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# EVALUATING THE HEALTH RISKS OF HEAVY METAL CONTAMINATION IN DRINKING WATER - A COMPREHENSIVE RISK ASSESSMENT

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***Abstract*** *Humans are frequently exposed to heavy metals (HMs) and pathogens via assimilation of contaminated drinking water all over the world, especially in developing countries. This study aimed to evaluate drinking water quality and related human health risks in spring and tap water in Hangu District, Pakistan. Standard methods were employed for gathering samples (10 from spring water and 10 from tap water) from the predominant drinking water sources in the study areas. The samples were assessed for human health risk, HMs, physicochemical, and microbiological evaluations. In the study site, the water supplies were polluted with faecal coliform, demonstrating high bacterial pollution. A total of 72 bacterial isolates were obtained from water samples, 40 from spring water, and 32 from tap water samples. The physiochemical parameters such as turbidity (2.10-4.65 NTU), total suspended solids (3.80-5.30 mg/L), electrical conductivity (498.3-569.9 µS/cm), COD (4-9 mg/L), BOD (6-11 mg/L), Total Dissolved Solids (477.8-638.3mg/L), pH (7.2-7.5), sulphate (187.2-269.8 mg/L), nitrate (0.43-0.62 mg/L), sodium (255.1-238.4mg/L), chloride (217.7-290.4 mg/L), Ca hardness (149.1-228.0mg/L), Mg hardness (122.6—6136.7mg/L) and total hardness (412.2-456.6 mg/L) were analyzed for both water samples. The HMs concentrations, such as lead, Manganese, Chromium, and Cadmium, were assessed in drinking water by Atomic Absorption Spectrometry and then compared with limits set by WHO. The HMs were noted as Cd (0.011-0.017 mg/L), Cr (0.017-0.088 mg/L), Mn (0.015-0.083 mg/L), and Pb (0.003-0.008 mg/L). According to the World Health Organization drinking water quality standard, the majority of physical and chemical characteristics fell within acceptable limits.. However, the values of sodium ions, nitrate, sulphate, and chloride ions were beyond the limits of the WHO in some water samples. The potential health risks of HMs to the native population were also studied. The concentrations of all selected metals were within permissible limits. Health risk indicators like the health risk index and the chronic daily intake were measured for children and adults. The CDIs of metals were observed in the order of Cr > Cd >Mn > Pb and Mn > Cr > Cd > Pb for spring and tap water, respectively. The HRIs of HMs were observed in order of Cd > Mn > Pb >Cr and Cd >Mn > Cr > Pb for spring and tap water, respectively. There was no health threat to the indigenous community because the HRIs of the HMs in the drinking water for adults as well as children were less than 1.*

**Keywords:** *Coliform bacteria; Antibiotic Susceptibility; Physiochemical parameters; Heavy metals; Health risk assessment*

## Introduction

Water is essential for long-term human, social, and economic development (Pan et al., 2023). Water has always been a valuable resource for various human activities, but its contamination is a major concern (Talema, 2023). Water, the top drinking fluid, is a major source of disease transmission. Obtaining clean and safe drinking water is the key concern in most underdeveloped nations because several water resources are non-potable without any treatment (Tortajada, 2020). Human beings depend on water resources for various purposes, particularly irrigation and drinking. The water needs have risen as industry and agriculture expanded due to human population and urbanization (Velmurugan et al., 2020). Drinking water is supposed to be the most sensitive to pollution from anthropogenic and geogenic activities like erosion and weathering of bedrock and mining, as well as agricultural activities (Balaram et al., 2023). These activities continue to worsen water quality, triggering the self-purification to disappear or lower, upsetting ecological equilibrium, and impacting day-to-day lives and activities of the local population (Mushtaq et al., 2020). The polluted water could degrade soil fertility and affect food crops (Khan et al., 2023), and using such water may be a reason for health problems in humans and other organisms (Farid et al., 2023).

Groundwater quality depends upon interactions in the aquifer between chemical components, gases, rock, and soil (Absametov et al., 2023). It is also influenced by natural processes like interactions with other aquifers, quality of recharge waters, velocity of groundwater movement, minerals deposition, and anthropogenic activities, like contaminants from human activities (Abanyie et al., 2023). The HMs are present naturally in groundwater at various levels, dependent on the sediments of nature and the kind of parent rock, which can affect human health (Ali et al., 2021). Though, HM contamination is of increasing concern as several sources of water are not safe anymore for drinking purposes (Prasad et al, 2022), furthermore, human actions like speedy urbanization as well as Industrialization can adversely affect water quality (Akhtar et al., 2021), resulting in increased spread of chemical and microbial contaminants on soil surfaces through the transportation of the pollutants, to the water receiving containers (Mostafidi et al., 2020). Consequently, microbial pollution has become a global issue (Tian et al., 2021). Low-quality drinking water may have long-lasting effects on the environment that can be deteriorated by land-use actions to shelter the food demand, which results in contaminants discharge (Obubu et al., 2022).

Toxic metals can easily enter the body through water or food ingestion, gathering in the tissues and then depositing in the circulatory system. Thus, the body cannot degrade the HM pollutants (Sonone et al., 2020). Certain water microorganisms, like Enterococci and E. coli, can result in waterborne epidemics and show the existence of faecal pollution (Some et al., 2021). The WHO reports point out that polluted water is responsible for approximately 80% of diseases worldwide and one-third of deaths in underdeveloped nations (WHO, 2004). Microbial quality is usually prioritized over the chemical quality of water in developing countries (Levallois and Villanueva, 2019). Therefore, microbial quality and purification evaluation should be encompassed in water management to protect the population from waterborne diseases (Chaúque et al., 2023). Many bacteria or coliforms are heterotrophs and are capable of living in water and surviving in water distribution systems (UNICEF/WHO, 2017). The indicator value of total coliform groups is used to assess the safety and cleanliness of distribution systems (Bai et al., 2023). The *E. coli* its indicator value can be utilized as an indicator of faecal (animal and human) pollution. Therefore, the presence of thermotolerant coliforms or *E. coli* shows a lack of disinfection, and should incentivize examination of impending sources of failure, like insufficient treatment in the distribution system (Vidal et al., 2019).

Pakistan, as a developing country, is also facing the issue of waterborne diseases due to poor water quality, improper sanitary conditions, and a lack of infrastructure services for water distribution. WHO suggested that before human consumption, drinking water must be cleaned and free from noxious pathogenic microbes and chemicals (WHO, 1975). However, in Pakistan, drinking water treatment is rarely done before use, and no international standards are scrutinized for microbiological, chemical, and physical limits. In Pakistan, around 70% of the population resides in rural areas with no appropriate water supply system. In Pakistan, the quality of drinking water is also affected by urbanization, which has risen from 31-34% in the previous few decades. Due to this increase in urbanization, the availability of safe drinking water decreased from 60% to 40% (Ullah et al., 2014), and approximately 60% of the deaths occur in Pakistan due to the consumption of polluted water (Nabi et al., 2019). In Pakistan, before water distribution, filtration is almost negligible. Furthermore, NEQs (National Environmental Quality Standards) or WHO standards are not followed for drinking water's physical, chemical, and bacteriological evaluations. The present study was conducted for physical, chemical, and bacteriological assessment of the drinking water of District Hangu.

## Materials and methods

### Study area

The district Hangu lies between 33° 31' 55.13" north latitude and 1° 03' 34.20"east longitude. The total population of this district was 518,811in 2017. The literacy rate according to the census of 2017 was 43.59% and agriculture is the major occupation of native populations. Hangu district has a steppe local climate with an average temperature of 20.7°C and 536 mm of rainfall annually.

### Sample Collection

Standard procedures planned by the APHA(1998) were used to collect water samples. The samples were taken from tap and spring water (delivered through house pipes) from Hangu District from January 2022 to April 2022**.** A Total of twenty water samples were obtained, ten samples from each tap and spring water. The spring water was obtained from Toyichina, Adda Jumat, and Gumbhat Jumat, while the tap water (sample) was collected from water tanks and different houses. Water having any color or sense of taste was excluded. Furthermore, samples with visible suspended solids added from outside were excluded. The water samples were allowed to run for a few minutes to escape outer contamination (dust particles). Samples were collected in clean, transparent 150-200 ml sterile plastic bottles (Islam et al., 2016). The collected samples were analyzed for physicochemical characteristics, microbial, and HMs (Ayaz et al., 2020).

**Microbiological evaluation**

Presumptive tests to assess coliforms' existence in water samples were executed by the most probable number (MPN) method (Sathiamurthy et al., 2021). The MPN of coliforms of the water sample (per 100 ml) was obtained from the combination of negative and positive outcomes acquired from the test tubes. The confirmatory tests were conducted to determine the existence of coliform bacteria in the samples. The bacterial isolates were identified on solid media by combining colonies and morphological characterization, and biochemical tests (Bitton and Ben, 2019).

**Gram Staining/Microscopy**

The Gram staining method was employed for microscopy of Bacterial Isolates. In microscopy, a smear of microbial isolates was prepared and fixed on a clean slide. Some drops of the crystal violet were put on a slide, then allowed to stand for one minute, and washed with distilled water. While in the second step, the smear was stained with Gram's iodine for 45 seconds and washed with distilled water. In the third step, 95% of ethyl alcohol was used as a decolorizer over the smear for 05 seconds and then washed with distilled water. After that, the smear was counterstained for 1 min with safranin and then washed with distilled water. This Gram staining method was followed for all microbial isolates, and bacteria were named as Gram-negative and Gram-positive. All the slides were studied on the basis of an oil emulsion objective lens (100X) (Becerra et al., 2016).

**Biochemical Tests**

The Gram-positive and negative bacteria were recognized via various biochemical tests such as Urease, Triple sugar iron, Oxidase, Coagulase, Catalase, Citrate, and Indole (Pindi et al., 2013).

**Coagulase Test**

The coagulase test is also known as the agglutination test, in which blood is taken in an Ethylene Dioxide Tetra Acetic Acid tube and centrifuged to separate the serum and red blood cells. A sterile slide was taken, and a drop of serum was poured on the slide. The bacterial colony was mixed well with serum. The result was observed as a fibrin thread formed between the cells, causing them to agglutinate or clump due to the coagulase enzyme. This test showed a positive result within 10-15 seconds (Umar et al., 2022).

**Catalase Test**

In the catalase test, one drop of hydrogen peroxide was placed on a sterile slide, and then the bacterial colony was emulsified. The production of bubbles shows positive results within 5 seconds, while the absence of bubbles shows negative results (Sher et al., 2020).

**Citrate Test**

The citrate test was applied to identify the capability of bacteria to use citrate as the chief source of growth and metabolism. In this test a pure and direct streaked inoculum is used in the slant of medium from bottom to top in a fish–tail–like motion. Hence, these slant test tubes were incubated at 37 °C and studied after 24-48 hours. Positive results were presented by color changes from green to blue, whereas green colors indicated negative results (Saimin et al., 2020).

**Urease Test**

For the urease test, urea agar was prepared, inoculated with a heavy inoculum of pure culture, and incubated for 18-24 hours. After incubation, the slant was observed after the due time. If a light color on the slant along with the butt is observed, it is considered positive (Sher et al., 2020).

**Triple Sugar Iron (TSI)**

The purpose of this test was to determine whether bacteria could ferment glucose, lactose, and sucrose. The goal was to acquire gas-producing and acid-producing microorganisms. A colony in the middle of the Triple Sugar Iron Media was inoculated with the sterile needle, streaked over the agar slant's surface, and then moved to the bottom of the glass tube. It was incubated at 37°C for 24 hours. A positive outcome is indicated if yellow appears in the butt and slant. Agar bubbles or ruptures showed that gas ($H_2$) was being produced, which is a good indicator (Saimin et al., 2020).

**Oxidase Test**

The oxidase test determines whether the bacteria are producing cytochrome C oxidase. One enzyme involved in the bacterial electron transport chain is the oxidase. The reagent used in this test is tetramethyl-p-phenylenediamine. The final product is purple if the bacteria have the enzyme. There is no colored final result if it is absent. Using a sterile stick, a smear is created when a few drops of the reagent are applied to the sterile paper and combined with the colony of test bacterial isolates. When the test is positive, a deep purple color is produced (Saimin et al., 2020).

**Indole Test**

In this test, the capability of an organism to degrade tryptophan to indole is indicated. Tryptophan undergoes hydrolysis and deamination via bacteria expressing tryptophanase that convert tryptophan to its by-products. The test is used to distinguish among the families of *Enterobacteriacae.* Indole is combined with Kovac's reagent. When the solution turns red, the result is considered positive (Saimin et al., 2020).

**Susceptibility Pattern**

For antibiotic susceptibility testing, Mueller-Hinton agar media and the disc diffusion method were used. Based on these tests, microbes were identified as antibiotic-resistant or sensitive. Hence, different antibiotics were used against these microbes, such as Amoxicillin, Ceftriaxone, Clarithromycin, Erythromycin, Clindamycin, Imipenem, Amoxycillin, Ciprofloxacin, Vancomycin, and Amoxicillin + Clavulanic Acid (Pamuk et al., 2019).

**Physicochemical Evaluations**

Physico-chemical characteristics were assessed in a water sample comprising electrical conductivity (EC), turbidity, pH, sulfate, total dissolved solids (TDS), chloride, BOD, COD, ammonia, TSS, and nitrate. Turbidity by nephelometry and TSS, TDS were obtained by the gravimetric method. The colorimetric method investigated the results for chloride, BOD, and COD by titration, sulfate by titrimetry, and nitrate. Conductivity and pH were analyzed using a Meter (Ayaz et al., 2020; Farid et al., 2023).

**Heavy Metals Analysis**

The HMs analyzed were cadmium (Cd), chromium (Cr), manganese (Mn), and lead (Pb). The HM extraction in the water samples was done by digestion with nitric acid ($HNO_3$) and hydrochloric acid (HCl) according to Khan et al. (2023). The HMs in the water samples were determined with an Atomic Absorption Spectrophotometer in CRL, University of Peshawar.

**Health risk assessments**

**Daily Intake of Chronic Metals**

The metals can enter the human body via several pathways, such as inhalation, dermal contact, and food intake. In contrast to oral intake, though, all other pathways are considered negligible (Muhammad et al., 2011). The CDI (μg/(kg·day)) of metal via water ingestion was measured by Eq. (1) (Khan et al., 2013).

$$CDI = \frac{Cm \times Iw}{Wb} \text{ ----------------------- (1)}$$

Cm represents the HM (μg/L) in water, Iw (Liter/day) is average ingestion of water per day (supposed to be 2 and 1 Liter/day for an adult and a child, respectively) (US EPA, 2011), while Wb is the average weight of individual (supposed to be 72 and 32.7 kg for an adult and a child, respectively) (Muhammad et al., 2011).

**Health risk indexes (HRIs)**

For estimation of health risks, the value of HRIs was measured by the formula below (Khan et al., 2013).

$$HRI = \frac{CDI}{RfD} \text{----------------------- (2)}$$

The values of RfD (oral toxicity reference dose, μg/(kg·day)) for Pb, Mn, Cr, and Cd are 3.6E+01, 1.4E+02, 1.5E+03, and 5.0E-01, respectively (US EPA, 2005). The HRI less than one is considered safe for human consumption (Anabtawi et al., 2022).

**Statistical analysis**

Different software programs accomplished statistical analysis, i-e, Microsoft Excel and Statistical Package for the Social Sciences (SPSS 16.0). Data are represented as mean values ± standard deviation. For confirmation of the statistical significance of the difference data were examined by SPSS with a t-test ($p < 0.05$). The mean values of the parameters were compared with WHO standards, and proper deductions were made.

**Results and Discussion**

**Microbiological Evaluation**

**Gram Staining**

For the identification of bacterial samples, the Gram staining technique was performed. Out of 20 water samples, a total of 72 bacterial species were isolated. A total of 40 bacterial species were isolated from spring water, of which 16 were Gram-positive and 24 were Gram-negative (Figure 1). From tap water samples, 32 isolates were obtained, of which 24 were Gram-positive and 08 were Gram-negative.

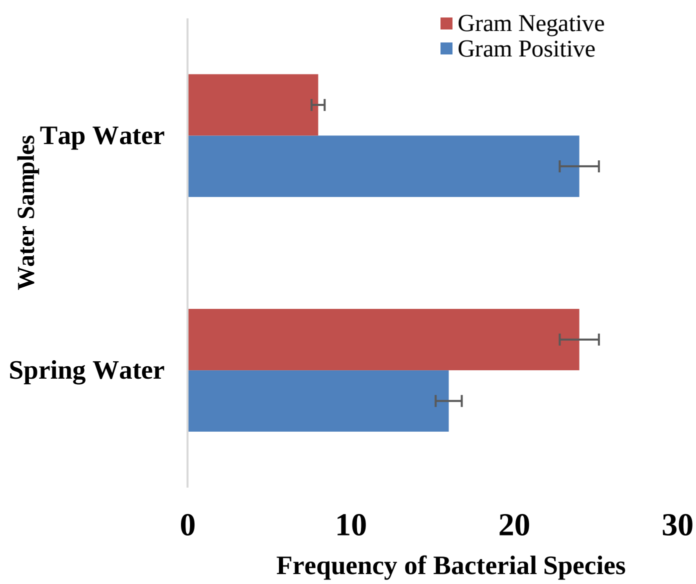


**Figure 1.** Frequency of Gram-negative and Gram-positive isolates

**Biochemical Characterization of Bacteria**

Different biochemical tests were performed to confirm the presence of bacteria. The bacterial species isolated and identified were *S. aureus*, *E. coli*, *P. aeruginosa*, *Shigella spp.*, and *Salmonella typhi* (Table. 1). The isolate that showed positive results for catalase, Citrate, and oxidase tests and negative results for coagulase tests was confirmed as *P. aeruginosa*. Those isolates showing positive results for catalase, coagulase, citrate, urease, and negative for indole production test and oxidase, were identified as *Staphylococcus aureus*. Similarly, the bacteria isolated, which showed negative results for citrate, urease and positive results for indole, were confirmed as *E. coli*. The species that showed negative results for citrate, urease, indole, oxidase, and positive for TSI were identified as *Salmonella typhi*. The positive for TSI and negative for citrate, urease, indole, and oxidase tests were confirmed as *Shigella*. Triple sugar iron (TSI) test performed for these bacteria showed that all the bacterial isolates of *E. coli*, *Shigella spp*, and *S. typhi* mentioned in were positive to this test, determining that these bacteria can ferment sugars but do not produce hydrogen sulphide (Table 1).

**Table 1. Biochemical Characterization of Isolated Bacteria**

| S.# | Catalase | Coagulase | Citrate | Urease | Indole | Oxidase | TSI | | | Bacteria Isolated |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Butt | Slope | Gas | |
| 1 | NA | NA | - | - | + | NA | Y | Y | NO | *E. coli* |
| 2 | NA | NA | - | - | - | - | Y | Y | NO | *Shigella spp* |
| 3 | + | - | + | - | + | + | NA | NA | NA | *P. aeruginosa* |
| 4 | + | + | + | + | - | - | NA | NA | NA | *S. aureus* |
| 5 | NA | NA | - | - | - | - | Y | R | No | *S. typhi* |

**Key:** - = negative, + = positive, Y=Yellow, R=Red, NA= Not applicable, No= Not appear

From spring water *S. aureus* 16(22.2%), *Salmonella typhi* 14 (19.4%), *Shigella spp.* 6 (8.3%), *P. aeruginosa*, and *E. coli 02* (2.7%) were obtained as shown in Table 4. Similarly, from tap water *Salmonella typhi* 04 (5.5%), *Shigella spp* 02 (2.7%), *S. aureus* 24(33.3), *P. aeruginosa* (1.3%), and *E. coli* 01 (1.3%) were isolated. The results indicated that spring water was more contaminated with bacterial pathogens than tap water samples (Figure 2).

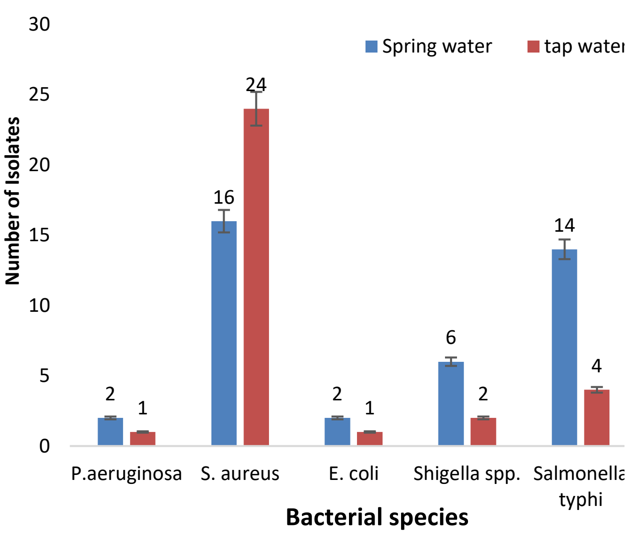


**Figure 2. Total isolated bacteria from water samples**

This study agrees with the findings of Doughari et al. (2007) that tremendously high bacterial species in water samples reveal that water has been polluted by microorganisms and is unhealthy for human use. Sometimes, bacteria find their way into the groundwater in dangerously high levels through seepage or runoffs. The main diseases that could arise from bacterial contamination of the drinking water include cholera, diarrhea, and typhoid. The groundwater at depth contains no or little bacteria that filtration could have eliminated as water infiltrates through soil. A higher number of bacteria of genera like *Shigella*, *Salmonella*, *Escherichia*, and *Enterobacter* was obtained from spring water, followed by tap water samples. The larger number of bacteria isolated from spring water samples, as observed in the current study, shows the existence of fecal coliform pollution and health risk for human consumption due to a large number of pathogens in water samples (Franciska et al., 2005). The possible reason for the microbial pollution of spring waters, as detected in the current study, may be due to the shallowness and openness of this water that permits easy entry of particles from the environment. The reason may also be poor sanitary conditions in the sites where such springs are situated. This contamination of water leads to an increase in the physicochemical parameters of the water samples. As observed in the current study, the physiochemical parameters and bacterial contamination show a positive correlation.

**Antibiotics Susceptibility**

Antibiotic susceptibility of 72 bacterial isolates i.e., *E. coli*, *P. aeruginosa*, *Shigella*, *Salmonella typhi* and *S. aureus* were carried out, and findings were obtained according to guidelines of Clinical and Laboratory standards Institute, and species were categorized as resistant sensitive (S) and (R). Based on this test, microbes were identified as antibiotic-resistant or sensitive. Hence, few types of antibiotics were used against these microbes, such as Clindamycin (CN), Erythromycin (E), Amoxicillin (AML), Imipenem

(IPM), Ceftriaxone (CRO), Clarithromycin (CLR), Vancomycin (VA), Ciprofloxacin (CIP), Gentamycin (GM) and Amoxicillin+ Clavulanic Acid(AMC) as shown in (Table 2 and Table.3).

**Pattern of Antibiotic Susceptibility in *E. coli***

Ciprofloxacin (CIP) had the lowest zone of inhibition of 10 mm for *E. coli*. Similarly, maximum zone of inhibition was shown by *E. coli* for Clarithromycin (CLR) (30mm) (Figure 3). The resistivity and sensitivity to ten commercially available antibiotics or antibacterial agents were determined by the disc diffusion method. The results showed the spring water *E. coli* were resistant towards antibiotics such as Clindamycin (50%), Erythromycin (50%), Amoxicillin (100%), Clarithromycin (100%), and Amoxicillin+ Clavulanic (100%). The study observed that *E. coli* isolated from spring water showed the highest resistance (100%) towards Amoxicillin, clarithromycin, and Amoxicillin+ Clavulanic Acidand highest sensitivity (100%) towards Imipenem, Ceftriaxone, Vancomycin, and Gentamycin. The findings are summarized in Table 2. The results presented that the *E. coli* isolated from tap water were highly resistant (100%) towards antibiotics such as Clindamycin, Erythromycin, Amoxicillin, Clarithromycin, and Amoxicillin + Clavulanic. The *E. coli* showed the highest sensitivity(100%) to some antibiotics such as imipenem, Cefrtiaxone, Vancomycin, Ciprofloxacin, and Gentamycin. The results are summarized in Table 3.

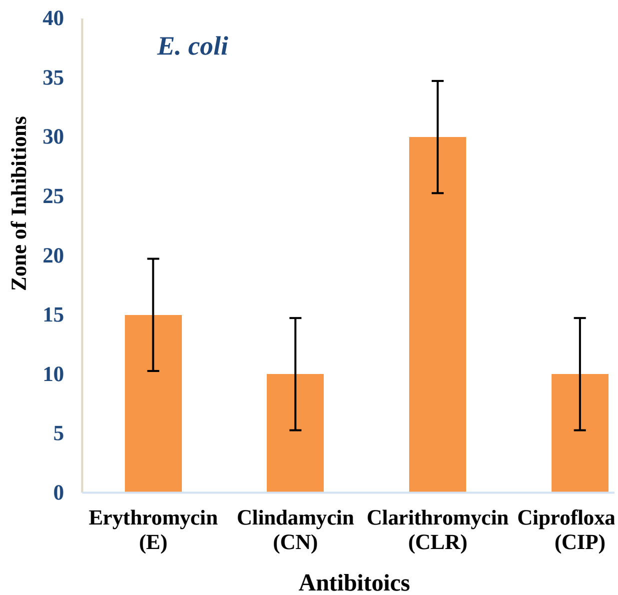


**Figure. 3 Antibiotic Susceptibility Pattern of *E. coli***

**Antibiotic Susceptibility Pattern in *P. aeruginosa***

In Figure 4, Ciprofloxacin (CIP) had the lowest zone of inhibition of 10 mm for *P. aeruginosa*, and the maximum zone of inhibition was shown against Amoxicillin (AMC) (30mm). The results showed that the spring water *P. aeruginosa* were resistant towards antibiotics such as Erythromycin (50%), Amoxicillin (50%), Clarithromycin (50%), and Amoxicillin+ Clavulanic (50%). The study observed that *P. aeruginosa* isolated from spring water showed the highest sensitivity (100%) towards Clindamycin, Imipenem, Ceftriaxone, Vancomycin, Ceprofloxacine, and Gentamycin. The results are summarized in Table 2. The results showed that the *P. aeruginosa* isolated from tap water were sensitive towards all the antibiotics used for the study, as shown in Table 3.

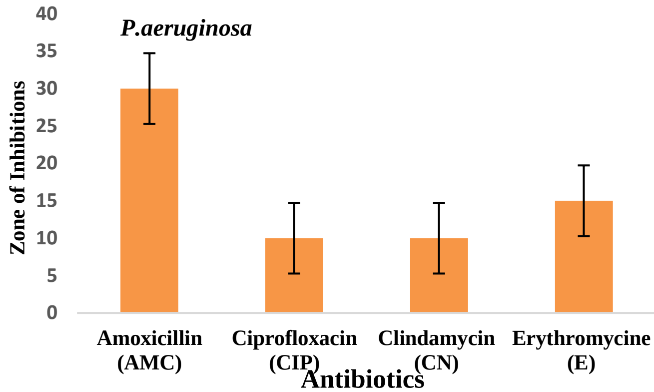


**Figure 4. Antibiotic Susceptibility Pattern of *P. aeruginosa***

**Antibiotic Susceptibility Pattern of *S. aureus***

Amoxicillin (AML), Clindamycin (CN), and Imipenem (IPM) had the lowest Zone of inhibition of 10 mm for *S. aureus*, and the maximum zone of inhibition was shown against Vancomycin (VA) (30mm) (Figure 5). The results showed the spring water *S. aureus* were resistant towards antibiotic such as Clindamycin (56.2%), Erythromycin (31.2%), Amoxicillin (25%), imipenem (25%), Cefrtiaxone (25%), Clarithromycin (31.2%), Vancomycin (25%), Ciprofloxacin (56.2%), Gentamycin (25%) and Amoxicillin+ Clavulanic (25%). The study observed that *S. aureus* isolated from spring water showed the highest resistance (56.2%) towards Clindamycin and Ciprofloxacin and the highest sensitivity (75%) towards Amoxicillin, Imipenem, Ceftriaxone, Vancomycin, Gentamycin, and Amoxicillin + Clavulanic. The results are summarized in Table 2. The results showed that the *S. aureus* isolated from tap water were resistance towards antibiotic such as Clindamycin (41.6%), Erythromycin (33.3%), Amoxicillin (33.3%), mipenem (25%), Ceftriaxone (37.5%) Clarithromycin (29.1%), Vancomycin (33.3%), Ciprofloxacin (37.5%), Gentamycin (33.3%) and Amoxicillin+ Clavulanic (33.3%). The *S. aureus* showed the highest sensitivity to Clarithromycin (70.8%). The results are summarized in Table 3.

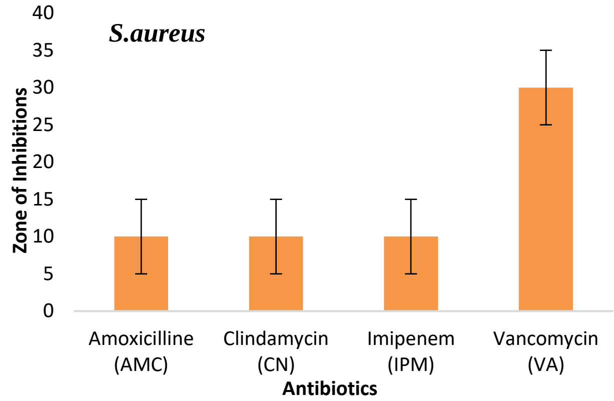


**Figure 5. Antibiotic Susceptibility Pattern of *S. aureus***

**Antibiotic Susceptibility Pattern of *Shigella spp***

Amoxicillin (AML), and Clindamycin (CN) had the lowest zone of inhibition of 10 mm for *Shigella spp*, and the maximum zone of inhibition was shown against Ceftriaxone (CRO) (30mm) (Figure 6). The results showed that the spring water *Shigella spp* were resistant towards antibiotic such as Clindamycin (33.3%), Erythromycin (83.3%), Amoxicillin (16.6%), imipenem (33.3%), Cefrtiaxone (33.3%), Clarithromycin (83.3%), Vancomycin (33.3%), Ciprofloxacin (33.3%), Gentamycin (33.3%) and Amoxicillin+ Clavulanic (16.6%). The study observed that *Shigella spp* isolated from spring water showed the highest resistance (83.3%) towards clarithromycin and the highest sensitivity (100%) towards Amoxicillin + Clavulanic Acid and Amoxicillin. The results are summarized in Table 2.
The results showed that the *Shigella spp* isolated from tap water were resistant towards antibiotic such as Clindamycin (50%), Erythromycin (50%), Amoxicillin (50%), imipenem (50%), Cefrtiaxone (50%), Clarithromycin (50%), Vancomycin (100%), Ciprofloxacin (50%), Gentamycin (100%) and Amoxicillin+ Clavulanic (50%). The *Shigella spp* showed 0% sensitivity to some antibiotics such as Vancomycin and Gentamycin. The results are summarized in Table 3.

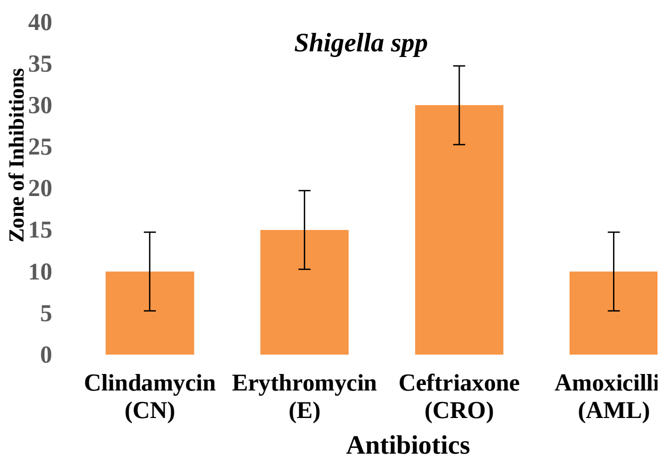


**Figure 6. Antibiotic Susceptibility Pattern of *Shigella spp***

**Antibiotic Susceptibility Pattern of *Salmonella typhi***

In Figure 7, Amoxicillin (AML), Imipenem (IPM), and Clindamycin (CN) had the lowest zone of inhibition of 10 mm for *Salmonella typhi*, and the maximum zone of inhibition was shown against Ceftriaxone (CRO) (30 mm) (Figure 7). The results showed the spring water *Salmonella typhi* were resistance towards antibiotic such as Clindamycin (35.7%), Erythromycin (64.2%), Amoxicillin (85.7%), imipenem (14.2%), Cefrtiaxone (14.2%), Clarithromycin (64.2%), Vancomycin (14.2%), Ciprofloxacin (35.7%), Gentamycin (14.2%) and Amoxicillin+ Clavulanic acid (85.7%). The study observed that *Salmonella typhi* isolated from spring water showed the highest resistance (85.7%) towards Amoxicillin and Amoxicillin+ Clavulanic Acidand the highest sensitivity (100%) towards Imipenem, Ceftriaxone, Vancomycin, and Gentamycin. The results are summarized in Table 2.
The results showed that the *Salmonella typhi* isolated from tap water were resistant towards antibiotic such as Clindamycin (50%), Erythromycin (25%), Amoxicillin (50%), imipenem (25%), Cefrtiaxone (25%), Clarithromycin (25%), Vancomycin (25%), Ciprofloxacin (25%), Gentamycin (25%) and Amoxicillin+ Clavulanic acid (50%)The *Salmonella typhi* were highly sensitive (75%) to Erythromycin, imipenem, Cefrtiaxone, Clarithromycin, Vancomycin, Ciprofloxacin, Gentamycin. The results are summarized in Table 3.

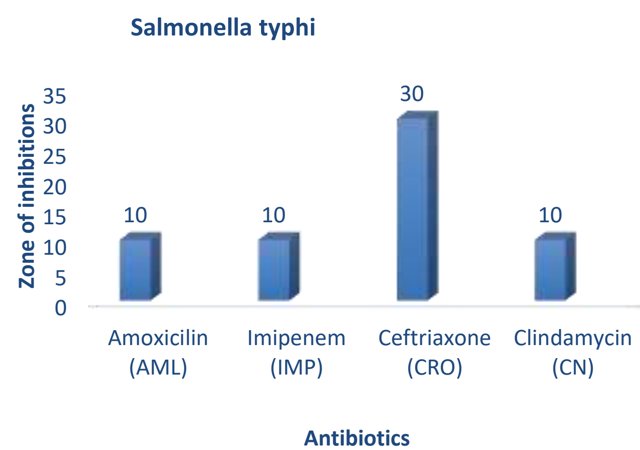


**Figure 7. Antibiotic Susceptibility Pattern of *Salmonella typhi***

**Table 6. Antibiotic Susceptibility Pattern of Bacterial Species against Spring Water**

| **Antibiotics** | | **Species** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***S. aureus* (n=16)** | | ***E. coli* (n=02)** | | ***S. typhi* (n=14)** | | ***Shigella spp* (n=06)** | | ***P.aeruginosa* (n=02)** | |
| | | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** |
| Clindamycin | **No.** | 09 | 07 | 01 | 01 | 05 | 09 | 02 | 04 | 0 | 02 |
| | **%age** | 56.2 | 43.7 | 50 | 50 | 35.7 | 64.2 | 33.3 | 66.6 | 00 | 100 |
| Erythromycin | **No.** | 05 | 11 | 01 | 01 | 09 | 05 | 05 | 01 | 01 | 01 |
| | **%age** | 31.2 | 68.7 | 50 | 50 | 64.2 | 35.7 | 83.3 | 16.6 | 50 | 50 |
| Amoxicillin | **No.** | 04 | 12 | 02 | 0 | 12 | 02 | 01 | 05 | 01 | 01 |
| | **%age** | 25 | 75 | 100 | 00 | 85.7 | 14.2 | 16.6 | 83.3 | 50 | 50 |
| Imipenem | **No.** | 04 | 12 | 0 | 02 | 02 | 12 | 02 | 04 | 0 | 02 |
| | **%age** | 25 | 75 | 00 | 100 | 14.2 | 85.7 | 33.3 | 66.6 | 00 | 100 |
| Ceftriaxone | **No.** | 04 | 12 | 0 | 02 | 02 | 12 | 02 | 04 | 0 | 02 |
| | **%age** | 25 | 75 | 00 | 100 | 14.2 | 85.7 | 33.3 | 66.6 | 00 | 100 |
| Clarithromycin | **No.** | 05 | 11 | 02 | 0 | 09 | 05 | 05 | 01 | 01 | 01 |
| | **%age** | 31.2 | 68.7 | 100 | 00 | 64.2 | 35.7 | 83.3 | 16.6 | 50 | 50 |
| Vancomycin | **No.** | 04 | 12 | 0 | 02 | 02 | 12 | 02 | 04 | 0 | 02 |
| | **%age** | 25 | 75 | 00 | 100 | 14.2 | 85.7 | 33.3 | 66.6 | 00 | 100 |
| Ciprofloxacin | **No.** | 09 | 07 | 0 | 02 | 05 | 09 | 02 | 04 | 0 | 02 |
| | **%age** | 56.2 | 43.7 | 00 | 100 | 35.7 | 64.2 | 33.3 | 66.6 | 00 | 100 |
| Gentamycin | **No.** | 04 | 12 | 0 | 02 | 02 | 12 | 02 | 04 | 0 | 02 |
| | **%age** | 25 | 75 | 00 | 100 | 14.2 | 85.7 | 33.3 | 66.6 | 00 | 100 |
| Amoxicillin+ Clavulanic Acid | **No.** | 04 | 12 | 02 | 0 | 12 | 02 | 01 | 05 | 01 | 01 |
| | **%age** | 25 | 75 | 100 | 00 | 85.7 | 14.2 | 16.6 | 83.3 | 50 | 50 |

**Table 3. Antibiotic Susceptibility Pattern of Bacterial Species against Tap Water**

| **Antibiotics** | | **Species** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***S. aureus* (n=24)** | | ***E. coli* (n=01)** | | ***S. typhi* (n=04)** | | ***Shigella spp* (n=02)** | | ***P. aeruginosa* (n=01)** | |
| | | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** |
| Clindamycin | No. | 10 | 14 | 01 | 00 | 02 | 02 | 01 | 01 | 0 | 01 |
| | %age | 41.6 | 58.3 | 100 | 00 | 50 | 50 | 50 | 50 | 00 | 100 |
| Erythromycin | **No.** | 08 | 16 | 01 | 0 | 01 | 03 | 01 | 01 | 0 | 01 |
| | **%age** | 33.3 | 66.6 | 100 | 0 | 25 | 75 | 50 | 50 | 00 | 100 |
| Amoxicillin | **No.** | 08 | 16 | 01 | 0 | 2 | 2 | 01 | 01 | 0 | 01 |
| | **%age** | 33.3 | 66.6 | 100 | 0 | 50 | 50 | 50 | 50 | 00 | 100 |
| Imipenem | **No.** | 06 | 18 | 0 | 01 | 01 | 03 | 01 | 01 | 0 | 01 |
| | **%age** | 25 | 75 | 00 | 100 | 25 | 75 | 50 | 50 | 00 | 100 |
| Ceftriaxone | **No.** | 09 | 15 | 0 | 01 | 01 | 03 | 01 | 01 | 0 | 01 |
| | **%age** | 37.5 | 62.5 | 00 | 100 | 25 | 75 | 50 | 50 | 00 | 100 |
| Clarithromycin | **No** | 07 | 17 | 01 | 0 | 01 | 03 | 01 | 01 | 0 | 01 |
| | **%age** | 29.1 | 70.8 | 100 | 00 | 25 | 75 | 50 | 50 | 00 | 100 |
| Vancomycin | **No.** | 08 | 16 | 0 | 01 | 01 | 03 | 02 | 00 | 0 | 01 |
| | **%age** | 33.3 | 66.6 | 00 | 100 | 25 | 75 | 100 | 00 | 00 | 100 |
| Ciprofloxacin | **No.** | 09 | 15 | 0 | 01 | 01 | 03 | 01 | 01 | 0 | 01 |
| | **%age** | 37.5 | 62.5 | 00 | 100 | 25 | 75 | 50 | 50 | 00 | 100 |
| Gentamycin | **No.** | 08 | 16 | 0 | 01 | 01 | 03 | 02 | 0 | 0 | 01 |
| | **%age** | 33.3 | 66.6 | 00 | 100 | 25 | 75 | 100 | 00 | 00 | 100 |
| Amoxicillin+ Clavulanic Acid | **No.** | 08 | 16 | 01 | 0 | 2 | 02 | 01 | 01 | 0 | 01 |
| | **%age** | 33.3 | 66.6 | 100 | 00 | 50 | 50 | 50 | 50 | 00 | 100 |

**Physicochemical Evaluation of Water Samples**

The mean values of water quality of documented physical and chemical parameters and the SD (standard deviation) of different sources (spring and tap water) were noted in Table 4. The spring water samples had the maximum pH mean value

(7.5 ± 1.54), while the tap water samples had the minimum pH values (7.2 ± 0.89). Higher average EC (569.9±8.12 µS/cm), TDS (638.3 ± 9.07 mg/L), turbidity (4.65 ± 0.76 NTU), BOD (11 ± 0.30 mg/L), COD (9 ± 0.11 mg/L), sulphate (269.8 ± 4.72 mg/L), nitrate (0.62 ± 0.02 mg/L), Mg hardness (136.7 ± 5.22 mg/L) and TSS (5.30 ± 1.69 mg/L) values were observed in spring water sources and lower in the tap water. Similarly, higher mean values of some parameters, such as sodium (255.1 ± 1.53 mg/L), chloride (290.4 ± 3.35 mg/L), Ca hardness (228.0 ± 3.86 mg/L), and total hardness (456.6 ± 4.73 mg/L) were determined for tap water and lower values for spring water samples. The values of TSS, sodium ions, nitrate, and sulphate exceeded the limits of the WHO. Whereas, sodium ions, chloride ions in tap water were beyond the limits of the WHO. Din et al. (2023a) carried out a study on the assessment of groundwater quality for irrigation and drinking purposes in the District Hangu. The study observed that the spring water source had the maximum mean pH value (7.64 ± 0.08), that was agreeing the pH result of the current study. Similarly, the mean TDS (596 mg/L), turbidity (9.30 NTU), and EC (917 µS/cm) values found were not consistent with the current results. Din et al. (2023a) also found that the values of sodium ions, chloride, and sulphide observed for spring water exceed the WHO limits. Ahmad and Ahmad (2012) analyzed the potable water characteristics and quality of the rural regions of District Hangu, Pakistan. In the study, all water samples were observed within the WHO guide values for turbidity and pH. Ullah et al. (2014) applied an integrated method for assessment of drinking water quality. The study found that the values of pH (7.3), TSS (5.6 mg/L), Total Hardness (338-524 mg/L), Ca Hardness (164-285 mg/L) and nitrate (0.52-0.93 mg/L) were in pact with the present study while the study found different results for EC (1176-1402 µS/cm), TDS (830-1198 mg/L), Mg hardness (155-212 mg/L), chloride (114-182 mg/L), sulphate (194-246 mg/L) and sodium (166-205 mg/L). The variations in the results may be attributed to the difference in the study area.

**Table 4. Physiochemical Characteristics of Spring and Tap Water**

| Parameters | Spring Water | | Tap water | | WHO limits |
|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | |
| **EC (µS/cm)** | 569.9 | 8.12 | 498.3 | 3.67 | 1000 |
| **pH** | 7.5 | 1.54 | 7.2 | 0.89 | 6.5-9.2 |
| **TDS mg/L** | 638.3 | 9.07 | 477.8 | 2.63 | 1000 |
| **TSS mg/L** | 5.30 | 1.69 | 3.80 | 1.05 | 5 |
| **Turbidity NTU** | 4.65 | 0.76 | 2.10 | 0.15 | <5 NTU |
| **BOD mg/L** | 11 | 0.30 | 06 | 0.19 | 250 |
| **COD mg/L** | 09 | 0.11 | 04 | 0.02 | 10 |
| **Sodium mg/L** | 238.4 | 2.88 | 255.1 | 1.53 | 200 |
| **Chloride mg/L** | 217.7 | 4.50 | 290.4 | 3.35 | 250 |
| **Nitrate mg/L** | 0.62 | 0.02 | 0.43 | 0.03 | 0.5 |
| **Sulphate mg/L** | 269.8 | 4.72 | 187.2 | 2.49 | 250 |
| **Ca Hardness mg/L** | 149.1 | 1.96 | 228.0 | 3.86 | 250 |
| **Mg Hardness mg/L** | 136.7 | 5.22 | 122.6 | 4.06 | 150 |
| **Total Hardness mg/L** | 412.2 | 6.10 | 456.6 | 4.73 | 500 |

### Heavy Metal Analysis

The collected water samples were assessed for HMs like Mn, Pb, Cd, and Cr using AAS. The Mn, Pb, Cd, and Cr concentrations in spring water were noted as 0.015, 0.003, 0.017, and 0.088 mg/L, respectively. The levels of Pb, Mn, Cr, and Cd analyzed in tap water were 0.008, 0.083, 0.017, and 0.011 mg/L, respectively. The results showed that Cd and Cr concentrations were higher in spring water than in tap water (Figure 8). Similarly, tap water was more polluted with HMs such as Mn and Pb than spring water. The study revealed that the metal concentration did not exceed the WHO drinking water limits. Din et al. (2023b) determined the HMs pollution and potential risk evaluation via groundwater drinking in the Hangu district and observed that the HM concentrations in groundwater were within acceptable guidelines set by the WHO, and suggested that children were more prone to health risks compared to adults. Bibi et al. (2016) assessed the HMs in drinking water of District Lakki Marwat, Pakistan, and found that the concentration of metals such as Pb and Cd were ranging 0-0.91mg/L and 0-0.007mg/L in water samples collected from different sites. Bibi et al. (2016) concluded that Cd and Pb were above the WHO standard value in some places. Moreover, according to bivariate analysis a strong positive (r =1, P value 0.001) correlation was observed between bacterial spp. and HMs.

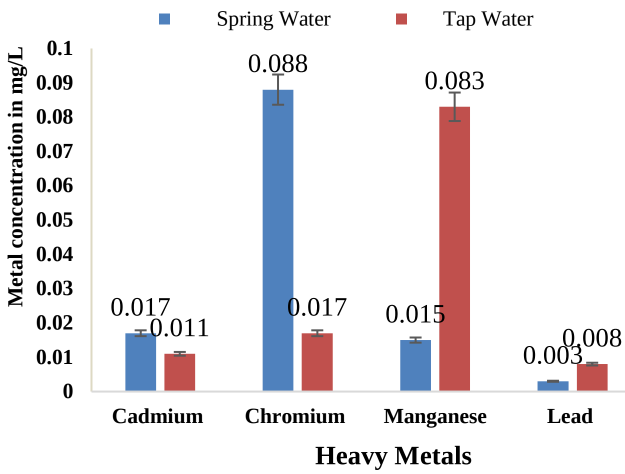


**Figure 8. Metal Concentration in Water Samples**

## Health Risk Assessments

### Chronic daily intakes of metals

The CDIs were detected in order of Cr > Cd >Mn > Pb and Mn >Cr > Cd > Pb through consumption of spring and tap water, respectively. The mean CDI of Cd for adults was 0.47 and 0.30 µg/(kg·day) through consumption of spring and tap water, respectively, while for children this was 0.52 and 0.34 µg/(kg·day) through intake of spring and tap water, respectively(table. 5). The Lowest Cd CDI (0.30 µg/(kg·day) was investigated for adults through ingestion oftap water, whereas the Highest Cd CDI (0.52 µg/(kg·day), through intake of spring water for children. The Cr CDIs adults were 2.44 and 0.47 µg/(kg·day) overingestion of spring and tap water, respectively; while this was 2.70 and 0.52 µg/(kg·day) overingestion of spring and tap water, respectively, for children. The lowest Cr CDI for adults (0.47 µg/(kg·day) was observed via consumption of tap water, while the highest Cr CDI (2.70 µg/(kg·day) was investigated through consumption of spring water for children (Table 5).

The Mn CDI for adults was 0.41 and 2.30 µg/(kg·day) overingestion of spring and tap water, respectively; while for children this was 0.46 and 2.55 µg/(kg·day) overconsumption of spring and tap water, respectively(table.5). For adults the lowest Mn CDI (0.41 µg/(kg·day), was investigated, while the highest Mn CDI (2.55 µg/(kg·day) was observed through intake of tap water for children. Through spring and tap water consumption, the Pb CDIs were 0.08 and 0.22 µg/(kg·day), respectively, for adults; whereas they were 0.09 and 0.24 µg/(kg·day) for children through ingestion of spring and tap water, respectively. The highest Pb CDI (0.24 µg/(kg·day)) was observed through tap water use for children (Table 5). Muhammad et al. (2011) assessed the health risks linked with HMs in the Swat potable water, and suggested that the CDI values were within safe limits. Muhammad *et al.* (2011) found that the CDI values were identified in the order of Mn > Pb > Cr > Cd > which were different from the current study.

**Table 5. Daily Intakes (µg/(kg·day) of Chronic Metals through spring and tap water**

| Metals | Individuals | CDIs(µg/(kg·day) | |
|---|---|---|---|
| | | Spring water | Tap water |
| Cd | Adults | 0.47 | 0.30 |
| | Children | 0.52 | 0.34 |
| Cr | Adults | 2.44 | 0.47 |
| | Children | 2.70 | 0.52 |
| Mn | Adults | 0.41 | 2.30 |
| | Children | 0.46 | 2.55 |
| Pb | Adults | 0.083 | 0.22 |
| | Children | 0.09 | 0.24 |

### Health risk indexes of metals

The HRIs of HMs were observed in the order of Cr followed by Mn >Cd > Pb and Mn > Cr >Cd > Pb over spring and tap water consumptions, respectively. For adults, the Cd HRIs were 9.4E-01 and 6.0E-01 through spring and tap water consumption, respectively. For children, the Cd HRIs were 10.4E-01 and 6.8E-01 through spring and tap water consumption, respectively. Similarly, for adults, Cr HRIs were 1.62E-03 and 31.1E-04 through spring and tap water consumption, respectively. However, for children, the Cr HRIs were 1.8E-03 and 3.4E-04 through spring and tap water consumption, respectively. The Mn HRIs for adults were 2.9E-02 through spring water consumption and 1.64E-02 through tap water ingestion. The HRIs of Mn were 3.2E-03 and 1.82E-02 through spring and tap water consumption, respectively, for children. For spring and tap water consumption, the HRIs of Pb were 2.3E-03 and 6.1E-03, respectively, for adults, and 2.5E-03 and 6.6E-03, respectively, for children (Table 6). The study results demonstrated that HRI values were within safe limits, as they were less than 1(HRI < 1), symptomatic of no health risk in the selected region. Khan et al. (2013) assessed the health risks related to HMs in the potable water of Swat, and found similar HIR values for Mn and Pb, similar to the current study, but the values obtained for Cd and Cr were much different from the current study. Similarly, the HRIs values obtained for Cd, Mn, and Cr were maximum in the current study compared to research studies carried out by Shah et al. (2012) and Muhammad et al. (2011). The HRIs of all metals show higher values than the values investigated by Nawab et al. (2016), in surface and ground water of District Shangla.

**Table 6. Health Risk Indexes of metals through drinking water**

| Metals | RfD | Individuals | HRI | |
|---|---|---|---|---|
| | | | Spring water | Tap water |

| | | | | |
|---|---|---|---|---|
| **Cd** | 5.0E-01 | Adults | 9.4E-01 | 6.0E-01 |
| | | Children | 10.4E-01 | 6.8E-01 |
| **Cr** | 1.5E+03 | Adults | 1.62E-03 | 3.1E-04 |
| | | Children | 1.8E-03 | 3.4E-04 |
| **Mn** | 1.4E+02 | Adults | 2.9E-03 | 1.64E-02 |
| | | Children | 3.2E-03 | 1.82E-02 |
| **Pb** | 3.6E+01 | Adults | 2.3E-03 | 6.1E-03 |
| | | Children | 2.5E-03 | 6.6E-03 |

## Conclusion and Recommendations

The study showed that the parameters such as TSS, sodium ions, nitrate, and sulphate values in spring water, while sodium and chloride ions in tap water were beyond the limits of WHO, other parameters were within the WHO-suggested permissible limits. The values of the samples, collected from the spring water, were higher for average EC, TDS, turbidity, BOD, COD, sulphate, nitrate, Mg hardness, and TSS compared to the tap water. Similarly, higher mean values of parameters, including sodium, chloride, Ca hardness, and total hardness, were determined for tap and lower for spring water samples. Tap water was safer as compared to spring water. The concentration of Cr was highest, followed by Cd > Mn > Pb in the spring water, while in the tap water samples, the order was Mn > Cr >Cd > Pb. All the selected metals were within their permissible limits set by WHO. The statistical results revealed that heavy metal pollution at various sites differs significantly ($P < 0.05$). According to the health risk assessment results, no health risk was detected in the study area (HRI < 1) based on US EPA (United States Environmental Protection Agency) standards. This study concluded that tap water was of higher quality compared to springs. The presence of fewer waterborne pathogens in the tap water samples is a sign of the microbial quality of tap water. Efficient water treatment methods that eliminate physicochemical parameters and HMs from water samples should be encouraged. Indiscriminate waste dumping into the environment can cause water pollution by infiltration into groundwater or runoff into surface water; therefore, appropriate waste dumping approaches should be stimulated. To attain Goal 6 of the United Nations Sustainable Development on sanitation and clean water by 2030, we recommend that regular treatment, monitoring, and proper education of stakeholders on drinking water treatment practices should be accompanied mostly in rural areas in the region, country, state, and continent.

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**Declaration**

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**Consent to Participate**

Not applicable

**Authors contribution**

MU and SK conducted research and wrote the initial draft of manuscript. SMS, MSK, and RA collected the literature and wrote the manuscript, and edited the manuscript in original. FS, HY, SY and AU make final editing in the manuscript. All authors have read and approved the final manuscript. The author have read and approved the final manuscript.

**Conflict of Interest**

The authors state that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial or personal manner concerning the development of this project, the gathering of data, the interpretation of the data, or the writing and publishing of this paper.

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**Consent for publication**

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