Total Hardness of Ami River water by Complexometric Titration in selected Industrial area of GIDA-Sector-13, Gorakhpur District. (Uttar-Parades) India.

**Abstract**

**Background:** The hardness of water varies depending on the resource treatment procedures, pipeline circumstances, and other factors. While not an urgent concern in water quality control, it can have significant effects on health, infrastructure, and industry. This study determines categorised, evaluates, and compares the seasonal fluctuation of hardness in river water samples from the four selected sites of GIDA-sector-13, Gorakhpur District. (U. P.) India, by complexometric titration.

**Materials and Methods:** Total 48 samples (four samples per site) of water were collected through stratified random sampling, and the hardness was then measured through complexometric titration.

**Results:** Total 48 samples are “very hard” river water, with fluctuating values from summer (475±34.15) and rainy (410±25.81) compared to winter (397.5±17.07). Out of 48 samples, 46 (95%) are hard, and only 2 samples are very hard (5%), categorised as hard to very hard as per the classification of USGS and BIS surface water quality standards (as per IS: 2296). The present study did not find any soft or moderately hard water.

**Conclusion:** It is urgent to treat the effluents before discharge and raise awareness among the public about the potential negative effects of using extreme hard water. Regular testing and analysis of water hardness levels in different regions can help identify areas that may require specific interventions for water treatment. Monitoring of water quality, especially in industrial settings, is crucial to prevent adverse effects associated with hard water.

**Introduction**

The presence of naturally occurring Ca2+ (Calcium) and Mg2+ (Magnesium) salts in water caused hard water. Water hardness is generally noticed as difficulty in soap lathering and the formation of foam when washing. Calcium (Ca2+) and magnesium (Mg2+) ions form insoluble salts with the soaps, causing precipitation of the soap foam (1). Dissolve polyvalent metallic ions from sedimentary rocks, seepage and runoff from soils are the main natural sources of hardness in water. Other polyvalent ions, such as aluminium (Al), barium (Ba), iron (Fe), and manganese (Mn), make a minor contribution to the total hardness of water is (2).

Hard water also reacts with dissolved carbonates (CO3) to form an insoluble calcium carbonate (CaCO3) precipitate in water. This “scale” (also called “lime-scale”) is able to build-up inside water pipes to such a scale that pipes completely blocked (3). Hard water is considered a trouble to agriculture, industries, and households that have to expend additional money on the additional soap and blocked pipe replacement (4).

Diverse geographic areas cover varying quantities of Ca2+ and Mg2+ salts in water, which causes soft rainwater to filter through various rocks, dissolving these molecules along the way. Magnesium (Mg 2+) is occurring in rocks, like magnesite, and dolomite, while calcium (Ca2+) is also rich in limestone and several basalts. The hardness measurement unit is milligrams per litre (mg/l) of calcium carbonate (mg/l CaCO3) (6).

Water with less than 50 mg/l of CaCO3 is considered soft, a quantity between 50 and 200 mg/L, is considered moderate, and a quantity greater than 200 mg/l is considered hard. WHO is monitoring the effects of hard water on health. Eczema can potentially cause in young children’s (5) and there might also be a relationship between cardiovascular health and water hardness; it is recommended to maintain the lowest amount of total hardness of drinking water of 150 mg/L calcium carbonate (CaCO3) (6). Polyvalent cations in water can lead to a range of health issues, including osteoporosis, nephrolithiasis, colorectal cancer, hypertension, coronary artery disease, stroke, insulin resistance, and obesity when used in excess (7).

According to a previous study, drinking water with calcium has significant prevention effects against the risk of gastric cancer. Additionally, magnesium showed a barrier against gastric cancer, but this was restricted to the group that consumed the highest concentrations of magnesium (10). Several investigations reported no correlation between water hardness and urinary stone formation (11). The hardness of water has been classified in terms of its equivalent CaCO3 mg/L, in the following way: USGS (12), (1) Soft: 0-60 mg/L, (2) moderately hard: 60-120mg/L, (3) Hard: 120-180 mg/L, (4) Very hard: >180 mg/L.

**Hard water effects on industries**

Hard water in the textile industry leads to soap wastage and fabric problems and causes difficulties in the crystallisation of sugar in the sugar industry and dye industries; precipitate formation is influenced by hardness that reacts with expensive dyes. In the paper industry, Ca, Mg, and Fe salts in water can have a negative impact on the standards of paper, and the hardness of the water can lead to the production of unwanted pharmaceutical products. Scale formation occurred in the boilers due to the salts present in the water boiled in the boilers. Scales stick to the walls of boilers due to the additional fuel required for boiling the water. Boiler walls come into contact with hard water or salt water, which generates cracks in the boiler walls. This can lead to raised pressure in the boilers and a boiler explosion (5). Hard water can formed a scale in pipes causes reduced...
the flow and pressure drop increase, this need to replaced or internally clean of pipes. The hardness of water in cooling towers reduced heat transfer and increased running costs (10). Hard waters are not suitable for washing, bathing, and laundering, in kettles and water heaters (11).

This study assesses the quality of Ami River water sources in GIDA under Gorakhpur conditions of water hardness measured in mg/L calcium carbonate (CaCO$_3$). So the study major motivation is that Sarya drain carried 8 major industries untreated effluents and ended up in the Ami River. These industries are Rayana paper mills, Sarya sugar mills, Azam rubber factory, Gallant Ispat steel factory, IGL ethanol factory, and other minor industries are used. Calcium sulfite is used as bleach and antibacterial in the manufacturing of paper; calcium silicates are used as rubber reinforcing; and calcium acetate is an important component of liming rosin for the production of metal soaps and synthetic polymers. These are the causes of the hardness of river water. In the local community located in the Bharsar (Adilapar) catchment area of Ami River, local people suffered from skin problems such as rashes, itching, high blood pressure, and diabetes due to the hard water used for bathing, washing, and consumption of fish and river water for agriculture purposes. The experiment recognised by the Natural Product Laboratory. Specifically, the study used the complexometric titration procedure in the analytical quantification of dissolved polyvalent cations concentration and compared it with the United States Geological Survey classification scheme (USGS) (12), for water hardness and BIS surface water quality standards (as per IS: 2296) (13), to evaluate the total hardness of river water from the four selected sites of GIDA Gorakhpur districts.

**Material and Methodology**

**Description of the study area**

The Ami is a plain fed river. The Tributary of the Rapti River flows through the Gangetic Plains, entering the Rapti River on the right bank. River Ami received industrial effluents through various sites in a main stretch beginning in Rudhauli to its end point at Sohgaura. Latitude in degrees, minutes, and seconds: 26°33′2″N, longitude in degrees, minutes, and seconds: 83°26′45″E. The Ami River has a serpentine length of 147 km, originates from villages Sikahara located in Dumariyanjh tehsil of Siddharth Nagar district, and serves as a lifeline for the people of Siddharth Nagar, Sant Kabir Nagar, Basti, and Gorakhpur districts in eastern Uttar Pradesh. Figure 1.

**Location**

Adilapar Village faces a high pollution load due to industrial discharges from GIDA through a Sarya drain (site1) into the river Ami. This is a mixed drain that collects treated effluents from eight major polluting industries and minor industries that are used. Calcium lactate, calcium diphosphate, and tricalcium phosphate compounds are used in food, medicine, and as polishing agents in toothpaste. Calcium sulfite is used in papermaking as bleach, calcium silicates are used in rubber as a reinforcing agent and calcium acetate is used in liming rosin and is also used in metallic soaps and synthetic resins formation. These calcium components influence the hardness of water. However, the drain does not have permission to release effluents, and any overflowing from these industries could end up in the drain. Requiring urgent attention from government agencies, pollution control boards and local people, the requires regular monitoring of the river water to certify that it ‘River remains a River. 22 km from Gorakhpur, near Bharsar, industrial sector, GIDA-13, Adhila Bazaar. Figure 1.

**Select 4 sampling sites**

Site 1. Near Ramilla Samiti (Effluent after Treatment)
Site 2. Semrahwa Baba Mandir (Just Entry Point into the River)
Site 3. 200 meter upstream river.
Site 4. 200 meter downstream river.

![Site 1. (Effluents After Treatment)](image1)

![Site 2. (Just Entry Point Into The River)](image2)

![Site 3. (200 Meter Up Stream)](image3)

![Site 4. (200 Meter Down Stream)](image4)

**Figure 1.** Pictures of the sampling location of the Ami River.
Sample Collection and Handling

A total of 48 samples were collected in 1000-ml plastic bottles, cleaned with liquid detergent, washed with distilled water, and air dried. No extra filtration or purification techniques were used prior to total hardness measurements (APHA) (14).

Complexometric Titration

The complexometric titration procedure can be measured using water hardness by adding a known concentration of the chelating agent EDTA through a burette to a sample containing an unknown amount of Ca²⁺ and Mg²⁺ ions. EDTA reacts, and capturing these metal ions creates a larger metal complex. So, even as there are calcium (Ca²⁺) and magnesium (Mg²⁺) ions in the solution, the EDTA reacts with them until all metal ions are used up. The end point of this titration is indicated by the colour change in the solution, assisted by the addition of an indicator (15–17). Indicator is a substance that has an evident colour changes on completion of a particular chemical reaction, which makes it easy to identify while the end point has been reached respectively (18). Figure 2.

Chelating agent (EDTA)

The most important chelating agent in analytical chemistry is ethylenediaminetetraacetic acid (EDTA) (20). The tetra-basic form of this acid forms complexes with virtually all metal ions. EDTA is a hexadentate ligand; each of the acid oxygen's and each of the amine nitrogen's can donate one electron pair. The metal ion is usually held in a one-to-one complex with EDTA, Figure 3. The complexes have four or five member rings, contributing significantly to their stability. Standardisation of EDTA titrant against a solution of the metal ion to be determined helps to eliminate any errors in endpoint selection (complete and line structure of EDTA in Figures 4 and 5).

EBT Indicator

Chelate metal is formed with the Eriochrome Black T molecule (Figure 6. EBT structure) with the disappearance of hydrogen ions from phenolate-OH and the formation of bonding between metal ions and oxygen atoms, as well as an azo-group. This indicator forms a stable red wine complex 1:1 with some cations, such as Mg²⁺, Ca²⁺, Zn²⁺, and Ni²⁺. Most of the EDTA titration occurs in buffer pH 8-10 a range where the dominant form of EBT is HIn blue coloured (21). Figure 6.

Figure 2. (a) molecule of EDTA; (b) metal complex of EDTA (the compound has a negative (-) charges, which are not shown in this reaction) (19).

Figure 3. Complete structure of EDTA (21)

Figure 4. Line Structure of EDTA (21)

Figure 5. EBT structure (Underwood 2002) (22)

Figure 6. EBT is blue in a buffer solution at pH 10. It turns red when Ca²⁺ ions are added.
Reagents Preparations

Ammonia solution: buffer (a): 16.9 grams of NH₄Cl in 143 ml of concentrated NH₄OH. Buffer (b): 1.25 gramms magnesium salt of EDTA dissolved in 50 ml distilled water. Mix both buffers solutions and dilute to 250 ml with distilled water. Dilute 10ml of the solution to 100 ml with distilled water (Supplementary material, flow chart 1).

Standard EDTA Solution 0.01M: 4 grams EDTA and 0.1 gramms magnesium bicarbonate (MgCl₂.H₂O) dissolved in 1000 ml distilled water (Supplementary material, flow chart 2).

Standardisation of EDTA ±0.01 M with CaCl₂ as a standard solution

accurately Weigh 1gram CaCO₃ and transfer to 250 ml conical flask. Then add 1:1 HCl till CaCO₃ dissolved completely. Add 200 ml distilled water and boil for 30 minutes then cool and add methyl red indicator. Add NH₄.OH.N drop wise till intermediate orange colour develops. Dilute to 1000 ml to obtain 1ml=1mg CaCO₃. (Supplementary material, flow chart 3).

Eriochrome Black indicator T: 0.4gm Eriochrome Black T, 4.5 gm hydroxymine hydrochloride add in 100ml 95% ethyl alcohol (Supplementary material, flow chart 4).

Titration procedure: (Supplementary material, flow chart 5) and Mechanism of complexometry titration with an EBT indicator and EDTA mention in, Figure 7.

Calculation

Total hardness (mg/L)= (T) × (1000)/V
Where,  
T= volume of titrant  
V= volume of sample

Statistical Analysis

MS Excel 2007, data values are evaluated using mean and standard deviations (mean±SD), and one way ANOVA was used in comparing water hardness means across different sites, and seasons with a significant value (P<0.5). A post hoc Turkey’s honestly with Bill Miller’s Open Stat software significant difference test was employed to determine which of the sites and seasons means significantly differed.

Result

Hardness of river water in different seasons

In the present investigation of total hardness from 2020 to 2021, the mean hardness value in water varied from 402.25 ± 2.98 mg/L to 454.5 ± 4.04 mg/L, in rainy conditions. Hardness mean value in winter ranged between 390.82±9.45 to 428.25±9.97 mg/L and hardness of water mean value in summer varied from 395.24 ± 8.60 to 461.25 ± 11.06, recorded at all four sites in the Ami River, Figure 8. The higher concentrations of hardness reported in winter and summer recorded higher concentrations of hardness compared to rainy, Figure.8. It might be due to the accumulation of the constituents in the absence of rainfall in these particular seasons. In the rainy season, the water bodies get diluted, which contributes to lower levels of concentration of the constituents. This value is classified as “hard” in the USGS-Water-Quality Information (WQI) classification scheme (12) and Indian standard (BIS) as per 2296:1992 surface water quality (13) above, it is evident that out of 48 samples, water is hard at 46 samples (95%) and exceptionally very hard at 2 samples (5%), respectively in (Table 1).

Categorization of water hardness as per USGS and compared with BIS

In rainy seasons, out of 16 samples collected, almost all the samples are hard (100%) in rainy season. More samples have a hardness range of 402.25 ± 2.98 to 454.5 ± 4.04, mg/L, i.e., hard water in the limit of the USGS standards mentioned, 250-500 mg/L, in (Table 1) it is evident that water is hard at 16 samples (100%) in rainy and all the samples are excessive as per BIS standard 2296:1992, which has a maximum limit 300 CaCO₃ mg/L. Each of the 16 samples is hard water (100%) in winter, as the USGS mentioned and exceeds the maximum level of BIS mentioned in, Table 1. Out of 16 samples, 14 are hard (95%) and 2 are very hard (5%) in summer, and all samples exceeded maximum permissible limits of BIS, respectively, in, Table 1. Altogether, out of 48 samples, were found to contain hard water (95%), There are only 2 samples (5%) that are found to be very hard water. The present study did not find any soft or moderately hard water in any of the 48 samples. Very hard and very soft water with extreme degrees of hardness are dangerous to human health (23).

One way ANOVA with Turkey HSD results

One way ANOVA does not show a significant value in rainy seasons, but they show significant p-value less than 0.05 in winter and a strong significant p-value of 0.001 in summer. They suggest that one or more sites are significantly different.

The turkey HSD test p-value of the corresponding of one-way ANOVA is lower than 0.001, which strongly suggests that one or more pairs of groups are significantly different. The turkey HSD test for each of the three groups (A vs. B) is not significantly different. Group (A vs. C) is a statistically significant difference, and group (B vs. C) is significantly different from each other.

Discussion

In light of these standards, the average hardness values are above 300 mg/L, in all the seasons and categorised hard water at all the sites and samples. Although out of 48 samples, mostly 46 were found hard (95%) and only (5%) samples were found very hard at site-1, in the summer. All sites have higher hardness concentrations than the prescribed limits of the BIS standard as per 1992 (24). Brewery plant untreated effluents had a high concentration level of 200 mg/L, exceeding the WHO (25-26) 100 mg/L drinking water limits. It can solidify water handling equipment, posing health risk (27). In a similar study conducted in Tipkur town, Tumkur district, the hardness value varied from 232 mg/L to 872 mg/L. Out of 15 samples, S5, S6, S9, S11, S14, and S15 exceeded the BIS (as per 1998) (28) prescribed hardness limit of 600 mg/L. Water with a hardness below 300 mg/L is potable, but higher than this limit causes gastrointestinal irritation. Normal water hardness does not pose any direct health problems, but a higher concentration of hardness above 600 mg/L may cause kidney
problems (29). The study of water quality in residential and industrial areas was carried out in GIDA Sector-13 and closed populated villages of GIDA Sector-5. A total of 8 samples were collected, India mark hand pump the hardness of ground water averaged between 217 mg/L and 426 mg/L, which is higher than the IS 10500:2012 permissible limit of hardness is 200 mg/L (30-31). Hard water is often responsible for causing eczema, especially in young children’s, because of soap and metal deposits that are difficult to remove from bathing water (32).

A study revealed that women aged 30-70 in Sangemini, central Italy, who drank high calcium water (318 mg/L), had significantly greater spine mineral density compared to those who drank low calcium water (<60mg/L). A water and food study showed an average calcium intake difference of 258 mg/L a day (33). Hard water constituent deposits can affect temperature control in cooking and backing equipment, impacting the consistency and quality of the final product. The use of hard water in canning or pickling can affect the stability and safety of food products by reacting with ingredients and impacting the efficiency of preservatives. Hard water can promote bacterial growth; inhibit the effectiveness of sanitising agents, and compromise food safety and quality (34). Freshwater Resources of Kanpur and its Suburbs; the hardness values range from 84 mg/L to 960 mg/L. No soft water find out of 19 stations, 8 were categorised moderately hard, 7 as hard, and only 4 stations were categorised as very hard water as per USGS standards. Hardness values exceeding 200 mg/L lead to scale formation in the pipeline distribution system, resulting in economic loss due to increased soap and fuel consumption (23). 120 samples of ground water were collected from one town and two different villages. A similar study concludes that of the 120 samples tested, 39 (32.5%) samples were moderately hard, 76 (63.3%) samples were hard water, and 5 (4.16%) samples were very hard water. No soft water was found out of 120 samples. Extreme degrees of hardness are dangerous to health (35).

Hard water also has harmful health impacts and directly affects many industrial processes, including boilers. Hardness in water is calculated by polyvalent cations (ion charges greater than +1). Water with low hardness values is ‘soft’, while those with high hardness values are referred to as ‘hard’. The total hardness in the study area varies between 164 to 1000 mg/L reported in the River Cauvery in and around Nerur (36). Jabalpur city of M.P., which showed some values higher than the permissible limit prescribed by W.H.O. (500 mg/L), the total hardness values were recorded in the range of 320 mg/L to 670 mg/L. Total hardness, especially Ca hardness, is a well-known modifying factor for the toxicity of heavy metals and other chemicals. Fish and shrimp populations are negatively affected by high levels of water hardness. It affects hatching, egg size, larval survival, mortality rate, etc. (37). The values of total hardness ranged from 156 (during April) to 670 mg/L (during October) in Perur Chettipalayam Lake, Coimbatore, Tamil Nadu (38). Hard water increased soap use result in soap residue or metal on the clothes or on the skin that is not easily rinsed-off and leads to contact irritations (39). Total hardness values are in the range of 127 to 134 mg/L in the riverbed-mining area of the Ganga River, Hardwar. Hardness value between 998 mg/L to 1182 mg/L in the west side industrial area and 950 mg/L to 1140 mg/L in the east side industrial area, Sholapur, Maharashtra (40). Total hardness for A (upstream) and samples B (downstream) fluctuated in the range of 360mg/L to 125 mg/L respectively, in Ateo Stream and Eleme Rivers State, (41). The standards are conformity with desirable limits of 500 mg/L to W.H.O. The average level of total hardness in surface water was found to be higher at sampling point, SR-3 (14.67 mg/L to 60.6 mg/L) and lower at sampling point, SR-2, (8.61 mg/L to 3.06 mg/L), in the Oruku River, Ota, in south-western Nigeria (42). The total hardness values for all the sites apart from the control ranged from 550-600 mg/L in Ateo Stream, Eleme, Rivers State, Nigeria, and were higher than the WHO limit (43). Exposure to hard water plays an important part in the etiology of atopic eczema, but specific causes are unknown. Several factors are associated with eczema flare-up, like dust, shampoo, nylon, sweating, wool, and swimming (44).

A limitation of this investigation is the limited number of samples and sites. The values are not compared to the origin sites of the Ami River or the end of the river sites. For further research, it is recommended to increase the sampling duration rainy, winter, and summer seasons, when assessing the levels of hardness using the EDTA method in the Ami River. The number of sampling stations in each river should be increased to capture the entire hardness level in the Ami River.

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### Table 1: USGS hardness classification of Ami river water samples for the study area, and values are comparing with BIS standards (SI: 2296:1992)

<table>
<thead>
<tr>
<th>Season</th>
<th>Sites</th>
<th>No of samples</th>
<th>Mean ±SD</th>
<th>Hardness classification</th>
<th>Hardness as (CaCO₃) mg/L</th>
<th>Representative Samples</th>
<th>% of samples</th>
<th>BIS (IS:2296:1992) 300 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy</td>
<td>Site-1</td>
<td>16</td>
<td>454.5±4.04</td>
<td>Soft</td>
<td>0-60</td>
<td>S-nil</td>
<td>100%</td>
<td>MH-nil</td>
</tr>
<tr>
<td></td>
<td>Site-2</td>
<td></td>
<td>426.83±5.91</td>
<td>Moderately</td>
<td>60-120</td>
<td>MH-nil</td>
<td>87.5%</td>
<td>H-nil</td>
</tr>
<tr>
<td></td>
<td>Site-3</td>
<td></td>
<td>407.08±5.18</td>
<td>Hard</td>
<td>250-500</td>
<td>H-nil</td>
<td>100%</td>
<td>V-nil</td>
</tr>
<tr>
<td></td>
<td>Site-4</td>
<td></td>
<td>402.25±2.98</td>
<td>Very hard</td>
<td>&gt;5000</td>
<td>V-nil</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Site-1</td>
<td>16</td>
<td>411.75±7.67</td>
<td>Soft</td>
<td>0-60</td>
<td>S-nil</td>
<td>100%</td>
<td>MH-nil</td>
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<tr>
<td></td>
<td>Site-2</td>
<td></td>
<td>402.25±2.98</td>
<td>Moderately</td>
<td>60-120</td>
<td>MH-nil</td>
<td>95%</td>
<td>H-nil</td>
</tr>
<tr>
<td></td>
<td>Site-3</td>
<td></td>
<td>404.83±3.41</td>
<td>Hard</td>
<td>250-500</td>
<td>H-nil</td>
<td>100%</td>
<td>V-nil</td>
</tr>
<tr>
<td></td>
<td>Site-4</td>
<td></td>
<td>426.5±9.97</td>
<td>Very hard</td>
<td>&gt;5000</td>
<td>V-nil</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>Site-1</td>
<td>16</td>
<td>461.25±11.08</td>
<td>Soft</td>
<td>0-60</td>
<td>S-nil</td>
<td>100%</td>
<td>MH-nil</td>
</tr>
<tr>
<td></td>
<td>Site-2</td>
<td></td>
<td>426.87±5.91</td>
<td>Moderately</td>
<td>60-120</td>
<td>MH-nil</td>
<td>100%</td>
<td>H-nil</td>
</tr>
<tr>
<td></td>
<td>Site-3</td>
<td></td>
<td>406.87±10.34</td>
<td>Hard</td>
<td>250-500</td>
<td>H-nil</td>
<td>100%</td>
<td>V-nil</td>
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<tr>
<td></td>
<td>Site-4</td>
<td></td>
<td>401.24±2.60</td>
<td>Very hard</td>
<td>&gt;5000</td>
<td>V-nil</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>48</td>
<td></td>
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</table>


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Figure 8. showing hardness of water fluctuating seasonally in the Ami river.
Potential sources of error in titration

There are some errors due to the preparation of chemical reagents. EDTA removes all metal ions in the water, not only calcium ions. This gives us a value that is not true for the concentration of calcium ions. This experiment causes an approximately error of 1%, but it is acceptable.

Conclusion

The study concludes with the total hardness of the tested samples. Based on these findings, it can be concluded that the Ami River has 95% hard water, 5% very hard water, and no soft water found out of 48 samples. All values are higher than the permissible limits (300 mg/L) of the BIS surface water quality standard (as per 2296:1992). The present analysis indicates that River Ami is in danger due to the negative effects of the different categorised of water hardness and their potential health impacts.

Authors contribution

Sarwat Jahan: conception, design, materials, survey, reviewing, literature search, lab experiment, data collection, processing, analysis and manuscript writing. Prof Ajay Singh: conception, revision of the submitted version and agree to be responsible for aspects designed for the work they performed.

Declaration of Competing Interests

Authors have no conflict with anyone and anywhere.

Data availability

Data will be provided upon request

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Supplementary material

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