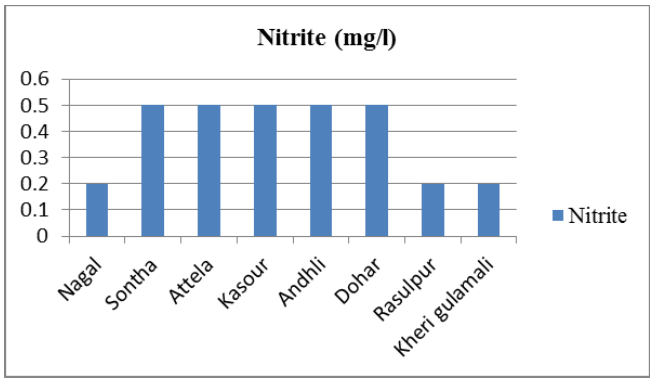

Fig.10: Nitrite in groundwater samples.

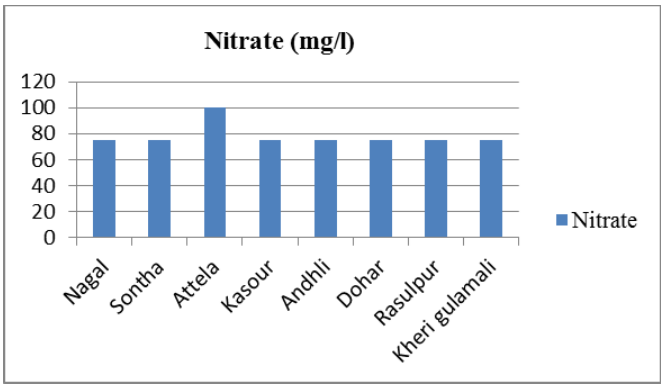

Fig.11: Nitrate in groundwater samples.

xi. Phosphate

In the study area phosphate is nil in all the eight groundwater samples (Table 1, Fig.12). As per BIS (IS 10500:2012) drinking water standards phosphate is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Phosphate is desirable in all eight groundwater samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

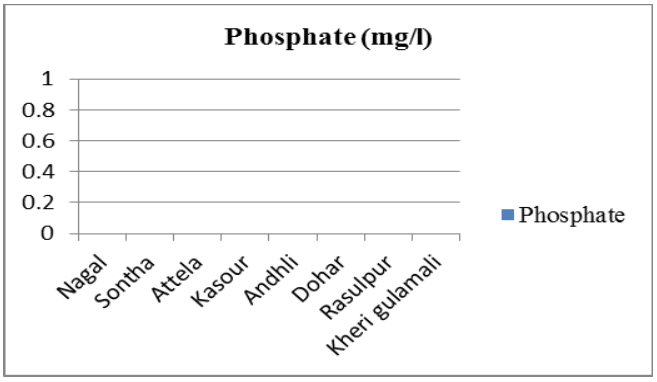

Fig.12: Phosphate in groundwater samples.

xii. Residual Chlorine

In the study area residual chlorine is nil in all the eight groundwater samples (Table 1, Fig.13). As per BIS (IS 10500:2012) drinking water standards residual chlorine is desirable if less than 0.2 mg/l, permissible between 0.2-1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Residual chlorine is desirable in all eight groundwater

samples (Nagal, Sontha, Attela, Kasour, Andhli, Dohar, Rasulpur, Kheri Gulamali).

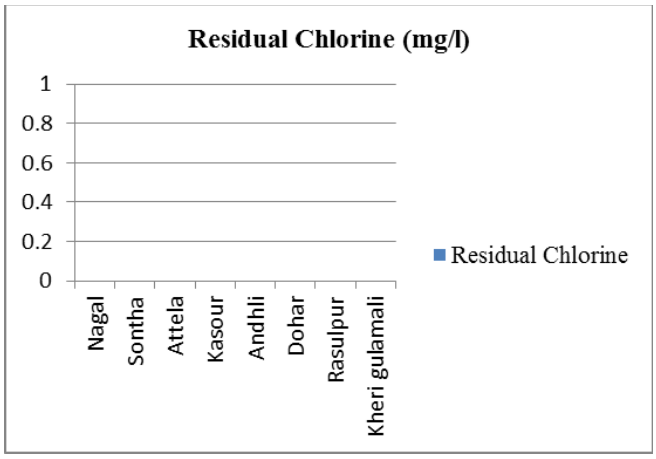

Fig. 13: Residual Chlorine in groundwater samples.

CONCLUSIONS

In the study area pH, chloride, iron, ammonia, nitrite, phosphate and residual chlorine are desirable in all eight groundwater samples. Alkalinity is permissible in all eight groundwater samples. Hardness is desirable in two groundwater samples and permissible in six groundwater samples. TDS is desirable in one groundwater sample and permissible in seven groundwater samples. Fluoride is permissible in six groundwater samples and non-potable in two groundwater samples. Nitrate is non-potable in all eight groundwater samples. The study is highly useful for planning and monitoring of groundwater quality in the study area.

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ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF CRUDE OIL DEGRADING NOVEL *PSEUDOMONAS AERUGINOSA* STRAIN MR21 FROM OIL POLLUTED MUMBAI HARBOR

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Abstract
Marine oil spill affects the marine flora and fauna to a greater extent in the marine ecosystem. Many marine bacteria have the ability to degrade petroleum hydrocarbons by producing biosurfactants and alkane hydroxylase enzymes. Biosurfactant enhances bioavailability of crude oil increasing the dispersion of hydrocarbons in the water by expanding the contact surface area due to emulsification. In this study, we have isolated and characterized crude oil-degrading *Pseudomonas aeruginosa* from oil-polluted Mumbai harbor water. The biosurfactant-producing ability of the strain MR21 was tested by different biosurfactant screening methods like the blue agar method, hemolysis, drop collapse assay, oil displacement method, surface tension reduction, and emulsification index. The strain MR21 showed a positive response for all these methods. Biochemical characterization indicated that this strain is able to hydrolyze casein and Tween 80 by producing proteases and lipases. The gas chromatography analysis indicated complete degradation of crude oil by strain MR21 after 30 days. The catabolic genes responsible for alkane degradation such as alkane hydroxylase gene (*alkB*) and flavin-binding monooxygenase gene (*almA*) were detected in this strain MR21 by polymerase chain reaction. This strain could be useful for bioremediation of marine oil spills in the environment.

Keywords
Biosurfactant, alkane hydroxylase, flavin-binding monooxygenase, proteases, lipases, emulsification.

1. INTRODUCTION

Man depends on petroleum hydrocarbons as the main source of energy for transportation and industry. Maritime transport of crude oil throughout the world by cargo is indispensable because many non oil producing countries like India imports crude oils from oil producing countries like Saudi Arabia and Iran. Oil spill caused by the accidents of oil carrying cargos are frequently happening throughout the world. Crude oil spills into the marine environment causes serious ecological damage to coastal fauna and flora. Crude oil is chemically complex in nature they are separated into saturates, aromatics, resins and asphaltenes [1]. Crude oil immediately undergoes transformation of its constituents when it's released into the marine environment. Microbial degradation is considered to be a major route for the breakdown of hydrocarbon components in the marine environment [2].

In 1946, Zobell recognized that many microorganisms have the ability to utilize hydrocarbons as a source of energy. After the supertanker Torrey Canyon sank in the English Channel, it triggered the scientific community's attention toward the problem of oil pollution in marine environment [3]. Diverse bacterial species capable of degrading crude oil have been isolated and characterized from oil polluted marine environment. Among the crude oil-degrading bacterial species the genus *Pseudomonas* plays an important role in degradation of spilled oil in sea. Oil degrading microorganisms have different mechanisms for uptake of these hydrocarbon molecules and diverse catabolic pathways to degrade hydrocarbons [4]. Bacteria have developed different hydrocarbon accession mechanisms to metabolize hydrocarbons, which include direct contact with these compounds in solid or liquid state, contact with pseudo-solubilized hydrocarbons by biosurfactant excretion and oil-water emulsions [5].

Biosurfactants are amphiphilic molecules that accumulate at interfaces, decrease interfacial tensions and form aggregate

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structures called micelles. Bacteria produce wide variety of high and low molecular weight Surface Active Agents (SAAS). The low molecular weight SAAS called biosurfactants and the higher molecular weight SAAS called bioemulsifiers. The best examples for low molecular weight biosurfactants are rhamnolipids produced by *Pseudomonas aeruginosa*. Rhamnolipids are more effective in lowering the interfacial and surface tension [6]. The genus *Pseudomonas* has different species producing diverse biosurfactant molecules [7-8].

Alkanes are a major fraction (>50%) of the crude oil. The ability of bacteria to degrade alkanes is generally determined by the presence of enzyme alkane hydroxylase which catalyze the initial step in the degradation of alkane. These alkane hydroxylase are widely present in gram-positive and gram-negative oil degrading bacteria and the encoding genes are usually denoted as *alkB* genes [9]. The alkane hydroxylase genes present in bacteria are classified into three groups based on phylogenetic analysis. The group (I) alkane hydroxylase encoded by *alkB1* catalyze the degradation of short chain n-alkanes (C_6-C_{12}). The groups (II) alkane hydroxylase encoded by *alkB2* catalyze the degradation of medium chain n-alkanes (C_8-C_{16}). The group (III) alkane hydroxylase encoded by *alkB3* catalyze the degradation of long chain n-alkanes (>C_{16}) [10].

Many long chain n-alkane degrading bacteria such as *Acinetobacter* and *Alcanivorax* genera produces an enzyme called flavin-binding monoxygenase which is responsible for long chain n-alkane degradation. The gene encoding for this flavin-binding monoxygenase named as *almA* gene [11]. In this study we have isolated and characterized a crude oil degrading *Pseudomonas aeruginosa* strain MR21 from oil polluted Mumbai harbor water and analyzed the presence of the alkane degrading genes in them.

2. MATERIALS AND METHODS

2.1 Sampling

Sea water sample was collected from Gateway of India-(GI) (18.921836°N 72.834705°E) in Arabian Sea near Mumbai. The samples were collected from the depth of 15cm in sterile 100 ml bottles and transported within 4 hours on ice to the Laboratory at Ambernath for isolation of crude oil degrading bacteria.

2.2. Isolation of crude oil degrading bacteria by enrichment culture method:

Modified Bushnell-Hass broth (MBHB) was used for isolation of biosurfactant producing bacteria. The chemical components of MBHB are (gl^{-1}) 1 g of KH_2PO_4, 0.2 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.02g of $CaCl_2$, 1g of NH_4NO_3, and 2 droplets of $FeCl_3$ 60% and 30g of NaCl. The pH was adjusted to 8.2 and autoclaved at 121^{0}C for 15 minutes. After the MBHM was cooled down to 40^{o}C, it was supplemented with 1% crude oil (v/v) (Bharat Petroleum Corporation, India) as the sole carbon and energy source. For solid modified Bushnell-Hass agar medium (MBHA) Agar, (Himedia, India) ($20g/l^{-1}$) was added to the solution [12]. 1ml of oil polluted seawater collected from Gateway of India in Arabian Sea near Mumbai, was added to 250ml Erlenmeyer flasks containing 50ml of MBHB & 1% crude oil (v/v) as carbon source and the flasks were incubated for 7 days at 30^{o}C on a orbital shaker (Orbitek Shaker, Scigenics, India) operating at 180 rpm. After a series of four subcultures, inoculum from the MBHB with 1% crude oil were streaked out to MBHM agar containing 1% (v/v) crude oil and phenotypically different colonies isolated on MBHM agar medium. One strain, which exhibited fast growth rate on crude oil was purified and designated as MR21 and stored in glycerol stock at -80 ^{0}C for further characterization [13].

2.3 Morphological and Biochemical characterisation of strain MR21

The morphological characterizations of the strain MR21 was carried out by Gram staining and hanging drop method. The colony characterisation was carried in *Pseudomonas* agar (fluorescein) media. In order to characterize the biochemical enzyme profile of the strain biochemical tests were carried out such as utilization of pure alkanes like hexadecane, tetradecane, octadecane, pristine, eicosane and utilization of polyaromatic hydrocarbon (PAH) like naphthalene, hydrolysis of casein, tween 80 and carbohydrate fermentations tests.These tests were done according to the Bergey's manual for identification taxonomy [14].

2.4 Biodegradation of hydrocarbons by DCPIP assay:

The conical flask containing 100 ml of Modified Bushnell Haas Broth (MBHB) with 1% diesel as carbon source and 1% 2, 6- dichloro phenol indophenol (DCPIP) redox indicator was inoculated with strain MR21 and incubated at 37°C on a rotary shaker The color change of MBHB containing DCPIP from blue to colorless indicated the biodegradation of hydrocarbon by bacterium. The bacterium which decolorizes DCPIP within 48 hours is considered to be efficient in degrading oil [15].

2.5 Biosurfactant screening methods for strain MR21

The strain MR21 was screened for their SAA-producing ability with nine different methods. The MR21 was grown in 500ml Erlenmeyer flasks with 100 ml of MBHB containing 1% (v/v) HSD as the carbon source. Flasks containing sterilized MBHB with 1% (v/v) HSD were inoculated with a loop full of bacterial culture grown in HSD containing marine agar plates, and the culture flasks were incubated in a rotary shaker for 3 days at 180 rpm and 37°C. After 3 days of incubation, the culture broth from each flask was centrifuged at 6000 rpm and 4°C for 15 min, and the supernatant was passed through a membrane filter paper of 0.22-µm pore size (Millipore). This cell-free culture broth was used to perform the drop-collapse assay, oil spreading assay, microplate assay, penetration assay, stable emulsification assay, and measurement of reduction in surface tension. The bacterial cells were used to perform the blue agar method, hemolytic assay, and bacterial adhesion to hydrocarbon (BATH) assay. All screening experiments were performed in triplicate, and the mean values were used as results. These tests were carried out as described below.

2.6 Blue agar plate method

The MBHM agar medium supplemented with 20 g l^{-1} hexadecane or diesel as carbon sources and cetyl trimethyl ammonium bromide 0.2 g l^{-1}, methylene blue 0.005 g l^{-1} were prepared. Single colony of MR21 was inoculated at the centre of blue agar plate and incubated at 37^{o}C for 24 hrs. A dark halo

around the colony was considered as positive for biosurfactant production. Biosurfactants that belongs to anionic class like rhamnolipids are detected by this method [16].

2.7 Hemolytic assay

The isolated single colony of strain MR21 was inoculated on Zobell marine agar (Himedia, India) containing 5% (v/v) blood and incubated at 37^{0}C for 24-48 hours. Hemolytic activity by bacterial culture was detected by clear halo zone around the colonies [12]

2. 8 Oil spreading assay

In 100mm glass Petri dishes (Borsosil, India) 40 ml of distilled water was added followed by addition of 100 µl of crude oil to the surface of the water, Then 10 µl of the cell free culture filtrates was added on the centre of the crude oil surface. The diameter of the clear zone formed by the oil displacement was measured [17].

2.9 Drop collapse assay

The drop collapse technique was carried out in the polystyrene lid of a 96-microwell plate (Nunc, USA) with slight modification [18]. The culture supernatant 100 µl was mixed with 5µl of methylene blue and added to the centre of wells of a 96-well microtiter plate lid. Then 5µl of diesel was added to the surface of the culture supernatant. Biosurfactant producing culture gave flat drops. For clear visualization of drop collapse and photographic purpose 5µl of methylene blue was added, which had no influence on the shape of droplets. The tests were carried out in triplicates in three separate microtiter plate lids.

2.10 Microplate assay:

The 100 µl of cell free supernatant of strain MR21 was added to a 96-well microplate (Nunc, USA). The plate was viewed underneath a paper with a grid. If biosurfactant is present in the cell free supernatant, the concave surface distorts the image of the grid below. The optical distortion of the grid provided a qualitative assay for the presence of biosurfactants [19].

2.11 Penetration assay:

Penetration assay was carried out as described in the literature [20]. The principle of the assay is the contacting of two insoluble phases which leads to a color change. The wells of 96-well microplate were filled with a 150 µl of a hydrophobic phase consisting of oil and silica gel. The paste was covered with a 10 µl of oil. Then, the 90 µl supernatant of the strain MR21 was colored by adding 10 µl of a red staining solution. The colored supernatant was placed on the surface of the paste. The presence of biosurfactants in the cell free supernatant facilitates silica entering the hydrophilic phase and the upper phase would change from clear red to cloudy white within 15 min.

2.12 Surface Tension

The surface tension (ST) of the culture supernatants was measured with a digital surface tensiometer (K12–Kruss Tensiometer, Germany) by Wilhelmy plate method [21]. The validity of the surface tension readings was checked with pure double distilled water (72mN/m) before each reading. The surface tension readings were taken in triplicate.

2.13 Emuslification Index

An equal volume of hexadecane was added to the same volume of cell free culture broth, mixed with a vortex vigorously for 2mins and left to stand for 24hrs. The emulsification activity was determined as the percentage of height of the emulsified layer (mm) divided by the total height of the liquid column (mm) [22].

2.14 Bacterial Adhesion to Hydrocarbon (BATH Test)

The strain MR21 was grown at 37°C with shaking at 150 rpm in Zobell marine broth for 24 h. The cells of strain MR21 was harvested at early logarithmic phase and washed twice with and resuspended in phosphate urea magnesium sulphate buffer (PUM). The chemical components of PUM buffer pH 7.1, are (g l-^{1}) 22.2 g of K_2HPO_4 $3H_2O$, 7.26 g of KH_2PO_4, 1.8 g of urea, 0.2g of $MgSO_4.7H_2O$. Bacterial cell suspensions were prepared in PUM buffer, 1.2 ml of suspension dispensed into 10 mm round bottom test tubes and hexadecane 0.2 ml was added to it. Following 10 min pre-incubation at 30°C, the tubes were vortexed uniformly on a vortex mixer (Mo Bio Laboratories, USA) for 2 min. After 15 min incubation the hydrocarbon phase accumulated at the upper phase, the aqueous phase was carefully removed with a micropipette and transferred to a 1 ml cuvette. The turbidity of the aqueous phase was determined at 400 nm using multimode microplate reader (Biotek Laboratories, Germany), before and after treatment. Results were recorded as the percentage absorbance of the aqueous phase after treatment relative to the initial absorbance of the bacterial suspension [23].

2.15 Determination of biodegradation of crude oil (ASTM) by *P. aeruginosa* strain MR21

The crude oil biodegradation potential of strain MR21 was quantitatively determined using crude oil quantitative mixture (ASTM-D5307). The biodegradation was carried in 500ml capacity conical flask containing 100ml of MBHB with 1% (v/v) crude oil quantitative mixture (ASTM-D5307) with 1% MR21 inoculum. The test and control were incubated for 30 days at 37 ^{0}C in orbital shaker (180RPM). After 30 days to determine the biodegradation rate the test and control flasks were extracted with equal volume of hexane thrice. The extracts were concentrated by rotary evaporator (Rotavapor – R110, Buchi, Switzerland). The residue was dissolved in 10ml of HPLC grade hexane and analysed by GC-FID[24].

2.16 Molecular identification of crude oil degrading strain MR21

The bacterial strain MR21 was identified by analysis of 16S rRNA for the taxonomic characterization. Total DNA extraction of bacterial strain MR21 was performed using a Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, USA). The complete bacterial 16S rRNA gene 1.5kb fragment was amplified by PCR from genomic DNA of strain MR21 using bacterial 16S rRNA gene universal forward primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and universal reverse 1492R (5'- ACGGCTACCTTGTTACGACTT-3') and species specific *P. aeruginosa* primers PA-SS-F and PA-SS-R [25]. The polymerase chain reaction (PCR)

amplification was performed in the total volume of 25µl in 200µl capacity thin wall PCR tube consisting of 7.5 µl of PCR grade water, 12.5 µl of 2X PCR master mix (MBI, Fermentas), 1 µl (10 pm) of each primer, and 3 µl of template DNA (50 pm).

Amplification was performed in a thermal cycler Mastercycler Epgradient PCR machine (Eppendorf, Hamburg, Germany). The PCR condition was, 94°C for 2 min, 94°C for 0.5 min, 52°C for 0.5min, 72°C for 1.5min, 30cycles and final extension at 72°C for 10 min. The species specific amplification of 16S rRNA for *P. aeruginosa* was performed as per recommended conditions of species specific *P. aeruginosa* primer[25]. The amplified 16S rRNA PCR product was purified using Invitrogen Pure Link Quick Gel Extraction and PCR Purification Combo Kit. The purified PCR products was sequenced using BigDye® Terminator V3.1 Cycle sequencing kit on automated capillary sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems). Partial 16S rRNA gene sequences were initially compared with sequences in the GenBank database using BLASTN [available at http://blast.ncbi.nlm.nih.gov] to identify their approximate phylogenetic affiliation and closest relatives. The 16S rRNA sequences of strain MR21was aligned with closest GenBank matches using CLUSTALW [26]. A phylogenetic tree was constructed using 1200 bp long aligned sequences with the neighbor-joining algorithm (P-Distance Method) in Molecular Evolutionary Genetics Analysis 2.1 software (MEGA, version 5) [27]. Bootstrapping was used to estimate reliability of the phylogenetic reconstructions (500 replicates).

2.17 Detection of alkane hydroxylase (*alkB*), flavin binding monoxygenase (*almA*) and rhamnosyl transferase I (*rhlB*) genes in strain MR21 by PCR:
The purified DNA of MR21 was screened to detect two catabolic genes that encode enzymes involved in alkane degradation pathway alkane hydroxylase gene *alkB* and flavin binding monoxynase gene *almA*. The *alkB* gene was amplified using two different reported primer sets (Mon F401 & MonR820) and *alkBf* and *alkBr* [28-29]. The *almA* gene was amplified using reported degenerate primer specific for *almA* (*alm*Adf & *almAdf*) [11]. The gene coding for rhamnosyl transferase I (*rhlB*) was detected in strain MR21 with *rhlB* specific primers KPD1 and KPD2 as per reported PCR condition [30].

3. RESULTS

3.1 Isolation, identification and characterisation of MR21

The bacterium was isolated by enrichment in the MBHB media with 1% crude oil. The morphological characterisation by Gram's staining and hanging drop method showed MR21 as motile rods (Figure. 1 & 2). The green fluorescent colonies were observed on *Pseudomonas* fluorescein agar. The isolated MR21 strain was able to utilize the different types of hydrocarbons. The hydrolytic enzyme assay revealed the production of hydrolytic enzymes like protease and lipase. The carbohydrate fermentation test showed that only few sugars like glucose, xylose and mannose were utilized by MR21 (Table1).

Table 1: Biochemical characterization of strain *Pseudomonas aeruginosa* MR21.

S. No	Biochemical Test	Results	S. No	Biochemical Test	Results
1	Gram staining	Gram –ve rods	30	Casein Hydrolysis	+
2	Motility	Motile	31	Tween 80 hydrolysis	+
3	Growth on Crude Oil	+		Carbohydrate Fermentation	
4	Growth on Diesel	+	32	Glucose	+
5	Growth on Kerosene	+	33	Mannitol	-
6	Growth on Petrol	+	34	Xylose	+
7	Growth on Lubricating oil	+	35	Inositol	-
8	Growth on Tetradecane	+	36	Sorbitol	-
9	Growth on Hexadecane	+	37	Rhamnose	-
10	Growth on Octadecane	+	38	Sucrose	-
11	Growth on Pristane	+	39	Lactose	-
12	Growth on Eicosane	+	40	Arabinose	
13	Growth on naphthalene	+	41	Adonitol	-
14	Growth on Sodium succinate	+	43	Salicin	-
15	Growth in the absence of sodium chloride	+	44	Maltose	-
16	Optimum temperature for Growth	30-45oC	45	Fructose	-
17	Pigment production	Pyoverdine	46	Galactose	-
18	Indole	-	47	Melibiose	-

19	Voges-Proskauer	-	48	Inulin	-
20	Citrate utilization	+	49	Sodium gluconate	-
21	Lysine utilization	-	50	Glycerol	-
22	Ornithine utilization	-	51	Dulcitol	-
23	Arginine utilization	+	52	Arabitol	
24	Nitrate reduction	-	53	Erythritol	
25	Malonate	+	54	Cellobiose	-
26	Urease	-	55	Melezitose	
27	Phenylalanine deamination	-	56	Raffinose	
28	H2S Production	-	56	Mannose	+
29	ONPG	-	57	Esculin hydrolysis	+

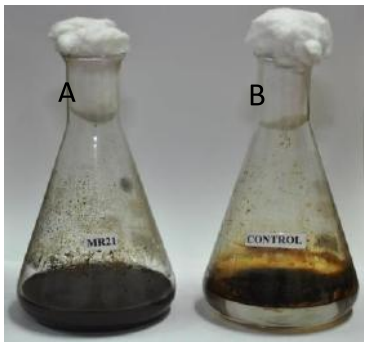


Figure 1. Enrichment of the Pseudomonas aeruginosa MR21 in crude oil.

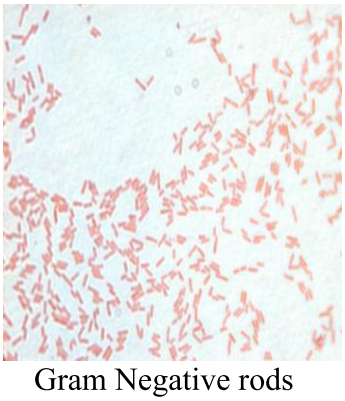


Figure 2. Gram's staining of Pseudomonas aeruginosa MR21.

3.2 DCPIP assay for hydrocarbon degradation:

The strain MR21 was subjected to hydrocarbon degradation by DCPIP assay. The assay showed the change in colour from blue to colourless within 48 hrs, indicating the utilisation/degradation of diesel as source of carbon (Figure 3a).

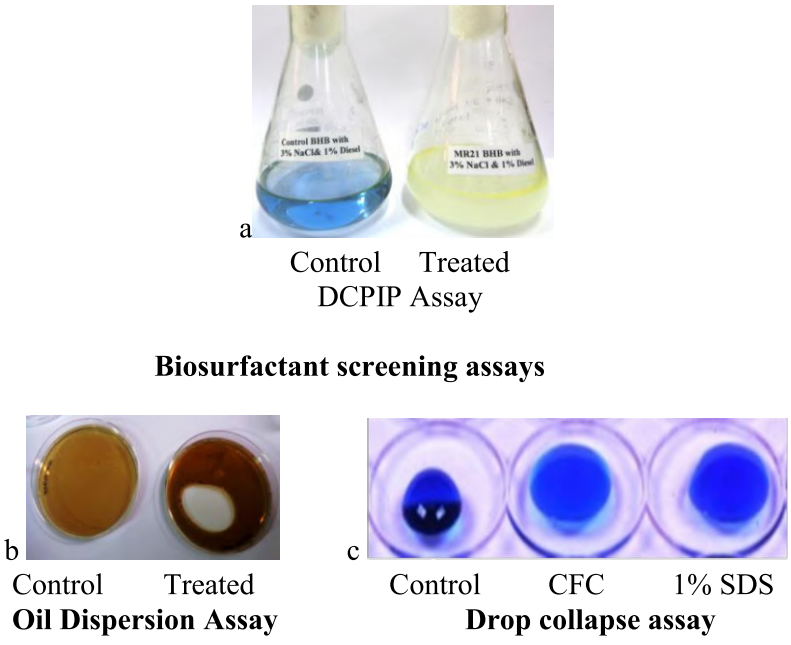


Fig.. 3: DCPIP assay for oil degradation by Pseudomonas aeruginosa MR21.

3.3 Biosurfactant screening of strain MR21:

The biosurfactant property was tested by various assays such as blue agar, hemolytic, drop-collapse, oil spreading, microplate, penetration, stable emulsification, ST measurement and BATH. The blue agar assay showed dark blue clear halo around the colonies, the hemolytic assay exhibited the zone of beta-hemolysis around the colonies. The MR21 showed positive results for the remaining assays as shown in Table 2 & Figure. 3b & c.

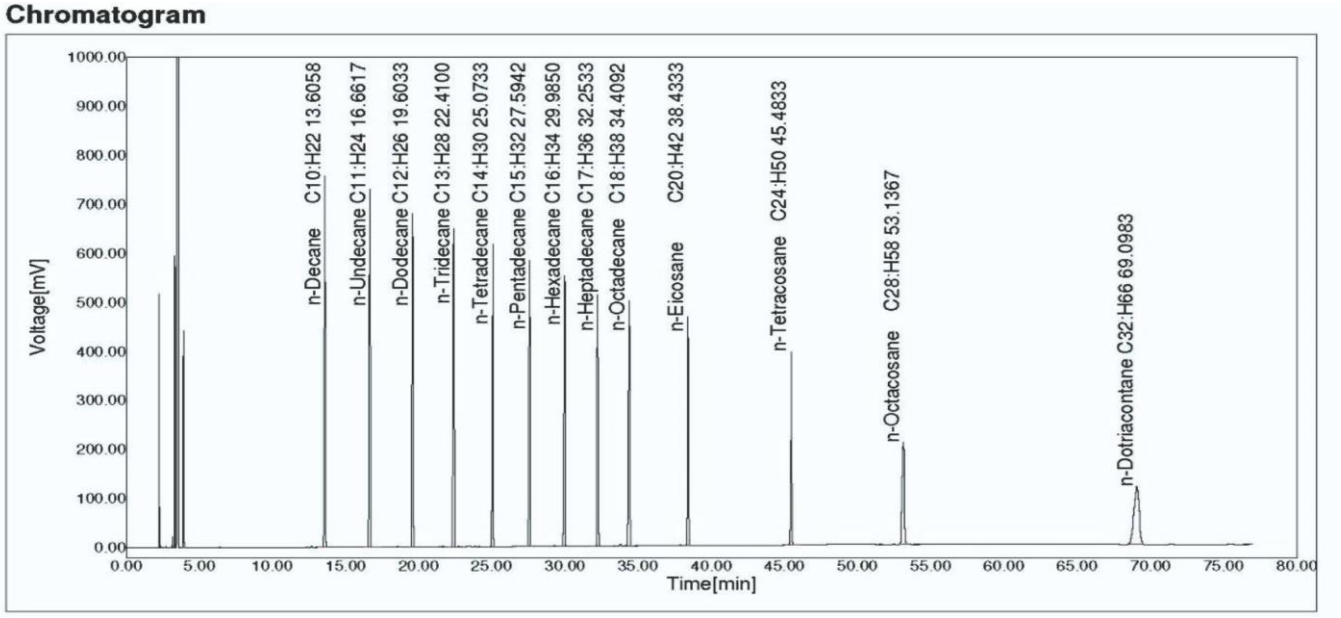
Blue agar plate	Hemolytic Test	Drop Collapse	Oil displacement (mm)	Microplate assay	Penetration assay	Surface Tension (mN/m)	BATH (%)	E24 (%)
+	+	+	48	+	+	28.2	65	70

Abbreviations: +, Positive response; -, Negative response

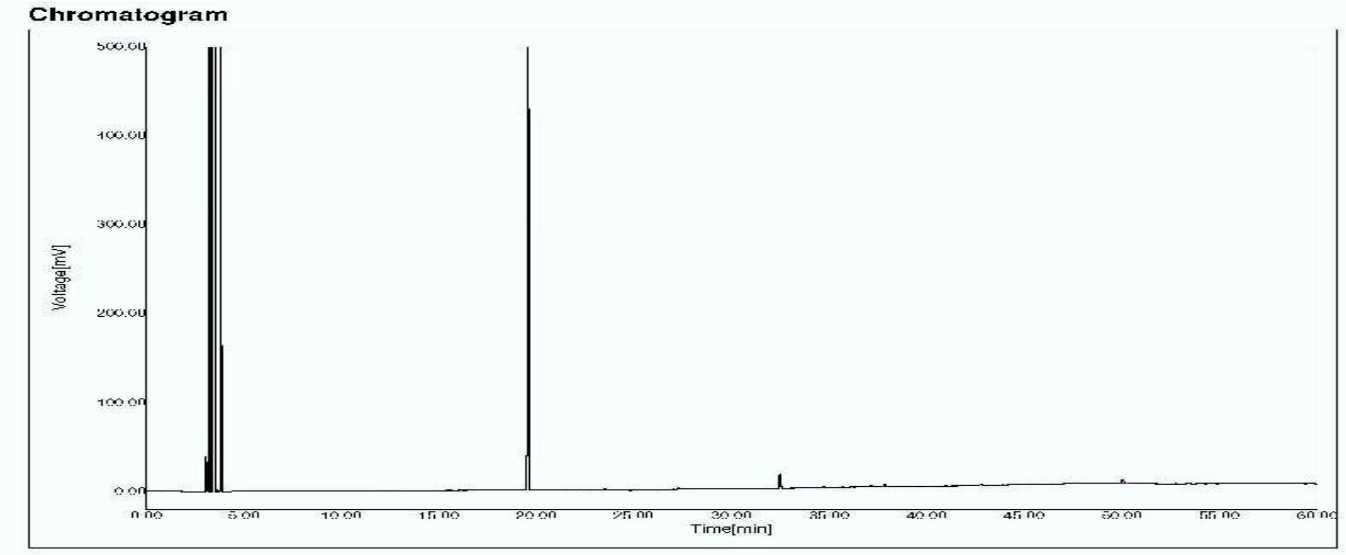
3.4 Determination of biodegradation of crude oil (ASTM) by *P. aeruginosa* strain MR21

The biodegradation of the crude oil was analysed by GC-FID showing the complete disappearance of the chromatogram peaks of the hydrocarbons of the crude oil ranging from C_{10} to C_{32}. This clearly indicated the complete biodegradation property of the MR21 strain. (Figure. 4a & b).

Fig. 4: GC -FID analysis of biodegradation of Crude Oil Quantitative Standard Mixture (ASTM-D5309) after 30 Days.



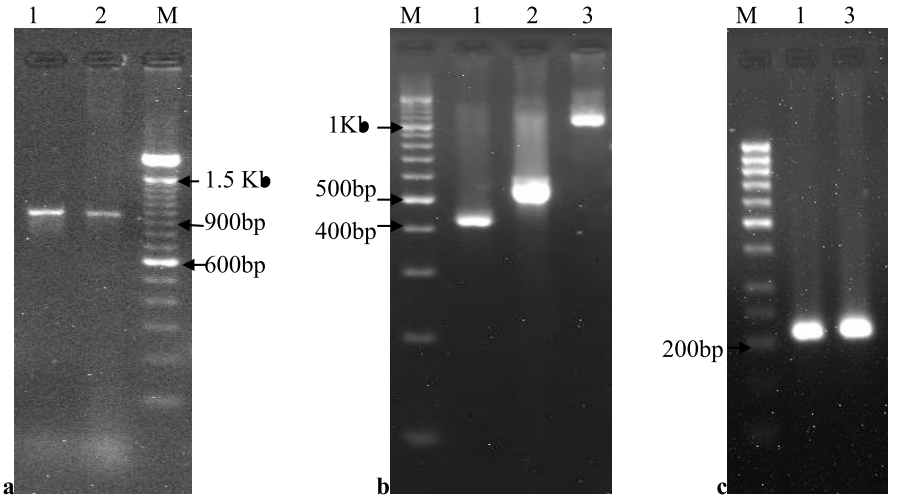
(a) Control



(a) Treatment

3.5 Molecular identification of MR21 strain:

The molecular identification was carried by the PCR amplification of universal 16S rRNA gene and species specific primers. The universal 16S rRNA gene amplification resulted in ~1500bp product and subsequently sequence characterisation revealed the homology with *P. aeruginosa* type strain (Figure 6a). The *Pseudomonas aeruginosa* strain MR 21 16S rRNA ribosomal RNA gene nucleotide was deposited in the GenBank database at the National Center for Biotechnology Information (NCBI), USA. The nucleotide accession numbers obtained was KY651215. This was further confirmed by *P. aeruginosa* species specific primers with amplification of 956bp PCR product (Figure 5a).



M-DNA ladder
Lane 1- ATCC10415
Lane 2- MR21
Lane 1 & 2-956bp

M- DNA Ladder
Lane 1- 420bp *alkB2* Gene
Lane 2- 550bp *alkB1* Gene
Lane 3- 1100bp *AlmA* Gene

M- DNA Ladder
Lane 1- ATCC10415
Lane 2- MR21
Lane 1&2- 226bp

Figure 5. (a) Molecular characterisation of *Pseudomonas aeruginosa MR21by P. aeruginosa species specific primer, (b) PCR amplification of alk1, alkB2 and AlmA Gene in P. aeruginosa strain MR21, (c) PCR amplification of* rhamnosyl transferase I (*rhlB*) in strain MR21 by PCR

3.6 Detection of alkane hydroxylase (*alkB*) gene, flavin binding monoxygenase gene (*almA*) and rhamnosyl transferase I (*rhlB*) in strain MR21 by PCR:

The bacterial DNA of MR21 was subjected for the detection of various genes responsible for the hydrocarbon degradation and biosurfactant production. The PCR amplification with two different *alkB* primer sets showed 420 and 550 bp products specific for the alkane hydroxylase gene. The PCR amplification of flavin binding monoxygenase specific gene (*almA*) resulted in 1100bp species specific product (Figure 5b). The gene coding for biosurfactant rhamnopilid was detected by amplification of *rhlB* gene of 226 PCR product (Fig 5c). The analysis of sequence characterisation has been described in Fig. 6. The PCR products of *alkB2* and *almA* were sequenced and their phylogenetic relationship with other reported gene was analysed with MEGA5. Both the genes were found clustered with reported *alkB /almA* genes of *P. aeruginosa* species (Figure 6b & c). The nucleotide sequence of *alkB2* and *almA* gene were deposited in the GenBank database at the National Center for Biotechnology Information (NCBI), USA. The nucleotide accession numbers obtained were LC179843 (*alkB2*) and LC179844 (*almA*).

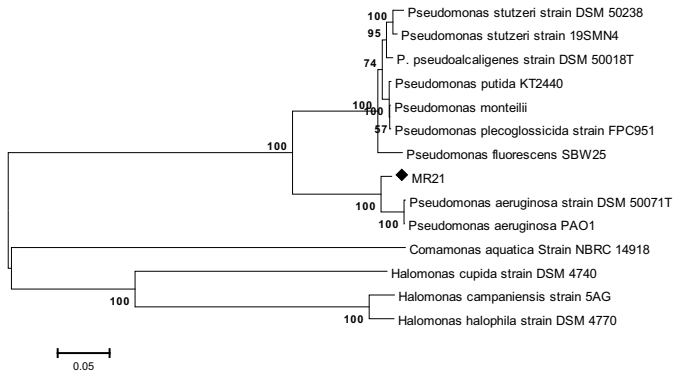
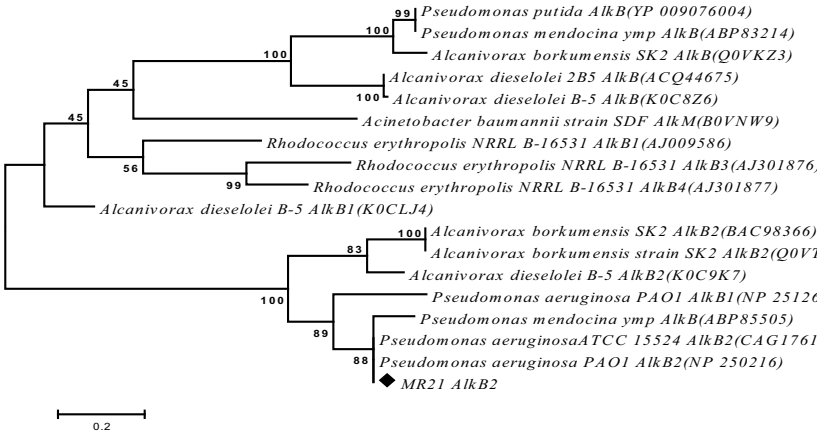
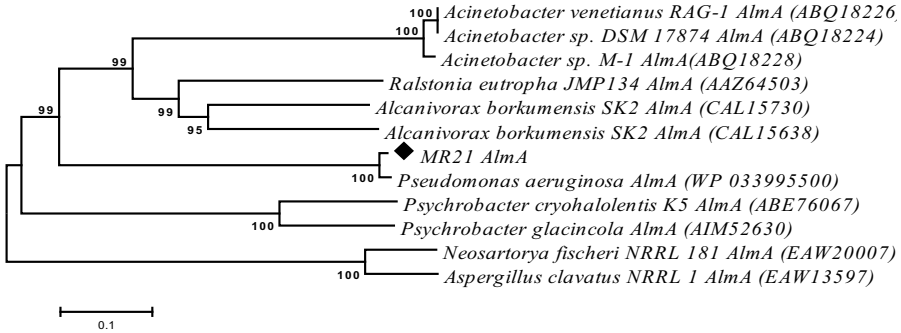


Figure 6: Evolutionary relationship of strain *P. aeruginosa* Mr21
a. Phylogenetic relationship of MR21 with related taxa based on 16S rRNA gene sequences. The evolutionary history was inferred using the Neighbor-Joining method in MEGA5. Bootstrap values (expressed as percentage of 500 replications) are shown at branch points. Bar indicates number of base differences per site.



b. Phylogenetic relationship of protein sequences for the partial alkane hydroxylase, AlkB (120 aa in total) from strain MR21 and other bacteria. The alignment was carried out with ClustalW and tree was generated with MEGA 5. Scale Bar, 0.2 substitutions per amino acid site.



c. Phylogenetic relationship of protein sequences for the partial flavin-binding monooxygenase, AlmA (333 aa in total) from strain MR21 and other bacteria. Scale Bar, 0.1 substitutions per amino acid site.

4. DISCUSSION

The *P. aeruginosa* MR21 strain a new marine isolate from Mumbai harbor, India was able to degrade medium and long chain alkanes (C_{12}–C_{32}), present in crude oil. In present study we have observed complete degradation (100%) of the wide range of alkanes where as the *P. aeruginosa*-AS, isolated from Caspian Sea has ability to degrade 12–100% of alkanes ranging from C_9-C_{25}. As compared to MR21 strain, the *P. aeruginosa*-AS has low oil degradation capability for medium and long chain alkanes [10]. Based on the 16S rRNA sequence alignment and phenotypic analysis it was understood that the MR21 strain is homologus with type strain *P. aeruginosa* DSM-50071T and PAO-1. The biosurfactant (rhamnolipids) producing property of MR21 was confirmed by blue agar method [16, 31-32]. This was further confirmed by detection of rhamnosyl transferase (*rhlB*) gene, a part of the rhamnolipids biosynthesis pathway unique to *P. aeruginosa* [10]. The oil degradation property of MR21 by DCPIP a redox indicator resulted in the change of blue colour to colourless [15, 33-34]. The biosurfactant produced by MR21 strain showed better oil displacement of 48mm, emulsification index value 70% and surface tension reduction to 28mN/m, than *P. aeruginosa-AS* which showed oil displacement of 21mm, emulsification index value 64% and surface tension reduction to 56mN/m [10].

Molecular characterisation of *P. aeruginosa* revealed presence of *alkB1*,*alkB2* and *almA* genes. The alkane hydroxylase enzyme catalyses the first step in aerobic degradation of alkanes by introducing the oxygen atoms derived from molecular oxygen into the alkane substrates which plays an important role in oil bioremediation. The presence of the *alkB1* and *alkB2* genes are responsible for the degradation of short and medium chain alkanes, while *almA* gene in MR21 is responsible for degradation of long chain alkanes [10-11]. The presence of these multiple alkane monoxygenase genes in the MR21 strain corroborates the efficient degradation of crude oil as confirmed by the GC-FID analysis. The present study revealed that the *P. aeruginosa* MR21 strain is a potential microorganism for bioremediation of oil spills in marine environment.

5. CONCLUSION

A crude oil-degrading strain, *P.aeruginosa* MR21 was isolated from oil polluted Mumbai Harbor efficiently degraded all major alkanes present in the crude oil. The multiple alkane degrading genes present in chromosome of strain MR21 completely metabolizes the alkanes in crude oil. The strain also produced anionic biosurfactant rhamnolipid which increases the bioavailability of crude oil for biodegradation in seawater. Thus, isolating functional microorganisms from oil polluted seawater can provide useful microbial resources for development of effective bioremediation technology for marine oil spills.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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B.
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CHALLENGES IN ACCESSING WATER AND SANITATION: A CASE STUDY OF GOLPURI, MEWAT, HARYANA

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Abstract
The development of any country is thought to be largely dependent on access to clean water and sanitary facilities. Any of them cannot be analysed in isolation because there has always been a strong connection between two. Every developing nation, like India, should be concerned when a sizable rural population continues to experience problems with water and sanitation. The government implemented numerous effective projects to address this issue. The Swacch Bharat Project (Gramin), started by the Honourable Prime Minister Shri Narender Modi ji, has built a significant number of toilets in rural regions throughout the course of this five-year period (2014-2019), although the issue of subpar sanitation in villages still exists. Rural women continue to experience pain and have several sanitization-related difficulties. The construction of toilets alone will not suffice to address this serious and deeply ingrained problem. The community's long-standing customs that have persisted may be one of the reasons.

Despite the efforts of the government and local NGOs, citizens in the Indian state of Haryana's Mewat area still experience sanitation issues. Also, we noted that the infection was more common in the female population than the male population. Those who utilised open defecation exhibited greater positive results for parasite infection. It was discovered during the study that the usage of toilets depends on a number of different criteria, including physical structure, accessibility (distance), water supply, and most crucially, habit or behaviour of users.

This study focuses on the problems with cleanliness and the waterfront that the locals in the study region confront. Even though open defecation continues to be a major source of water contamination and the spread of communicable diseases have immediate negative effects on public health, our research supports the hypothesis that the area's significant female participation in livestock and agricultural management as well as improper waste disposal practises may all contribute to the area's infection prevalence.

Keywords
Water, Sanitation, Open Defecation.

INTRODUCTION

Sanitation is most essential for health and wellbeing. Open defecation is directly linked to poor sanitation conditions. This results in improper disposal of human excreta hence poor environment Quality. The spread of contagious bacterial and viral illnesses is associated with poor environmental quality and sanitation. Both human health and a country's socioeconomic development are significantly impacted by this. Most rural people at risk are women and children. The health of the rural population is adversely affected by unscientific living conditions like improper sanitation, open defecation, shortage of water, and poor quality of water, lack of proper solid & liquid waste management. In majority of the villages and especially in North India people do not have safe access to sanitation facilities. Lack of sanitation is responsible for causation and spread of several diseases and illness conditions.

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For millions of people and animals, diseases brought on by worms and germs in faeces are a constant source of suffering. Cholera is a severe sanitation-related sickness that can spread quickly and cause unexpected death in large numbers of individuals. Children are particularly vulnerable to sickness due to poor sanitation. Approximately 892 million people globally defecate in the open (Saleem et al. 2019). This can transmit several infectious diseases and women are the most vulnerable. The human excreta are reported to host viruses, virions, parasitic eggs, protozoan cysts, and bacteria.

Another study focusing on the importance of gender-responsive sanitation facilities has also been investigated (Caruso et al. 2017). These results demonstrate that toilets may restrict social freedoms, highlighting the fact that sanitation encompasses more than just a physical structure and calls for wider social standards that are currently disregarded by large-scale sanitation initiatives. A recent study highlighted that in many countries, social or cultural norms prevent girls and women from using the same sanitation facilities as male relatives or prohibit the use of household facilities on the days women and girls menstruate (Wendland et al. 2018). According to a review on the "Health and Social Impacts of Open Defecation on Women," women and girls are frequently at a disadvantage because of many socio-cultural and economic factors that prevent them from having the same rights as men. (Saleem et al. 2019). Studies have also shown that actions like carrying water and going long distances to find appropriate urination places are signs of additional burdens that can be physically taxing and unpleasant for women, especially pregnant women.

Swachh Bharat Abhiyan initiated by the government of India on the birthday of Mahatma Gandhi is a noble initiative. In 2014, the prime minister announced the goal of eliminating open defecation by 2019. Under the Government's Swachh Bharat Mission, a large number of individual household latrines have been built in rural areas, but we still have a long way to go in order to achieve the Swachh Bharat Mission. In May 2019, more than 5, 60,041 out of 6, 50,000 villages; 617 districts and 30 states have been declared open defecation free (ODF). This speed of construction is quick. We cannot question the authenticity of government data. However, based on our experiences and previous studies, we can say that there is a lacuna in the whole process of collecting data and declaring a village or a panchayat ODF. First of all, the government is primarily focused on building toilets and pays little attention to empowering the public with knowledge about use and upkeep. Second, there is less emphasis on behaviour modification among rural populations. Thirdly, prior to 2014, the government incentivized below poverty line (BPL) families to make toilets, while other program studies demonstrated that the BPL list itself was subjected to large inclusion and exclusion errors. Fourthly the people who got the incentive to build toilets tricked the officials by showing the photographs of the same pit time and again and got the money credited in their account. Hence, we can say that ground realities are different than what appears on the paper. People are still unaware and ignorant about the ill effects of defecating in the open. We will present a social study of the area and demonstrate our study with pertinent data.

Objectives of the study

The study aimed to:

- To analyse the quality and access to basic sanitation in the area.
- To analyse the problems associated in absence of good quality toilets and access to clean drinking water.

Study and Sampling Area

District Nuh is one amongst 22 districts of Haryana. Residents of the area are predominantly MEO, Muslim community. Residents primarily work in agriculture, but a significant portion also perform labour and driving duties. Initially the district name was Mewat (came in Existence in 2005) with headquarters Nuh. Later in 2003 the district name changed from Mewat to Nuh. The district has a total area of 1874 sq.km with 357 village panchayats and 531 villages (Figure 1). Mewat has a very low literacy rate, especially for ladies. For Muslim women in Mewat, the literacy rate ranges 1.76 % to 2.13 %, the lowest in the country (Census, 2011).

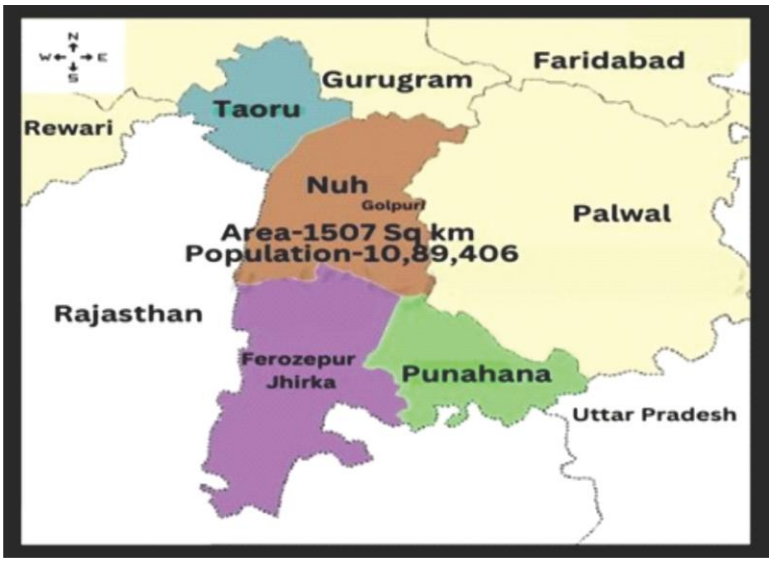

Figure 1: Map of the study area, Nuh.

Not very far from Gurgaon, this area is still lagging on sanitation and hygiene front. After independence, Mewat remained a backward region (Aspirational Districts Baseline Ranking, March 2018, NITI Ayog) also National Family Health Survey (NFHS, 2015-16) indicates the district to be low on every parameter. The majority of those who suffer in this area are women. The women in the neighbourhood are uneducated about their social rights and socially and economically underdeveloped. They spent the most of their time taking care of household duties and working in the fields to cultivate crops. In fact, there are times when they are only permitted to use restrooms after men (as per field reports). We have selected Golpuri village as our study area in district Nuh which is situated on the eastern side with a distance of 6 km from district Nuh.

Methodology

Nuh district consists of several villages. We selected Golpuri village a women-oriented study area. This study mainly focuses on women, so our study participants were women either one-on-one or in groups. We have taken a sample size of around 50 houses, and the method of selection was simple random sampling. We have framed a Questionnaire having series of questions related to availability of toilets, on sanitation practices, attitudes towards OD, latrine ownership and use, and the reasons for non-adoption and/or non-use despite the financial assistance and social marketing efforts of SBA-G, water availability and use. Other tools and techniques used in the study include (a) Key Informant Interviews (b) Community meetings (c) Focus group discussions and (d) Non-participant observation. The first step was to arrange a discussion with Key informants of the area that involved a series of exploratory, open-ended discussions, in groups or one-on-one (Figure 2). Stakeholders for this activity include sarpanch of Gram Panchayat, Aanganwadi workers, Asha workers, active women group, caste leaders etc. Door to door campaigns and community interactions were organised in our second step. This activity helped us to understand the problem in depth. As sometimes women hesitate to discuss her problems in groups, this activity was the way to build our rapport with them. Our third step involved Focus group discussions organised with women as well as with men. A man is considered as the main decision maker in the family so his viewpoint is also found important to draw conclusions. Our fourth and main step was a questionnaire interview with 50 families where the main respondents were the women of the family. This survey gives a clear picture about toilet usage and maintenance.

Fig. 2: Survey and meetings held with people in the Golpuri Village, District Nuh.

Some major observations found during the survey and discussions were as follows:

- Village surveyed has no proper drainage system.
- Most of the toilets found were single pitted toilets.
- Community has misconceptions and issues related to cleaning of pits/ toilets.
- Whole village has only one hand pump and the sources for the water is hard in nature and cannot be used for drinking purposes.
- Village has a presence of a pond that which is in a pathetic condition.
- There was no primary Health centr found in Golpuri.
- Sanitation infrastructure in the school was not found in good condition.
- The water boosting station and drainage system in the village was unscientific (Figure 3).

RESULTS AND DISCUSSION

The present research deals with challenges in accessing sanitation and drinking water among the community of Golpuri village, Nuh Haryana. During the visits we found a lack of proper sanitation, infrastructure, water, and hygienic usage of the village. There were many misconceptions

Fig. 3: Water boosting station and drainage system in the village.

amongst the villagers related to use of sanitation facilities. Our findings indicate a clear picture related to the myths and taboos about the sanitation and challenges in accessing these facilities.

(A) Toilet Availability and usage

We visited the village and conducted the preliminary analysis as per our methodology mentioned above. We have targeted 50 houses for an interview. Interviews were organised one-on- one in individual houses where women partners were interviewed related to their toilet usage and problems associated with that. The outcome of the study reveals that results of SBM-G are very clearly seen on ground. There is a drastic increase in toilet numbers and usage but still people defecate in the open and live in unhygienic conditions. It might be due to some loopholes and lacunas in the implementation part of the project. Out of 50 houses surveyed we found 18 toilets were poorly maintained and 20 have structural issues. In 8 houses we did not find any toilet. Only 4 toilets were found that were in good condition. In other words, we can say that people have facilities but use and maintenance was not proper (Figure 4).

We observed the following reasons for less usage of sanitary infrastructure.

- During the visit, a sizable village community group was at work as labour, and the house's financial situation was not favourable. They found it difficult to set aside money for sanitation in this situation.
- Many homes are cramped since there wasn't enough room for building. For two or three households, there was just one toilet available in that situation. The usage is constrained by this constraint. This condition restricts the usage.
- Many toilets were found with structural damages with time they became defunct.
- The majority of the toilets were single pit toilets, and locals had anticipated increased usage would result in early pit filling.
- Water is once again a key issue in the area; without it, toilets could not be cleaned or maintained.
- Many elderly people reported they feel more comfortable in the field as compared to a closed congested room (toilet)
- Toilets had little to no upkeep, yet there were many users because of huge families. Due to these issues, using the restroom was not a healthy option but rather a place for infection.
- Lastly, the survey also indicated that villagers have minimal knowledge on proper sanitation behaviour.

(A) Water availability and Use

Without this vital natural resource water, human life is unimaginable. The primary supply of fresh water used by humans for many purposes is groundwater; if it becomes polluted, we may have problems. A report on drinking water quality by Khurana et al. claims that there is a significant

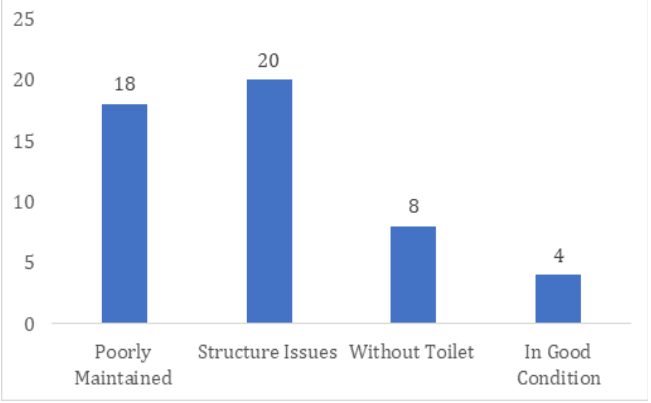

Figure 4: Condition of toilets in Golpuri.

health cost associated with poor water quality. Around 37.7 million Indians are thought to be affected by waterborne diseases every year, 1.5 million children are thought to die from diarrhoea alone, and 73 million working days are thought to be missed as a result of waterborne illness. An estimated $600 million in economic costs are incurred annually as a result.

Nuh region, contains saline water. During our visits to Golpuri we observed that other than toilet facilities, water was the next major concern in the area (Figure 5).

We observed several issues related to water given below:

- All surveyed families have issues with water supply. As per locals there was no water supply from government sources although the village is covered with a water pipeline.
- Village has a government water chamber and booster station but with no water (Figure 3).
- For drinking water needs purchase of 20 litres water bottle in Rs 20/- was common among most families.
- For domestic use tanker water costs approximately Rs 700/- per 1000-liter tanks.
- Villages have only one hand pump which is hardwater. This water is used for washing clothes and utensils.
- For years villagers have been using pond water for washing and cleaning as it is easily accessible but the condition of the pond was very pathetic.
- They are having issues with the toilets as a result of the water shortage.
- Residents are completely unaware about the reuse and treatment of water and their financial condition did not allow them to make these arrangements.

Women in rural areas are still suffering and facing many challenges on the sanitation front. A study on rural women in Odisha revealed that carrying water, cleaning, bathing, managing menstruation, and changing clothes were all included in sanitation behaviours in addition to excrement and urination (Sahoo et al. 2015). Women endured environmental, social, and sexual challenges when they participated in these activities. Another study by Tinna Khanna & According to Madhumita Das (2016), many girls and women in rural regions lack a place to change their clothing and are unable to routinely wash themselves due to a lack of water and restrooms as well as the stigma and shame associated with menstruation.

Figure 5: Water Sources in the village.

Water is essential for economic, environmental and social needs and therefore the right to access to clean and fresh water is the basic human right. However, most rural areas of the developing countries have no improved water supply and the impact was further aggravated as a result of high population growth. According to a study conducted by Fahmida et al. (2018) in Bangladesh reported that child development is positively linked to improved water sanitation and hygiene. The group with combined water, sanitation, hand washing and nutrition interventions has shown significant improvement in the development of child in comparison to all other group intervention. Ground water of the study area found with high TDS levels which make unfit for drinking and create problem in other domestic uses also. A study conducted by Ravindra et al. (2018) for water quality assessment of three lakes of Haryana viz; Tikkar Taal, Karan Lake and Brahma Sarovar, reported four parameters (DO, BOD, Iron and EC) which were beyond the permissible limits when compared with standards given by ICMR/BIS. Also, TDS is as high as 500 ppm in one of the samples. This study found the water of all these lakes are unfit for drinking as they have poor water quality index. As per finding of a study by

Maninder et al. (2009) in Haryana on prevalence of Anaemia in women from the Jat community suggested that age-related decline in the mean Hb level may be mainly attributed to consumption of poor quality and quantity of diet with increasing age. High salinity is found in some parts of study area and is reported to intrude the fresh water zones. Over-exploitation is resulting in the intrusion of saline groundwater towards the fresh groundwater, speeding up the depletion (Priyanka et al., 2016). Due to scarcity of water the young girls and women also compromise on menstrual hygiene. Lack of hygiene and exposure to fecal contents on daily basis multiplied their risk to infections and consequent sickness. The health workers in the village revealed that diarrhea cases as well as vector borne diseases, particularly malaria and dengue, were widespread among children and adults alike. (Narang, 2014). A study on the positive relationships between infrastructure-based approach and reduction in cases of diarrhea and other related morbidities in rural areas, provided it is universal and includes both in-home water and sanitation facilities too (Dufflo, et al., 2015). Similar findings are observed in our study area.

Limitations/Challenges

- Women were completely unaware about the government schemes subsidy and are completely dependent on men for decisions.
- Because of widespread misconceptions about restrooms, it can be challenging to bring up the subject in public at times.
- Toilet use is a matter of habit, and habit takes time to change.
- Women are expected to perform both at home and outdoors in rural areas, yet they are never given enough attention when it comes to their health or wellbeing.
- Lack of awareness on related topics also becomes a major concern when we talk about safe and hygienic access.
- The rural populace has a pervasive misperception and lack of knowledge about how to use and maintain toilets.
- The government is working hard on the water front as well, but there are issues because of unauthorised water connections made by locals.

Recommendations

Toilet coverage has significantly improved over the past four years. The significance of having sanitation facilities is now understood by the community. The government also manages a lot of programmes and initiatives, but if the outcomes do not match expectations, there might be a problem. We have some recommendations for improving the situation.

- As we have shown, there is a significant disconnect between possessing the facility and how individuals really behave while using it. Thus, a focused strategy that ensures toilet usage is needed.
- Programs related to behaviour change, personality development, social stigma redressal and financial literacy should be organized.
- All programs must ensure women participation so that they can understand the problem and situation.
- Following construction, a monitoring procedure for at least six months should be in place to assess usage.
- Use of mass media and Information, Education and Communication (IEC) is found to be effective in dissemination of information.
- Technologies must be used to improve the quality of pond water as with time it is becoming the main source for spreading infections.

CONCLUSIONS

India has progressed on many fronts over the decades since independence in 1947. Our purchasing power, per capita income, literacy, and average longevity has been rising substantially. However, on the other hand India has performed badly in areas of sanitation. The sanitation is not only the absence of garbage and waste material strewn around but also the access to toilet facilities, safe drinking water and connectivity to the drainage system. Although open defecation occurrences have decreased due to improvements in toilet facilities in rural areas over the past three to four years, it is nonetheless true that some people still defecate in the open. This nation urgently needs to practise good sanitation and hygiene.

There should be a community driven approach while making policies related to hygiene and sanitation. One's decision to use the restroom is entirely their own, and we cannot compel them to do so. But we can reach a large audience by employing efficient communication technologies. To a considerable extent, government made headway in providing amenities, but efforts are still needed to make usage of these services a habit.

The authors declare that they have no conflict of interest.

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MIRACULOUS HEALTH BENEFITS OF CUMIN (*CUMINUM CYMINUM* L.)

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Abstract
Cumin is most widely used for culinary and medicinal purposes. It is generally used as a food additive and flavoring agent in many cuisines. Cumin has also been widely used in traditional medicine to treat a variety of diseases, including hypolipidemia, cancer, and diabetes. The literature presents ample evidence for the biological and biomedical activities of cumin, which have generally been ascribed to its content and action of its active constituents, such as terpens, phenols, and flavonoids. The present paper provides an overview of phytochemical profile, biological activities, and ethnomedical and pharmacological uses of Cumin.

Keywords
Cuminaldehyde, Thymol, Pyrazines, Thymoquinon, Insomnia, Bronchitis, Lactation , Anticarcinogenic activity, Hypolipidermia.

INTRODUCTION

Cumin (Cuminum cyminum L.) is a small annual and herbaceous plant belonging to the Apiaceae family. It is a multipurpose plant species cultivated in the Middle East, India, China, and several Mediterranean countries, including Tunisia.Dubbed as the second most popular spice in the world (next to black pepper), cumin comes from a small flowering herbaceous plant from the Apiaceae or Umbelliferae family, which also includes parsley, fennel and hemlock. It is native to Asia, Africa, and Europe, but it is widely used in cooking throughout the world. It is the second most popular spice after black pepper. The plant, which grows about 1 to 2 feet tall, is actually native to the Middle-East Asian region, but is now grown all over the world. The aromatic seeds are the part of the plant that's most widely utilized. These cumin "seeds," which are actually the plant's small dried fruits, look very similar to caraway seeds. They're yellow-brown, oblong-shaped and longitudinally ridged. . Today, cumin is a spice that's highly valued in different cuisines. Mexicans, Indians and North Africans love using it to add color and flavor to their dishes. Cumin is also a primary component of curry powder, blended with other herbs and spices. [1-2]Cumin seeds' health benefits mainly come from their phyto-chemicals, which are touted to have carminative, antioxidant and anti-flatulent properties. Cumin is a good source of energy, vitamin A, C, E and B6, thiamine, riboflavin, niacin, antioxidant carotenes lutein, zeaxanthin and minerals like iron, manganese, copper, calcium, magnesium, phosphorous, and potassium.. This spice also works as an expectorant that loosens mucus and phlegm in the respiratory tract. One curious characteristic of cumin is that although it's a stimulant, it can also work as a relaxant, making it potentially helpful for alleviating insomnia. [2-4] Research is still ongoing, but animal studies found that cumin may help reduce the risk of hypoglycemia. Cumin may have anticancer properties, as it stimulates the secretion of chemopreventive and detoxifying enzymes from the glands. This study provides scientific support for the anti-stress, antioxidant, and memory-enhancing activities of cumin extract and substantiates that its traditional use as a culinary spice in foods is beneficial and scientific in combating stress and related disorders. Because of its strong aroma, only a small amount of cumin essential oil is used in recipes to provide these with a powerful punch. Cumin essential oil is also attributed to its bactericidal, carminative, digestive, diuretic and antiseptic properties for numerous other benefits. It is

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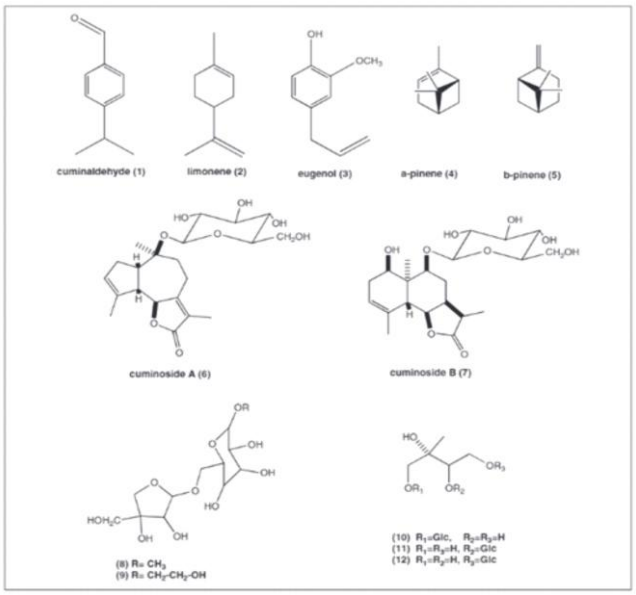
also rich in protein and amino acids, carbohydrates, dietary fiber and a reasonable amount of fats & fatty acids. . Cumin is known for the benefits it offers, more than its taste or flavor, as it helps in losing weight, improving digestion and immunity, and treating skin disorders, boils, piles, insomnia and respiratory disorders. [5-6]

Fig. 1: Cumin herb and Inflorescence.

Phytochemistry

Phytochemical analysis showed that Cuminum cyminum contained: alkaloid , anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid. Nutrient contents of cumin (in 2 g of seeds) were included: calories 7.50, calories from fat 4.00, calories from saturated fat 0.28, protein (g) 0.36, carbohydrates (g) 0.88, dietary fibre (g) 0.22, total fat (g) 0.44, saturated fat (g) 0.04, monounsaturated fat (g) 0.28, polyunsaturated fat (g) 0.06, water (g) 0.16, Ash (g) 0.16, vitamin A (IU) 25.40, vitamin A (RE) 2.54, α-carotenoid (RE) 2.54, beta carotene (µg) 15.24, thiamin – B1(mg) 0.02, niacin – B3(mg) 0.10, niacin equiv 0.10, vitamin C 0.16, vitamin E alpha equiv 0.02, vitamin E (IU) 0.04, vitamin E (mg) 0.02, folate(µg) 0.20, vitamin K (µg) 0.11, calcium(mg) 18.62, copper(mg) 0.02, iron(mg) 1.32, magnesium (mg) 7.32, manganese (mg) 0.06, phosphorus (mg) 9.98, potassium (mg) 35.76, selenium(µg) 0.10, sodium (mg) 3.36, zinc (mg) 0.10, palmitic acid (g) 0.02, oleic (g) 0.28, linoleic acid (g) 0.06 and omega 6 fatty acids (g) 0.06. Organic acids (aspartic, citric, malic, tartaric, propionic, ascorbic, oxalic, maleic and fumaric acids) were isolated from seeds of Cuminum cyminum. Cumin fruits contained 2.5 to 4.5% volatile oil and 10% fixed oil. It appeared that the constituents of Cuminum cyminum essential oil were differ according to the area from which the Cuminum cyminum samples were taken. The major compounds in the Turkish cumin (Cuminum cyminum) seed oil were cuminaldehyde (19.25-27.02%), p-mentha-1,3-dien-7-al (4.29-12.26%), p-mentha-1,4-dien-7-al (24.48-44.91%), γ-terpinene (7.06-14.10%), p-cymene (4.61-12.01%) and β-pinene (2.98-8.90%). Cuminaldehyde, γ-terpinene, o-cymene, limonene and β-pinene were determined to be the major constituents of Syrian Cuminum cyminum . The major compounds in cumin essential oil of Egyptian cultivars were cumin aldehyde (35.25%), tetradecene (12.25%), γ-terpenene (12%), β-ocimene (9.72%), p-mentha-2-en-ol (9%), α-terpinyl acetate (5.32%), αterpinolene (3%), lmonine (0.5%), myrcene (0.2%), β-pinene (0.9%) and α-pinene (0.19%). Tunisian variety of Cuminum cyminum contained cuminlaldehyde (39.48%), gamma-terpinene (15.21%), Ocymene (11.82%), beta-pinene (11.13%), 2-caren-10-al (7.93%), trans-carveol (4.49%) and myrtenal (3.5%) as major components. Analysis of the fruit oil of Cuminum cyminum from Delhi showed that the major constituents were transdihydrocarvone (31.11%), γ-terpinene (23.22%), p-cymene (15.8%), α- phellandrene (12.01%) and pmenth-2-en-7-ol (3.48%) and cuminlaldehyde constituted only 0.58%. Analysis of cumin oil samples from four different German regions showed that the major compounds in all samples were monoterpenes beta -pinene, p-cymene, gamma –terpinene, the terpenoid aldehydes, cuminic aldehyde and the isomeric menthadien carboxaldehydes. However, Li and Jiang found that Chinese cumen seed oil contained cuminal (36.31%), cuminic alcohol (16.92%), γ-terpinene (11.14%), safranal (10.87%), p-cymene (9.85%) and β-pinene (7.75%) as major components. Thymol (40.65%), γ-terpinene (24.51%), b-pinene (5.38%), a-pinene (3.47%), camphene (2.31%), terpinene- 4 – ol (2.00%), cuminaldehyde (1.79%), a-thujene (1.45%), a-terpinolene (1.17%), myrcene (1.07%), limonene (1.04%), α-phyllanderene (0.94%), acetoxylinalool (0.57%) and sabinene (0.37%) represented the major components isolated from cumin essential oils from Kurdistan mountain of Iran(91) . 20 compounds from the Cuminum cyminum (seeds) oil including: α-pinene 2.14, sabinene 1.01, β-pinene 4.89, β-myrcene 1.45, α-terpinene 0.84, p-cymene 1.77, limonene 0.24, -terpinene 1.07, α-terpinolene 0.08, Camphor 0.12, Terpinen-4-ol 0.04, α-terpineol 2.47, geraniol 0.07, geranyl acetate 4.11, β-caryophyllene 3.44, α-phellandrene 1.09, cuminaldehyde 60.01, thymol 2.04, β-farnesene 3.01 and caryophyllene oxide 6.12. However, 32 compounds from Cuminum cyminum oil including: isobutyl isobutyrate 0.8, a-thujene 0.3, a-pinene 29.1, sabinene 0.6 , myrcene 0.2 , d-3-carene 0.2, p-cymene 0.3, limonene 21.5, 1,8- cineole 17.9, (E)-ocimene 0.1, g -terpinene 0.6, terpinolene 0.3, linalool 10.4, a-campholenal 0.03, transpinocarveole 0.07 , d-terpineole 0.09, terpinene-4-ol 0.5, a-terpineole 3.17, trans-carveole 0.4, cis-carveole 0.07, geraniol 1.1, linalyl acetate 4.8, methyl geranate 0.2, a-terpinyl acetate 1.3, neryl acetate 0.09, methyl eugenol 1.6, b-caryophyllene 0.2, a-humulene 0.2, spathulenol 0.07, caryophylleneb epoxide 0.1, humulene epoxide II 0.08 and acetocyclohexane dione-2 0.4(93) . 49 components were identified in the essential oil constituents of the Cuminum cyminum fruit grown in Delhi, which represented 99.78% of total detected constituents. The essential oil was characterized by the presence of

monoterpene (79.61%), sesquiterpene (2.66%), aromatic (16.55%) and aliphatic compounds (0.66%). Among thirty four monoterpenes detected, there were fourteen hydrocarbons (41.28%), twelve alcohols (5.76%), six keto compounds (31.92%), one aldehyde (0.54%) and two esters (0.11%). The predominant monoterpene hydrocarbon was γ-terpinene (23.22%) followed by α-phellandrene (12.01%), α-pinene (1.78%) and α-terpinene (1.24%).



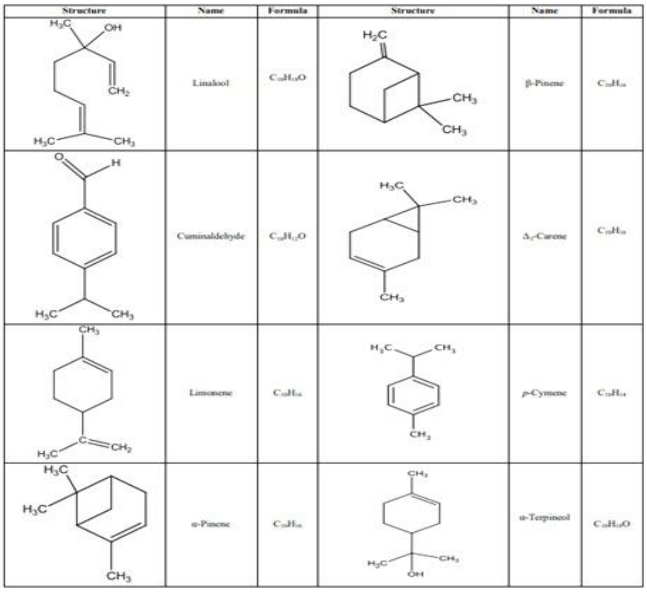


Fig. 2: Structures of phytoconstituents in Cumin.

Among twelve monoterpenic alcohols, p-menth-2-en-7-ol (3.48%) was the major alcoholic constituent and trans-dihydrocarvone (31.11%) was the prominent monoterpenic ketone in the essential oil. The sesquiterpenes identified in the oil were teresantalol (2.62%) and karvaknol (0.04%). The aromatic compounds detected were p-cymene (15.87%), 8a-methyl octahydro-2(1H)- naphthalenone, 2-isopropyl-5-methyl phenol, pcymen-7-ol, o-cymen-5-ol, p-cymen-3-ol, 6-allyl-4,5-dimethoxy-1,3-benzodioxole and 2,a,8,8-tetramethyl decahydrocyclopropanal [d] naphthalene. The aliphatic compounds included 1-(1, 2, 3-trimethyl-2-cyclopenten1-yl) ethanone, 3-isopropyl phenol, 2-methyl-4-isopropyliden-cyclopentan-1-al, 1-methyl-4-iso propyl-3-cyclohexen-1-ol, 2-isopropenyl-5-methyl-hex-4-enal, 4-isopropyl cyclohex-1,3-dien-1-yl) methanol, 4- isopropyl-1-cyclohexen-1-carbaldehyde, hexadecylene oxide and (3,4-dimethyl-2-oxo-cyclopenten-1-yl) acetic acid(88) . Analysis of the methanolic extract of the fruits of Cuminum cyminum led to the isolation of five terpenic and steroidal constituents, they were characterized as 1,4,5,8-tetrahydroxynaphthyl geranilan-10 -al 1 -oate, lanost5,20 (22)-dien-3α-olyl ndocosanoate, labdan-6α,16,20-triol-16-(10 ,11 -dihydroxy anthraquinone-2 -oate), stigmast-5-en- 3β-O-D-arabinopyranosyl-2 -benzoate and lanost-5,24-dien-3β-ol 3β-O-D- arabinopyronosyl-2 noctadec- 9 , 12 - dienoate. The characteristic odour of cumin was attributed to the presence of sminaldehyde, 1, 3-p-menthadien-7al, 1-4-pmenthadien-7-al. 14 free amino acids were also isolated from the seeds. While, flavonoid glycosides isolated from the plant were included apigenin-7-glucoside, luteolin-7-glucoside, luteolin-7-glcuronosyl glucoside, luteolin and apigenin. Total polypheols in cumin were 4.98± 0.31. (mg GAE/g DW). Phenols (salicylic acid, gallic acid, cinnamic acid, hydroquinone, resorcinol, P-hydroxybenzoic acid, rutin, coumarine, quercetin) were isolated from seeds of Cuminum cyminum. However, Cuminum cyminum roots, stems and leaves, and flowers were investigated for their total phenolics, flavonoids, and tannins contents. In all Cuminum cyminum organs, total phenolics content ranged from 11.8 to 19.2 mg of gallic acid equivalents per gram of dry weight (mg of GAE/g of DW). Among the polyphenols studied, 13 were identified in roots, 17 in stem and leaves, and 15 in flowers. The major phenolic compound in the roots was quercetin (26%), whereas in the stems and leaves, p-coumaric, rosmarinic, trans-2-dihydrocinnamic acids and resorcinol were predominant. In the flowers, vanillic acid was the main compound (51%). A total of 19 phenolic compounds were successfully identified during the ripening of cumin seeds. Rosmarinic acid was the major phenolic acid for the unripe seeds, while, half ripe and full ripe seeds were dominated by pcoumaric acid. [5-20]

Fig. 3: Images of cumin seeds and powder.

Traditional Health Benefits

Cumin is extremely good for digestive problems. The very aroma, which comes from an organic compound called Cuminaldehyde, the main component of its essential oil,

activates the salivary glands in our mouth, which facilitates the primary digestion of food. Next is thymol, a compound present in cumin, which stimulates the glands that secrete acids, bile, and enzymes responsible for complete digestion of the food in stomach and intestines. Cumin is also carminative, which means that it relieves you from gas troubles, and thereby, improves digestion and appetite. Due to its essential oils, magnesium, and sodium content, cumin promotes digestion and also gives relief from stomach-aches when taken with hot water. The main cause behind piles (hemorrhoids) is constipation added with infections in the wound in the anal tract, which is also caused by constipation. Cumin because of its dietary fiber content, and carminative, stimulating, antifungal and antimicrobial properties, acts as a natural laxative in powdered form. These characteristics are due to the presence of essential oils comprised mainly of cuminaldehyde and certain pyrazines. [4-6] It is important to note that it is capable to clear up all of the symptoms and causes of hemorrhoids. Although research is still ongoing, early studies report that cumin, among a number of other spices, can have a powerful effect in preventing diabetes by reducing the chances of hypoglycemia. The animals that were tested showed a sharp decline in hypoglycemia when fed cumin seeds in their diet. They also showed a decrease in glucosuria, which is a condition where the urine contains too much glucose, also resulting in hypoglycemia and diabetes. Some of the components of cumin essential oil are hypnotic in nature and have tranquilizing effects, which also help to relieve stress and anxiety that commonly cause insomnia. The presence of caffeine (the stimulating agent), and the richly aromatic essential oils (the disinfectants) make cumin an ideal anti-congestive combination for those suffering from respiratory disorders such as asthma and bronchitis. It acts as an expectorant, meaning that it loosens up the accumulated phlegm and mucus in the respiratory tracts, and makes it easier to eliminate those from the system via sneezing or coughing up and spitting. The common cold is a viral infection which affects our body frequently when our immune system becomes weakened. Again, the essential oils present in cumin act as disinfectants and help fight viral infections which can cause the common cold. Cumin also suppresses the development of coughing in the respiratory system since it dries up the excess mucus. Cumin is rich in iron and has a considerable amount of vitamin C, which is essential for a healthy immune system and keeps infections from forming or becoming worse. Cumin is rich in iron and thus very good for lactating mothers or pregnant women, as well as for women who are undergoing menses. Moreover, cumin is said to help ease and increase secretion of milk in lactating women due to the presence of thymol, which tends to increase the secretions from our glands, including milk, which is a secretion from the mammary glands. Also, cumin is more beneficial if taken with honey. Cumin has a remarkable amount of calcium (> 900 mg/100 grams) which accounts for over 90% of our daily requirement of calcium. This calcium is an important constituent of milk and hence cumin is very good for lactating mothers. As stated above, cumin is very rich in iron (> 66 mg/ 100 grams) which is more than 5x the daily requirement of iron for an adult. [5-7] So, cumin can be a nutritious additive to the daily diet of anemic people. It can help reduce the symptoms of anemia like fatigue, anxiety, cognitive malfunction, and digestive issues. The amount of iron in cumin leads to an increased hemoglobin production and subsequent prevention of anemia, but the increased blood flow has other benefits as well. When your blood circulation is at its best, adequate amounts of oxygen are able to reach the organs and the brain, leading to an optimal performance of those bodily systems. The Proper amount of oxygen and iron in the brain lead to increased cognitive performance and a decrease in cognitive disorders like Alzheimer's disease and dementia. Almost everyone knows that vitamin-E is good for the maintenance of skin and the prevention of premature aging symptoms. It keeps the skin young and glowing. This vitamin is also present in abundance in cumin. The essential oils present in cumin have disinfectant and antifungal properties. This prevents any microbial and fungal infection from affecting the skin. Not all skin issues are disorders or infections, some of them are simply signs of aging. Vitamin E acts as an antioxidant in this regard and combats the free radicals that attack the skin and result in signs of premature aging like wrinkles, age spots, and sagging skin. This, combined with the antibacterial capacity of cumin, makes for healthy, beautiful skin that lasts far into your old age. Boils are outlets for the removal of toxic substances and foreign matters such as microbes from the body. This means that they are the symptoms which show that a high amount of toxic substances have accumulated in the body. Those who regularly use cumin in food have a significant reduction in the occurrence of boils, rashes, pimples, and other signs of excess toxin content. Components such as cuminaldehyde, thymol, and phosphorus are good detoxifying agents which help in the regular removal of toxins from the body. The healthy way of removing toxins is through the excretory system, not through boils. As discussed above, an abundance of iron, the presence of essential oils, vitamin C, and vitamin A in cumin boosts our immune system in a number of ways. Vitamin C is one of the most powerful antioxidants that we have in our body, and it stimulates the function and activity of white blood cells. [8-11]As an antioxidant, vitamin C fights the detrimental effects of free radicals, which are the dangerous byproducts of cellular metabolism. They are constantly being created in the body, and therefore, must be eliminated. Antioxidants neutralize free radicals that lead to many diseases, including, but not limited to, cardiovascular diseases and cancer. Cumin itself has detoxifying and chemo-preventive properties, and accelerates the secretion of detoxifying and anti-carcinogenic enzymes from the glands, as it also does to other secretions. Furthermore, it has beneficial antioxidants like vitamin C and vitamin A within its chemical makeup, in addition to those essential oils. Besides having countless other benefits, the antioxidants have anti-carcinogenic properties too, and those found in cumin are particularly good for colon cancer prevention. [12-20]

Culinary Uses

Spices play an important role in making a dish more flavorful. Indian cuisine is especially known to have some of the healthiest traditional spices as its main ingredients. Cumin is one such spice that forms an integral part of various dishes in the Indian cuisine. Cumin is traditionally used as a spice in Indian cooking, either as whole seeds or in powdered form. It

is a major component in a curry's preparation and other food products. Derived from cumin seeds, this oil is used as a scent in cosmetics including creams, perfumes, and lotions. It is used to add flavor to alcoholic beverages and desserts. Cumin seeds are used to make medicines that help in treating problems like diarrhea, colic, inflammation, bowel and muscle spasms and gas. When ground cumin is mixed with honey and pepper, it works as an aphrodisiac.

DISCUSSION AND CONCLUSION

Besides its culinary uses, this aromatic spice is known for its medicinal properties since ancient times. Being an excellent source of iron, it aids in digestion, boosts the immune system and has anti-carcinogenic properties. Black cumin seeds contain about 100 chemical compounds including vitamins, proteins, carbohydrates, minerals and fatty acids. They are known for their healing qualities. Thus, this spice has a rich history and was particularly favored by the ancient Egyptians, Greeks and Romans. Regular usage of cumin in your food helps in keeping your skin free from boils, rashes, pimples etc. This is because it has components such as Cuminaldehyde, Thymol and phosphorus which are good detoxifying agents. Cumin has a high content of vitamin E which keeps your skin healthy and glowing. Besides, the essential oils, cumin have disinfectant and anti-fungal properties which protect your skin from fungal and microbial infections. Cumin is also a good source of dietary fiber which helps in the cleaning process and removes toxins. Vitamin E present in cumin triggers the anti-ageing processes within the body, thus preventing pre mature ageing symptoms. It acts as an antioxidant to combat the free radicals that attack the skin and cause signs of ageing like wrinkles, age spots and sagging skin. Oil extract from cumin is a great stimulant, carminative, antioxidant and diuretic. It is often used for massage in aromatherapy and scalp treatments to get rid of dandruff. Cumin helps to lower blood sugar levels and thus helps in maintaining proper blood content levels in the body. This is a great boon for people suffering from Diabetes. Cumin seeds are very rich in iron, which makes it an essential natural health ingredient. Cumin seeds contain Thymoquinone, which reduces inflammatory processes and other mediators that cause asthma. They also act as a bronchodilator .This is achieved by its anti-oxidant characteristics that fight against impurities and free radicals. This helps in making the body's immunity better in combating diseases. Cumin seeds are rich in iron which is necessary for the formation of haemoglobin in the blood. This in turn is required for the transportation of oxygen in the body. Consuming cumin seeds will keep one protected from anaemia. Cumin is healthy for women of all age groups and is known to influence a healthy menstrual cycle. Cumin is helpful in treating colon and breast cancer. The seeds contain thymoquinone, dithymoquinone, thymohydroquinone and thymol which are anti-carcinogenic agents. Cumin is rich in Vitamin C and has anti-fungal properties. These help to cure cold and other respiratory problems. Cumin seeds are also suggested for kidney health. Good metabolism process helps to keep all the other body processes in check. Iron present in cumin helps to properly maintain our metabolic activity. Enzymes present in cumin help to breakdown foods and thus aid in digestion. In traditional medicine, cumin was used to treat hoarseness, jaundice, dyspepsia and diarrhoea. Its seeds were used for stomachic, diuretic, carminative, stimulant, astringent and abortifacient properties. The oil of cumin was used in perfumery and as a seasoning in curry powders, soups, stews, sausages, cheeses, pickles, meats and chutneys. In America, Africa and India the drug is used as an abortive and as an emmenagogue. In Indonesia, it was used in cases of bloody diarrhea and headache (paste is applied to the forehead). It was also taken orally for rheumatic ailments. In India, cumin was used as an abortifacient, for kidney and bladder stones, chronic diarrhea, leprosy and eye disease. In Unani system of medicine, the fruits of Cuminum cyminum were used as an astringent, carminative, emmenagogue, for the treatment of corneal opacities, ulcers, boils, styes and to relieve cough and inflammation. Phytochemical analysis showed that Cuminum cyminum contained: alkaloid , coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin and steroid. The previous pharmacological studies revealed that Cuminum cyminum exerted antimicrobial, insecticidal, anti-inflammatory, analgesic, antioxidant, anticancer, antidiabetic, antiplatelet aggregation, hypotensive, bronchodilatory, immunological, contraceptive, anti-amyloidogenic, anti-osteoporotic, aldose reductase , alpha-glucosidase and tyrosinase inhibitory effects, protective and central nervous effects. This review highlights the chemical constituents and pharmacological effects of Cuminum cyminum.

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SAVE THE ENVIRONMENT (STE) was founded and registered on 19th November 1990. In 1992 with the collaboration of WWF (India), the organization started working to combat arsenic poisoning problem of water in the arsenic prone areas of West Bengal. Since then STE has been involved in various projects related to combat arsenic problem in India.

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