

# International Journal of Environment and Health Sciences





## SAVE THE ENVIRONMENT (STE)

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## **A PEER REVIEWED & REFEREED JOURNAL**

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#### AIMS AND OBJECTIVES OF IJEHS:

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...

The year 2020 was a difficult year for the mankind, but at the same time, it made us all realize that the power of unity and discipline is of utmost importance while managing the severest of crises. The COVID-19 pandemic impacted public health and environment alike. However, the year 2021 is being deemed to be the 'Year of Recovery'. All of us need to join hands together to tackle the aftermath of the pandemic and to ensure that we stay firm in our efforts to create a sustainable environment.

Surveys suggest that in order to ensure a better respiratory capacity and overall improved health, the necessity of clean air and pure water needs to be addressed more now than ever before. Perils linked to environmental risk factors have to be managed for a bluer and greener earth. In this outlook, propagating awareness for environmental sustainability has become the need of the hour. Formation of regulatory bodies and authorities to disseminate societal alertness towards environmental safety is on the rise.

With this perspective, the International Journal of Environment and Health Sciences (IJEHS) proposes to provide a reliable platform to discuss technologies and strategies for management of aforesaid environmental matters, especially for the current post-COVID-19 period. 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 year. With this, I wish all our readers a Very Happy New Year, 2021 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

## International Journal of Environment and Health Sciences

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# A. Environmental Sciences Section



## PHYSICO-CHEMICAL AND BACTERIAL STATUS OF DRINKING WATER SOURCES HARBORING HOSPITAL AREAS: A CASE STUDY OF TEZPUR HOSPITAL AREAS

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#### Abstract

Access to potable water is one of the major concerns in the rural parts of North East India. Additionally, the water sources in and around the areas harbouring operational hospitals are prone to a plethora of organic and inorganic contaminants, and therefore the likelihood of any deterioration in their quality cannot be negated. To investigate such a possibility, samples were collected from 13 different potential areas surrounding the hospitals of in and around Tezpur town, Assam, India and several physico-chemical parameters were determined. The total hardness of 12 out of 13 samples was found to be significantly lesser than the standard range of Bureau of Indian Standards (BIS). Similarly, TDS of 11 samples, resistivity of 7 and pH of 2 samples were found to be out of the acceptable range. Salinity and conductivity of 2 out of 13 samples were found to be very high. Analysis for turbidity revealed a vast range of data (0.09-418 NTU), which is way out of the permitted range. The amount of dissolved oxygen was lowest for the sample collected from hand pumps. Furthermore, only 3 samples showed any significant presence of Iron. Surprisingly, only 4 samples indicated the presence of bacterial contamination. Therefore, this study will be useful in taking proper remedial measures for water decontamination.

#### Keywords

Physico-chemical parameters, Bacterial analysis, WHO, BIS, NE (North-East).



**Graphic abstract** 

#### INTRODUCTION

Water is a vital component for the sustainability of human life. But undertaking optimum measures for sustainably good water quality for human use is a matter of high serendipity and entropy [1]. Drinking water should be clear, odorless and preferably free from all harmful micro-organisms, especially bacteria.As per a report published by WHO, nearly 80% of harmful diseases in human beings are caused due to polluted drinking water[2][3].Poor sanitation, food sources are becoming intrinsic to the occurrence of pathogens exposure. Poor water quality, sanitation and hygiene are the reasons for 1.7 million deaths a year worldwide [4]. According to theWorld

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Health Organization (WHO) 90% of such deaths are reported in children and effectively all are in developing countries [5][6].

In several states of a developing country like India, more than 90% of the population depends on ground water for drinking and other purpose [7]. It is a herculean task to restore back the optimum quality of water, once it gets contaminated-[8]. The decisive reasons behind the deterioration of urban water. primarily are agricultural, urban conducts, input of untreated industrial as well as hospital effluents, domestic wastewater (like used detergent, excreta and faecal sludge), even uncontrolled landfills, thereby posing tremendous threat to human health [9]. The unbalanced physico-chemical parameters and presence of harmful micro-organisms in drinking water are reasons behind causing several adverse effects in human body [10]. The ground water in North-Eastern states of India (especially Arunachal Pradesh, Assam and Nagaland), are usually found to be high on the iron and fluoride contents [11]. The people from several parts of Assam are facing lots of struggles for a drop of good quality water [12]. The microbial contaminations of water sources are occurring manly due to the industrial waste and domestic waste, except the educated people the rural people are consuming this contaminated water without testing [13][14]. The different physical, chemical and bacterial parameters of water can affect the water quality and the comparative study of these different parameters can be done by mathematical model called water quality index (WQI) [15][16].

The main aim of this study was to investigate the physicochemical parameters and bacterial contamination in drinking water samples collected from areas surrounding the hospitals, in and around Tezpur town hospitals, Assam. These parameters were compared limits as mentioned in the BIS.

#### MATERIALS AND METHODS

Samples were collected from 13 different locations in and around Kanaklata Civil Hospital (KCH), Tezpur and Tezpur Medical College & Hospital (TMCH), Bihaguri, in sterile sample collection amber bottles (1000 ml) and sterile polypropylene centrifuge tubes. Standard procedures were followed while collection & storage of samples, parameters and physical state of water samples such as temperature and pH were measured at the sampling site using a standard thermometer and a portable pH meter (model: EuTech pH610), respectively. The latitude and longitude of all the sampling sites along with the source were recorded (Table-1) using GPS system (model: Garmin GPS72H), sampling location map (fig.1) made by Google earth 6.1 and QGIS 2.12 software. The bottles were then taken to the laboratory in contaminant free ice-boxes in order to avoid the outside contamination and the change in physical, chemical and biological parameters in the samples. Samples were stored at 4°C temperature until further analyses as per standard procedure used for water analysis (APHA, 2005). AR grade reagents and ultrapure de-ionized water were used for all the analyses wherever required. TDS, EC, and Salinity were measured by multi parameter (EuTechCD650). Turbidity was measured using turbidity meter (EuTech TN100) and total hardness was checked by complexometric titration using Erichrome Block –T as indicator (EDTA method). Iron was estimated using colorimetric method in a UV-Vis spectrophotometer (AnalytikjenaSPECORD 205). Fluoride was measured by ion meter (Thermo scientific ORION 4 STAR). Bacteriological analysis was performed using the Bacteriological Field Test Kit (H<sub>2</sub>S strip method developed by DefenceResearch Laboratory, DefenceResearch &Development Organization) using mentioned protocol. The bottles containing H<sub>2</sub>S strips were filled with 20 ml of test water sample and incubated at 37°C for 72 hours. Any change in colour was monitored and recorded every 24 hours.

#### **RESULTS AND DISCUSSION**

Figure 1 describes the map locations from where the samples were collected while Figure 2 on the other hand shows photographs of some sites of collection, giving us an idea of the immediate surroundings various physico-chemical parameters were analyzed and the detailed results are mentioned below. The source, location and physical properties like color and odour of the sample enclosed in Table 1. The light brown color was observed in some samples (GN3, GN4, GN9 and GN13) and sample GN8 had dark brown color. None of the collected samples have odour.



Figure 1: Location map of water collection points.



Figure 2: Photographs of some collection sites. (a-h) in and around TMCH; and (i) inside KCH.

Five types of water sources, i.e. tap, hand pump, well, bore well, filter tank), were selected in and around the operational hospitals in Tezpur town. The preliminary observations like latitude, longitude, color and odour were recorded and the same is presented in table .1. Some samples viz., GN3, GN4, GN9 & GN13 showed a light brown colour while GN8

appeared as dark brown in colour; nonetheless all samples were found to be odourless (Table. 1). The temperatures recorded at the time collection of water sample was nearly 35°C for all locations. The physico-chemical parameters of the collected water samples are also mentioned in table. 2.

Sl. No.	Sample code	Water source/	GPS L	ocation	Odour	Color
		Locality	Latitude	Longitude		
1.	GN 1	Tap water	26.68093	92.65326	NO	NO
2.	GN2	Hand pump	26.68093	92.65326	NO	NO
3.	GN3	Well water	26.68246	92.65020	NO	Light brown
4.	GN4	Bore well water	26.68246	92.65020	NO	Light brown
5.	GN5	Filter Tank water	26.68246	92.65020	NO	NO
6.	GN6	Well water	26.68246	92.65020	NO	NO
7.	GN7	Well water	26.67827	92.65329	NO	NO
8.	GN8	Hand pump	26.67671	92.65361	NO	Dark brown
9.	GN9	Hand pump	26.67678	92.65369	NO	Light brown
10.	GN10	Filter Tank water	26.67678	92.65369	NO	NO
11.	GN11	Well water	26.65266	92.77362	NO	NO
12.	GN12	Well water	26.65268	92.77367	NO	NO
13.	GN13	Tap water	26.62893	92.79705	NO	Light brown

Table 1: Sampling Sites and Sources of Collected Water Samples along with Preliminary Observations\*.

	Parameters											
	рН	Conductivity (µs/cm)	TDS (mg/L)	Turbidity (NTU)	Salinity (mg/L)	Total Hardness (mg/l)	D.O. (mg/L)	Resistivity (KΩ)	F-(mg/l)	Fe(mg/l)		
BIS Standard (desirable- permissible)	6.5-8.5	-	500-2000	1-5	-	200-600	>5	1-5	1.0-1.5	≥0.3		
Sample Code												
GN1	7.01	178.8	170.2	3.03	166.6	75	6.30	2.94	0.159	Tr#		
GN2	6.57	174.1	166.1	0.94	162.2	87.5	6.62	3.01	0.149	Tr#		
GN3	6.44	82.4	78.66	42.7	78.72	25	5.11	6.36	0.125	Tr#		
GN4	6.07	94.44	90.09	113	89.5	37.5	3.41	5.55	0.149	Tr#		
GN5	6.68	102.2	97.61	1.60	96.60	37.5	7.07	5.12	0.147	Tr#		
GN6	6.67	448.8	428.2	15.39	425	75	5.33	1.16	0.114	Tr#		
GN7	6.61	601.2	572.5	5.96	573.1	150	5.12	0.87	0.186	Tr#		
GN8	6.11	181.2	172.8	418	168.4	62.5	5.02	2.89	0.142	0.35		
GN9	6.25	89.87	85.5	54	85.24	37.5	4.74	5.85	0.151	0.72		
GN10	6.44	88.75	84.65	0.54	84.45	37.5	7.76	5.90	0.137	Tr#		
GN11	5.91	232.9	222.2	0.09	217.2	75	5.55	2.25	0.098	Tr#		
GN12	6.96	606.0	578.1	0.66	580	200	7.43	0.86	0.128	Tr#		
GN13	7.07	151.7	144.9	4.8	141.5	50	5.70	3.45	0.302	0.23		

Table 2: Physico-Chemical Parameters of Collected Water Samples\*

\* Red highlighted texts represent values that are out of BIS standard range

\* Tr<sup>#</sup>- trace amounts ( $\leq 0.3$ )

Dissolved oxygen (DO) of sample GN10, collected from filter tank (Figure 2h), and was found to be the highest (7.76 mg/l) among all collected samples. Total hardness of the sample GN12 was found to be the highest (200 mg/l). Total dissolved solids (TDS) for the sample GN12 was found to be 578 ppm. It was logical that sample GN12 also possess highest electrical conductivity (EC = 606.0  $\mu$ s) and salinity

(580 ppm) and naturally very less resistivity (resistivity =0.865 K $\Omega$ ). Sample GN7, which was collected from wells, shows nominal variations in the respective parameters, even though they were collected from two distant locations. For example, values of total hardness, TDS, EC, salinity and resistivity for GN7 was measured as 150 mg/l, 572.5 mg/l, 601.2  $\mu$ s, 573 ppm and 0.8733 K $\Omega$ , respectively

Table.	3:	Comparison	of	<b>Physico-Chemical</b>	Parameters	among	Water	Samples	Collected	from	Hospitals	with
Surroi	ındi	ings Areas of H	Iosp	pitals and BIS stand	ards of water.							

Parameters	Water sam hospitals (GN1, G	nples from GN2, GN11, GN13)	Samples from sur hospitals (GN3, GN	BIS Standards		
	maximum	minimum	maximum	minimum	Max.	Min.
pН	7.07(GN18)	5.91(GN11)	6.96(GN12)	6.11(GN8)	8.5	6.5
TDS (mg/l)	222.2(GN11)	144.9(GN13)	578.1(GN12)	78.66(GN3)	2000	500
EC (µs)	232.9(GN11)	51.7(GN13)	606 (GN12)	82.4(GN3)	-	-
Salinity (ppm)	217.2(GN11)	141.5(GN13)	580 (GN12)	78.72(GN3)	5	1
Total hardness (mg/l)	87.5(GN2)	50(GN13)	200 (GN12)	25(GN3)	-	-
DO (mg/l)	6.62(GN2)	5.55(GN11)	7.76 (GN10)	3.41(GN4)	>5	5
Fluoride (mg/l)	0.302(GN13)	0.098(GN11)	0.186 (GN7)	0.114(GN6)	1.0	-
Iron (mg/l)	0.23(GN13)	Tr	0.72 (GN9)	Tr	-	0.3

A comparison of various physico-chemical parameters with that of the BIS standards is presented in Table3 and it was observed that the physico-chemical parameters of the water samples, which are collected from hospitals have fewer amounts of TDS, total hardness, DO, Salinity and less electrical conductivity compared to the samples collected from surrounding areas of hospitals. Few of the analyzed physico-chemical parameters of water samples collected from hospitals are present below the acceptable limit of BIS standard (Fluoride, Iron, Total Hardness, and TDS) (BIS standards). However, resistivity and pH lies in the range of acceptable and permissible limits of BIS standard.

#### pН

The pH of any water sample is a measure of its acidic or basic property [4]. It is one of the major parameters for determining the quality of water, as it provides information in regard to effects on solubility of various metallic contaminants. pH fluctuation in water bodies are related to the discharge of industrial, hospital, home hold and human waste [16]. Change in pH further leads to change in physico-chemical and biological parameters of water. Higher pH contributes to the formation of tri-halo-methane which are toxic [4]. Alkaline pH value is witnessed due to presence of alkaline earth metals like sodium andpotassiumwhich interact with soluble CO forming carbonates and bicarbonates resulting in shifting of the pH above 7.0 (BIS standards). The highest value of pH among all the samples was recorded as 7.07 (assumed in the neutral pH range) for GN13 and the lowest value came up as 5.91 (acidic pH) for the sample GN11 (table 2).



Figure 3: pH values of the samples collected from different location.

#### **Electrical Conductivity**

Electrical conductivity (EC)represents the ability of water to conduct electrical current which in return provides a general indication of water quality with respect to amount of total dissolved solids like presence of cations and anions, their concentration, mobility,etc [17]. Therefore, an augmentation in EC is evident usually with an increase in the concentration of dissolved salts in a particular water sample [2]. Conductivity of water also varies with changes in temperature. The highest EC value among all the collected samples was recorded as 606  $\mu$ s for GN12, whereas the lowest came as 82.4  $\mu$ s for GN3 (table 2).



Figure 4: Electrical conductive values of the collected samples.

#### Total Dissolve Solids (TDS)

TDS is general proportional to degree of pollution. It is considered as an indicator of salinity of water and describes all solids dissolved in water [18] [19]. TDS comprise inorganic salts (Ca, Mg, Na, K, HCO<sub>3</sub>, Cl, and SO<sub>4</sub>) and some small amounts of organic matter dissolved in water. TDS can be influenced by changes in pH because it leads to precipitation of some of the solutes as well as affects solubility of suspended matter. Water containing more 500 mg/L of TDS is not considered desirable for drinking [10]. The highest value of TDS among all collected samples was measured as 578 mg/l (GN12), and the lowest one was 84.65 mg/l (GN10). TDS will effects the Electrical Conductivity of water that is why the sample GN12 with higher TDS causes high EC and the sample GN10 has lower TDS with less EC (table 2).



Figure 5: Total Dissolved Solids (TDS) values of the samples (Red column- within the BIS limit and Blue column-below the BIS limit).

#### Turbidity

Turbidity is a measure of the degree to which the water loses its transparency due to the presence of suspended particulates [20][15]. It is caused due to presence of suspended particles and/or colloidal matter which reduce the transmittance of light through water. It may be caused by inorganic or organic matter or a combination of both. High turbidity being an indicator of presence of large number of suspended solids, significantly reduces the aesthetic quality of water source [20]. It increases the cost of water treatment for drinking and food processing. Generally, turbidity causing factors can be either natural or human induced. Human activities can also cause erosion resulting in an increase in turbidity. Although substances resulting in high turbidity may not be intrinsically harmful, but significantly interfere with any disinfection process during water treatment and may provide a medium for microbial growth[2]. These microbes include bacteria, viruses and parasites which cause symptoms such as nausea, cramps, diarrhea etc. Among all the collected water samples, the highest value of Turbidity was found to be 418 TNTU for GN8 and lowest value was 0.09 TNTU for GN11 (table 2).



Figure 6: Turbidity values of the samples (Red columnhigh values above the limit and Blue column-within the limit, Yellow column- below the limit).

#### Salinity

Salinity of any water body is correlated to its TDS, since it indicates the presence of dissolved salts [7]. Small amounts of dissolved salts in natural waters are required for the life of aquatic plants and animals but higher quantities lead to severe health issue like increased blood pressure or hypertension leading way to cardiovascular diseases [7][21]. Among the collected water samples, the maximum value of salinity is 580ppm for GN12 and minimum value is 78.72 ppm for GN3. Salinity is related to EC&TDS-if salinity will increase then EC&TDS of water will also increase.



Figure 7: Salinity values of the samples (Red column- high values above 150 mg/L and Blue column-below 150 mg/L).

#### Fluoride

Fluoride is required in low concentration by human body to prevent dental diseases but very high concentration of fluoride causes fluorosis which affects the teeth and bones [22][23]. Moderate amounts lead to dental effect but long-term ingestion of large amount of fluoride results in skeletal disorders[22]. The highest amount of fluoride content was found for the sample GN13 (from civil hospital) and was found to be 0.302 mg/l. The lowest amount was found for the sample GN11 (from CHRISTIAN HOSPITALS, 0.09 mg/l).

Fluoride content in all the samples was found to be quite similar and within the range of 0.09-0.302 mg/l although, below the desirable limits prescribed by WHO and Indian BIS standards Fluoride content in all the samples was found to be guite similar and within the range of 0.09-0.302 mg/l although, below the desirable limits prescribed by WHO and Indian BIS standards (WHO standards 2011) (BIS standards 2010). Iron content in all the samples were in negligible amount, except for the samples GN8, GN9 and GN13. Iron content for these samples were determined as 0.35 mg/l, 0.72 mg/l and 0.23 mg/l respectively. Comparing the values with BIS standards, it can be ascertained that the amount of iron present in sample GN13lies within the limits of BIS standards, but the same for GN8 and GN9 lies above the limits of BIS standards. The pH of the sample GN18 was found to be 7.07 and was the highest among all collected samples. Highest Turbidity was obtained for the sample GN8 which was measured as 418 NTU.



Figure 8: Fluoride content of the samples (all samples have below the lower limit of BIS standards).

#### Iron

Iron is an important element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bio-availability and ranges from 10 to 50 mg/day [24][25]. Groundwater may hold iron (II) at concentrations up to several milligrams per liter without discoloration or turbidity in the water when directly pumped from a well [26]. Taste is not usually noticeable at iron concentrations below 0.3 mg/liter, although turbidity and color may develop in piped systems at levels above 0.05–0.1 mg/L[27]. Laundry and sanitary ware tends to stain at iron concentrations above 0.3 mg/L. In drinking-water supplies, iron (II) salts are unstable and are precipitated as insoluble iron (III) hydroxide, which settles out as a rust-colored silt. During the study, maximum amount of iron was found in GN9 (0.72 mg/l) and minimum amount was found in GN13 (0.23 mg/l).



Figure 9: Iron content of the samples (Red column- in limit of BIS standards and, Yellow column- below the limit).

#### Screening for bacterial contamination

According to the recent BIS slandered reports [15] and literatures [28][29] the microbial contamination is the main reason for water borne diseases like diarrhea. Likewise, as per the reports it is anticipated that in 2015, an estimated 1.3 million people lost their lives from diarrheal diseases and among them 499,000 were children, younger than five years old [28]. So,

Microbial contamination, which is reducing water quality is linked to causing inflammation, anemia, stunting and it's becoming the matter of concern [29][30].

The analysis of bacterial contamination can be seen from the images provided in Figure 10. This test was carried out by using the water testing field kit which was designed by DRDO-DRL Tezpur. The kit is provided with testing facilities for both physio-chemical and bacteriological parameters. The test is semi quantitative and are based on accept/reject basis.No bacterial contamination was detected in the first 24 hours of incubation as all samples maintained the characteristic yellow colour. Only after 48 hours of incubation, samples GN10 and GN12 showed a transition to the colour black (Table. 4). Post 72 hours incubation bacterial contamination was detected in 2 more samples (i.e., GN5 & GN6). This concludes that the remaining samples are free from bacteria, and also we can say that the The analysis of bacterial contamination can be seen from the images provided in Figure 10. This test was carried out by using the water testing field kit which was designed by DRDO-DRL Tezpur. The kit is provided with testing facilities for both physio-chemical and bacteriological parameters. The test is semi quantitative and are based on accept/reject basis.No bacterial contamination was detected in the first 24 hours of incubation as all samples maintained the characteristic yellow colour. Only after 48 hours of incubation, samples GN10 and GN12 showed a transition to the colour black (Table. 4). Post 72 hours incubation bacterial contamination was detected in 2 more samples (i.e., GN5 & GN6). This concludes that the remaining samples are free from bacteria, and also we can say that the water samples (GN1, GN2, GN11 and GN18) which are collected from hospitals were free from bacterial



Figure 10: Analyses for Bacterial contamination using DRDO Water Testing Kit: (a) No Bacterial contamination after 24 hours incubation; (b) Contamination in sample GN10 and GN12 after 48 hours; and (c) Bacteria contamination observed in GN5 and GN6 after 72 hours.

contamination, due to the presence of less turbidity inbecause those samples which were collected from hospitals have less turbidity this is one of the reason for absence of bacteria in those samples. It is interesting to find that samples like GN3 and GN4, although having high turbidity did not display any bacterial contamination, whereas samples like GN10 and GN12 having very less turbidity did show the presence of bacterial contamination. A probable reason may be attributed to the location of the samples' sources where certain inconsistent external factors might have played a role.

S. No.	Time taken for incubation	Sample with bacterial contamination
1.	24 hours	None
2.	48 hours	GN10,GN12
3.	72 hours	GN5,GN6

Table 4: Results of bacterial contamination analysis.

#### CONCLUSION

From this present study it can be concluded that, the physicochemical parameters of different water samples which were collected from in and around the hospitals in Tezpur town, Assam, had substantial variation. Samples GN7 and GN12 have greater amounts of TDS and DO in comparison with other samples. Because of this greater amount of DO, the metal ions present in the water sample get oxidized with accompanying increase in pH value. That is why these two samples have slightly higher value ofpH.

Samples GN5, GN6, GN10, and GN12 showed the presence of bacterial contamination. Compared to other samples, the water samples which are contaminated with bacteria, have significantly very high value of DO. Because of this higher amount of DO in water, bacteria in the water samples can have a chance to sustain for longer time as we have observed for the samples GN5, GN6, GN10, and GN12. Since there was no substantial uniformity of values obtained from all the undertaken parameters that could virtually connect the influence of hospitals on its nearby biota. Therefore, from this study it can be concluded that the hospitals may have minimum to nil influence on the quality of drinking water surrounding hospital areas.

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## **GEOMICROBIOLOGY: AN EMERGING SCIENCE AND WAY OUT TO** ENVIRONMENTAL AND HEALTH PROBLEMS

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#### Abstract

Geomicrobiology involves the role of microorganisms in geological environments and geochemical processes. Extreme environments such as caves, hot springs, deep-sea hydrothermal vents, marine environments, extreme cold environments such as Arctic and Antarctic, deserts, and heavily polluted environments, host diverse microbial species of varied interest. Researchers from various fields are partnering to investigate such microorganisms for a variety of environmental and health applications. It is estimated that our planet still has 99.99 percent of its microbial diversity unexplored. So, to delve deeply into the exploration of microbial diversity and its functions, the metagenomic approach acts as a lens for a broader picture than traditional culturing methods. The metagenomics approach entails the direct investigation of microorganisms in specific environmental samples. Several such discoveries have made significant contributions to medicine, pharmaceutical industries, bioremediation, biodegradation, biomineralization, biomining, biofuel production, space exploration, and other fields. Thus, this article discusses the identification of extreme microbes and their possible potential applications in the environment and health.

**Keywords** Geomicrobiology, Extreme Environments.

#### INTRODUCTION

Geomicrobiology is a cutting-edge area of researchthat involves the interactions of microbes with earthly processes. Microorganisms though invisible are the rulers of the planet. Microorganisms isolated from extreme environments have a wide range of applications in environmental and health sciences. Our planet has room for every kind of extremities like boiling heat (Thermophiles) and freezing temperatures (Psychrophiles), highly acidic (Acidophiles) and basic conditions (Alkaliphiles), extremely high pressure (Barophiles) and low pressure, regions of high radiation intensities, nutrient-rich and deficient conditions, and also the combination of two or more such extremes. Microbes that live in harsh environments have a unique metabolic potential that allows them to survive in such extremities. Indeed, these microorganisms cannot survive in the absence of the extreme conditions to which they have adapted. To identify unknown microbial diversity conventional culture-dependent methods

are not enough, because these methods can be used only for the predefined hypothesis of known diversity. So to overcome the roadblock of conventional culture methods, the meta-analysis of the environmental samples i.e the cultureindependent methods will pave the way. Recently the search for novel microbes in extreme environments has become the frontier research subject. To unravel the estimated 99.99 percentage of microbial diversity, metagenomics analysis will act as a lens to zoom in on the untapped organisms.

The main objective of this review paper is to discuss the identification and the applications of microorganisms isolated from extreme environments in the field of environment and healthcare.

#### Culture dependent and independent methods for characterization of geomicrobiome

The microbial diversity in environmental samples can be

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extracted in two ways, as shown below.

- Culture dependent method
- Culture-independent method

#### Culture dependent methods

Various agar plate and liquid media methods are employed in the traditional approach to recover the microbiome from the intended environmental sample. Different microbial species require different compositions of medium for their growth. So, culture-dependent methods are commonly used for predefined microbial community targets. The typical culture media should contain energy sources (Light, carbohydrates, energy from oxidation, etc.), sources of carbon, nitrogen, sulphur, and phosphorus, minerals like calcium (Ca<sup>2+</sup>) magnesium (Mg<sup>2+</sup>), and sodium (Na<sup>+</sup>), vitamins, and growth factors (Amino acids, blood, and its derivates, antioxidants, etc.). Different types of culture media used for the isolation of bacteria and fungi and their target species are depicted in Table 1. After the isolation of microbes via culture, the microbial communities can be characterized using either traditional biochemical methods or DNA extraction followed by sequencing.

Culture media for bacteria **Target species** Culture media for fungi **Target species** Nutrient agar To isolate non-fastidious Birdseed agar Cryptococcus neoformans organisms like klebsiella pneumonia, Morgnella morganni, Providencia alkalifaciens, Salmonella typhimurium Histoplasma capsulatum Enterobacteriaceae Mac Conkey agar Brain-heart infusion agar and *Blastomyces* dermatitidis To isolate various Mannitol salt agar Staphylococcus aureus CHROMagar Candida candida species medium Chocolate agar To isolate pathogenic Czapek-Dox agar Aspergillus and Penicillium species. bacteria To isolate most fungal Thayer-Martin agar Neisseria gonorrhoea Potato dextrose agar species Cetrimide agar Pseudomonas aeruginosa Potato flake agar To isolate saprophytic and pathogenic fungi Hektoen enteric agar Salmonella sp. and Sabouraud dextrose agar To isolate clinically Shigella sp. important fungi

Table	1:	Culture	media	for	bacteria	and	fungi	with	the t	target	species.
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#### Culture-Independent methods

The steps involved in the culture independent method for the investigation of microbial diversity are given below(Simon and Daniel, 2011):

- Culture independent method involves the direct extraction of DNA and RNA from the environmental sample based on the need.
- In the case of DNA, the extracted DNA can be amplified for the construction of a metagenome library for bioprospecting application.
- To assess the taxonomic diversity, the extracted metagenomic DNA from the target sample is subjected to sequencing of phylogenetic marker genes.
- The extracted DNA can also be sequenced to assess the taxonomic diversity and their metabolic potential.

• To assess the microbial community functions and the active members of the microbial community the extracted RNA should be subjected to mRNA and rRNA analysis respectively.

# Applications of Extremophiles in Environment and Health

The metabolic potential and the structural stability of the extremophiles have immense applications in environmental and health sectors. Scientific exploration of these resources is still in the infant stage of its development. Table 2 depicts the various applications of extremophiles.

#### **Applications in Environment**

Environmental health is the price we pay for all of the modern conveniences. Since the beginning of the green revolution and industrial revolution, the quality of earth's products such as air, water, soil, and other natural resources has begun to deteriorate. Thus it is our primary responsibility to restore

	** *			
S. No.	Microbes	Habitat	Applications	Reference
1	Desmodesmus sp. MAS1			(11)
2	Heterochlorella sp. MAS3	Soil and Lake water	Bioruel production	(Abinandan et al., 2018)
3	Nesterenkonia sp.	Hypersaline lake in Iran	Butanol, ethanol, and acetone production	(Amiri et al., 2016)
4	Pseudomonas fluorescens AH-40	Crudeoil polluted soil	Bioremediation of phenanthrene contaminated sites	(Mawad et al., 2020)
5	Streptomyces sps.	Bay of Bengal	The biosynthetic and bioactive compound	(Ghosh et al., 2020)
6	Penicillium sp. (GBPI_P155)	The soil of the Indian Himalayan region	Natural colour pigment (Orange)	(Pandey et al., 2018)
7	Bacillus licheniformis	Geothermal springs of	Thermostable enzyme	(Panosvan et al. 2020)
	Anoxybacillus favithermus	Armenia and Nagorno	production	(1 unosyun et un., 2020)
	Parageobacillus toebii	Karabakh		
8	Pyrodictium delaneyi Su06	Deep-sea hydrothermal vent	Iron oxide reduction	(Kashyap and Holden, 2021)
9	Rhodothermus marinus	Hot spring	Biorefinery	(Kristjansdottir et al., 2020)
10	Geomyces sp.F09-T3-2	Marine sponges	Pectinolytic activity	(Poveda et al., 2018)
11	Penicillium glabrum SF-7123	Antarctic marine	Anti inflammatory and anti-neuroinflammatory	(Ha et al., 2020)
12	Haloferax volcani	Dead sea	Antioxidant, bioprocessing, bioremediation, carotenoid synthesis	(Haque et al., 2020)
13	Streptomyces sp. AMA50	Marine environment (Thailand)	Antifungal activity	(Sangkanu et al., 2021)
14	Serratiopeptidase (Isolated from Serratia marcescens)	The intestine of Bombyx mori L.	Anti-inflammatory, fibrinolytic effects	(Jadhav et al., 2020)
15	Archaeoglobus fulgidus	Hot springs, high-temperature oil fields	Biodegradation of Phthalic acid ester's (PAEs)	(Zhang et al., 2020)

Table 2: Application of extremophiles in various environmental and health applications.

environmental health by remediating and utilizing it sustainably. Fighting fateful harsh conditions like highly polluted environments, acid mine drainage, clearing landfills, restoring the degraded lands, and so on with the geologically extreme microbes have gained importance among environmental scientists. It is also of significant interest to employ them as an environmentally acceptable option for biofuel production, bioremediation, biodegradation, biomining, bioleaching, bioplastics, and for a variety of synthetic and toxic chemicals used in industrial applications.

#### **Biofuel Production**

Replacing fossil fuels with eco-friendly alternatives will be the greatest contribution to achieve sustainable development goals (SDGs). The production of biofuels started with edible biomasses like sugarcane, wheat, maize, animal fats, and so on, and the second generation fuels gradually shifted to nonedible biomasses like waste, wood, straw, grasses, bagasse, etc. The third-generation biofuel production employs various microorganisms and microalgae as an efficient alternative to the previous generation of biofuels. To develop cost-effective and energy-efficient biofuels as a superior replacement for fossil fuels while simultaneously addressing rising energy demand is the need of an hour.

#### Enzymes

Extremophiles create extremozymes, which are stable enzymes that can resist extremes in temperature, pH, light intensity, and so on. Extremozymes have the potential application in biotechnological advancements and in the case of biofuel production enzyme activity is an important process in the conversion of the raw biomass into biofuels. For instance, microalgae are extensively used for biofuel production as they can accumulate 50- 70% of lipids by  $CO_2$  fixation (Saharan et al., 2013). The lipids can be further degraded by cellulose-degrading enzymes and thus producing bio-oils. Many acidophilic, halophilic, and thermophilic fungus and bacteria are also employed in the production of green energy generations.

#### Biomining

Biomining uses microorganisms to recover metals and ores from the environment, as well as to clean up metalcontaminated sites. The mining sites and mine drainages are well known for their acidic pH. The extreme acidity will degrade the lands and also the plant growth is inhibited thus causing land and resource degradation. Mining for the commercially important metals and ore in a sustainable way is the major challenge. Extremophiles will be the best solution to the challenge as they can resist a wide range of temperatures and pH fluctuations. Acidophilic microbes, for example, may keep their internal pH lower than other microbes, allowing them to endure such acidic environments without internal cell damage (Chen, 2021). Widely employed extremophiles in biomining include Acidothiobacillus ferrooxidans, Pyrococcus furiosus, sulfolobus metallicus, Metalosphaera sedula, etc.

#### Bioremediation

Microbes are known as potential remediators as they can degrade even complex chemical compounds and heavy metals.The bioremediation process though has many advantages it has some disadvantages such as the slow rate of degradation of pollutants. To overcome this, it is important to understand the microbial communities involved in the bioremediation process at the molecular level. Microbes that thrive in geological extremes are the ideal cleaners for manmade extremes. As they have the ability to adapt and respond to the increased contaminants in the surrounding environment. Extremophiles who live in hostile environments have developed mechanisms to mobilize, immobilize, convert, absorb, or biodegrade pollutants in an eco-friendly way. They also have the potential to transform toxic organic pollutants into nontoxic end products within a short period of time irrespective of their half-life (Dua et al., 2002; Reiger et al., 2002; Sinkkonen and Paasivirta, 2000). Bacillus safensis, Haloferax mediterranei, Nitrosopumilus maritimus, Pseudomonas stutzeri, Halococcus salifodinae, Halobacterium noricense, methanothrix soehngenii, Methanococcus mazei, Halofera mediterranei, Rhodanobacter sp. are some of the examples of extremophiles used in the field of bioremediation to remediate petroleum products, heavy metals, pesticides, wastewater and radionuclides (Kaushik et al., 2021).

#### Applications in Health

Modern medical science and technological advancements have cured a variety of previously incurable health conditions.Microorganisms that are resistant to contamination and fluctuation have a huge role in medicine. Extremophiles producing bioactive compounds, antimicrobial peptides, and enzymes are of great commercial interest and are considered a safe alternative for synthetic compounds.

#### **Cure for Protein Misfolding Diseases**

Proteins are highly susceptible to external and internal stress conditions. This stress may eventually lead to protein denaturation and paves way for various degenerative and neurodegenerative diseases like Alzheimer's disease, cystic fibrosis, Huntington's disease, Parkinson's disease. Recently, the stability of extremophiles and the extremozymes and extremolytes produced by them have gained importance in the field of regenerative medicine. Marine ecosystems serve as a reservoir for a variety of interesting neuroprotective compounds. In particular, Alzheimer's disease (AD) is a booming problem among the old-age population. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) are considered as the important target enzymes to treat or prevent AD. The alkaloid compound derived from a marine bacterium Rapidithrix thailandica (Marinoquinoline A) (Sangnoi et al., 2008) and fungus Acrostalagmus leteoalbus TK-43 (Acrozines A-C) --(Cao et al., 2019) are proved to have an inhibitory effect on AChE.

#### **Cure for Antibiotic Resistance**

The widespread use of antimicrobial compounds has caused the targeted microbial communities to adapt and resist the drugs. Therefore, antimicrobial/antibiotic resistance is the major concern face by health care sectors and remains a barrier to treat the diseases effectively. The development of new antimicrobials will be the breakthrough in the fieldof drug discovery. Since microorganisms have the capability to adapt and resist any kind of harsh conditions, the need for new antimicrobial compounds is in growing demand. Extremophiles present in the caves, marine environment, highly saline environment, and other extreme environments are the reservoir for the brandnew bioactive compounds that will be the promising key to break the challenge of antimicrobial resistance.

Peptides, diketopiperazines, and other bioactive compounds produced by extremophiles are of main interest for antimicrobial compounds. To name some microbes that are identified from extreme environments and are currently involved in the field of drug discovery are *Penicillium* sp. *Trichoderma velutinum–(Singh et al., 2018), Penicillium* granulatum MCCC 3A00475(Niu et al., 2018), Acremonium persicinum SCSIO 115—(Luo et al., 2019), Aspergillus syndowii SP-1(Li et al., 2017).

#### Other health care applications

The other medicinal applications of extremophiles include:

- The carotenoid pigment isolated from extreme halophilic archaeon *Halobacterium salinarum* is used as artificial retinas (Charlesworth and Burns, 2015).
- Radiation-resistant extremophiles can produce extremolytes and extremozymes which have the potential application as sunscreen, antioxidants, antiproliferative, anticancer agents, DNA repair mechanism, etc (Aguilera et al., 2002; Asgarani et al., 2000; Babu et al., 2015; Russo et al., 2008).
- Extremophiles are also used as alternative vaccine delivery vehicles.
- Health supplements
- Protein stabilizers
- Anti inflammatory and neuroinflammatory
- Skincare products
- Drug delivery

#### CONCLUSIONS

Geomicrobiological studies provide vast resources of microorganisms that can be explored for countless environmental and medicinal applications. Human beings are the sole responsible for the fateful extreme conditions like landfills, disposal of biomedical and electronic waste, polluted environments, radiation environments, land exploitation, and wastelands due to intensive agriculture practices and pesticide usage. As a result, climate change is the compliment we have gained for environmental destruction. Biomedical waste disposal is one of the major reasons for the development of antibiotic-resistant microbial strains. Therefore, understanding the microbes and their metabolism at the molecular level is the best of all solutions. Though the exploration of extremophiles and their applications in different fields have gained importance, it still needs a drift for active commercial applications. Thus, the metagenomics analysis of geomicrobiome will surely be the way out to the majority of environmental and health problems.

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# WATER QUALITY STUDY IN MORNI TOWN, PANCHKULA DISTRICT, HARYANA, INDIA

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#### Abstract

Water is necessary for survival of living beings. In the modern developmental activities water is one of the natural resource under pressure of anthropogenic activities. In hilly areas it is also polluted due to anthropogenic activities and direct mixing of polluted water with fresh water due to flow of polluted water through joints and fractures. In the present study water quality at Morni town located on Lower Siwalik hill has been studied. Five water samples have been collected during June 2018 and analysed using field water testing kit prepared by Tamilnadu Water Supply and Drainage Board, Chennai for twelve chemical parameters. The results of chemical analysis of water samples shows that pH varies 6.5 -7, alkalinity 100 mg/l to 150 mg/l, hardness 140 mg/l to 230mg/l, chloride 20mg/l to 50mg/l, total dissolved solids (TDS) 336 mg/l to 516 mg/l, fluoride 0.5 mg/l to 1.5mg/l, Iron 0 mg/l to 10mg/l, ammonia 0.5mg/l to 2.0 mg/l, nitrite 0.2 mg/l to 0.5 mg/l to 0.5 mg/l. The water quality is potable in three water samples and non-potable in two water samples (Kila Ghat and Handpump-2). The study is useful for monitoring the water quality for drinking purpose.

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#### **Keywords**

Water, quality, potable, non-potable, Morni, Haryana.

#### INTRODUCTION

Water is prime requirement for survival of living beings. Suitability of water for different uses like drinking, agriculture and industrial purposes mainly depends on its quality. For all the different uses of water for drinking purpose its quality should be as per drinking water standards of Bureau of Indian Standards in India. The availability of good quality water in present context of developmental activities has become rare because of anthropogenic activities in all types of terrain whether plain or hills. Workers have done work on water quality in different types of terrains to understand the water quality status (Goel et al. (2018), Ismail (2015), Oiste (2014), Satyanarayana et al. (2013), Sujatha et al. (2012), Wali et al. (2018)).

#### Study area

Morni town is located on Lower Shiwalik Hills in Panchkula district of Haryana. The geo-coordinates of the town is latitude 30.7° N and longitude 77.08° E and located at an altitude of 1267m above mean sea level.

#### Objective

The main objective was to study water quality for drinking purpose in Morni town, Panchkula district, Haryana.

#### **MATERIALS AND METHODS**

Total five water samples were collected from different locations in Morni Town in the month of June 2018 in 120 ml plastic bottles. Water samples were analysed using Field Water Testing Kit prepared by Tamilnadu Water Supply and Drainage Board, Chennai for twelve chemical parameterspH, alkalinity, hardness, chloride, total dissolved solids, fluoride, iron, ammonia, nitrite, nitrate, phosphate and residual chlorine (Table 1). Chemical analysis results of water samples were compared with BIS Drinking Water Standards (IS 10500:2012) (Table 2). The chemical analysis results were entered in the excel and prepared bar graphs for each chemical parameter.

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Water Sample	Latitude	Longitude	рН	Alkalinity	Hardness	Chloride	TDS	Fluoride	Iron	Ammonia	Nitrite	Nitrate	Phos- phate	Resi- dual
Kila Ghat	30.689847	77.082847	6.5	100	150	30	336	1.5	2	0.5	0.5	20	0	0.5
Water Tank	30.689836	77.082807	6.5	130	150	30	372	1	0.3	0.5	0.5	45	0	0
Hand pump-1	30.688037	77.086669	6.5	150	200	30	456	1.5	1	0.5	0.2	20	0	0
Hand-2 pump	30.686457	77.088832	7	150	230	50	516	0.5	10	2	0.5	45	0	0.2
Jal Ghar	30.687817	77.087525	6.5	130	140	20	348	0.5	0	0.5	0.5	20	0	0

 Table1: Water sample analysis results (in mg/l except pH).

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

S. No.	Parameter	Pota	able	Non-Potable
		Desirable	Permissible	
1.	рН	6.5 to 8.5		< 6.5 to > 8.5
2.	Total Hardness (mg/l)	<200	200-600	> 600
3.	Iron (Fe) (mg/l)	<0.3		> 0.3
4.	Chlorides (Cl) (mg/l)	<250	250-1000	> 1000
5.	Total Dissolved Solids (TDS) (mg/l)	<500	500-2000	> 2000
6.	Nitrate (NO3) (mg/l)	< 45		> 45
7.	Fluoride (F) (mg/l)	< 1.0	1.0-1.5	> 1.5
8.	Residual Chlorine (RC) (mg/l)	< 0.2	0.2-1	> 1.0
9.	Ammonia (mg/l)	< 0.5		> 0.5
10.	Alkalinity (mg/l)	< 200	200-600	> 600
11.	Nitrite (mg/l)	<1.0 mg/l	-	>1.0 mg/l
12.	Phosphate (mg/l)	<1.0 mg/l	-	>1.0 mg/l

#### **RESULTS AND DISCUSSION**

#### pН

pH in the water samples varies 6.5 to 7. In all the five water samples pH is desirable as per drinking water standards (Figure 1, Table1 and Table2).



Figure 1: pH in water samples.

#### Alkalinity

Alkalinity in the water samples varies 100 mg/l to 150 mg/l. In all the five water samples alkalinity is desirable category as per drinking water standards (Figure 2, Table1 and Table2).



Figure 2: Alkalinity (mg/l) in water samples



Figure 3: Hardness (mg/l)in water samples.

#### Hardness

Hardness in the water samples varies 140 mg/l to 230 mg/l. In Handpum-2 hardness is permissible (230 mg/l) and in other four water samples hardness is desirable as per drinking water standards (Figure 3, Table1 and Table2).

#### Chloride

Chloride in the water samples varies 20 mg/l to 50 mg/l. In all the five water samples chloride is desirable as per drinking water standards (Figure 4, Table1 and Table2).



Figure 4: Chloride (mg/l)in water samples.

#### Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS) in the water samples varies 336 mg/l to 516 mg/l. In Handpum-2 TDS is permissible (516 mg/l) and in other four samples Total Dissolved Solids is desirable as per drinking water standards (Figure 5, Table1 and Table2).



Figure 5: Total Dissolved Solids (TDS) (mg/l) in water samples

#### Fluoride

Fluoride in the water samples varies 0.5 mg/l to 1.5 mg/l. In all the five water samples fluoride is desirable as per drinking water standards (Figure 6, Table1 and Table2).



Figure 6: Fluoride (mg/l)in water samples.

#### Iron

Iron in the water samples varies 0 mg/l to 10 mg/l. In two water samples-Kila Ghat (2mg/l) and Handpum-2 (10 mg/l) iron is non-potable and in other three water samples iron is desirable as per drinking water standards (Figure 7, Table1 and Table2).



Figure 7: Iron (mg/l)in water samples.

#### Ammonia

Ammonia in the water samples varies 0.5 mg/l to 2 mg/l. In one water sample-Handpum-2 (2 mg/l) ammonia is nonpotable and in other four water samples ammonia is desirable as per drinking water standards (Figure 8, Table1 and Table2).



Figure 8: Ammonia (mg/l)in water samples.

#### Nitrite

Nitrite in the water samples varies 0.2 mg/l to 0.5 mg/l. In all the five water samples nitrite is desirable as per drinking water standards (Figure 9, Table1 and Table2).



Figure 9: Nitrite(mg/l) in sample locations.

#### Nitrate

Nitrate in the water samples varies 20 mg/l to 45 mg/l. In all the five water samples nitrate is desirable as per drinking water standards (Figure 10, Table1 and Table2).



Figure 10: Nitrate (mg/l)in sample locations.

#### Phosphate

Phosphate in all the five water samples is nil. In all the five water samples phosphate is desirable as per drinking water standards (Figure 11, Table1 and Table2).



Figure 11: Phosphate (mg/l)inwater samples.

#### **Residual Chlorine**

Residual Chlorine in the water samples varies 0mg/l to 0.5 mg/l. In all the five water samples residual chlorine is desirable as per drinking water standards (Figure 12, Table1 and Table2).



Figure 12: Residual Chlorine (mg/l) at water samples.

#### CONCLUSIONS

In the study area water quality is non-potable in water samples Kila Ghat (iron 2 mg/l), Handpump-2 (iron 10 mg/l; ammonia 2 mg/l) and potable in water samples Water Tank, Handpump-1, Jal Ghar. The study is highly useful for monitoring the water quality for drinking purpose in the study area.

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# B. Health Sciences Section



### PREECLAMPSIA AND STERILE INFLAMMATORY MOLECULES

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#### Abstract

Preeclampsia (PE), as a pregnancy-specific diseases, has become one of the main causes of maternal and fetal morbidity & mortality worldwide. It is also one of the major risk factor for pre term birth. PE is typically characterized by hypertension, proteinuria, and an excessive maternal systemic inflammatory response. Recent evidences suggests the notion that natural killer T (NKT) cells (a small, but significant immunoregulatory T cell subset of human peripheral blood lymphocytes) play pivotal roles in pregnancy. NKT cells with unique transcriptional and cytokine profiles exist in different peripheral tissues acting as mediators between the innate and adaptive immune systems. NKT cells secrete Interleukin-4 (IL-4) and Interferon-y (IFN-y) which might regulate the balance between Type 1 T helper (Th1) and Type 2 T helper (Th2) responses. Sterile inflammatory molecule and damage-associated molecular pattern (DAMP) released from various cells during stress has been implicated in inflammation. Studies showed that there is a direct relationship between inflammation and preeclampsia. Here, we intend to summarize the concept of the emerging link between sterile inflammatory molecules and PE. Further more, we will discuss the possible therapeutic strategies that target sterile inflammatory molecules for the PE.

#### Keywords

PE, sterile inflammatory molecules, circulating nucleicacids, Decorin.

#### Introduction

Preeclampsia (PE) is a pregnancy-specific hypertensive disorder characterized by hypertension, proteinuria and other systemic disturbances at or after 20 weeks of gestation (1). It is one of the leading causes of maternal and fetal major adverse events including death and iatrogenic pre term birth (2). The precise mechanisms of PE's pathogenesis, early and accurate diagnosis of this disease remains a challenge till date. It is widely acknowledged that the placenta is the central organ in the pathogenesis, and PE is caused by maternal responses to Placentation and associated with an increased inflammatory state. It has been further proposed that PE is an excessive maternal inflammatory response to pregnancy, involving pro-inflammatory cytokines such as tumor necrosis factor, inter leukin and HMGB1 (3). A recent study reported a presence of maternal adverse events including eclampsia without any traditional clinical and laboratory findings such as hypertension and proteinuria. Hence, there is an urgent need to investigate practical, routinely used, and inexpensive markers for early and accurate diagnosis and risk stratification of PE.

PE presents as gestational hypertension (systolic  $\geq$  140mmHg or diastolic  $\geq$  90 mmHg) and signs such as proteinuria, renal insufficiency, thrombocytopenia, hepatic dysfunction, and pulmonary edema (4). This can result in increased morbidity and mortality rates in affected mothers

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and fetuses, if left un treated. The only known treatment for PE is the delivery of both fetus and the placenta, often resulting in premature birth, leading to deleterious consequences. Since the treatment for PE involves removing the placenta, many studies have thought that an abnormal placenta plays a causal role in the pathogenesis of PE (5-7).

PE affects 2-8% of pregnancies (8) with eclampsia accounting for 50,000 casualties per year worldwide. It is known that a fetus does not have to be present for preeclampsia to develop, as this disorder arises in cases of molar pregnancies within creased placental size and no fetus(9). PE is a major cardiovascular risk for the duration of the pregnancy, post-parturition and in later life, while also being related to maternal malfunction of the vasculature (10-11). Also, endothelial dysfunction may contribute to elevate the peripheral resistance of blood vessels, which forms an essential component of the maternal syndrome (12-13). Although the etiology of pre-eclampsia is still poorly understood, a deficient supply of blood to the placenta giving rise to abnormal placentation is considered to be one of the main features of the disease. Inadequate placentation and poor perfusion are thought to provoke the secretion of many anti-and pro-angiogenic mediators, as well as inflammatory cytokines in to the maternal vasculature.

Inflammation is essential for successful female reproduction. Inflammatory processes are implicated in every step of fertility from the menstrualcycle (ovulation, menses) to early pregnancy (implantation, decidualization) and later during labor (myometrial activation, cervical ripening, weakening of fetal membrane), whereas quiescence of these mechanisms is maintained by local immune cells during gestation to allow maternal tolerance of fetal antigens allograph. However, untimely inflammatory triggers shifting the immunological balance towards activation can lead to adverse pregnancy out comes including preterm birth.

Failure to mount a local inflammatory response in early or late gestation can also lead to adverse conditions, including miscarriages. Evidence shows that impaired inflammatory response is implicated in numerous female reproductive tract pathologies including menstrual disorders(14), endometriosis-associated infertility (15), recurrent miscarriage (16-17), intrauterine growth restriction (18), PE (19-20) and preterm labor (21-22). Infertility has an estimated global prevalence of 9% with >72 million infertile women worldwide (23), whereas preterm birth and PE, the two leading causes of perinatal mortality and morbidity, have an estimated prevalence of>11% (24) and 3-5% (25-26), respectively. Therefore, understanding the mechanisms where inflammation is untimely triggered in the uterus is primordial to develop effective therapeutics to improve fertility and decrease poor obstetrical outcomes.

Inflammation linked reproductive disorders do not require infections. A majority of patients suffering from PE, preterm

labor or other inflammatory diseases during pregnancy display no clinical signs of infection either. Although observational and causal data accumulated for over30 years have linked infection to preterm labor, preterm birth without infection is more prevalent (27). Furthermore, antibiotics are inefficient to prevent preterm labor in women with infection (28),suggestingthatinfection-induced pro labor effects arise from inflammation (self) rather than infection (non-self).

The development of PE is typically a "*two-stage*" process (29): the first stage is the incomplete remodeling of spiral arteries leading to poor placentation and the second stage is the resulting stress, especially hypoxia-induced oxidative stress (30-31), leading to the symptoms typical of late-stage PE. However, the actual originating causes of either of these two stages essentially remains obscure, despite the emergence of many theories. Thus, PE is also known as the "*disease of theories*"(32).

Normal pregnancy is associated with intensive changes in the maternal cardiovascular system that enables adequate oxygen delivery and nutritive ingredients to the fetus. Physiological vascular adaptation (increased blood volume, increased cardiac minute volume and reduced vascular resistance) is followed by increased endogenous production of nitric oxide (NO) and improved response of smooth muscles on the reaction of NO (33-34). Impaired response of the blood vessels on vasoconstrictor agonists during the pregnancy can be partly regulated by NO (35). Recently, several in vitro studies assessing the role of NO in vascular reactivity in pregnancy and PE, observed that the biosynthesis of NO increased during pregnancy, especially in the second trimester, with its peak in the third trimester (36-38). However, some studies showed that there were no changes in the biosynthesis of NO in normal pregnancy compared with non-pregnantwomen (39-40) suggesting that the biosynthesis of NO during normal pregnancy still remains controversial.

#### **Risk Factor of PE**

PE occurs with increased frequency among young, nulliparous women. However, the frequency distribution is bimodal, with a second peak occurring in multiparous women greater than 35 years of age. A number of maternal risk factors have been recognized to identify high-risk pregnant women, including preconception obesity, chronic hypertension, family history, and more. It is hypothesized that an increase in adipose tissue, which is a rich source of pro-inflammatory cytokines and complement proteins, causes an aggravated systemic inflammatory response, angiogenic imbalances in circulation and the placenta, and abnormal placental development resulting in PE (41). Lykke et al. found that PE, spontaneous preterm delivery, or fetal growth deviation in a first single ton pregnancy predisposes women to those complications in their second pregnancy, especially if the complications were severe (42).

In a cohort study of 536,419 Danish women, delivery between 32 and 36 weeks of gestation increased the risk of PE from 1.1% to 1.8% in the second pregnancy, whereas delivery before 28 weeks increased the risk of PE to 3.2%. PE in a first pregnancy, with delivery between 32 and 36 weeks of gestation, increased the risk of PE in a second pregnancy from 14.1% to 25.3% (42). Some risk factors contribute to poor placentation, where as others contribute to increased placental mass and poor placental perfusion secondary to vascular abnormalities. In addition to those discussed above, PE risk factors also include some disorders such as hydatidi form mole, agestation altrophoblastic disease, thrombophilia, urinary tract infection, diabetes mellitus, collagen vascular disease, etc.

Some studies have referred to obesity as arisk factor for PE and showed that the relationship between maternal weight and PE is a progressive risk and varies from 4.3% in women with a BMI<19.8, up to13.3% for women with a BMI  $\geq$  35 (43).

#### Signs & Symptoms of PE

PE can develop without any presenting symptoms or show up as swelling and/or edema, proteinuria and high blood pressure. Other symptoms include, headache, nausea and vomiting, abdominal and shoulder pain, lower back pain, blurring of vision, hyperreflexia, and shortness of breath with anxiety.

PE is a serious condition of pregnancy, and can be particularly dangerous because many of the signs are silent while some symptoms resemble the normal effects of pregnancy. The disease is sometimes referred to as a silent killer because most women do not realize that their blood pressure is going up until it's too late.

#### *High blood pressure (Hypertension)*

High blood pressure during pregnancy is one of the indicators that PE may be developing. It is traditionally defined as blood pressure 140/90 or greater, measured on two separate occasions atleast four hours apart. During pregnancy, arise in the diastolic blood pressure by 15 degrees or more, or a rise in the systolic blood pressure by 30 degrees or more can be a cause for concern.

#### Proteinuria

In PE, when the urine contains proteins, due to the loss of kidney function, it is termed as proteinuria.

#### Nausea or Vomiting

These are significant when the onset is sudden and after midpregnancy. Morning sickness usually disappears after the first trimester, so the sudden appearance of nausea and vomiting after mid pregnancy may be linked to PE.

#### Changes in Vision

Vision changes are one of the most serious symptoms of PE. They may be associated with central nervous system irritation or be an indication of swelling of the brain(cerebral edema). Common vision changes include sensations of flashing lights, auras, light sensitivity, or blurry vision or spots.

#### Stages of PE

For many years PE has been considered to be at wo-stage disease. The first stage comprises poor placentation and the second stage is the clinical expression of the disease i.e., hypertension and proteinuria. Stage 1 is preclinical and without symptoms, occurring between 8-18 weeks of pregnancy when the uteroplacental circulation is established by spiral artery remodeling. This results in dysfunctional perfusion of placental intervillous space with oxidative and hemodynamic stress molecules. The damaged placenta releases excessive pro-inflammatory and anti-angiogenic factors into the maternal circulation.

The precursors of poor placentation are immunological reactions generated from the adaptability of the mother's immune system to the foreign entity i.e., the fetus. Placentation begins after 8 weeks when the uteroplacental circulation, previously closed by tropho blast plugs in the spiral arteries, begins to open. Defective placentation may arise from premature opening, and perfusion of the intervillous space by oxygenated arterial blood. When defective placentation extends over about 10 weeks, it presents as PE. The second half of pregnancy is associated with excessive or deficient placental derived factors in the mother's blood, secondary to placental damage, before the appearance of clinical signs. Superimposition of a second and later spiral artery lesion, called acute a therosis further reduces utero placental perfusion and predisposes spiral artery thrombosis, which underlies the occurrence of placental infarcts and hence PE (44).

#### Mild PE

Women with mild PE are hospitalized for further evaluation and, if indicated, a pretermdelivery would be necessary. A

#### **CLASSIFICATION OF PE**

Mild Preeclampsia	Severe Preeclampsia
Blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic but <160/110 mm Hg	Blood pressure ≥160 mm Hg systolic or ≥110 mm Hg diastolic on 2 occasions at least 6 hours apart while the patient is on bed rest
Proteinuria ≥300 mg/24 h but <5 g/24 h	Proteinuria of 5 g or higher in 24-hour urine specimen or 3+ or greater on 2 random urine samples collected at least 4 hours apart
Asymptomatic	Oliguria <500 mL in 24 hours Cerebral or visual disturbances Pulmonary edema or cyanosis Epigastric or right upper quadrant pain Impaired liver function Thrombocytopenia Fetal growth restriction

Figure 1: (Current Diagnosis & Treatment Obstetrics & Gynecology, 11th Ed. 26. Hypertension in Pregnancy by David A. Millwe, MD)

complete blood count and levels of serum transaminases, lactate dehydrogenase, and uric acid should be checked weekly to twice weekly. Delivery is indicated if the cervical status becomes favorable, antepartum testing is abnormal, the gestational age reaches 40 weeks, or evidence of worsening PE is seen. Women with mild PE before 37 weeks' gestation are evaluated with bed rest, twice-weekly antepartum testing, and maternal evaluation as described (45). Corticosteroids are administered if the gestational age is <34 weeks; amniocentesis is performed as needed to assess fetal pulmonary maturity. Any evidence of disease progression constitutes an indication for hospitalization and consideration of delivery. The benefit of prophylactic in trapartum magnesium sulfate in preventing convulsions in patients with mild PE has not been conclusively demonstrated (46).

#### Severe PE

Severe PE mandates hospitalization. Delivery is indicated if the gestational age is 34weeks or greater, fetal pulmonary maturity is confirmed, or evidence of deteriorating maternal orfetal status is seen. Acute blood pressure control may be achieved with hydralazine, labetalol, ornifedipine. The goal of antihypertensive therapy is to achieve a systolic blood pressure <160 mmHg and a diastolic blood pressure <105 mm Hg (47). Four large randomized controlled trials comparing magnesium sulfate with other methods of treatment to prevent convulsions in women with severe PE have demonstrated that magnesium sulfate is associated with a significantly lower rate of eclampsia than either no treat mentor nimodipine. Lucas and colleagues reported no seizures among 1049 preeclamptic women receiving magnesium sulfate prophylaxis. Expectant management is contraindicated in the presence of fetal compromise, uncontrollable hypertension, eclampsia, DIC, HELL Psyndrome, cerebraledema, pulmonaryedema, or evidence of cerebral or hepatic hemorrhage. When severe PE is diagnosed before 24 weeks of gestation, the likelihood of a favorable outcome is low (48).

#### **Pathogenesis of PE**

The pathogenesis of PE is not fully elucidated but much progress has been made in the last decades. The placenta has always been a central figure in the etiology of PE because the removal of the placenta is necessary for symptoms to regress. Pathologic examination of placenta from pregnancies with advanced PE often reveals numerous placental infarcts and sclerotic narrowing of arterioles. The hypothesis that defective trophoblastic invasion with associated uteroplacental hypo perfusion may lead to PE is supported by animal and human studies (49). Thus, a two-stage model was developed: incomplete spiral artery remodeling in the uterus that contributes to placental ischemia (stage 1) and the release of antiangiogenic factors from the ischemic placentain to the maternal circulation contributes to endothelial damage (stage2) (Figure 2). During implantation, placental trophoblasts invade the uterus and induce the spiral arteries to remodel, while obliterating the tunica media of the myometrial spiral arteries; this allows the arteries to accommodate increased blood flow independent of maternal vasomotor changes to nourish the developing fetus (50). Part of this remodeling requires that the tropho blasts adopt anendothelial phenotype and its various adhesion molecules. If this remodeling is impaired, the placenta is likely to be deprived of oxygen, which leads to a state of relative ischemia and an increase inoxidative stress during states of intermittent perfusion. This abnormal spiral arteryre modeling has been shown to be the central pathogenic factor in pregnancies complicated by intrauterine growth restriction, gestational hypertension, and PE. However, one limitation to this theory is that these findings are not specific to PE (51).



Figure2: 2-stage model of PE pathogenesis.

#### **Angiogenic Factors**

In 2003, Maynard et al. showed that soluble fms—like tyrosine kinase 1 (sFlt-1) was up-regulated in the circulation of preeclamptic women. sFlt-1 is a splice variant of the vascular endothelial growth factor (VEGF) receptor, which does not contain the cytoplasmic and membrane domains of the receptor. This allows it to circulate and bind to VEGF and placental growth factor (PIGF), essentially antagonizing their binding to cell surface receptors. When sFlt-1 was injected into rats using an adeno virus, they developed significant hypertension and albuminuria and histologic changes consistent with PE (i.e., glomerular enlargement, endotheliosis, and fibrinde position within the glomeruli). Thus, sFlt-1 seems to be a key mediator in the development of PE (52).

A second placenta–derived protein, soluble endoglin (sEng), was also found to be upregulated in PE. sEng, a circulating coreceptor of TGF- $\beta$ , can bind to TGF- $\beta$  in the plasma. Antagonizing TGF- $\beta$ , a proangiogenic factor, is analogous to

sFlt-1 antagonizing VEGF. In fact, elevated levels of sEngin the circulation have been shown to induce signs of severe PE in pregnant rats (53). The true significance of these angiogenic markers and their ability to predict adverse maternal or fetal outcomes, showed that in a group of women with the clinical diagnosis of PE an elevated level of sFlt-PIGF ratio (angiogenic form) is associated with worse maternal and fetal out comes compared with in women with a lower ratio (non angiogenic form).

#### Nitric Oxide Pathway

The nitric oxide (NO)/nitric oxide synthase (NOS) system is also disrupted in PE. NO is a potent vasodilator that acts to induce relaxation in vascular smooth muscle cells via a c-GMPpathway. Decreased levels of NO and increased levels of arginase (which degrades a precursor molecule in the NOS pathway) have been reported in PE (54-55). A deficiency in NO has been shown to correlate with met abolic derangements seen in PE, such as hypertension, proteinuria, and platelet dysfunction. NO deficiency induces the uteroplacental changes characteristic of PE in pregnant mice, including decreased uterine artery diameter, spiral artery length, and uteroplacental blood flow. These findings suggest that an intact NOS system is essential for normal spiral artery remodeling and pregnancy.

#### **Oxidative Stress**

From the start of pregnancy, the placenta assumes a state of oxidative stress arising from increased placental mitochondrial activity and production of reactive oxygen species, mainly superoxide anion. In PE, a heightened level of oxidative stress is encountered (56) from the placenta, when free radical synthesis occurs with maternal leukocytes. The superoxide–producing enzyme NADPH oxidase is present in placental trophoblast and women with early onset of PE have been found to have higher superoxide production compared with those with late-on set disease (57).

#### **Sterile Inflammation and PE**

Sterile inflammation is triggered when Damage associated molecular patterns (DAMPs) activate Pattern recognition receptors (PRRs) to mount an acute immune response in order to solve the adverse triggering insult. Since DAMPs are endogenous intracellular molecules primarily released as a result of non-programmed cell death to convey danger cues in the first few hours of an injury, they are also referred to as alarm ins. Candidate alarmins include, but are not limited to: high mobility group box1(HMGB1), Decorin, von Willebr and factor(VWF), Heat shock proteins (HSP) and uric acid. Placental hypoxia elicitsa variety of cellular and inflammatory responses in PE, elevating circulating levels of many pro-inflammatory factors (Figure 3).



Figure3: Sterile inflammation and PE.

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#### HMGB1

HMGB1 is a highly conserved non-histone protein (25 kDa) with cytokine-like activity in the extra cellular space. It is ubiquitously expressed in the nucleus where it plays a role in DNA replication, transcription and repair, and nucleo some stabilization. Although originally discovered in nucleus, HMGB1 is also found in cytosol, mitochondria and on membrane surface, and can be released to the extracellular milieu through active (secretion) and passive pathways: (I) active pathways are triggered by pathogenic products (e.g. bacteria, viruses) or other stressors (e.g., oxidative stress, cytokines), which has been shown in immune cells and non-immune cells; where as (ii) passive release is observed following tissue injury and cell death, especially necrosis and in specific cases of apoptosis—including when triggered by sterile injury events (e.g., hypoxia, senescence, auto immune disease).

HMGB1 fundamentally resides in the nucleus under normal circumstances, stabilizing nucleo somes and facilitating gene transcription (58). However, under different conditions, it is hyperacetylated, translocated from the nucleus to the cytosol, and then secreted actively or passively (50). It has been shown that HMGB1 passively diffuses from various cells to the extracellular space during cellular necrosis or damage (60). It is also actively released by macrophages, monocytes, and dendritic cells upon activation (61). Once HMGB1 accumulates in the extracellular milieu, it conveys danger signals by triggering inflammatory pathways via Toll-like receptors. A study showed that the mechanism of HMGB1-induced inflammation is mainly mediated via the MyD88-dependent pathway(62).

HMGB1causes endothelial dysfunction (63) and promotes leukocyte activation and thrombosis (64). Increased HMGB1 expression has been reported in the placenta or placental explant-derived extra cellular vesicles from PE patients (65-66). Hypoxic trophoblastic cells in case of PE are rich sources of numerous alarmins such as uric acid, cell-free fetal DNA, HMGB1, and IL-1 $\alpha$ ; when they die, they release these alarmins into the extracellular environment which can result in a sterile inflammatory response (67). HMGB1 is expressed by trophoblasts and can be found either in their nucleus or cytoplasm (68). When the placentae are exposed to preeclamptic blood serum of the mother, there is increase in levels of cytoplasmic HMGB1 in the syncytiotropho blast (69). Praderv and et al. showed that circulating levels of HMGB1 are higher in third-trimester PE than in normal pregnancies (70). This evidence suggests that HMGB1 plays a role in the development of PE.

#### Decorin

Decorin is a small leucine-rich proteoglycan produced by connective tissue and highly expressed in reproductive tissues such as chorionic villusmesenchymal cells and decidual cells of the pregnant endometrium. Biological functions of DCN include: collagen assembly, myogenesis, tissue repair and regulation of cell adhesion and migration by binding to ECM molecules or antagonizing multiple tyrosine kinase receptors (TKR) including EGFR, and VEGFR-2(71). DCN restrains angiogenesis by binding to thrombospond in-1,TGF  $\beta$  and VEGFR-2. DCN actions at the fetal-maternal interface include restraint of trophoblast migration, invasion and uterine angiogenesis. DCN over expression in the decidua is associated with PE and may have a causal role by compromising endovascular differentiation of the trophoblast and uterine angiogenesis, resulting in poor arterial remodeling. Elevated DCN level in the maternal blood has been suggested as a potential biomarker in PE (72).

In a study, Gogiel *et al.* reported increased DCN levels of the umbilical cord vein wall inpatients with PE (73) and Siddiqui et al. found that increased DCN levels were predictors of PE even before the on set of clinical symptoms (74). The link between DCN and PE can be attributed to the impaired proliferation and migration of trophoblasts and endothelial dysfunction, which are thought to be responsible for adverse pregnancy outcomes.

#### vWF

Von Wille brand factor(vWF), a multi-domain multi merized glycoprotein, plays an important role in primary hemostasis. vWF is a carrier protein for blood clotting factor VIII (FVIII) and this interaction is required for normal FVIII survival in the circulation. Under physiological conditions, the vascular endothelium produces many substances which are closely associated with hemostasis, fibrinolysis, the synthesis of growth factors, and the regulation of vessel tone and permeability. One such substance that is synthesized by, and stored in, endothelial cells is vWF. vWF is also known to have an important function in plate let adhesion an daggregation. However, vWF is as ensitive marker and levels can be influenced by many pathological conditions, including the acute phase response (75).

A study showed that VWF levels are elevated in healthy pregnancy compared with the non pregnant state, and further increases in pregnancies complicated by PE and that pregnancies complicated by severe PE exhibit VWF biology that is distinct from healthy pregnancy(76). This speculates that PE-associated VWF differences could play a direct role in the vascular pathogenesis and end-organ damage of PE.

#### HSP90

In pregnancies complicated with PE, diminished placental function increases the apoptosis in placenta endothelial cells, where an increase of HSP90 protein levels has been reported when compared to that observed in normotensive controls. HSP 90, the most abundant chaperone in eukaryotic cells accounting for 1–2% of cell proteins in most tissues, is involved in multiple biological processes such as cellproliferation, differentiation, and apoptosis.

#### Uric acid

Uric acid (160 Da) is a product of the metabolic breakdown of purine nucleotides by xanthine oxidase, with normal blood concentration range between 40-60 µg/ml. Upon achieving concentrations >70 µg/ml, uric acid forms needle-like, immunostimulatory monosodium urate (MSU) crystals, which cause acute inflammation. In the last few years, uric acid has been vastly regarded as an alarmin of sterile inflammation because of the high cytosolic concentration ( $\approx$  4mg/ml) released upon cell death, which reacts with extracellular sodium to form MSU in the immediate vicinity of cellular injury.

#### CNAPS

Circulating nucleic acids in maternal plasma and serum (CNAPS) is a group of extra cellular nucleic acids including cell free DNA (cfDNA), cffDNA (fetal origin), total circulatingRNA, placental RNA, and other novel species.

Multiple pathways have been associated with the pathophysiology of PE, including inadequate placental development and systemic maternal endothelial dysfunction. As a link between impaired placentation and endothelial function, both maternal and fetal-derived cell-free deoxyribonucleic acid (cfDNA) fragments have been found in the circulation of pregnant women (77), and are known to be elevated in PE compared to normal pregnancy (78-79). In addition, increased cfDNA levels in maternal circulation correlate with disease severity in PE, and are associated with poor maternal-fetal outcomes (80-81).

Cellular damage and/or tissue-associated hypoxia leads to elevated RNA fragments, extracellular RNA (eRNA), in the circulation released from the disrupted, damaged cells (82). Asper background literature survey, we found that eRNA initiates cascades related to vascular diseases (83-84) i.e., that of blood coagulation along with inflammatory processes (85). Identification of endogenous ligands (DAMPs) by contemporary studies has created a substantial interest due to their high significance in SI. Tissue hypoxia can stimulate apoptosis and necrosis of affected tissue resulting in liberation of extra cellular nucleic acids in to circulation.

The exact underlying mechanism leading to the release of extracellular DNA into the maternal circulation and its consequencesare not completely clear, butexisting evidence indicates that fetal cell trafficking is perturbed in placental tissue early in gestation, and predates the manifestation of clinical symptoms in PE (86). It has been hypo the sized that increased circulating levels of fetal cfDNA may be due to ischemia/hypoxia within the intervillous space resulting in enhanced oxidative stress, apoptosis and/or necrosis of trophoblastic cells (87). Recent studies suggest that fetal cfDNA may activate specific receptors in maternal immune cells and platelets, which in turn trigger the production and secretion of pro-inflammatory cytokines and additional cfDNA release, resulting in a systemic humoral and cellular immuneresponse in PE (88). Interestingly, cfDNA may also act on the maternal vasculature, possibly contributing to endothelial and vascular dysfunction and then hypertension in PE (89).

Munchel *et al.* examined circulating RNA in pregnant women who developed early-onset PE and found 49 transcripts of maternal, placental, and fetal origin that classified as mall but in dependent cohort of pregnant women with early-onset PE (90). Several studies have suggested that dysregulated noncoding RNAs in the maternal-fetal interface participate in the regulation of proliferation, invasion, and apoptosis of trophoblasts, thereby promoting the pathogenesis of PE(91-92).

#### CONCLUSION

PE is a major cause of maternal mortality & morbidity particularly in low- and middle-in come countries(LMICs). Multi-faceted factors including hypoxia, inflammation of undetermined mechanisms impact the on set & progression of this disease which cause immunological alteration of placental micro-environment & impaired angiogenesis. PE, characterized by hypertension and inflammation, is accompanied by multiple symptoms including proteinuria, inflammatory response and oxidative stress which have been implicated as the key features in the pathophysiology of PE. As previously reported, hypertension and proteinuria are the characteristic symptoms of PE which has also been proven in our clinical assessment.

One of the contributors of PE is placental hypoxia leading to oxidative stress. As NO is a potent vasodilator, it relaxes the smooth muscle contraction of blood vessels. When its level in circulation decreases, the contraction of blood vessels increases which leads to elevation in the blood pressure. As a result of hypoxia, NO levels decrease in the blood vessels causing hypertension in the PE patients.

Inflammation is strongly associated with placental dysfunction and complications related to pregnancy such as preterm birth (PTB), low birth weight, fetal growth restriction (FGR), still birth and PE. Our knowledge of the actions of DAMPS at the maternal- fetal interface is still in early stages. Some of the important DAMPs that play a vital role in pathophysiology of PE areHMGB1, DCN, vWF, HSP90, circulating nucleic acids, ands 100b. As an inflammatory cytokine, HMGB1 plays an important role in endothelial permeability under oxidative stress and promotes the pathological process of inflammation. Placental is chemia may promote the release of a variety of factors, such as vWF, into the maternal circulation synthesized by the endothelium. vWF has been suggested to be a marker of generalized endothelialdys function, which leads to the clinically recognized symptoms of PE, which include hypertension, proteinuria, thrombocytopenia and impaired

liver function. Decorin (DCN) is a protein that limits the invasion and endovascular differentiation of extravillous trophoblast cells during early placentation and is found in creasing quantities in maternal blood and placental tissue in PE patients. Apart from hypertension and proteinuria PE also manifests with liver and endothelium dysfunction (93). It is deduced that in PE there is release of different mediators from liver and blood vessel endothelium which causes vasoconstriction and liver hypoxia, leading to the diagnosis of systemic disorders such as neuronal edema, cardiovascular disorder, liver disorders etc.

In normal pregnancy, the utero-placental spiral arteries in the myometrium undergo structural modifications where the musculo-elastic tissue of the tunica media is replaced by invading trophoblastic cells surrounded by a thick layer of fibrinoid material. The affected vessels undergo progressive vasodilatation. This phenomenon is described as the "physiological changes of spiral arteries". These changes are responsible for the low vascular resistance of the placental bed and allow a large increase in blood flow to the intervillous space. In pre-eclampsia, the endovascular trophoblast does not invade the myometrium. Physiological changes are confined to the decidual segment. Therefore, the spiral arteries in the placental bed are less dilated than normal pregnancy and they remain responsive to vasomotor influences. The impaired intervillous blood flow results in inadequate perfusion and ischemia of the placenta especially in the second half of pregnancy (94). The histological changes in PE/eclamptic placentas include infarcts, increased syncytial knots, hyper vascularity of the villi, cytotrophoblast proliferation, thickening of the trophoblastic membrane, obliterative enlarge dendothelial cells in the fetal capillaries, and atherosis of the spiral arteries in the placental bed (95).

Future research can be focused on large-scale genomic and proteomic approaches for the identifying of novel target molecules and diagnostic biomarkers, which will aid in formulating effective therapeutic interventions to prevent PE and/ordetect and treat it at an early stage.

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### IMPACTS OF 'WORK FROM HOME' MODEL ON HUMAN HEALTH

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#### Abstract

The coronavirus (COVID-19) pandemic has rendered a large proportion of the workforce unable to commute to work, to mitigate the spread of the virus. This has induced a shift in the working arrangements of millions of employees to Work-from-Home (WFH). The aim of this study was to evaluate the impacts of WFH on holistic health of workers and analyze the significant stressors among each category of social, mental and physical well-being. Approximately 30% participants reported substantial continuity of WFH considering the physical-mental issues, whereas more than 50% preferred WFH considering social-impacts. Subjective responses from two online surveys were analyzed to understand factors associated with holistic health. Vulnerable population was identified under each human-variable. While some unhealthful behaviors appeared to have been aggravated, other more healthful behaviors also emerged. Due to the flexibilities offered, it is possible that WFH may continue to some degree in future. To make this arrangement feasible and contribute effectively towards productive work, organizations will require to implement formalized decisions on continuity of WFH model based on the individual impact analysis considering demographic and professional variables of different workers. Based on the study recommendations have been made for longitudinal studies to investigate the dynamics of remote-work.

#### Keywords

COVID-19, Physical and mental Health, Social Health,Work from Home (WFH), Impacts, Demographics, Occupations.

#### INTRODUCTION

COVID 19, a global pandemic caused by a highly infectious virus - corona virus resulted in unprecedented fatalities. The World Health Organization (WHO) laid guidelines to adopt strict measures in the form of nationwide lockdowns, social distancing, and restriction on movements to combat the disease. To comply with these guidelines and to avoid economic run-down, as a consequence of this companies, organizations and institutions revolutionized the prevailing work arrangements and the concept of 'Work from Home' (WFH) emerged. "WFH is a shared working arrangement in which a worker fulfils the essential responsibilities of his/her job while remaining at home, using information and communications technology (ICT) and in turn the employer ensures business continuity and employment" (ILO, 2020). Due to restrictions on anthropogenic activities like industrial projects, vehicular movement, construction projects, tourism and other common transportation activities, the world witnessed a 'never before' stagnant phase.

According to the Central Pollution Control Board (CPCB), Air Quality Index (AQI) is a tool to measure the level of seven air pollutants (PM<sub>2.5</sub>, PM<sub>10</sub>, CO, NH<sub>3</sub>, NO<sub>2</sub>, SO<sub>2</sub> and Ozone) by transforming the weighted values into a single number (CPCB, 2020). Metro-cities not only reported improvement in AQI, but also demonstrated a decline in adverse health effects due to reduction in air pollutants including aerosols that cause premature mortality through lung cancers, respiratory illness, breathing discomfort and cardiopulmonary diseases (Partanen, Jean, & Matthews, 2018). Reduction in dumping of industrial effluents enhanced the self-cleansing capacity of the Indian rivers. Reports claim 40-50% improvement in water quality of the river Ganga with

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Biochemical Oxygen Demands (BOD) levels below 3ppm, Dissolved Oxygen (DO) levels above 8 ppm, making it fit for drinking standards(South Asia Network on Dams, 2020). Due to restricted human interaction with nature in a country like India, WFH proved to be a blessing for the environment, resulting in flourishing of flora, fauna and human-health.

WFH curtails frequent commute time, enhances flexibility and permits working population to work when they are most productive. It also offers workers to get more time to take care of their families and fulfill their responsibilities. (International Labor Organization). But along with the benefits of WFH, negative impacts on physical, mental and social health follow(Xiao, et al., 2021) (Oakman, et al., 2020) (Eddy, 2021). Thus WFH model impacts holistic health of humans. This study was taken up to explore the impact of WFH on various health aspects among people of varied age-groups, different gender, varied work profiles and different family background. The specific objectives of this study are:

- To identify the type of jobs suitable for working from home.
- To predict the scope of WFH considering each aspect of holistic health individually.



Figure 1: Flowchart of the Preliminary Survey.

- To quantify the intensity factors of all impacts and analyze the human variables potentially affected by each impact.
- To suggest the feasibility of WFH modelwithin the Indian work culture based on the study.

#### Methodology

A descriptive study was carried out from December 2020 to May 2021. Data was collected through two online structured survey questionnaires– Preliminary and secondary surveys using Google forms. The aim of the former survey was to evaluate feasibility of WFH considering all segments of holistic health individually, while the purpose of latter study was to conduct a subjective impact analysis to understand effects of WFH on people with varied work-profiles and demographics.

#### **Preliminary Survey: Introductory analysis**

A structured google form was prepared. To attain maximum responses, the link for former survey was circulated through emails, social media platforms and call requests. 1572 responses were received from preliminary survey. 92 forms were eliminated that did not meet inclusion criterion of spending most of time working. A total of 1480 valid responses were evaluated for the study. The first part of the questionnaire included mandatory questions on professional details to be filled by the participants, responses of which resulted in list of occupations that are suitable to incorporate WFH model into its work culture. The second part included ves/no type mandatory questions on socio-economic, physical and mental aspects for continuing WFH, each attached with an optional blank to justify corresponding answer stating pros or cons. The flowchart of the preliminary survey is given in Figure 1.

#### Secondary Survey: Descriptive Analysis

Analyzing the characteristics of participants of primary survey, sampling was done to get responses for understanding the correlation between WFH impacts and demographic, socio-economic and professional variables of shortlisted respondents from primary survey. The aim of the secondary survey was to propose a feasible WFH model for future considering impact on each human variable. The link for secondary survey was recirculated among those participants through emails and social media. To ensure unbiased analysis, from the received responses, a mixed bag of 100 ideal participants was chosen as the sample size for descriptive survey using SQL Database software. The flowchart of the descriptive survey and secondary survey is given in Figure 2 and Figure 3.



Figure 2: Flowchart of sample-size for descriptive survey.



Figure 3: Flowchart of Secondary Survey.

Initial part of secondary survey included mentioning personal and professional details. The next section included Multiplechoice-Questions on social, mental and physical aspects of WFH, where participants were supposed to tick-mark all those checkboxes applicable, against the listed pros-cons within each category of holistic health. The last open-ended question in both surveys helped in determining feasibility of WFH in the future of work culture. The questionnaire was reviewed and approved by a senior professor of Health and Environmental science.

#### RESULTS

#### Outcomes of Primary Survey Work profiles suitable for WFH

From the responses of introductory survey, we got a list of professions that can incorporate online mode of remote work.

The occupations suitable with WFH are broadly classified and given in Figure 4.

Sr.No.	Work profiles suitable for WFH
1	Business, creatives and legal professionals
2	Engineering and Architecture, Technicians
Э	Education and Arts
4	Healthcare and social services
5	Computer science, Basic science and research
6	Service sector and physical occupations

Figure 4: Occupations suitable for 'Working from home'

## Responses to scope of WFH with respect to individual aspect of holistic health

From the primary survey, majority of the people preferred WFH option in view of the economic (60%) and social (51%) impacts, whereas several respondents stated refusal on continuity of WFH considering physical (66%) and psychological (70%) impacts (Figure 5).



#### Scope of Continuity of WFH based on:

Figure 5: Evaluating the scope of WFH in the long run considering Economic, Social, Psychological and Physical aspects.

#### **Outcomes of Secondary Survey**

#### Characteristics of Participants of Secondary survey

Impacts on Holistic health due to WFH are subjective. Hence an even distribution of occupations among the survey participants was ensured to avoid bias and understand the correlation between people with different demographics, different work culture and their respective views on impacts of WFH.Demographics broadly included personal details like gender and age-group as well as socio-economic background of participants (Figure 6). Professional details encompassed current work-profile and history (Figure 7)

#### **Responses of secondary survey**

All factors of social, mental and physical impacts were quantified with secondary survey. The graphical representations are presented in Figure 8, Figure 9 and Figure 10.

Major stressors of social impacts include problem in Worklife balance(43%) and communication issues (34%), while potential enablers include family reunion (58%) (Figure 8).

More than half(55%) of the respondents experienced laziness and irregular routine during WFH.As per the respondents, sleep disorders(37%) and stress(31%) add up further to the mental and psychological impacts. Though fewer participants reported problems such as anxiety(22%) and depression(20%), they are potentially detrimental psychological impacts (Figure 9).

More than 50% reported weight gain and strain in eyes under the physical impacts category. Musculoskeletal issues such as back pain (47%), neck pain (33%), and wrist pain (12%) add up for the ergonomic impacts. Increased screen time leads to visual symptoms including dark circles (35%) and headache (36%).Nearly one-third participants claim improvement in physical health due to home-made food (Figure 10).

#### Feasibility of WFH in the future of Work culture

Combining the outcomes of both the introductory and analytical survey, nearly one-third of participants suggest a strong negation for the proposal of WFH being a New-normal in future. Two-thirds of respondents want WFH model to be permanently incorporated into the future work culture. Approximately 40% demand hybrid work culture in future with conditional continuity of WFH and going to workplace only whenever required (Figure 11).

Responses from surveys depict a surge in number of holistichealth issues post transition to WFH. Summary of metaanalysis demonstrates the category of variables more affected, corresponding to each impact (Figure 12).

From the Kiwiat diagram(Figure 13), prominent conclusion can be drawn that working-females report more health issues than males, and hence are more vulnerable for health impacts of WFH.

Radar plot of Figure 14 represents the workers' population having significant health impacts due to WFH. People of agegroups 40-60 years are more susceptible to the impacts of WFH than the young.

Results from Kiwiat-diagram state that employees belonging to Joint-families report more health issues than those from

	Demographics of Participants																
Gender Age Group					Type of Location of residence			Family size		Participants having Children		Economic Status					
Male	Female	20-30	30-40	40-50	50-60	Rural	Suburban	Urban	Nuclear (4 members or less)	Joint (more than 4 members)	Infants / kids	Teens	Poor (<2.5 LPA)	Lower income (2.5 - 5 LPA)	Middle class (5 - 10 LPA)	Upper middle class (10 - 50 LPA)	High income (>50 LPA)
50	50	25	30	30	15	14	31	55	52	48	37	49	6	17	30	29	18
	Total Participants : 100																

Figure 6: Demographics distribution of 100 participants.

Professional Details of Participants												
Occupation							Type of Work		Type of worker			
Business, Creatives and Legal Professionals	Engineering and Architecture, Technicians	Education and arts	Healthcare and Social services	Computer science, basic science and research	Service sector and Physical occupations	Full-time (6 to 8 hours or more)	Part time ( less than 6 hours)	Permanent	Temporary or contract basis	Migrant workers (Working in other city or area)	Yes	No
11	26	18	14	20	11	73	27	67	24	19	11	89
Total Participants : 100												



nuclear families (Figure 15). In contrast, those having children above 13 years are less vulnerable from the impacts of WFH than those having infants.

From the radar-chart, it can be observed that the lower- and middle-income group show more vulnerability from the impacts of WFH as compared to the higher economic class. (Figure 16)



Figure 8: Intensity of each factor of Social Impacts.

Histogram of Figure 17 highlights the occupations more susceptible from the impacts of WFH on their holistic health. Health sector is least affected, whereas the service sector, businesses and engineering professions are at a higher risk.



Figure 9: Intensity of each factor of Mental and psychological Impacts.



Figure 10: Quantification of intensity of each factor of Physical and ergonomic impacts due to WFH.



Figure 11: Responses to feasibility of WFH Model in future.

	Summary of Meta-analysis on association of Holistic health impacts with different human variables											
	Variables	Personal Va	ariables		Socio-econ	omic Variable	25	Professional Variables				
	mpacts	Gender	Age- group	Type of Location	Family size	Participants having children	Economic status	Occupation	Type of work	Type of Worker	Prior WFH experience	
	Family Reunion		30-60			Yes			Full-time	Migrant		
Social Impacts	Social isolation	Males	40-60	Urban				Service sector and Physical occupations				
	Communication issues		40-60			No	Average	Education and arts, sales and marketing			No	
	Work-life balance	Females	30-50	Urban		Yes		Engineering and Architecture, Technicians	Full-time			
	Increasing expectations	Males and Females			Joint	Yes	Average			Temporary		
	Increasing arguments among family											
	Irregular routine	Females		Urban	Joint	Yes			Full-time	Permanent		
	Sleep disorders		50-60		Nuclear		Average		Full-time			
acts	Laziness and fatigue		20-30		Nuclear	No						
ogical Imp.	Lack of self confidence	Females	50-60	Rural		No teens		Education and arts; Service sector and physical occupations			No	
sycho	Fear of losing jobs								Part-time	Temporary and Migrant	No	
ЧÞ	Lack of time for onself	Females			Joint	Yes	Average		Full-time		No	
lan	Anger issues, frustration	Females	40-60									
Menta	Depression and trauma		20-30		Joint	No	Low or average	Engineering and Architecture, Technicians		Temporary and Migrant	No	
	Tension free atmosphere as everyone is around	Males			Nuclear and Joint	Yes	Low or average			Migrant		
s	Weight gain		40-60	Urban			Average	Computer science, basic science and research	Full-time			
mpac	Hairfall	Female		Urban	Joint and Nuclear		Average					
mici	Dark circles		20-30					Computer science,				
d Ergonol	Strain in eYes		40-60					research				
	Headache		30-50									
al an	Backpain											
/sic:	Neckpain		40-60				Average	Service sector and			No	
Ч	Wrist pain			0.001			orLow	Physical occupations				
	Muscular pain											
	Improvement in physical health	Males								Migrant		

Figure 12: Meta-analysis indicates those human variables that are predominantly impacted.

40



Figure 13: Working- Women are more susceptible to the health issues due to WFH.



Figure 15 : Employees belonging to Joint -family are more prone to holistic health issues due to WFH and those having infants are more vulnerable than those having only teenager children.



Figure 17: Vulnerability of Professions reporting prominent health issues due to WFH.

#### DISCUSSION

64% females reported problems in work-life balance, while only 22% males claimed that issue. In order to satisfy the dual roles, working-women fulfill their responsibility for household chores, compromising sleep and skipping meals to



Figure 14: Impacts of WFH are predominant on holistic health of workers of age-group 40-50 and 50-60.



# Figure 16: Lower- and middle-income groups are more vulnerable to health impacts due to WFH as compared to the higher economic class.

meet to work-targets and deadlines, in turn affecting their physical and psychological health. In order to balance both tasks simultaneously on daily basis, women undergo stress (62%) and report symptoms of depression(34%). Our outcomes align with findings of other researchers that working-women are more vulnerable to developing depression while WFH during the pandemic (Sato, et al., 2021) (Solomou & Constantinidou, 2020).

One more implication from our study is that people with lowor middle-income group report 34% more health issues than those from higher economic class. Individuals with lowerincome cannot afford the technological and ergonomic requirements of WFH, and also lack job-security which can subsequently lead to anxiety and a phenomenal increase in stress levels (Wilson, et al., 2020) (Stanton , et al., 2020). Those who do not have a dedicated workspace with appropriate adjustments are more likely to report musculoskel et al and visual symptoms (Kelishadi & Sadegh, 2020) (Xiao, et al., 2021). A study by Michelle M. Robertson, 2016 suggests Human Factors/Ergonomics (HF/E) considerations for remote-working (Robertson & Mosier, 2016). WFH allows workers to set-up a dedicated workplace and adjust the IEQ factors (lighting, temperature, humidity, air quality, noise, ergonomics, etc.) according to their personal preferences to improve productivity and wellbeing (Robertson & Mosier, 2016) (Vimalanathan & Thangavelu, 2014) (Rodrigues, et al., 2017).

The results of this study capture the deleterious role that family-work conflict and a chaotic environment play on WFH outcomes. Ironically individuals from a joint family report 65% more health-impacts than those from nuclear families. Over burden of expectations from intergenerational family, distracting environments and lack of privacy due to overcrowded homes may reduce engagement and motivation to work, exerting a negative influence on the productivity and psychological segment of employees (Galanti, et al., 2021). Unlike the traditional assumption of having a toddler at home can be a positive predictor of well-being, working-parents having infants or kids had higher chances of reporting holistic health issues (Xiao, et al., 2021). Our outcomes further support this inter-relationship. The meta-analysis suggests that workers having infants reported 56% more healthimpacts than those who do not have infants(Figure 12, Figure 15). To minimize the distractions from children while WFH, it is recommended by other authors to have a private space and appropriate work-space at home. Besides that having at least one teenager lowered the chance of reporting new health issues. Studies suggest that inspite of being a source of distraction, teenagers are comparatively more independent, require less supervision, and can abate in household tasks (Rao & Indla, 2010)(Xiao, et al., 2021).

There are some suggestions for subsequent research in this field that derive from the current study. Indeed, our model, although including many variables, gives only a trivial account of the multiple dynamics that underlie the complex phenomenon of the WFH. Based on this, we believe it is important that ensuing studies take into consideration, a more representative sample and a more precise research design, particularly among professional and personal resources. Such meta-analysis can open important horizons for future longitudinal studies, which still have much to investigate on the complex reality of remote work and its outcomes in terms of employees' well-being and health.

#### **CONCLUSION AND RECOMMENDATIONS**

Overall outcomes from this study indicate that the impacts of WFH on individuals' holistic health vary considerably.

Significant stressors in social impacts include work-family conflict and overburden of expectations. Due to difficulty in unplugging from work, work-life boundaries are blurred. Visual symptoms due to prolonged screen-exposure and musculoskeletal problems due to poor postures in addition to lack of consideration of ergonomics, led to decline in physical health of workers. Swap in the modus operandi may cause issues such as inferiority-complex, irritability, loneliness, fear of losing jobs, and chronic stress. Ongoing work-family conflict can lead to detrimental psychological issues such as insomnia, burnout, identity crisis, depression and emotional exhaustion.

Significant findings emerging from the meta-analysis of this study imply that impacts of WFH vary considerably with lifecourse stage, gender, profession type and socio-economic status. From the systematic review, the variables vulnerable to deterioration of holistic health were of either criterion – Age: exceeding 40 years (61%); Gender: Females (55%); family-type: Joint (62%); Children: Infants (69%); Annual income: lower- or middle-income groups (60%).This study highlights factors that impact holistic health and well-being of workers while working from home and provides a foundation for considering the best for a positive WFH experience.

In this study we investigated the feasibility of WFH in foreseeable future. In contrast to the predictions, though 30% oppose WFH being incorporated into future work culture, nearly 70% respondents' desire continuity of WFH.

Despite this being a time-bound analysis, a systematic methodology was adopted for conducting the survey. A further strength was the undertaking of a formal quality appraisal with preliminary survey and database, to reduce the likelihood of biasedness and ensure existence of all permutations and combinations of diverse demographics, work profiles and their respective association with WFH. Our study demonstrates the impact of WFH on the physical and mental well-being of workers. However there are limitations as far as the interpretation and use of these outcomes is concerned. The outcomes are not meant to directly depict conventional WFH circumstances, since the data was obtained within a limited span during the pandemic and may not illustrate the health-status or experiences, once the WFH model becomes progressively routine. While the implications of the data are valid, our sample included over-representation from workers in India, application of the results may not be widely generalizable overseas and may vary from the experiences of individuals with different backgrounds. Though the respondents characterized wide range of workprofiles, the occupational genres of study are neither representative of all professions nor do these facts state all work-profiles within each category.

To circumvent the impacts of WFH on holistic health,

keeping in mind their flexibility and productivity, we would like to recommend conditional continuity of WFH by suggesting incorporation of a hybrid model into the future work culture with appropriate consideration of work-home boundaries, division of tasks and role-clarity, training sessions for managers, facilitation of co-worker networking, performance-indicators, management and technical support. Depending on the occupation, a hybrid model can be prepared and implemented wherein the authorities can divide the staff into groups and incorporate rotating shifts of employees working on-site and employees Working-from-Home. This way everyone gets equal opportunities and work-life balance. This will help in revolutionizing the Indian work-culture, keeping in mind the health and safety of employees.

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