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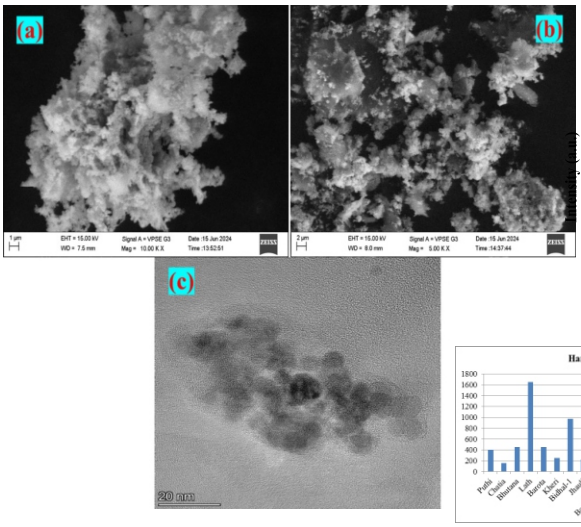

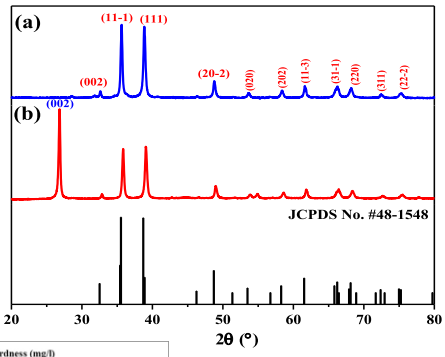

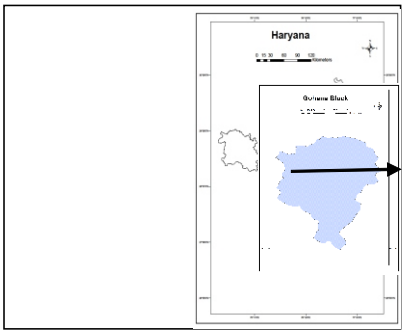

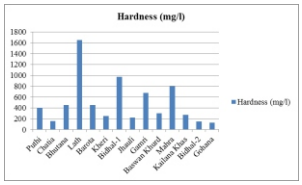

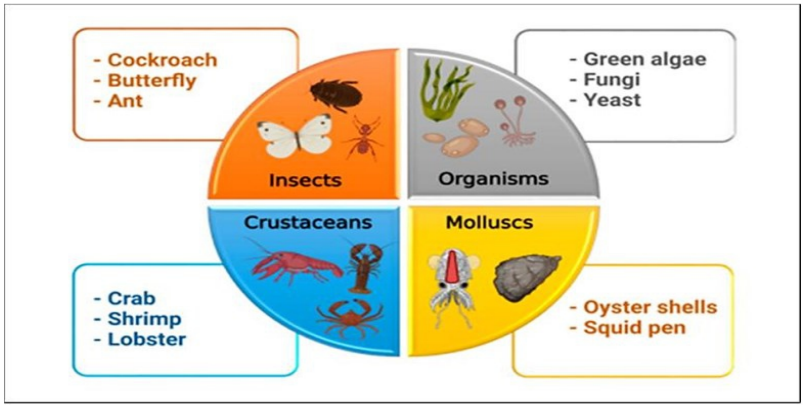

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The IJEHS is an official publication of Save The Environment (STE). It publishes peer reviewed quarterly, original articles (Research paper, Review articles, Short Communication, Case studies, etc.) related to all fields of Environment and Health Sciences.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

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International Journal of Environment and Health Sciences

From The Editor's Desk…

As we welcome the New Year 2024, the time has come to work together for creating a sustainable and environment-friendly earth around us by making the most of this recovery phase. New policies are being formulated for improving air, soil and water quality which will further improve the health status of public as well as the environment quotient. Undoing the economic losses and health crisis incurred in the past two years, by implementing more responsible actions will be the main pledge.

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

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

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

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

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

We thank all authors who have contributed to the journal and have consistently been with us in the past years. With this, I wish all our readers a Very Happy New Year, 2024 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
Executive Editor-In-Chief

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GROUNDWATER QUALITY ASSESSMENT FOR DRINKING PURPOSE IN GOHANA BLOCK OF SONIPAT DISTRICT, HARYANA

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Abstract
Water is necessary for drinking, irrigation and industrial purposes. Increasing population, urbanisation, industrialisation and agricultural practices put pressure on the availability and quality of water. The present study area Gohana block is located in Sonipat district of Haryana state. The geo-coordinates of the study area are latitudes 28.95^0 N to 29.18^0 N and longitudes 76.65^{0}E to 76.87^0 E and covers an area of 315.44 sq. km. Geologically alluvium and geomorphologically alluvial plain are present in the study area. The main objective was to assess groundwater quality for drinking purpose in the study area. In the study area fourteen groundwater samples were collected in 250 ml double capped plastic bottles. Geo-coordinates of sample locations were noted with the help of mobile GPS. Chemical analysis of fourteen groundwater samples were done using Tamilnadu Water Supply and Drainage (TWAD) Board,Chennai prepared Field Water Testing kit for twelve chemical parameters viz. pH, alkalinity, hardness, chloride, total dissolved solids (TDS), fluoride, iron, nitrite, nitrate, ammonia, phosphate and residual chlorine. Results of groundwater samples analysis were compared with BIS drinking water standards (IS 10500:2012) to know groundwater quality for drinking purpose. In the study area pH ranges 6.5 to 8, alkalinity 80 mg/l to 530 mg/l, hardness 130 mg/l to 1650 mg/l, chloride 20 mg/l to1500 mg/l, TDS 312 mg/l to 3768 mg/l, fluoride nil to 5 mg/l, iron nil to 3 mg/l, ammonia nil to 1 mg/l, nitrite 0.5 mg/l to 2 mg/l, nitrate 45 mg/l to 100 mg/l, phosphate nil to 1 mg/l and residual chlorine ranges nil to 0.2 mg/l.The study is highly useful for planning and monitoring of groundwater quality for drinking purpose in the study area.

Keywords
Groundwater, quality, drinking, assessment, Gohana, Sonipat, Haryana.

1. INTRODUCTION

On the Earth water is available in plenty but the useable especially drinking purpse is very less. Further increasing population, irrigation practices and industrial uses put pressure on the available fresh water. In urban areas surface and groundwater availability is decreasing day by day.Good quality water reduces health issues like fluorosis. Shekhar and Sarkar (2013), Spanos et al. (2014),Vijaya Lalitha et al.(2016), Asadi and Kumar (2017), Kaur et al. (2017), Khan and Jhariya (2017), Nourbakhsh and Yousef (2017), Zidi et al. (2017), Khelif and Boudoukha (2018) had done work on assessment of groundwater quality for drinking purpose in different areas.

2. STUDY AREA

Gohana block is located in Sonipat district between the latitudes 28.95^0 N to 29.18^0 N and longitudes 76.65^0 E to 76.87^0 E (Fig.1). The study area covers an area of 315.44 sq. km. Geologically alluvium and geomorphologically alluvial plain are present in the study area.

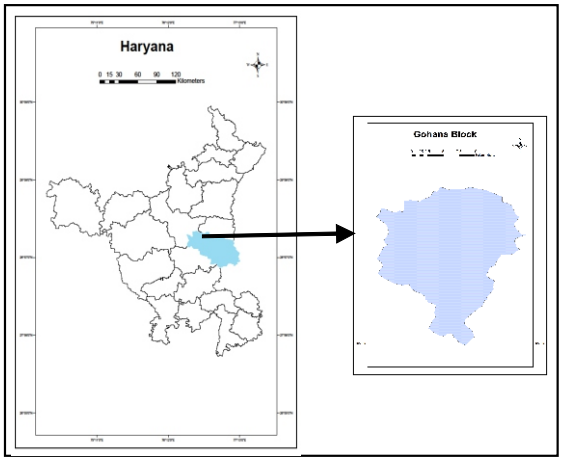

Figure1: Location map of the study area.

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Color versions of one or more of the figures in this article can be found online at www.stenvironment.org

3. OBJECTIVE

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

4. MATERIALS AND METHODOLOGY

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

Table 1: Results of groundwater samples analysis.

S. No.	Sample Location	Latitude	Longitude	pH	Alkalinity	Hardness (mg/l)	Chloride (mg/l)	TDS (mg/l)	Fluoride (mg/l)	Iron (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Residual Chlorine (mg/l)(mg/l)
1	Puthi	29.07	76.69	7.5	250	400	250	1080	5	0	1	0.5	100	0	0.2
2	Chatia	29.08	76.88	8	290	160	50	600	5	0	0.5	1	100	0	0.2
3	Bhutana	29.19	76.62	7.5	450	450	200	1320	5	0	1	1	100	0	0
4	Lath	29.08	76.78	7	290	1650	1200	3768	1.5	0	1	0.5	75	0	0
5	Barota	29.12	76.74	7.5	220	450	100	924	1.5	0	0.5	0.5	100	1	0
6	Kheri	29.11	76.76	7	170	250	120	648	1.5	0	0.5	1	75	0.5	0
7	Bidhal-1	29.07	76.81	7.5	390	970	800	2592	5	3	0.5	0.5	75	0.5	0
8	Jhauli	29.09	76.80	7.5	200	220	150	684	2	0	0	0.5	75	0	0
9	Gamri	29.17	76.76	7.5	420	680	1500	3120	2	0	1	0.5	75	0	0
10	Baswan Khurd	29.09	76.68	7	270	300	20	708	0	0.3	1	2	45	0	0.2
11	Mahra	29.09	76.69	7.5	370	800	50	1464	1	0	1	0.5	100	0	0
12	Kailana Khas	29.17	76.76	7.5	300	270	30	756	5	0	0.5	0.5	75	0	0.2
13	Bidhal-2	29.07	76.80	7.5	530	150	60	888	3	0	0.5	1	100	0	0
14	Gohana	29.13	76.69	6.5	80	130	50	312	0.5	0	1	0.5	45	0	0

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

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

5. RESULTS AND DISCUSSION

5.1 pH

In the study area pH ranges 6.5 to 8 (Table1, Fig.2). As per BIS (IS 10500:2012) drinking water standards pH is desirable between 6.5 to 8.5 and non-potable if less than 6.5 and more than 8.5 (Table 2). pH is desirable in all the fourteen groundwater samples (Puthi,Chatia, Bhutana, Lath, Barota, Kheri, Bidhal-1. Jhauli, Gamri, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2, Gohana).

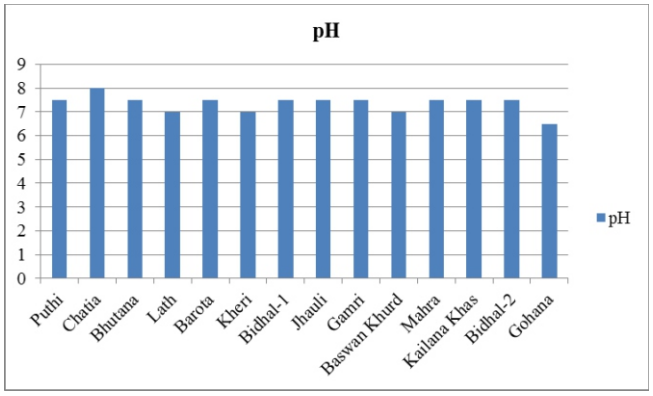

Figure 2: pH in groundwater samples.

5.2 ORIGIN AND DISTRIBUTION

In India, forested areas have long been set aside and preserved due to the religious convictions of the local communities. This practice dates back many centuries. Indian society is made up of several cultures, each of which has its customs for protecting the environment and the animals that live there. India is full of sacred trees, particularly in the areas inhabited by native populations. India's first Inspector General of Forests, Brandis, admitted that sacred forests existed (Brandis, 1897). The sacred groves have a historical.

5.3 Alkalinity

In the study area alkalinity ranges 80 mg/l to 530 mg/l (Table1, Fig.3). As per BIS (IS 10500:2012) drinking water standards alkalinity is desirable if less than 200 mg/l, permissible between 200 mg/l-600 mg/l and non-potable if more than 600 mg/l (Table 2). Alkalinity is desirable in one groundwater sample (Gohana) and permissible in thirteen groundwater samples (Puthi, Chatia, Bhutana, Lath, Barota, Kheri, Bidhal-1, Jauli, Gamri, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2).

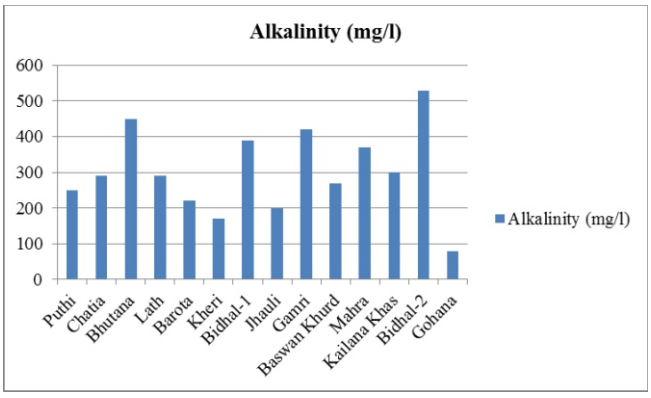

Figure 3: Alkalinity in groundwater samples.

5.4 Hardness

In the study area hardness ranges 130 mg/l to 1650 mg/l (Table1, Fig.4). As per BIS (IS 10500:2012) drinking water standards hardness is desirable if less than 200 mg/l, permissible between 200 mg/l - 600 mg/l and non-potable if more than 600 mg/l (Table 2). Hardness is desirable in three groundwater samples (Chatia,Bidhal-2,Gohana), permissible in seven groundwater samples (Puthi, Bhutana, Barota, Kheri, Jhauli, Baswan Khurd, Kailana Khas) and non-potable in four groundwater samples (Lath, Bidhal-1, Gamri, Mahra).

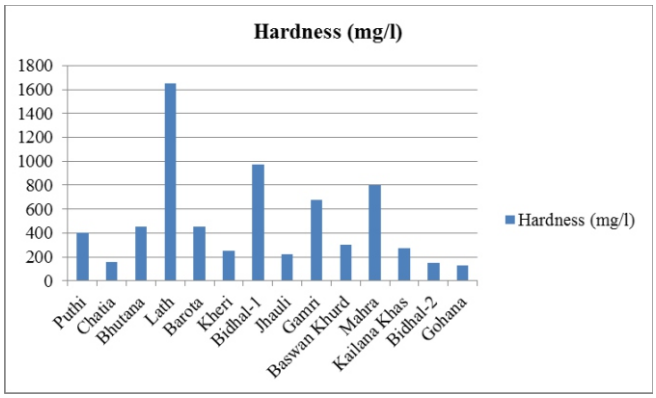

Figure 4 : Hardness in groundwater samples.

5.5 Chloride

In the study area chloride ranges 20 mg/l to 1500 mg/l (Table1, Fig.5). As per BIS (IS 10500:2012) drinking water standards chloride is desirable if less than 250 mg/l, permissible between 250 mg/l - 1000 mg/l and non-potable if more than 1000 mg/l (Table 2). Chloride is desirable in ten groundwater samples (Chatia, Bhutana, Barota, Kheri, Jhauli, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2, Gohana), permissible in two groundwater samples (Puthi, Bidhal-1) and non-potable in two groundwater samples (Lath.Gamri).

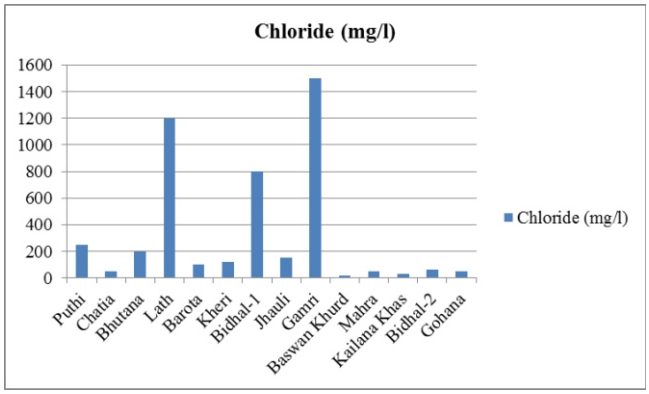

Figure 5: Chloride in groundwater samples.

5.6 Total Dissolved Solids (TDS)

In the study area TDS ranges 312 mg/l to 3768 mg/l (Table1, Fig.6). As per BIS (IS 10500:2012) drinking water standards TDS is desirable if less than 500 mg/l, permissible between 500 mg/l -2000 mg/l and non-potable if more than 2000 mg/l (Table 2). TDS is desirable in one groundwater sample (Gohana), permissible in ten groundwater samples (Puthi, Chatia, Bhutana, Barota, Kheri, Jhauli, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2) and non-potable in two groundwater samples (Lath, Gamri).

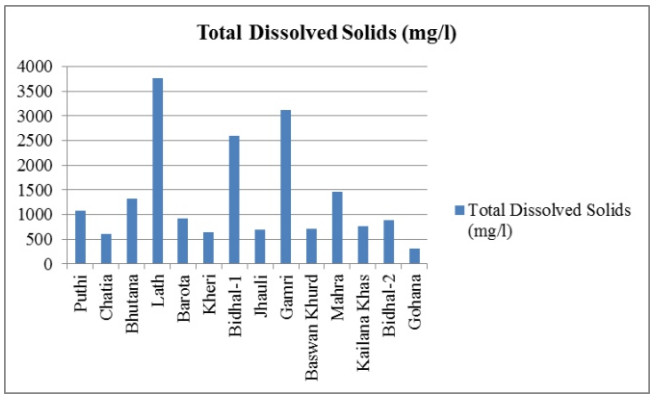

Figure 6: TDS in groundwater samples.

5.7 Fluoride

In the study area fluoride ranges nil to 5 mg/l (Table1, Fig.7). As per BIS (IS 10500:2012) drinking water standards fluoride is desirable if less than 1.0 mg/l, permissible between 1.0 mg/l -1.5 mg/l and non-potable if more than 1.5 mg/l (Table 2). Fluoride is desirable in two groundwater samples (Baswan Khurd, Gohana), permissible in four groundwater samples (Lath, Barota, Kheri, Mahra) and non-potable in eight groundwater samples (Puthi, Chatia, Bhutana, Bidhal-1, Jhauli, Gamri, Kailana Khas, Bidhal-2).

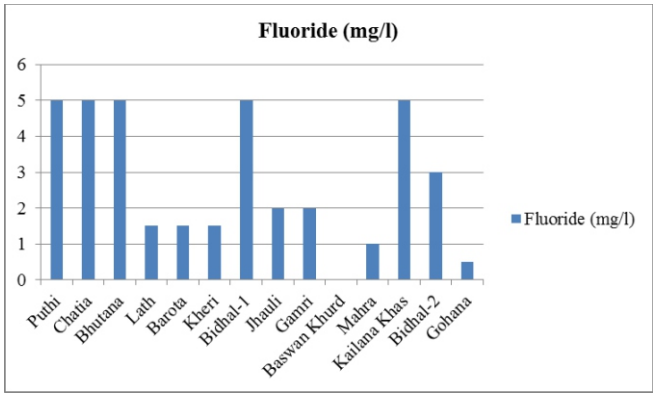

Figure 7:Fluoride in groundwater samples.

5.8 Iron

In the study area iron ranges nil to 3 mg/l (Table1, Fig.8). As per BIS (IS 10500:2012) drinking water standards iron is desirable if less than 0.3mg/l and non-potable if more than 0.3 mg/l (Table 2). Iron is desirable in thirteen groundwater samples (Puthi, Chatia, Bhutana, Lath, Barota, Kheri, Jhauli, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2, Gohana) and non-potable in one groundwater sample (Bidhal-1).

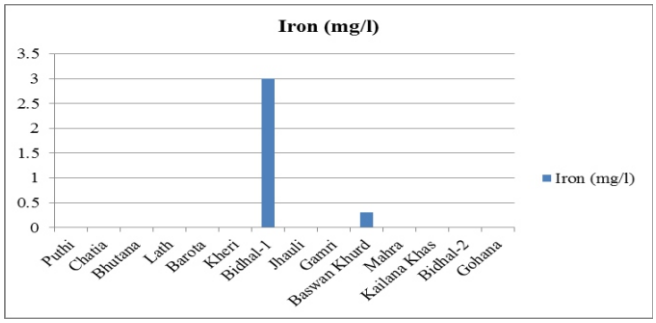

Figure 8:Iron in groundwater samples.

5.9 Ammonia

In the study area ammonia ranges nil to 1 mg/l (Table1, Fig.9). As per BIS (IS 10500:2012) drinking water standards ammonia is desirable if less than 0.5 mg/l and non-potable if more than 0.5 mg/l (Table 2). Ammonia is desirable in seven groundwater samples (Chatia, Barota, Kheri, Bidhal-1, Jhauli, Kailana Khas, Bidhal-2) and non-potable in seven groundwater samples (Puthi, Bhutana, Lath, Gamri, Baswan Khurd, Mahra, Gohana).

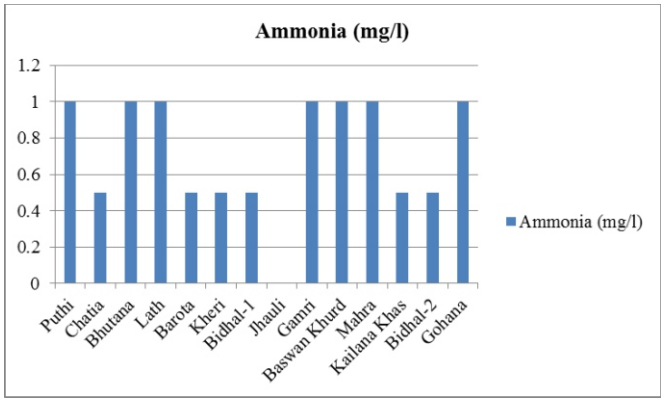

Figure 9:Ammonia in groundwater samples.

5.10 Nitrite

In the study area nitrite ranges 0.5 mg/l to 2 mg/l (Table1, Fig.10). As per BIS (IS 10500:2012) drinking water standards nitrite is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Nitrite is desirable in thirteen groundwater samples (Puthi, Chatia, Bhutana, Lath, Barota, Kheri, Bidhal-1, Jhauli, Gamri, Mahra, Kailana Khas, Bidhal-2, Gohana) and non-potable in one groundwater sample (Baswan Khurd).

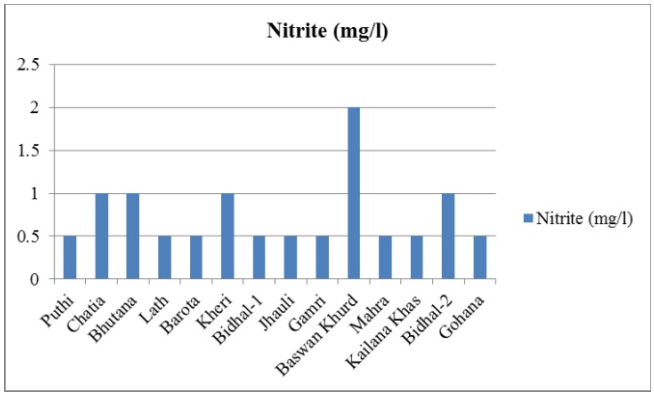

Figure 10:Nitrite in groundwater samples.

5.11 Nitrate

In the study area nitrate ranges 45 mg/l to 100 mg/l (Table1, Fig.11). As per BIS (IS 10500:2012) drinking water standards nitrate is desirable if less than 45 mg/l and non-potable if more than 45mg/l (Table 2). Nitrate is desirable in two groundwater samples (Baswan Khurd, Gohana) and non-potable in twelve groundwater samples (Puthi,Chatia, Bhutana, Lath, Barota, Kheri, Bidhal-1, Jhauli, Gamri, Mahra, Kailana Khas, Bidhal-2).

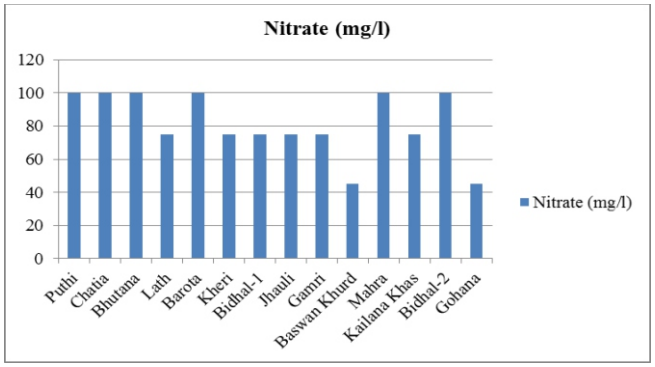

Figure 11: Nitrate in groundwater samples.

5.12 Phosphate

In the study area phosphate ranges nil to 1 mg/l (Table1, Fig.12). As per BIS (IS 10500:2012) drinking standards phosphate is desirable if less than 1.0 mg/l and non-potable if more than 1.0 mg/l (Table 2). Phosphate is desirable in all fourteen groundwater samples (Puthi,Chatia, Bhutana, Lath, Barota, Kheri, Bidhal-1, Jhauli,Gamri, Baswan Khurd, Mahra, Kailana Khas, Bidhal-2, Gohana).

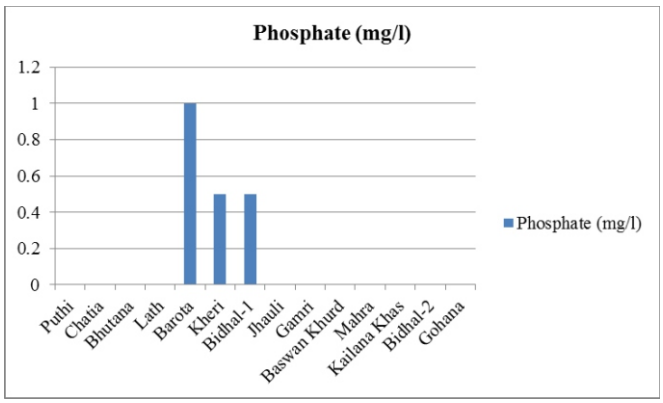

Figure 12: Phosphate in groundwater samples.

5.13 Residual Chlorine

In the study area residual chlorine ranges nil to 0.2 mg/l (Table1, Fig.13). As per BIS (IS 10500:2012) drinking water standards residual chlorine is desirable if less than 0.2 mg/l, permissible between 0.2 mg/l-1 mg/l and non-potable if more than 1.0 mg/l (Table 2). Residual Chlorine is desirable in ten groundwater samples (Bhutana, Lath, Barota, Kheri, Bidhal-1, Jhauli, Gamri, Mahra, Bidhal-2, Gohana) and permissible in four groundwater samples (Puthi,Chatia, Baswan Khurd, Kailana Khas).

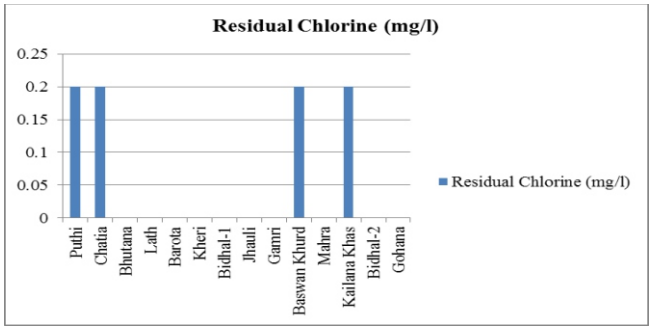

Figure 13: Residual Chlorine in groundwater samples.

6. CONCLUSIONS

In the study area pH is desirable in all fourteen groundwater samples. Alkalinity is desirable in one groundwater sample and permissible in thirteen groundwater samples. Hardness is desirable in three groundwater samples, permissible in seven groundwater samples and non-potable in four groundwater samples. Chloride is desirable in ten groundwater samples, permissible in two groundwater samples and non-potable in two groundwater samples. TDS is desirable in one groundwater sample, permissible in ten groundwater samples and non-potable in two groundwater samples. Fluoride is desirable in two groundwater samples, permissible in four groundwater samples and non-potable in eight groundwater samples. Iron is desirable in thirteen groundwater samples and non-potable in one groundwater sample. Ammonia is desirable in seven groundwater samples and non-potable in seven groundwater samples. Nitrite is desirable in thirteen groundwater samples and non-potable in one groundwater sample. Nitrate is desirable in two groundwater samples and non-potable in twelve groundwater samples. Phosphate is desirable in all the fourteen groundwater samples. Residual Chlorine is desirable in ten groundwater samples and permissible in four groundwater samples. The study is highly useful for planning and monitoring of groundwater quality for drinking purpose in the study area.

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GREEN SYNTHESIS, CHARACTERIZATION OF CUO AND RGO/CUO NANOCOMPOSITES VIA REFLUX METHOD

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Abstract
This study focuses on the green synthesis of CuO and rGO/CuO via the reflux technique. The produced materials can be confirmed by conducting various analytical techniques. The analysis revealed particle sizes of approximately 29.35 nm for CuO and 23.56 nm for rGO/CuO. Internal and surface morphology studies confirmed the well-developed particles formed on rGO sheets. UV-Vis's analysis provided absorbance data and band gap values, while elemental analysis confirmed the presence of copper, oxygen, and carbon. FT-IR spectra identified metal-oxygen bonds within the 600-400 cm ^{1} range in both CuO and rGO/CuO. These materials demonstrate broad potential applications, including photocatalysis, antioxidant and antifungal activities, sewage and industrial dye water treatment, energy storage, and corrosion resistance.

Keywords: rGO; CuO; Green synthesis; Reflux method; Characterization.

1. INTRODUCTION

In ancient Egypt, from 2000 BCE, copper was employed for water purification and sterilization. Additionally, it was used for treating wounds, showcasing its significant role in early medical practices. The antibacterial properties of copper have been recognized for centuries. During the Roman Empire, copper was commonly used in cooking pots, which inadvertently helped reduce bacterial contamination in food and water. Copper's ability to kill bacteria and other microorganisms stems from its oligodynamic effect, where even small amounts of copper ions disrupt essential microbial processes, leading to cell death. This ancient application highlights an early, albeit indirect, understanding of copper's antimicrobial properties. Copper was also employed to prevent the spread of communicable diseases. Notably, Japanese soldiers placed small pieces of copper in their water flasks throughout the Second World War to avoid dysentery [1].

However, copper ions can raise concerns about their impact on workers, organizations, and ecosystems [2]. In this context, several studies have explored copper-based nanomaterials as reservoirs for controlled ion release, aiming to mitigate the continuous discharge of Cu^{+} in the nature [3]. CuO exhibit a oxidation rate than their bulk counterparts when exposed to air due to both physic and chemical instability [4]. CuO are constant, highly durable, a extended shelf life equated to other materials or chemical compounds [5]. However, as soon as nanomaterials are reintroduced to interest in biological media, they tend to convert unbalanced, foremost to the increased combination of antibacterial agents and a subsequent reduction in their effectiveness. In this context, a practical solution is incorporating them into films or supportive matrices, enhancing their stability and effectiveness [6]. For instance, an appropriate material is graphene, which is only one atom thick, solid, and flat, consisting of sp^{2}-bonded carbon atoms in a hexagonal 2D lattice [7]. In the most informed approaches for synthesizing CuO/graphene nanocomposites, copper salts are introduced into ethanol-covering exfoliated graphene. Following synthesis, the resulting nanoparticles typically range from 25 to 200 nm [8]. Recently, CuO/graphene nanocomposites have

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been synthesized through a straightforward method involving mixing graphene with CuO nanoparticles by a usual size of approximately 60 nm [9]. Graphene oxide (GO) stands out as an oxidized graphene derivative among the various graphene-related compounds. Graphene oxide offers a large surface area, the potential to incorporate numerous initiated functional groups, respectable water dispersion, and a moderately simple research process. Its biocompatibility further enhances its suitability as a support material, making it an excellent platform for nanomaterials [10]. This material enhances the constancy of nanoparticles and improves their antibacterial performance, which is attributed to the formation of ultrafine particles.

Additionally, GO can boost the adsorption assets for Cu (II) ions, exhibiting a high adsorption capacity for copper ions. Studies show that graphene oxide can improve the adsorption capacity by approximately 30% [11]. Cu salts are precursors, along with an aqueous graphene oxide (GO) dispersion in an alkaline solution. The mixture is then reduced, assisted by oxidation in the presence of a complexing agent, using methods such as microwave irradiation, NaOH, or heat [12]. In these studies, the size of CuO nanoparticles ranged from 30 to 400 nm [13]. Therefore, a reduction in the size of nanomaterials can significantly enhance their efficiency and performance [14-15]

The current investigation is a modest green synthesis scheme for CuO and a reflux method for synthesizing rGO/CuO nanocomposites. The materials have been categorized us ing several practices, such as XRD, SEM, EDAX, FTIR, UV-Vis, and HRTEM analysis. The prepared materials include tenders in catalysis, photodegradation, microbial activity, antifungal treatments, and anticancer therapies.

2. MATERIAL SYNTHESIS

2.1. Preparation of CuO using Ashwagandha plant fuel

A silica crucible was used to dissolve 1 g of $Cu(NO_3)_2$ in a very minimal of water. Half a gram of dried Ashwagandha herb was used as fuel. The mixture was kept in furnace at 600°C for one hour under oxygen-rich open combustion conditions. The corresponding reaction equation is shown below (Eq. 1) [16].

$$Cu(NO_3)_2 * 3H_2O + \text{Ashwagandha (fuel)} \rightarrow CuO + CO_2(g) + H_2O \ldots\ldots (1)$$

2.2. Synthesis of rGO/CuO via reflux method

0.1 g of rGO was sonicated for 30 minutes and added to an RB flask. Separately, 0.25 g of CuO was mixed with 30 mL of water in the RB flask. The combination was stirred magnetically at 400 rpm and heated to 90°C for 30 minutes. Subsequently, 1% sodium sulfite and 1 g of sodium citrate were added to the reaction mixture and stirred for one hour. Finally, 2 M NaOH was added into the mixture, which was stirred for 30 mins. The solution was then allowed to settle for a few minutes, passed through Whatman filter paper, and dried for 30 minutes at 60°C in the oven [17].

3. RESULTS DISCUSSION

3.1. XRD

The XRD of CuO and rGO/CuO are presented in Fig. 1. CuO was synthesized using the SCM. The diffraction peaks observed at 31.59°, 36.75°, 38.32°, 48.78°, 52.56°, 58.21°, 60.27°, 65.43°, 68.34°, 72.34°, and 75.34° resemble the Miller planes (110), (11-1), (111), (20-2), (020), (202), (11-3), (31-1), (220), (311), and (22-2), respectively. These peaks line up well with the usual JCPDS card number 48–1548, confirming the formation of CuO [18]. In the XRD of rGO/CuO, a prominent peak observed at 24°-25° agrees with the (002) plane of rGO, indicating its successful incorporation into the composite. This high-intensity peak confirms the presence of rGO alongside CuO, synthesized by the reflux method. The remaining peaks correspond to CuO, with no additional peaks observed, thereby confirming the purity of the composite material and the absence of impurities [19]. The size was determined using the Eq. (2),

$$D = \frac{K\lambda}{\beta Cos\theta} \ldots\ldots (2)$$

For CuO, the size was found to be 29.35 nm, whereas for rGO/CuO, it was 23.56 nm.

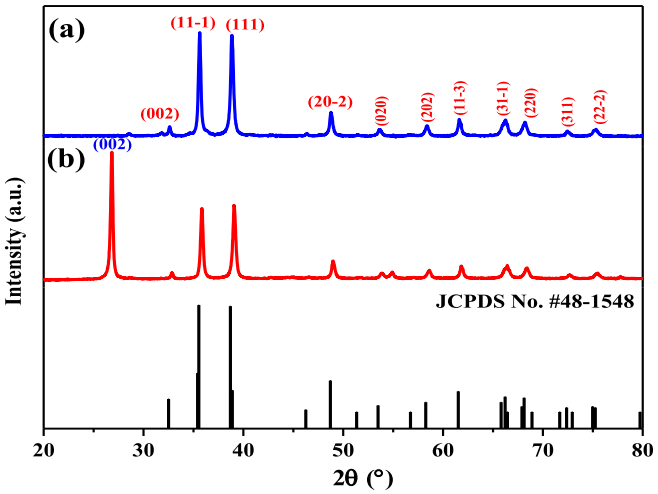


Figure 1. (a, b) XRD of CuO and rGO/CuO.

3.2. SEM and TEM

The SEM image of CuO and rGO/CuO was displayed in Fig. 2(a, b). In Fig. 2(a), the SEM of CuO particles exhibits an even distribution throughout the whole surface area of a spherical form. Additionally, nearly vacant spaces are observed within the compound, which could be attributed to the synthesis process [20]. Fig. 2(b) illustrates an SEM image of rGO/CuO, where the graphene sheet matrix is uniformly decorated with CuO particles. The surface morphology analysis further approves the practical synthesis of the rGO/CuO, as such uniform dispersion indicates the effective integration of CuO onto the graphene layers. In Fig. 2(c), the internal reveals well-defined spherical particles distributed across the surface of the rGO sheets. The CuO are closely packed and clustered, indicating strong interaction and integration with the rGO matrix [21].

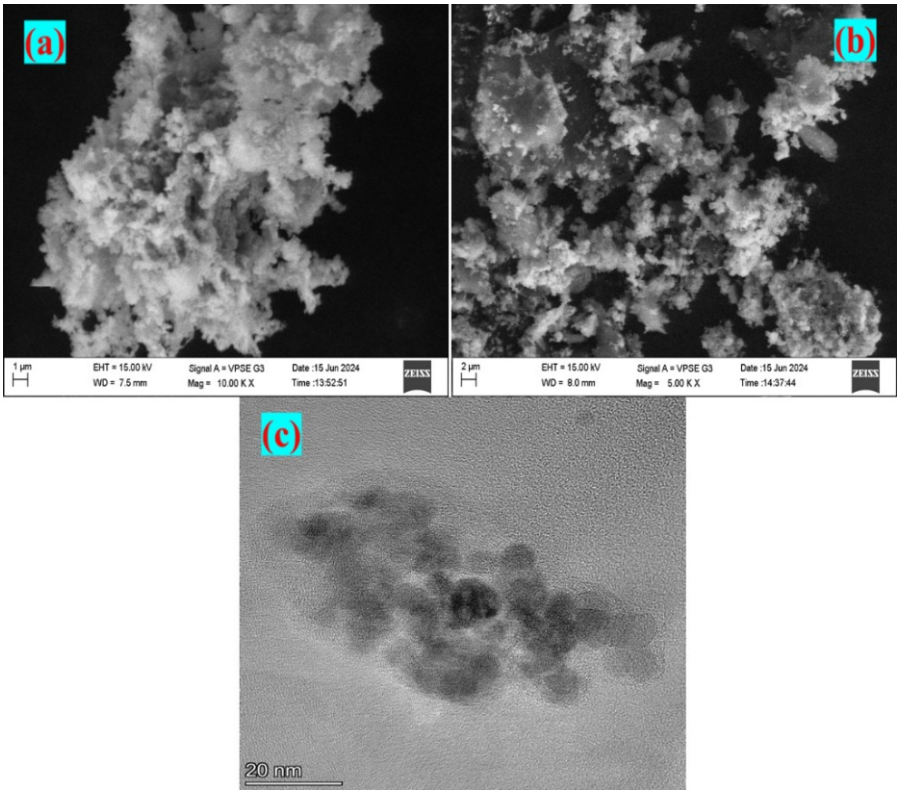
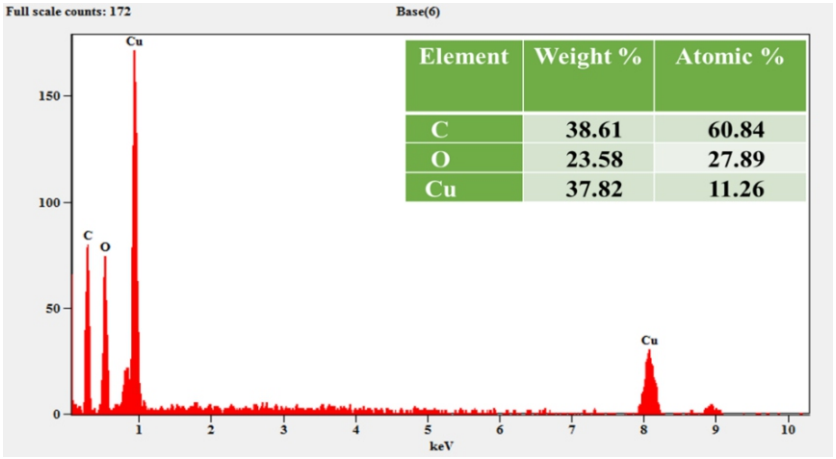


Figure 2. SEM of (a, b) CuO, rGO/CuO and (c) TEM of rGO/CuO.

3.3. EDX

EDX analysis of the synthesized rGO/CuO composite, in Fig. 3, displays prominent peaks for Cu, C, and O, confirming the production of rGO/CuO via the reflux technique. The absence of any additional peaks in the spectrum indicates the composite's purity and validates the synthesis process's effectiveness [22].



Element	Weight %	Atomic %
C	38.61	60.84
O	23.58	27.89
Cu	37.82	11.26

Figure 3. EDX image of rGO/CuO.

3.4. UV-Vis spectroscopy and Bandgap

The UV studies and band gap analysis in Fig. 4. The absorption spectra confirmed the Energy band gap using the Kubelka Munk curve under Eq. (3) [44].

$$\mathbf{F(R)} = \frac{\mathbf{(1-R^2)}}{\mathbf{2R}} \ldots\ldots\ldots\ldots\ldots \mathbf{(3)}$$

$$\boldsymbol{(F(R)hV)^2 = C\left(hV - E_g\right)} \ldots\ldots\ldots\ldots\ldots \mathbf{(4)}$$

The plot of (F(R)hν)2 vs photon energy (hν) and the energy band gap Eg are shown in Fig. 4. According to Eq (4), the expression (F(R)hν)2 is used to determine the optical band gap. From the band gap values shown in Fig. 4(a, b) (inset), it has been noted that the CuO and rGO/CuO have band gaps of 4.78 and 1.43 eV [23].

3.5. FTIR

The C-O vibrations are responsible for the peak at 1117 cm¹ in the rGO/CuO seen in Fig. 5. The peak shows the existence of

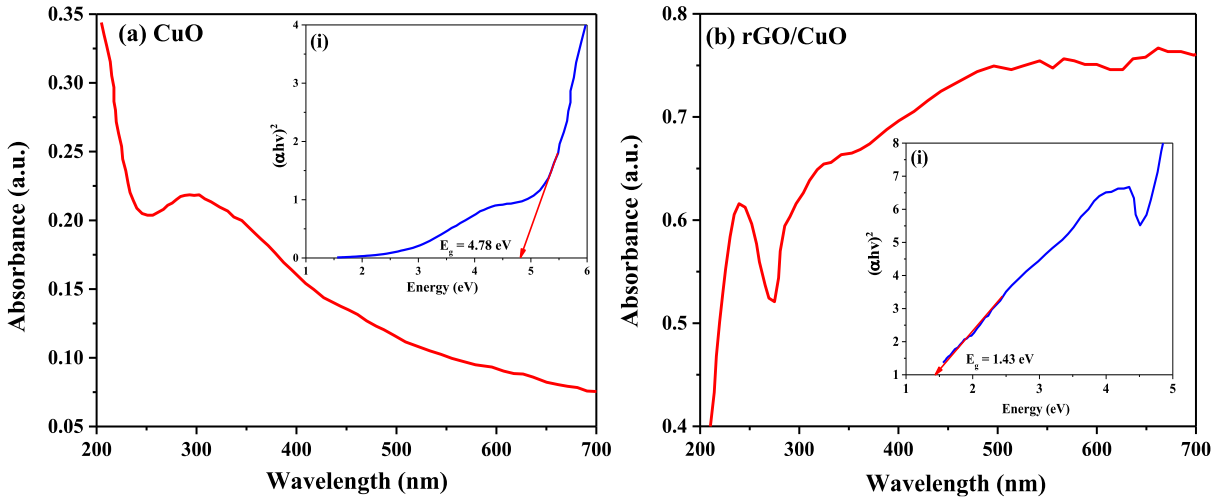


Figure 4: (a, b) UV-Vis and (inset) Tauc's plot of CuO and rGO/CuO.

C-C bonds inside the GO sheets at 1566 cm $^{-1}$. O–H vibrations are observed around 3389 cm $^{-1}$, though weak in the rGO [24]. The peak at 882 cm $^{-1}$ is attributed to Cu-OH bond vibrations, further confirmatory the successful synthesis of rGO/CuO. The absence of CuO-specific peaks and a peak at 432 cm $^{-1}$, corresponding to Cu-O, validate that CuO has been effectively into the rGO sheets [25].

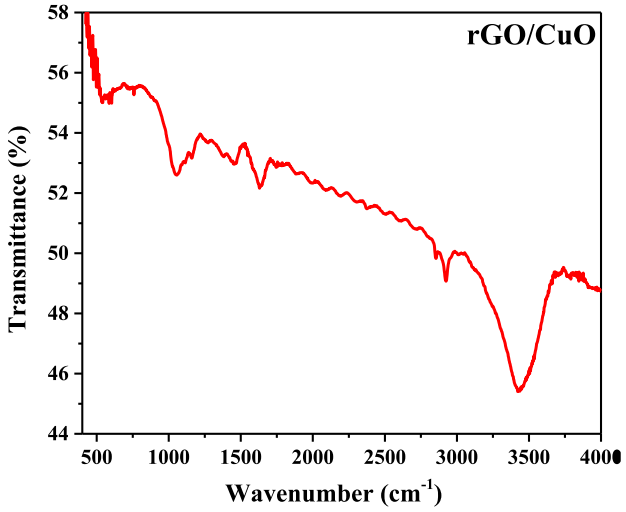


Figure 5 IR of rGO/CuO.

4.CONCLUSION

The green approach of CuO and rGO/CuO by means of the reflux method has proven to be a practical approach for producing nanomaterials with promising applications in various fields. The characterization results confirm the successful synthesis of well-defined CuO and rGO/CuO particles with optimal sizes and surface morphologies. These materials exhibit an extensive choice of potential uses, particularly in photocatalysis, environmental remediation, sensor, and corrosion resistance. Future work will optimize the synthesis process and explore their execution for practical purposes, such as wastewater treatment and energy devices, to maximize their impact on sustainable technologies.

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ASSESSMENT OF PHYSIOCHEMICAL, HEAVY METALS, AND BIOLOGICAL CHARACTERISTIC OF BELLANDUR LAKE WATER BODIES IN KARNATAKA

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Abstract
The significance of water in lakes goes well beyond being just a liquid; it plays a crucial role in temperature regulation, supports aquatic ecosystems, and enables vital processes such as nutrient cycling. The study provides an in-depth examination of the lake's physical, chemical, and biological parameters in Karnataka, India, including Bellandur Lake, highlighting its overall health and pollution levels. The analysis of water quality parameters indicates differing conditions and varying degrees of pollution within these freshwater ecosystems. While specific parameters, such as pH and faecal coliform counts, meet acceptable standards, others point to potential environmental concerns, including high levels of turbidity, total dissolved solids, and chemical oxygen demand. Moreover, the presence of unpleasant odours and disagreeable tastes further emphasizes the influence of pollution sources on the sensory qualities of the water. The findings reveal the unique characteristics of heavy metal presence in Bellandur Lake, suggesting potential mineral enrichment and contamination sources in the other. The fluctuating levels of faecal coliform highlight the need for ongoing monitoring and management. This research emphasizes the urgency of adopting remedial measures to improve water quality and maintain the ecological balance of these lakes. Continuous vigilance and proactive measures are crucial to reducing pollution risks and ensuring the long-term sustainability of the region's freshwater reservoirs.

Keywords: Water; Physiochemical; Heavy metals; Biological Characteristic; Bellandur lake.

1. INTRODUCTION

Among all the resources accessible to humanity, water holds a position of utmost importance. The necessity of conserving water bodies, particularly freshwater sources, is being increasingly recognized worldwide, as water is crucial for sustaining life. Seventy-one per cent of the Earth's surface is covered by water, earning it the nickname "blue planet." However, most of this water is saline, making freshwater incredibly scarce and valuable. Freshwater exists primarily in groundwater, ice, snow, glaciers, and snowfields, comprising only about 2.5% of the Earth's total water. This freshwater is found in moist soil, seas, lakes, and flowing streams. A concern about global water scarcity has emerged, with water being considered a potential cause of future conflicts, particularly in developing nations. According to 2006 data from the WHO, around 36% of urban Indians lack access to safe drinking water, and the situation is even more dire in rural areas, where approximately 65% of the population faces the same issue [3]. As we all know, life is not possible without water, especially freshwater resources. Good-quality potable water is essential for various sectors, including agriculture, industry, and commerce. Access to water was a key factor for the first settlers, and this remains true today. The history of public water supply demonstrates that its expansion is closely tied to the growth of urban environments [1].

Although the Earth is covered with water on and beneath its surface, only a tiny fraction-around one per cent is fresh and potable. Most of the Earth's water, approximately 1.4 billion cubic kilometres (326 million cubic miles), is found in the seas or ice in glaciers and polar caps. The solids dissolved in the ocean water are approximately 35 g per liter (4.5 ounces

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per gallon). For these reasons, it cannot be used in agriculture or industry and is not edible for human beings. While the water is abundant, there is strong evidence that it is not available where and when it is required most. This means that high-quality water in many areas is scarce due to the increased city and industrial development rate and the pollution that comes with it. As the population and economic growth are rapidly growing, India stands before the significant problem of scarcity of water resources among all natural resources. As for the case of most freshwater bodies worldwide, their water quality has been degraded by pollution. Water in its various phases: oceans, seas, rivers, lakes, clouds, rain, snow, fogs, etc. is indispensable to life of any type. Water pollution has become a significant issue as population growth and advancements in agriculture, urbanization, and industrialization have limited freshwater sources. He pointed out that the shortage of this vital resource is significant in many regions of the Earth. Most wastewater undergoes little or no treatment and is discharged into water bodies such as rivers, lakes, and estuaries. In addition to their crucial role as water reservoirs, lakes serve many other functions in Earth's biosphere: they are habitats for flora and fauna, help stabilize the microclimate, enhance the landscape's beauty, and provide countless recreational opportunities for people. Contributing to their persistence, heavy metals are relatively non-volatile and can bioconcentrate from water and sediments. Additionally, they can bio-magnify through the food chain, making heavy metals one of the most significant threats among all pollutants [2].

This study focuses on assessing the water quality of Bellandur Lake in Bangalore, Karnataka, India. The aim was to gather information on the contamination status of the lake, including physicochemical contamination and the presence of selected heavy metals.

2. STUDY AREA

Bangalore, the capital of Karnataka, is over 400 years old. The city was founded by the late Magadi Kempegowda in 1537. Bangalore is the state's capital today and is a major cultural, economic, administrative, and industrial hub. Bangalore, located in southern India, sits 920 meters above mean sea level. Geographically, it lies at a latitude of 12° 95' north and a longitude of 77° 57' east. The city experiences a temperate climate, making the weather favourable throughout the year. The growth in population, particularly among working-age individuals, can be attributed to the infrastructural developments that accompanied the rise of the software industry in the early 1990s. As a result, the city has earned the title of the "Silicon Valley of India," the country's leading exporter of IT services [4]. Bangalore had a population of 16,391 at the start of the twentieth century (1901), and by 2001, this number had grown to 5,686,844. The city's population density of 11,000 people per square kilometre reached 8,425,970, according to the preliminary reports of the 2011 Census of India. According to recorded history and traditions, the Kempe Gowdas, who first established Bangalore, constructed the city's initial tanks with the help of bunds. These bunds were created by blocking the natural valley systems in the area. In addition to enhancing the city's aesthetic appeal, most of the lakes and tanks were artificial to meet the needs of irrigation, water supply, and fishing. In ancient Indian culture, the water from these lakes was also used as "Dhobi Ghats," where clothes were washed and sun-dried by dhobis, or washermen. The lakes have also contributed to replenishing the water sources, such as wells, that people in the surrounding areas depend on for their water supply. Unfortunately, the lakes typically seen in Bangalore have fallen victim to urban development. This vast reserve is the largest in Bangalore, covering an area of 892 acres. Located at 12°58' north latitude and 77°35' east longitude, the dam sits 921 meters above mean sea level. It has a catchment area of 287.33 square kilometres and an extent of 110.94 square miles. Bellandur Lake spans over 3 km in length and 2.75 km in width, with a storage capacity of 17.66 million cubic feet. Located about 20 km southeast of Bangalore in a notable bio-geographical isolated region, it is one of the largest artificial lakes in Southeast Asia. Bellandur Lake once symbolized Bangalore's beautiful and healthy water supply. The lake's aquatic life functioned as a natural treatment plant for the storm runoff that accumulated there. In this sense, Bellandur Lake acted as the city's organ, preventing the bioaccumulation of organic waste. Indeed, the lake was home to a remarkable diversity of animals and aquatic life, attracting migratory birds from across the country. In addition to being a historic hub for the fish trade, the lake served as a crucial source of drinking water for half of the city's population. In other words, the area surrounding Bellandur Lake in Bangalore was a sensitive and fragile ecosystem. The Bellandur Lake is part of the broader Bellandur drainage system, which drains the southern and southeastern parts of the city. The current Bellandur Lake can be seen as a receptor for three generations of tanks. The eastern stream, originating from the northern end near Jayamahal, drains the entire eastern area. Another significant network is the central stream, historically located in the centre of the city's transportation maps, particularly around the K.R. Market area. This stream extends into the middle part of the city, playing a key role in the drainage system. The second chain is the western stream, which flows through the southwest and ultimately drains into Bellandur Lake. Further down the plateau, water from the Varthur Lake, located to the east, flows into the Pinakani River basin. As far back as three decades ago, eighteen different villages relied on the water from Bellandur Lake for their sustenance. The supplying tanks of Bellandur Lake suffered from link breakage in the 1980s due to urbanization. As a result of this chain failure and unchecked commercial, residential, and industrial expansion, the lake no longer collected sufficient rainwater. Instead, it became overloaded with water containing untreated sewage and effluents. The reduced fish population in the lake led to problems for fishermen and other aquatic life. In the 1990s, some property near the lake was designated as a bike route. Bangalore's IT boom also rose during this time, contributing to rapid urbanization. Recently, weeds have invaded a large

portion of Bellandur Lake. The water has become opaque and dark in colour, accompanied by a foul smell. It is important to note that most birds once inhabited the area are now rarely seen. The significant foaming at the outflows suggests that effluents are downstream of the lake. Exploration of the lake has primarily focused on the levels of physicochemical parameters, and some selected heavy metals [5-6].

3. METHODS AND MATERIALS

The water samples from Bellandur Lake were collected in December 2024 and will be used in subsequent calculations by researchers. Water samples from the lake were collected using the grab sampling method with a 500 ml polyethene bottle. The collected samples were promptly delivered to the analytical laboratory for testing. The analytical laboratory promptly received all collected samples, initiating analysis without delay. Some of the heavy metals, along with various physicochemical parameters, were analyzed using standard methods. The Color and temperature were recorded on the spot.

4. RESULTS AND DISCUSSION

Water temperature is important because it determines the metabolic processes organisms in water can undergo. In water, the rate of chemical reactions increases with temperature while the solubility of gases decreases. Additionally, flavours and odours intensify as the temperature rises. The water temperature ranged between 24.2 and 24.4 ° C. Due to the abundance of weeds and algae, the current investigation found that the lake water had a brownish-black tint. Turbidity refers to the measure of how cloudy or murky the water appears. In simple terms, water appears turbid when particles are suspended in it. Various factors contribute to turbidity, including tiny animals and plants, organic matter, fine sand particles, small clay particles, and plankton. Turbidity is recognized as a limiting factor in the biological productivity of water bodies. In this investigation, turbidity values were recorded at 14.89 NTU. This exceeds the acceptable consumer limit below 5 NTU across all sites examined. The electrical conductivity of water is influenced by the total dissolved solids present. According to international drinking water quality standards set by the World Health Organization, the electrical conductivity of drinking water should not exceed 500 µS/cm. Total dissolved solids (TDS) are a critical criterion for assessing potable water quality, as they directly influence its suitability for consumption. TDS contributes a distinct taste to water; at higher concentrations, it can render the water unpleasant to drink. Daily water consumption containing more than 450-500 mg/L of TDS may lead to gastrointestinal discomfort. Typically, high TDS levels impart a repulsive taste that dominates the sensory perception of the water. Additionally, elevated TDS levels are associated with the apparent hardness of water, often leading to precipitation within pipes, plumbing fixtures, water valves, and filters. The findings from this research indicate that the TDS levels significantly exceed the BIS-prescribed limit of 765 mg/L. The Bureau of Indian Standards (BIS) recommends a working pH range of 6.0 to 8.7 for most applications, sectors, and industries, along with other probable maximum contaminant levels for water quality. The pH of the water samples analyzed in this study ranged from 7.56 to 7.58, falling within the acceptable range prescribed by BIS standards. Additionally, the total hardness of all sampling sites was tested and found to comply with the BIS standard, measuring 235.4 mg/L. According to BIS standards, the calcium hardness was measured at 53.98 mg/L in the sample, within the permissible limit [7-8]. Similarly, magnesium hardness at these locations was recorded at 29.32 mg/L, meeting the acceptable standards. If hardness levels increase significantly, such as forming encrustations in water delivery systems, it can produce undesirable effects on domestic water usage. According to BIS standards, sampling points with alkalinity levels exceeding 457.21 mg/L are considered safe and ideal. Excessive alkalinity can negatively affect food taste, which can be particularly problematic when preparing food products. In this study, the permissible limit set by the BIS standard was exceeded, as sulfate levels were found to be 52.43 mg/L. Additionally, dissolved oxygen plays a crucial role in influencing nitrate content, as it is affected by the activity of nitrifying bacteria in the water. In this research, nitrate levels ranged from 12.61 mg/L, exceeding the recommended limit set by BIS standards. Such elevated nitrate levels can potentially lead to health issues like methemoglobinemia, commonly known as blue baby syndrome. The high nitrate concentration could be attributed to the lake's evaporation, which concentrates nutrients, decaying macrophytes and increased phytoplankton production. Chloride levels were within the recommended range at the sample stations according to the BIS standard, at 183.4 mg/L. The phosphate concentration was recorded at 6.7 mg/L. According to the BIS standard, the fluoride concentration at the sample site is 5.2 mg/L, above the allowable limit. An increase in dissolved oxygen concentrations to about 4.9 mg/L is likely due to effluent discharge, which introduces oxidizable organic matter. This organic matter consumes dissolved oxygen through biochemical oxygen demand (BOD) processes and vegetation degradation, especially at higher temperatures. Biomass can thrive and sustain itself only when dissolved oxygen concentrations reach at least 4-5 mg/L, while fish risk dying when concentrations drop to or remain below 2-3 mg/L. Therefore, it is recommended to use more environmentally safe and efficient materials in construction to prevent oxygen concentrations from falling below specified levels. Wastewater typically has low dissolved oxygen content, and while previous studies using ICMR standards may have detected higher DO levels, the levels observed in this study are lower. Biomass can thrive and sustain itself only when dissolved oxygen concentrations reach at least 4-5 mg/L, while fish risk dying when concentrations drop to or remain below 2-5 mg/L. Therefore, it is recommended that more environmentally safe and efficient materials be used in construction to prevent oxygen concentrations from falling below specified levels. Wastewater typically has low dissolved oxygen content, and while previous studies using ICMR standards may have detected higher DO levels, the levels observed in this study

are lower. The reduction in dissolved oxygen levels can be attributed to the high biochemical oxygen demand (BOD) readings, which were above the recommended 5.0 mg/L according to ICMR standards. The chemical oxygen demand (COD) value was also 72.56 mg/L. The COD test measures the amount of oxygen required to neutralize all reagents in the water, including non-biodegradable ones, indicating the extent of pollution and the potential impact on water quality. COD is a key parameter for evaluating the overall pollution level of water. As the concentration of organic materials rises, the COD of the water also increases. Research in this area found that the iron levels in the analyzed lake water samples were 1.5 mg/L, exceeding the permissible limit of 1.0 mg/L and the desirable limit of 0.3 mg/L. This excess iron can result in undesirable taste and appearance of the water, promote the growth of iron bacteria, and negatively impact water supply structures and domestic usage. Nickel concentrations ranging from 0.1 mg/L exceed the BIS standard of 0.02 mg/L, posing a risk of allergic reactions. Similarly, chromium concentrations between 0.06 mg/L surpass the BIS standard of 0.05 mg/L, with higher carcinogenic levels. The measured copper concentrations, ranging from 0.08 mg/L, exceed the permissible BIS limit of 0.05 mg/L. Copper levels above this threshold can cause side effects such as discolouration, corrosion, and an unpleasant taste in food and beverages. Additionally, the BIS standard sets the acceptable dissolved lead limit at 0.01 mg/L. However, within the observed range of 0.04 to 0.08 mg/L, lead in water becomes hazardous. Zinc and Arsenic were absent in the sample collection [9-10].

Table 1: Characteristics of Lake water.

Sample Parameters	**Bellandur Lake sample**
T (°C)	24.2
pH	7.56
Colour	Brownish Black
EC	1132
Turbidity	14.89
Total hardness (mg/l)	235.4
TDS (mg/l)	765
DO (mg/l)	4.9
COD (mg/l)	72.56
BOD (mg/l)	25.42
Mg^{+2} (mg/l)	29.32
Alkalinity (mg/l)	457.21
Ca^{+2} (mg/l)	53.98
Cl- (mg/l)	183.4
SO_4^{-2} (mg/l)	52.43
$PO4^{-3}$ (mg/l)	6.7
NO_3^{-} (mg/l)	12.61
F- (mg/l)	5.2
Pb_{+2} (mg/l)	0.12
Cd_{+2} (mg/l)	0.4
Fe_{+2} (mg/l)	1.5
Ar_{+2} (mg/l)	0
Zn_{+2} (mg/l)	0
Cu_{+2} (mg/l)	0.08
Cr_{+2} (mg/l)	0.03
Ni_{+2} (mg/l)	0.1

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CONFLICT OF INTERESTS
No conflicts of interests were disclosed.

5. CONCLUSION

Based on the findings of this study, it can be concluded that the lake water quality exhibits significant deviations from standard guidelines. Turbidity and electrical conductivity exceed the permissible limits recommended by BIS and WHO, respectively. TDS are above the desirable limit but remain within the permissible range per BIS standards. At specific sampling points, hardness and nitrate concentrations surpass the desirable limits, while alkalinity and fluoride levels exceed permissible limits according to BIS standards. Dissolved oxygen levels fall below ICMR recommendations, and elevated values of BOD and COD indicate higher organic pollution. While iron is a significant contaminant, other heavy metals such as zinc, cadmium, nickel, chromium, copper, and lead were detected in a few samples, raising concerns about localized pollution hotspots.

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UNVEILING THE IMPACT OF *AZOLLA FILICULOIDES* SUPPLEMENTATION ON POULTRY GROWTH AND FEED EFFICIENCY

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Abstract
Azolla filiculoides, a nutrient-rich aquatic fern, was evaluated for its potential as a dietary supplement to enhance weight gain and feed conversion efficiency (FCR) in poultry. The study was conducted over an 18-week period using 300 chicks, randomly divided into six groups of 50 each. The groups included a control (0% A. filiculoides) and five experimental groups supplemented with varying levels of A. filiculoides (5%, 10%, 15%, 20%, and 25%). The results demonstrated a significant improvement in growth performance and feed efficiency with increasing supplementation levels. The 25% A. filiculoides group achieved the highest weight gain of 1.8139 kg and the lowest FCR of 1.02, compared to the control group, which recorded a weight gain of 1.212 kg and a higher FCR. A clear inverse relationship was observed between FCR and weight gain, indicating enhanced feed utilization efficiency with higher inclusion levels of A. filiculoides. The lower supplementation levels (5% and 10%) showed minimal effects on growth performance, whereas higher levels (15%, 20%, and 25%) significantly improved weight gain and feed efficiency. These findings highlight the potential of A. filiculoides as an economical, sustainable, and nutritionally effective feed additive for poultry. The study underscores its viability in reducing feed costs while optimizing growth performance, particularly at the 25% inclusion level, making it a promising candidate for sustainable poultry nutrition systems.

Keywords:
Azolla filiculoides, Sustainable poultry nutrition, Broiler growth performance, Feed conversion ratio (FCR), Cost-effective poultry feed, Poultry production efficiency

1. INTRODUCTION

Poultry production is a vital contributor to global nutrition, economic stability, and food security, providing an affordable source of high-quality protein essential for human health (Donma & Donma, 2017; Jilo & Hasan, 2022). In India, poultry farming supports rural livelihoods, poverty alleviation, and gender equality, particularly benefiting smallholders and women (Dadheech, 2014; Al-Khalaifah & Al-Nasser, 2022). However, the industry faces rising production costs, primarily due to the high prices of conventional feed ingredients like soybean meal (SBM) and maize, which constitute up to 75% of total production expenses (Rout *et al.*, 2017; Dinani *et al.*, 2018). This has necessitated the exploration of cost-effective and sustainable feed alternatives.

Chick weight plays a crucial role in poultry production, influencing growth, hatchability, and overall productivity. Heavier chicks, often derived from heavier eggs, show better growth rates, higher meat productivity, and improved feed efficiency, especially during early stages of life (Mbajiorgu & Ramaphala, 2014; Fedorova & Vakhrameev, 2023). This relationship is critical for broiler production, where early chick weight serves as a strong predictor of uniformity and performance (Singh & Nagra, 2006). Managing chick weight through careful selection of egg weight and early monitoring is essential for maximizing productivity and profitability in poultry farming. SBM is a widely used protein source in poultry diets due to its high protein content and balanced amino acid profile (Dei, 2011; Gaur & Pandey, 2020). However, rising costs and reliance on imports make SBM

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economically unsustainable for small-scale farmers in India. Additionally, anti-nutritional factors (ANFs) in raw soybeans, such as protease inhibitors, require further processing to improve digestibility, which increases production costs (Nahashon & Kilonzo-Nthenge, 2011; Lambo *et al.,* 2023;). These challenges have prompted the search for sustainable and cost-effective alternatives.

Feed costs, accounting for 60-75% of total poultry production expenses, remain a significant challenge in India (Rout *et al.,* 2017; Dinani *et al.,* 2018). The high prices of maize and soybean meal, commonly used at inclusion rates of 55-65% and 25-30%, respectively, have driven interest in alternative feed ingredients (Dinani *et al.,* 2019). Options like rice gluten meal and crude rice bran oil have shown potential to reduce costs by 7-9% while maintaining performance (Anitha *et al.,* 2009). The use of feed additives and nutrient optimization further enhances feed efficiency and reduces expenses (Mandal *et al.,* 2005; Abbas, 2023). In this context, *Azolla filiculoides*, a fast-growing aquatic fern, has emerged as a promising alternative to SBM. Rich in protein, amino acids, and minerals, Azolla supplementation has been shown to reduce feed costs while improving growth performance and feed conversion ratios (Jadhav & Pattar, 2024; Chekol, et al., 2024). Inclusion of up to 15% Azolla in poultry diets has demonstrated enhanced weight gain and feed efficiency without adverse effects on health (Kumar *et al.,* 2018; Adil *et al.,* 2022). Furthermore, Azolla's low input requirements and environmental benefits make it a sustainable option for reducing production costs in poultry farming (Yadav & Chhipa, 2016; Hafeez *et al.,* 2024;).

Given its rapid growth, low input requirements, and environmental benefits, Azolla holds promise as a sustainable replacement for SBM in poultry feed. This study aims to evaluate the efficacy of Azolla as a cost-effective feed ingredient, focusing on its impact on growth performance, feed efficiency, and economic viability, contributing to sustainable poultry production in India.

2. MATERIALS AND METHODS

2.1 Cultivation of *A. filiculoides*

The cultivation of *A. filiculoides* was carried out at a site within Mr. Raghavendra Bhat's coconut plantation in Mittlakatte, Davangere district, chosen for its accessibility and ease of maintenance. Three cement tanks, each measuring 6x3x2 feet, were constructed and prepared with a nutrient-rich slurry of fine soil, cow dung, and vermicompost. The tanks were filled to three-fourths capacity with water and stocked with a fresh culture of *A. filiculoides* sourced from Krishi Vigyan Kendra, Davangere. Shading was provided with a green net to reduce evaporation and prevent contamination, while water temperature and pH were regularly monitored. The ponds were emptied and replenished with fresh cultures every six months to sustain optimal growth. Under suitable conditions, *A. filiculoides* was ready for harvest within two weeks. Harvesting involved using a mesh tray, followed by washing to remove residual odors. The harvested biomass was sun-dried for 2-3 days and stored in airtight bags to ensure long-term usability.

2.2 Study Area and Experimental Setup

The experiment was conducted at Balaji Poultry Farm, owned by Mr. Ramakrishna, on Hadadi Road, Davangere, Karnataka. A total of 300 chicks were randomly divided into six groups of 50 each, with one control group receiving regular feed and five experimental groups supplemented with *A. filiculoides* at 5%, 10%, 15%, 20%, and 25% inclusion levels. The chicks were housed in clean, disinfected, and well-ventilated sheds, with separate enclosures for each group to ensure uniform conditions. Clean water and feed were provided ad libitum, and temperature and humidity were closely monitored to maintain optimal health and growth conditions.

2.3 Feed Preparation

The poultry feed was formulated with maize as the primary energy source, supplemented with soybean meal, DDGS, rapeseed (35-42% protein), DORB, and salt to provide essential nutrients. Growth and protein synthesis were supported by lysine and DL-methionine (DLM), while additives such as acidifiers, enzymes, toxin binders, trace minerals, phytase, choline chloride, vitamin premix, liver powder, limestone powder, de-oiled groundnut cake (GN), and di-calcium phosphate (DCP) enhanced digestion, mineral balance, and overall health. The feed was further supplemented with sun-dried and powdered *A. filiculoides* at 5%, 10%, 15%, 20%, and 25% inclusion levels, replacing an equivalent portion of the regular feed, with the control group receiving unsupplemented feed.

2.4 Experimental Design

A feeding trial was conducted from the 6^{th} to the 18^{th} week to evaluate the growth performance and feed efficiency of broiler chicks. Initially, 300 chicks were weighed individually, and they were randomly divided into six groups of 50 chicks each. Feed was supplied in two shifts daily, at 7:30 AM and 6:30 PM, with fresh water provided *ad libitum*. Weekly, body weights and feed intake were recorded to monitor growth and consumption. Feed conversion ratio (FCR) was calculated at weekly intervals to assess the efficiency of feed utilization across the treatment groups.

2.5 Data Collection and Analysis

The data were statistically analysed to compare the growth performance and feed efficiency among the different groups.

3. RESULTS

3.1 Harvesting *A. filiculoides*

In a short span of 2 to 3 weeks, *A. filiculoides* demonstrated exceptional biomass accumulation, rapidly colonizing the pond surface with a dense, green mat. This species, renowned for its robust and adaptive growth characteristics, thrived under optimal environmental conditions, highlighting its capacity for accelerated expansion (Figure 1).

3.2 Growth Performance

3.2.1 Weekly Body Weight

The progressive increase in body weight of chicks fed with varying levels of *A. filiculoides* supplementation, compared to the control group, over 18 weeks was observed. In the control group, the chicks exhibited a final body weight of 1.212 kg**,** the lowest among all groups. The inclusion of 5% *A. filiculoides* resulted in a slightly higher final body weight of 1.0486 kg, showing minimal improvement compared to the control. With 10% *A. filiculoides* supplementation, the final weight reached 1.208 kg**,** further indicating moderate enhancement in growth performance.

Significant improvements were observed in groups with higher *A. filiculoides* inclusion levels. Chicks fed with 15% *A. filiculoides* achieved a final weight of 1.2458 kg**,** and those with 20% *A. filiculoides* reached 1.4054 kg**,** demonstrating substantial growth benefits. The most pronounced improvement was observed in the 25% *A. filiculoides* group, with a final body weight of 1.8139 kg, reflecting a 49.6% increase compared to the control (Figure 2). The results highlight a clear positive correlation between the level of *A. filiculoides* supplementation and the body weight of the chicks, with higher supplementation levels (15%, 20%, and 25%) yielding significantly better growth. This demonstrates the potential of *A. filiculoides* as an effective feed additive to enhance growth performance in poultry.

3.2.2 Feeding Efficiency, Weight Gain and FCR

The results demonstrate that increasing *A. filiculoides* supplementation in poultry feed significantly enhances growth performance and feed efficiency. The average body weight progressively increased from 1.212 kg in the control group to 1.8139 kg in the 25% supplementation group, with intermediate weights of 1.315 kg, 1.51 kg, 1.69 kg, and 1.72 kg for 5%, 10%, 15%, and 20% supplementation, respectively. Similarly, weight gain improved from 0.869 kg in the control to 1.56 kg in the 25% group. Feed intake showed an inverse trend, reducing from 0.75 kg in the control group to 0.525 kg in the 25% group, indicating better feed utilization. The FCR also improved with higher supplementation, decreasing from 2.01 in the control group to 1.02 in the 25% group, with intermediate FCRs of 2.13, 1.98, 1.56, and 1.49 for 5%, 10%, 15%, and 20% supplementation, respectively. These results highlight the efficacy of *A. filiculoides* in enhancing growth and feed efficiency while reducing feed consumption in poultry (Table 1).

Figure 1: Preparation and feeding of *A. filiculoides* for broiler chicks.
(A) Harvested *A. filiculoides* being prepared for feeding.
(B) Control group chicks housed in clean, well-ventilated cages and fed with conventional poultry feed.
(C) Poultry feed mixed with sun-dried *A. filiculoides*, ready for feeding.
(D) Experimental group chicks consuming feed supplemented with *A. filiculoides*.

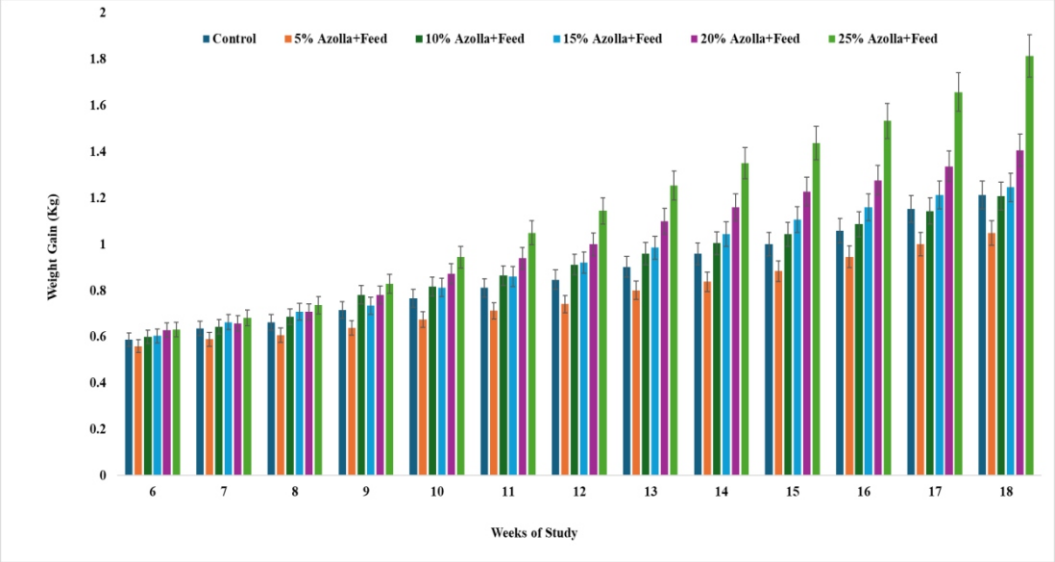

Figure 2: Weight gain of broiler chicks fed with varying levels of *A. filiculoides* supplementation over 18 weeks. Error bars represent standard errors for each group.

Table 1: Effect of *A. filiculoides* supplementation on growth performance and feed efficiency in broiler chicks. The table summarizes the average body weight (Kg), weight gain (Kg), feed intake (Kg), and feed conversion ratio (FCR) of chicks under different dietary treatments.

The Studied Characters		Treatments of *A. filiculoides*				
	Control	5%	10%	15%	20%	25%
Average Body Weight (Kg)	1.212	1.315	1.51	1.69	1.72	1.8139
Average Weight Gain (Kg)	0.869	0.959	1.13	1.23	1.3	1.56
Average Feed Intake (Kg)	0.75	0.67	0.852	0.595	0.697	0.525
FCR	2.01	2.13	1.98	1.56	1.49	1.02

4. DISCUSSION

A. filiculoides, a fast-growing aquatic fern, is a cost-effective and nutritionally rich alternative to soybean in poultry diets, offering high protein, amino acids, vitamins, and minerals suitable for broilers and layers (Alagawany *et al.*, 2023; Jadhav & Pattar, 2024; Chekol, et al., 2024). Studies show that Azolla can reduce feed costs by up to 30% while maintaining growth performance comparable to soybean-based diets (Jadhav & Pattar, 2024). In broilers, supplementation up to 15% improves feed efficiency and body weight without adverse health effects (Adil *et al.*, 2022; Najim *et al*., 2022), and in quails, it enhances growth rates and feed conversion ratios (Hafeez *et al.*, 2024). Economic analyses indicate reduced costs and comparable weight gains when replacing up to 10% of soybean meal (Paudel *et al.*, 2015; Kumar *et al.*, 2018). While Azolla can reduce egg production in some cases, it lowers nutrition costs, making it suitable for backyard systems (Marjan, 2024). Optimized inclusion can balance cost savings and nutritional adequacy, improving egg quality without affecting production in layers (Abate *et al.*, 2020). Azolla presents a sustainable, economical feed alternative with environmental benefits like bioremediation (Khushbu *et al.*, 2022; Paryanto *et al.*, 2023; Yoldashev *et al.*, 2024).

The findings of this study strongly correlate with the existing literature, underscoring the potential of *A. filiculoides* as a cost-effective and nutritionally rich feed supplement for poultry. The progressive increase in body weight observed with higher levels of *A. filiculoides* aligns with previous studies highlighting Azolla's role in enhancing weight gain. For instance, broilers fed with 5% Azolla demonstrated improved body weight and FCR compared to controls (Najim *et al.*, 2022), consistent with the observed improvement at 5% *A. filiculoides* inclusion in this study. Similarly, the notable weight gain at 10% and higher supplementation levels corroborates findings from Kumar *et al.* (2018), who reported that *Azolla pinnata* can replace up to 10% of soybean meal without compromising growth performance. Similar to our results, where *A. filiculoides* supplementation significantly improved body weight and FCR at higher inclusion levels, Arram *et al.* (2023) observed notable enhancements in growth performance and feed efficiency with 5% and 10% *A. pinnata* supplementation, emphasizing its potential as a sustainable feed additive.

The substantial growth benefits at 15%, 20%, and 25% supplementation levels observed in this study resonate with Tawasoli *et al.* (2020) and Al-Shwilly (2022), where Azolla inclusion at moderate-to-high levels resulted in significant weight gain in Vanraja poultry birds and broilers. Furthermore, the pronounced improvement in feed conversion ratio (FCR) with increasing *A. filiculoides* levels, particularly at 25%, aligns with studies indicating that Azolla inclusion enhances feed efficiency across various poultry breeds (Yadav & Chhipa, 2016; Mishra *et al.*, 2016). For example, Kadaknath poultry and Pratapdhan chicks demonstrated improved FCR at 7.5% and 15% Azolla meal inclusion, respectively, indicating its broad applicability across poultry types (Yadav & Chhipa, 2016; Borkar *et al.*, 2021). The reduction in feed intake observed with higher *A. filiculoides* inclusion is also supported by previous findings that Azolla improves nutrient utilization, resulting in lower feed consumption without affecting growth (Paudel *et al.*, 2015; Kumar *et al.*, 2018). The efficiency of *A. filiculoides* as a feed additive is further emphasized by the FCR improvement, which decreased progressively with higher inclusion levels. This finding aligns with Al-Shwilly (2022), who reported significant FCR improvements in broiler Ross chicks at 15%-45% Azolla inclusion.

Interestingly, while some studies, such as Hartati *et al.* (2023), noted a plateau in growth benefits at higher Azolla inclusion levels, this study demonstrates continued improvement up to 25% supplementation. The variability in effectiveness, as noted by Rana *et al.* (2017), highlights the importance of optimizing supplementation levels based on the specific breed and production goals. Additionally, the economic benefits of Azolla supplementation, such as the reported 30% feed cost reduction (Jadhav & Pattar, 2024), further support its viability as an alternative feed ingredient. Overall, the results of this study align with and expand upon existing literature, reinforcing the efficacy of *A. filiculoides* in improving growth performance, feed efficiency, and economic sustainability in poultry production. The findings validate its potential as a sustainable replacement for traditional feed ingredients like soybean meal, offering both nutritional and financial advantages while supporting environmentally friendly poultry farming practices.

5. CONCLUSION

The study establishes *A. filiculoides* as a highly effective and sustainable alternative to conventional feed ingredients in poultry diets. The inclusion of *A. filiculoides* significantly enhanced growth performance, feed efficiency, and weight gain, with higher supplementation levels (15–25%) yielding optimal results. These findings not only align with existing literature but also highlight the dual benefits of cost reduction and improved nutritional outcomes. By replacing traditional protein sources like soybean meal, *A. filiculoides* addresses both economic challenges and environmental concerns in poultry farming. This research reinforces its role as a versatile, eco-friendly feed additive capable of supporting sustainable poultry production systems. Further studies could explore its integration with probiotics and its impact on broader biochemical and immunological parameters.

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CONFLICT OF INTERESTS
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MARINE BASED CHITOSAN BIOPOLYMER AS POTENTIAL BIOSOAP, ECO-FRIENDLY ALTERNATIVES

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Abstract
Marine-origin polysaccharides have been used in recent research because they are readily available, reasonably priced, biocompatible, and biodegradable. Since it can be made from leftover marine crustaceans, chitosan is becoming more and more valuable in a variety of applications. The structural component of fungi, insects, and crustaceans, chitin is the second most common biopolymer on Earth, behind cellulose. Chitin can be deacetylated to produce chitosan, a deacetylated derivative of chitin. Because the amino groups that give the polymer its many characteristics are present, it is a functionally versatile biopolymer. Though it has been employed in many industrial applications, biodegradable chitosan soap is one of the more recent uses for it. The properties of chitosan have been enhanced through a variety of techniques, including the use of plasticizers and cross-linkers, the embedding of fillers like fibers, whiskers, and nanoparticles, and the blending of the polymer with other natural and synthetic polymers as well as with natural extracts and essential oils. To get this biopolymer to industrial levels for use in biosoap and bioplastic applications, however, a lot more research is still required. Foamability, Total Alkali, TFM, pH, Hardness, and Antimicrobial are the characteristics of biosoap.

Keywords
Chitosan, Biosoap, Marine Biopolymer, Biodegradable, Tissue Engineering.

1. INTRODUCTION

One of the best cleaning agents for water is soap [1]. The saponification reaction is the procedure used to make soaps. Salts of potassium and sodium are utilized in the making of soap. Soaps are frequently used for skin cleansing and washing. The ability of soaps to wash is attributed to the presence of fatty acids, which can be derived from both plant and animal sources. These sources include oleic acid, lauric acid, palmitic acid, and stearic acid, which have both saturated and unsaturated fatty acid chains [2]. The proportion of chloride, free alkali, pH, total fatty acid, and moisture content are among the chemical characteristics of soap [3]. Utilizing renewable biomaterials is one environmentally friendly way to lessen waste generation and environmental deterioration [4-8]. Products manufactured from biopolymers have found innovative and imaginative uses in the bioeconomy and biotechnology throughout the past few decades [9]. Natural resources have played a significant role in human existence as a source of food and therapeutic elements to prolong human life in medicine [10].

Marine-derived biopolymers, utilized in tissue engineering, are among the many materials under investigation that may prove to be useful restorative therapy choices for the regeneration of damaged bone function and/or treatment of bone abnormalities [11-13]. They possess a number of attributes that make them ideal for use in the development of bioengineering-based medical technologies [14]. Among the earliest biodegradable materials utilized in clinical treatment were natural polymeric polymers. The bioactive properties of natural polymers allow them to interact with cells more effectively, enhancing the way that cells behave in biological

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systems [15]. The three primary types of natural polymers are proteins, polysaccharides, and polynucleotides (chitin, chitosan, alginate, gelatin, silk, and elastin) [16]. In tissue engineering applications, the use of natural polymers as architectures makes biological sense. Natural medications derived from marine sources are therefore more targeted and effective in treating a wide range of human illnesses [17]. Simple production and extraction processes are necessary for engineering materials, and polymeric polymers derived from marine minerals meet these criteria [18].

Natural biopolymers have several applications, including the production of implants and surgical equipment as well as medications, because of their intrinsic biocompatibility and biodegradable architectures. Institutions in charge of health control, surveillance, and inspection are also more likely to accept these chemicals [19]. Biological properties of marine biomaterials generally promote the synthesis of novel bioactive compounds with specific pharmacological properties that are important to the pharmaceutical industry. Moreover, they are usually biocompatible and biodegradable [20]. Marine compounds are special because they possess two essential characteristics in the world of pharmacy: a high bioavailability and a target affinity [21]. Tissue engineering, which includes implanting biomaterials and has applications in tissue regeneration, is one of the scientific fields with the most active research presently. The process of enhancing, maintaining, fixing, or regaining different biological tissues' functionality [22].

Chitin is a common biopolymer that is present in algae, crustacean exoskeletons, fungi, and insects. A few fungi (Mucoraceae) contain chitosan, which is less common in nature [23]. In the past, chitin obtained from crustacean sources was chemically deacetylated to create the bulk of chitosan samples that are currently sold commercially [24]. Important bioactive compounds, such as chitin and chitosan, offer a wide range of incredibly useful properties, such as antioxidants, wound healing, bacterial resistance, and the removal of certain contaminants. Because chitin and related components are renewable resources, they find application in a wide range of industries, including agriculture, cosmetics, pharmaceuticals, packaging, and nutrition [25]. Chitosan, which is less common in nature, is present in a number of fungi (Mucoraceae). Most commercially available chitosan samples were previously produced by chemically deacetylating chitin derived from crustaceans [26].

To eliminate dirt from surfaces like skin, fabrics, and other substances, soap is a chemical that dissolves in water. In addition to being crucial ingredients in lubricants and utilized in textile spinning, soaps are mostly employed as surfactants for cleaning, washing, and bathing. The process of processing vegetable or animal oils and fats with a strong alkaline solution yields cleansing soaps. A metal radical reacts with fatty acids or fatty glycerides to form soap, which is a chemical compound or mixture of chemical compounds. For washing, bathing, and cleaning purposes, soaps are primarily employed as surfactants [27]. An enormous amount of waste crustacean shell is produced annually as a major by-product of the seafood industry. This waste can be used to make value-added chitin, which can then be turned to chitosan through a relatively straightforward deacetylation process. Chitosan is far less expensive than other biopolymers since it is derived from a bio-waste product utilizing numerous energy-efficient techniques. Still, chitosan is a far better option for food packaging applications due to its unique qualities. Many industries, including natural cosmetics, soap, biomedical, agriculture, textiles, water treatment, photography, chromatography, electronics, paper, and food have already employed chitosan.

After chitin is deacetylated, a white, viscoelastic polymer known as chitosan is created. Chitosan is both biodegradable and biocompatible. Products made from chitosan that have had polymers with distinct properties produced by altering their basic structure [28]. Chitosan has attracted a lot of interest in bone regeneration because to its excellent qualities, which include antibacterial activity, sustained drug release, environmental friendliness, and exceptional biocompatibility [29]. Many skin irritations, such as flaking, dryness, redness, itching, and rash, are more frequently brought on by the chemicals included in commercial soaps; these chemicals can include petroleum compounds and chemical scents. Total fatty matter (TFM), moisture content, caustic alkali, pH, and free alkali were among the physical and chemical characteristics of chitosan-based soaps that were examined in order to assess if they would irritate skin.

2. MATERIALS AND METHODS

2.1 Sources and Structure

The most widely used and cost-effective method for separating chitosan is chitin deacetylation. Knowing that natural compounds, like chitin and chitosan, are derived from the remnants of other natural materials is one of the most crucial things to know. They originate from the trash produced by aquatic life. It should be highlighted as a result that natural resources only come in a certain amount [30, 31]. Many different types of animals and fungi rely on chitin as its structural component. Table 1 lists some popular chitin sources along with their chitin contents.

Table 1: Various sources for chitin extraction with percentage content in each by dry mass. (Hamed, Ozogul, & Regenstein, 2016). [32].

Organism	Chitin Content (%)	References
Crustaceans		Arbia, Arbia, Adour, & Amrane, 2013 [33]; Synowiecki & Al-Khateeb (2003) [34]
Nephro (lobster)	69.8	
Euphausia superb (krill)	24.0	
Homarus (lobster	60.0 -75.0	
Crangoncrangon (Shrimp)	17.8	
Lepas (goose barnacle)	58.3	
Fungi		
Aspergillus niger	42.0	Synowiecki & Al-Khateeb (2003) [34]
Penicillium notatum	18.5	
Penicillium chrysogenum	19.5 - 42.0	
Saccharomyces gutulata	2.3	

Figure 1 depicts the procedures for producing chitin from natural resources. Chitin is made in three steps: the protein is extracted in the first step using an alkaline solution; the minerals are removed in the second step using an acidic solution; and the color is added in the third step. For the substance to acquire specific qualities, each step is crucial. The use of enzymes and acids generated by microorganisms is another method for producing chitin with exceptional quality and purity.

The largest amine polysaccharide polymer after natural cellulose is chitin, which was initially identified in mushrooms in 1811 [36]. As illustrated in Figure 2, chitin is present in the skin and skeleton of snails, invertebrates, crustaceans, and insects, including crab, king crab, crayfish, lobster, and shrimp, despite the fact that it fortifies the cell walls of many fungi. Due to its biological characteristics, practical applications, and biodegradable and biocompatible nature as polymers generated from renewable natural sources, chitin and its derivatives are highly valuable economically [38].

Chitosan is generally employed in biomaterials, especially drug delivery systems and synergistic applications to augment

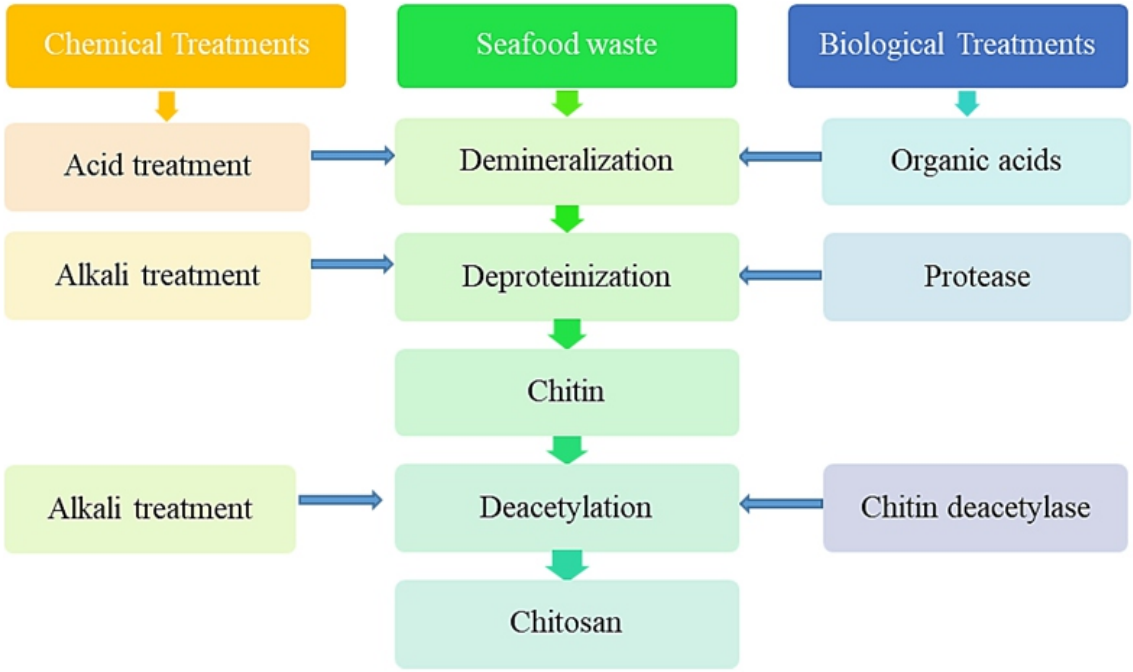

Figure 1: Chemical and biological procedures are used to produce chitin and chitosan. Reproduced with the permission from [38], Copyright 2019 Wiley Periodicals, Inc.

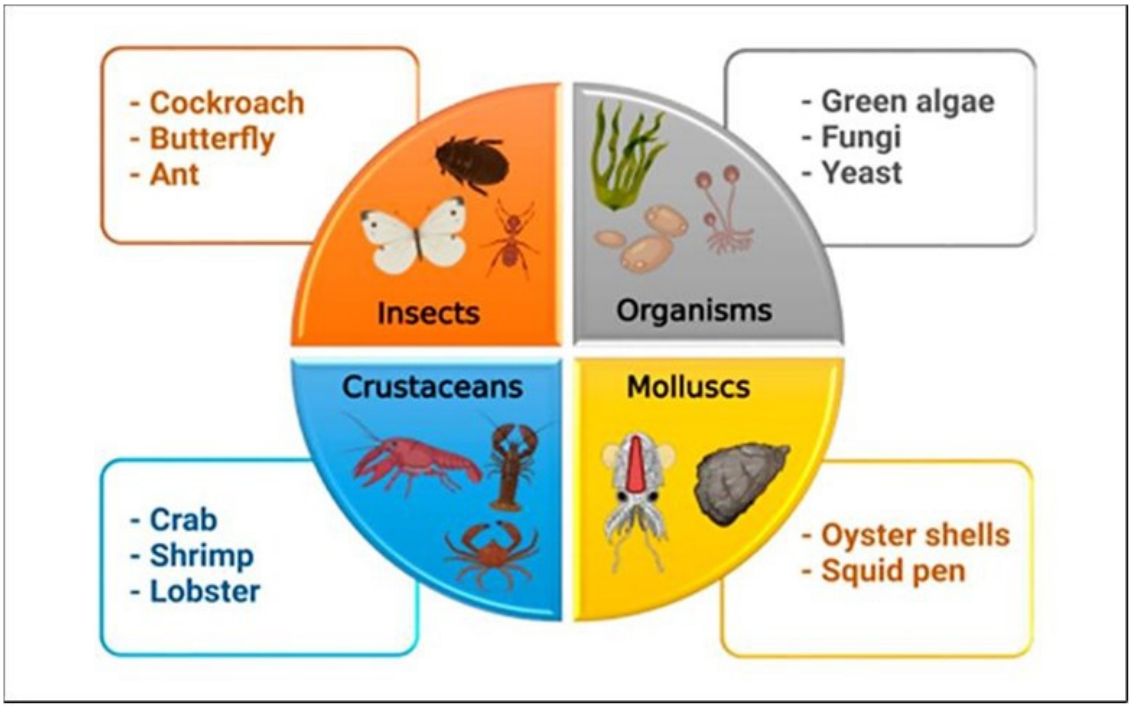

Figure 2: Sources of the chitosan. Reproduced with the permission from [37], Copyright 2014 Elsevier Inc.

the therapeutic advantages of other chemicals. How to make chitosan from chitin is shown in Figure 3.

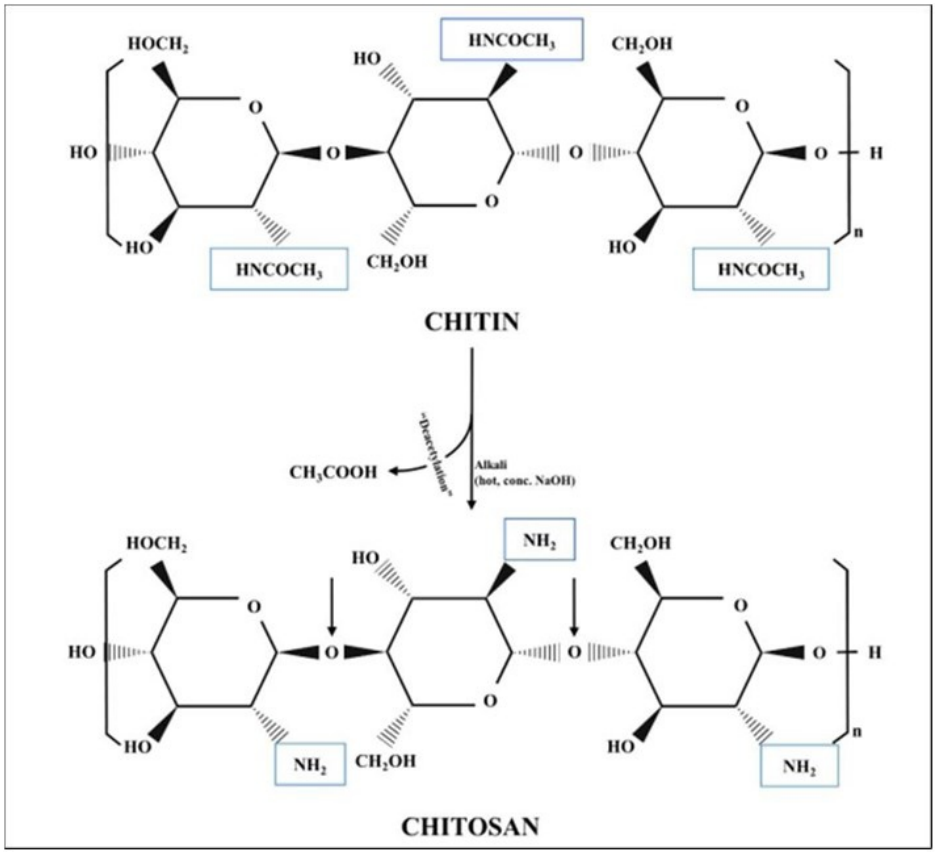

Figure 3: Production chitosan from chitin. Reproduced with the permission from [39], Copyright 2000 American Chemical Society.

2.2 Preparation of Soap by Hot Process Method

Chitosan of analytical grade was obtained from Sigma Aldrich and utilized in the purification process. To accelerate the interaction between the alkali and the fat, 3g of the Chitosan powder (A), measured oil, Alovera gel, glycerin, milk protein, flavor, and coloring agents were added to a 250 mL beaker. To make a 0.2 N NaOH solution, a fixed volume of distilled water was combined with a calculated weight of NaOH. The aqueous base solution was gradually added to the oils at a temperature of 33-43 °C, and the mixture was constantly stirred until it started to thicken. After that, the mixture was put into a plastic mold and given time to harden. For sample (B), the sample procedure was repeated. Photographs of Soap A and Soap B, respectively, in Figures 4 (a) and 4 (b), show the computed weights of the two samples in Table 2.

Table 2: Weight of Soap Mixtures.

Samples	Chitosan Powder gm	Oil mL	Caustic lie mL	Gel gm	Glycerine gm	Colouring Agents mL	Flavour mL	Protein gm
Soap A	3	Coconut oil-175	150	5	5	Blue colour-5	5	5
Soap B	3	Almond oil-175	150	5	5	-	5	5

Figures 4 (a) & (b): Photographs of Soap A and Soap B.

3. RESULTS AND DISCUSSION

3.1 Characterization of the Soap Prepared

3.1.1 Foamability Test

Each soap sample weighed around 2.0g. It was then dissolved and transferred to a 250 mL measuring cylinder that held 100 mL of distilled water. To create foam, the liquid was violently agitated for approximately five minutes. For roughly ten minutes, the measuring cylinder was left to stand. After that, a measurement and record of the foam's height in the solution were made [40]. Comparing the manufactured soaps with commercial soap, the same process was performed.

3.1.2 Determination of pH

Around 10 mL of distilled water was added to a beaker containing 5g of each soap combination, which was weighed and dissolved. The pH reading of the pH meter was taken after it was immersed into the solution [41]. The control sample underwent a same treatment.

3.1.3 Determination of Total Alkali

5g of each soap sample were combined with 2.5 mL of 1M H2SO4 solution and 10 mL of neutralized alcohol. The mixture was then heated until the soap sample was completely dissolved. Phenolphthalein was used as an indicator for titrating the solution against 1 M NaOH.

The following relation was used to compute the total alkali:

The total alkali was calculated using the relation:

$$\% \text{ Total alkali} = (VA\text{-}VB)/W \times 100\%$$

Where: V_A = volume of acid used (cm^3),
VB= volume of base (cm^3),
W= weight of soap (g) [40]

3.1.4 Determination of Total Fatty Matter

50 mL of distilled water was used to dissolve around 5g of each soap sample, which was then heated. After the mixture was heated to a transparent consistency, 10 mL of H_2SO_4 was added. After adding 2g of wax, the mixture was heated again until the fatty acid content solidified. The following phrase

was used to determine the outcome when the solution was allowed to cool and solidify into a cake. The cake was taken out, dried, and weighed.

$$\%\,TFM = (A\text{-}Z)/W \times 100\,\%$$

Where, A = weight of the obtained cake,
Z = weight of the wax, and
W = weight of the soap [42].

3.1.6 Determination of Hardness

A needle was inserted into the surface of each soap sample, and a lead fishing weight was fastened to it. In both the manufactured soap blend and the control sample soap, the needle's entry point was measured and noted. Calculate the values of Foamability, pH, Percentage of total alkali (%), Percentage of Total fatty matter (%), Hardness in Table 3.

Table 3: Foamability, pH, Percentage of total alkali (%), Percentage of Total fatty matter (%), Hardness.

Samples Produced	Soap Foam (cm)	pH	Alkali (%)	TFM (%)	Hardness
Soap A	12.1	9.1	0.32	71	0.57
Soap B	13.4	8.8	0.19	66	0.84

3.1.5 Antimicrobial Test for the Soap Produced

The manufactured soap made from neem and soy beans underwent an antimicrobial test in the microbiology lab of Ahmadu Bello University in Zaria's Department of Pharmaceutical Sciences. The test specimen organisms from the lab that were used for this were;

- *Escherichia coli*
- *Staphylococcus aureus*
- *Salmonella typhi*

Sensitivity Test

The isolated bacteria were prepared into Muller Hinton Agar (MHA) glass plates after standardization. Five wells were excavated in the sterile plates using an 8 mm diameter, sterilized cork borer spaced the same distance apart. The labels on the wells were based on the various soap concentrations that were made (100 mg/mL, 50 mg/mL, 25 mg/mL, 125 mg/mL, and the control "CP" mg/mL). The wells were filled with sterilized filter paper and designed to hold approximately 0.1 m of each of the soap's concentrations. The soap was relocated for an 18-24 hour incubation period in an incubator after being left at room temperature for approximately one hour, or the pre-diffusion period. Following the incubation time, when the extract was absorbed into the surrounding media, the plates were closely monitored. It was found that the existence of microbiological activity corrupted the organism and fostered growth. A transparent ruler was used to measure the zone of inhibition, and the findings were obtained and discussed.

Table 4: Sensitivity test of the each of the organisms on various concentrations of the biosoap extract.

Organisms Test	Zone of inhibition (mm) at varying concentrations (mg/mL)			
	100	**50**	**25**	**12.5**
E. coli	13.00	10.00	0.00	0.00
S. aureus	12.40	11.00	0.00	0.00
S. typhi	13.00	12.00	10.00	0.00
Control	33.00	31.00	30.00	30.00

4. CONCLUSION

Soap made with chitosan Using the hot process approach, soap B was effectively made. The results showed that compared to branded soaps, chitosan soap was better suited for human skin. Consequently, chitosan-based soap has favorable physicochemical qualities that make it suitable for usage as medicinal or cosmetic soap. Therefore, chitosan soap may also help to prevent acne and protect the skin. Chitosan is proving to be a low-cost and safest alternative to the use of chemical fungicides and bactericides against various plant pathogens. It is user and eco-friendly in nature. It can be incorporated with bio-control agents owing to its compatibility.

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CONFLICT OF INTERESTS

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

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