Kurdistan Region – Iraq
Ministry of Higher Education and Scientific
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College of Science

The study of Biological, chemical and physical contamination of spring water in Akre, Kurdistan region, Iraq

A Research Project

Submitted to the Department of Environmental Science and Health, College of Science, Salahaddin University-Erbil in partial fulfillment of the requirement of Bachelor degree of science

By

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Abtract

In the current study there are spring (ground) water that affected by earth quick and spring water far away from it (not affected by earth quick) about 18 sample collection in different sides of the same place in Akre city for each types (triple collecting of sample), observed the two types of bacterial isolated from the spring water that affected by earth quick were (*Streptococcus agalactiae, Staph.epidermidis, E.coli,and Klebsiella pneumonia)* and one type of algae (cyanobacteria) and coliform bacteria, That diagnosis of those isolated bacteria by using macroscopic and microscopic examination in addition of physical and chemical test for all the samples isolated . After comparison between both types of water sample the earth quick have affective on changing water in three parameters (biological, physical and chemical).

Keywords: *Spring water; Quality Assessment; Physicochemical parameters; WQI; Akre district, gram stain, bacteria, coliform, and algae.*

The aims of the present study are:

1. To evaluate Biological, chemical and physical parameters of springs (ground) water collected from Akre districts –Kurdistan Region of Iraq.
2. Make a compression between the spring (ground) water affected by earthquake and other spring water normally (in various places) but at the same region to classify the springs for drinking purposes, then obtained mean values with the WHO standards to classify the springs for drinking purposes.
3. Estimate the quality status of springs water by using WQI assessments.

Introduction:

Water is an essential requirement of human and industrial developments and it is one the most delicate part of the environment (Das and Acharya, 2003).

Water pollution is the pollutants of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater) as well as highlighted as water quality degradation that alter its physicochemical characteristics of water and impede its normal use. The contaminants originated either as solid, liquid or gaseous form have various effects depending on their amount, potential danger and fragility in the environment where they are released. These pollutants can have irrespective of the sources of pollutants whether human or a natural origin may be responsible for water pollution by decomposition of organic debris. The saltwater invades whereas landslides, earthquakes, volcanic dust eruption causes water blackening (Manzoor, A.S., 2013).

An earthquake is a natural disaster that causes enormous destruction. Numerous places have observed changes in water quality brought on by earthquakes during the previous ten years. For example, secondary hazards degrade the quality of water in sources like spring water and groundwater (Makoto et al., 2020).

The region of accumulation of heavy metals within fish varies with the route of uptake, heavy metals and species of fish concerned. Their potential use as bio monitors is therefore significant in the assessment of bioaccumulation and biomagnification of contaminants within the ecosystem. Many dangerous chemical elements, if released into the environment, accumulate in the soil and sediments of water bodies. (Abida et al., 2008).

Water may be called polluted when the following parameters stated below reach beyond a specified concentration in water.

i) Physical parameters. Colour, odour, turbidity, taste, temperature and electrical conductivity constitute the physical parameters and are good indicators of contamination. For instance, colour and turbidity are visible evidences of polluted water while an offensive odour or a bitter and difference than normal taste also makes water unfit for drinking.

ii) ii) Chemical parameters: These include the amount of carbonates, sulphates, chlorides, fluorides, nitrates, and metal ions. These chemicals form the total dissolved solids, present in water.

iii) iii) Biological parameters: The biological parameters include matter like algae, fungi, viruses, protozoa and bacteria. The life forms present in water are affected to a good extent by the presence of pollutants. The pollutants in water may cause a reduction in the population of both lower and higher plant and animal lives. Thus, the biological parameters give an indirect indication of the amount of pollution in water. (Cabral., 2010).

The present study was conducted on springs water located within the Akre district. Akre district is a region located in the northeast of Iraq. It is located in Dohuk Governorate-Kurdistan region. The total area of the district is (1134) km2.

It was formed in 1877 by the Ottoman Empire and the city of Akre became the center of the district. Akre district is the center of three sub-districts, (Dinarta, Girdasin, and Bjeel) with an estimated population of 150,000 people Fig.1(a, b). Akre is located north of latitude 37.4 and east of longitude 44.8, a height of 665m above sea level. Akre is about 110 km southwest of Erbil, 100 km to the east of center Duhok, and 90 km north of center Mosul, Ninewa.

The geography of the area is mountainous, and the climate is considered semiarid, characterized by hot, dry summers and, cold, wet winters, and is usually snowy with more rainfall in the north than in the central and southern parts. The major water sources are springs and rivers, and a great proportion of the population obtains water from springs for drinking and domestic purposes (Malaika and Raswol, 2014).

The studied sites located in Akre district namely (spring water affected by earth quick and other spring(ground) water normally) .

Figure: 1, C, D. Show the map of the place that collected the samples in this study.

Figure (1): (a): Map of Iraq (b): Map of Duhok Governorate (c): Akre district showing

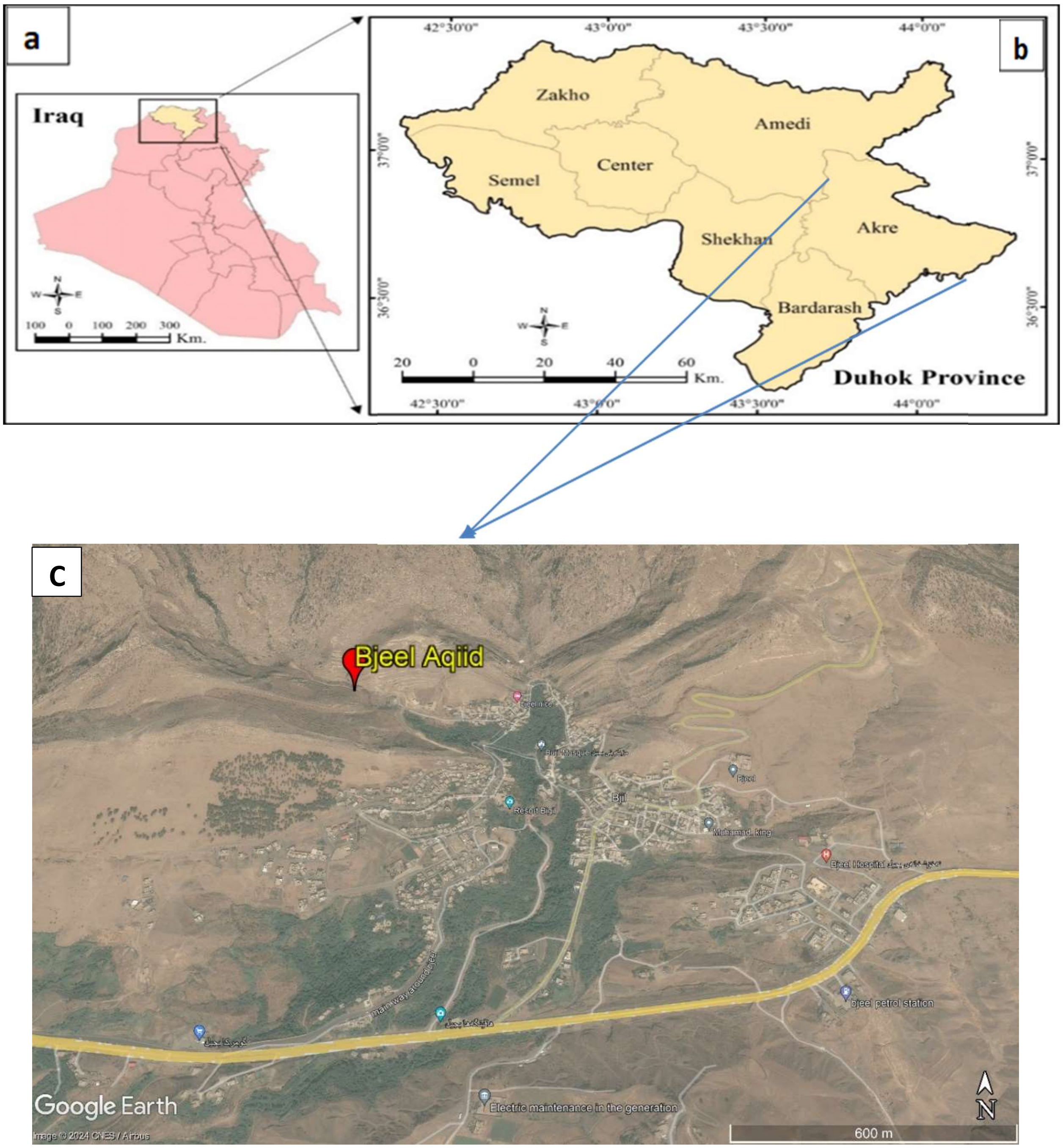

Figure C (spring water affected by earth quick in Akre region).

Figure D: spring water normally unaffected in Akre region).

Material and methods

In this study there were two groups (spring water affected by earth quick (Figure.1,C and spring water normally (Figure.1,D) Eighteen samples collecting of spring water as a triple times and different period (15 days) from different place and the same region for both types (affected and unaffected by earth quick of spring water then diluted the all collecting samples by using serial dilution method then growth on the two types of media (Nutrient agar and MacConkey agar) to detect a biological change like (bacteria).

1**.** Sampling Area Sampling was from the Akre Kurdistan region of ground water, (Figure 1,C,D). Water and sediment samples from Akre were collected for analyses.

2. Sampling Points Water and sediment samples were collected from five point designated as S1 to S5. Samples were collected at each the point of Akre from different places and for comparing using another place far away but at the same region (Akre).(St1,St2, St3, St4, and St5) these collection also as a different points.

3**.** Sample Collection Water samples were collected in dark glass containers pre sterilized by oven.

4. Examination in laboratory For all samples that collected three parameters as Biological, chemical and physical test performed:

A. Biologicals examination: by using serial dilution for each of the samples (S1, S2, S3, S4, S5, S6, S7, S8, S9, St1,St2, St3, St4, S5, S6, S7, S8, and St9). Then growth on two types of culture media (MacConkey agar and Nutrient agar) for 48 hr. at 37^0 C.

S: SAMPLE (affected), St: as astandard (unaffected).

Diagnosis microscopic:

To identified microscopically using gram stain and under microscope we can observe the shape and color of bacteria for distinguished between gram-positive and gram-negative bacteria. By using Gram stain is a technique test separated bacteria into two groups Gram-positive and Gram negative (Benson, 2001), show in figure (2).

The procedure of gram stain:

1. Place the prepared fixed smear on a slide rack then floods the slide with crystal violet.
2. Wait 15 seconds then rinse the slide with water.
3. Flood the slide with Gram's iodine.
4. After 15 seconds rinse the slide with water.
5. Apply the decolorizer to the slide.
6. Rinse the slide immediately with water.
7. Flood the slide with counterstain.
8. Wait 15 seconds then rinse the slide with water.
9. Blot the slide with absorbent paper. Be careful not to wipe the cells off the slide.
10. Allow the newly stained slide to air dry completely.
11. View the slide under oil using the oil immersion objective for a total magnification of 1000X.
12. Record results based on your laboratory's criteria.

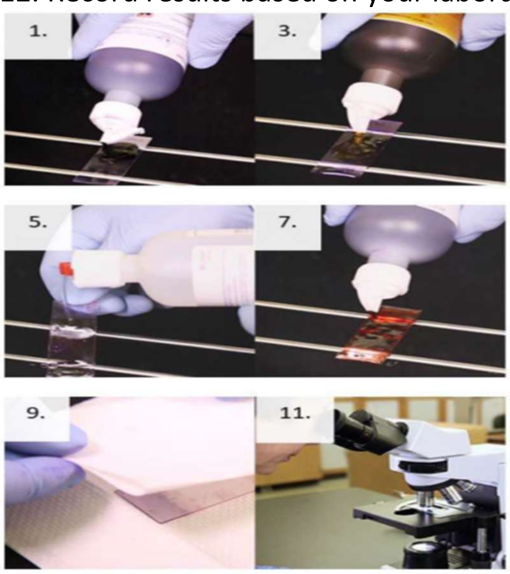

Figure 2: the gram stain method for distinguish gram +ve and gram –ve bacteria.

Diagnosis macroscopic

By using two types of culture media to diagnosis and colony characteristics on each of them, (all samples: s1,s2,s3,s4………..s9,st1,st2,st3,st4,……. st9).after incubation for 48 hours at 37^{0} C then observing of colony forming on the media and study of properties of colony.

That can be observed from the colony characteristics of colony growth on both media used (nutrient agar and MacConkey agar) then calculating the number of bacteria by using this Formula: Number of bacterial colonies CFU of water (ml) = number of colony /dilution factor *100. by using serial dilution method for each of sample then growth on two types of media (Nutrient agar , MacConkey agar). Show in figure (5).

Nutrient agar: is a general purpose liquid median supporting growth of a wide range for non-fastidious organisms.
MacConkey agar: is a different medium for the selection and recover of Entero bacteris cade and related enters Gram -negative rods (Ifeanyi et al .2014).

Serial dilution method: Using the five test tube each of them contain equal of distilled water (9ml) take 1 ml from original water sample to the first tube then from the first tube transfer 1ml to another tube respectively from 10^-3 and 10^-5 transfer 1 ml by using Micropipette into the Media (Nutrient agar and MacConkey agar) incubated for 24 hr. at 37°c incubation.

2.Physicochemical examination:

The water samples were analyzed to evaluate some physicochemical properties of water quality including Turbidity, pH, EC, TDS, Chloride (Cl^-), Sulfate (SO_4), Alkalinity, Total Hardness, Calcium (Ca^{+2}), Magnesium (Mg^{+2}), Sodium (Na^{+2}), Potassium (K^+), Nitrate (NO_3). Water samples were collected using glass dark bottles and transported as soon as possible to the laboratory to be analyzed within 48 hours for some physicochemical properties. All the procedures carried out for the examination of water samples were according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The turbidity was measured by (Palin test Micro 950 – Turbidity meter). pH , EC and TDS were measured by (PH and Conductivity meter, Model Jenway-3540), Chloride (Cl^-) by (Argentometric method), Sulfate (SO_4) by spectrophotometer, Total hardness(TH), Calcium (Ca^{+2}), Magnesium (Mg^{+2}) and Alkalinity (TA) were analyzed by the titrimetric method according to (APHA, 1998), Sodium (Na^{+2}), Potassium (K^+) were measured by flame photometer, Nitrate (NO_3) was analyzed by colorimetric method using digital ultraviolet spectrophotometric screening method (JENWAY 6305 Spectrophotometer).

STATISTICAL ANALYSIS

The analytical results of the physicochemical parameters of springs were subjected to descriptive statistical analysis, table with the aid of standard statistical methods (Gupta, 2009).

For computing WQI three steps were followed. In the first step, each of the 12 parameters has been assigned a weight (wi) according to its relative importance in the overall quality of water for drinking purposes. The maximum weight of 5 has been assigned to the parameter nitrate due to its major importance in water quality assessment. Alkalinity was given the minimum weight of

1 as it plays an insignificant role in the water quality assessment (Srinivasamoorthy et al., 2008) In the second step, the relative weight (RW) was computed by the following equation (Horton, 1965).

$$RW = wi / \sum_{i}^{n} wi$$

Where, RW is the relative weight, **wi** is the weight of each parameter and **n** is the number of parameters. Calculated relative weight **(RW)** values of each parameter are also given in (Table 1).

Table (1): WHO standards weight (wi) and calculated relative weight (Wi) for each parameter.

Parameters	Unit	WHO	Weight (wi)	RelativeWeight (RW)
Turbidity	NTU	5	3	0.0909
pH		6.5 – 8.5	4	0.1212
EC	µs/ cm	1000	3	0.0909
TDS	mg/ L	500	3	0.0909
T. Alkalinity	$mgCaCO_3$/ L	200	1	0.0303
T. Hardness	$mgCaCO_3$/L	200	2	0.0606
Calcium (Ca^{2+})	mg/L	100	2	0.0606
Nitrate (NO_3^-)	mg/L	50	5	0.1515
Magnesium (Mg^{2+})	mg/L	30	2	0.0606
Chloride (Cl^-)	mg/L	250	2	0.0606
Sodium (Na^+)	mg/L	200	3	0.0909
Potassium (K^+)	mg/L	10	3	0.0909

Table (2): Water quality classification based on WQI value

Water Quality Index Level	Water Quality Status
<50	**Excellent**
50-100	**Good**
100-200	**Poor**
200-300	**Very Poor**
>300	**Unsuitable**

RESULTS AND DISSCUSION

In the present study, four isolates of bacteria were identified among about
10 spring water that affected by earthquake and no observation of bacteria in the samples that collected from spring water which no under affected on earthquake that samples were collected from different places, The samples were processed using serial dilution method then cultural techniques. the results showed that, four isolates of bacteria were obtained 2Gram Positive and 2 Gram negative which were included

1-Streptococcus agalactiae G+ve
2-E-coli G-ve
3-Klebsiella sp premmonide G-ve
4.Streptococcus agalactiae G+ve

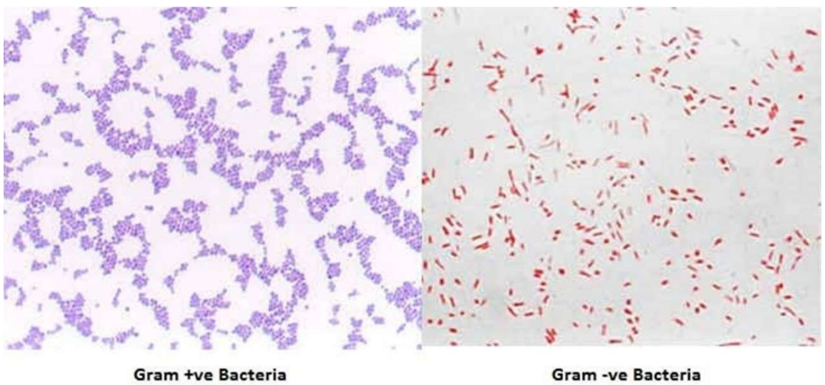

Figure 3: The two types of the isolated bacteria in spring(ground) water that affected by earthquick (Bjeel area) in Akre region (A-*Streptococcus agalactiae,* B- *E-coli*).

Color and Turbidity

Color contamination of water bodies is caused by metals, dye pollution, soil particles, and by the occurrence of water bloom caused by earthquake. Color contamination of a water body is a problem because of the harm it. And when it used as drinking water or for other domestic uses, its color distresses the users. Typical algae species that color water resources such as spring water reservoirs include Cyanobacteria (blue-green algae). Shown as figure (4).

Figure (4): Cyanobacteria isolated from spring water that affected by earthquake (Bjeel area) in Akre region

After proceeded the serial dilution of all the sample isolates (spring water affected by earthquake) and cultured on the media from two diluted factor 10^{-3} and 10^{-5} the number of colony per unit of litter(19% ,23%, 26%, and 32%). Figure (5). In addition of these types of bacteria indicated another microorganisms as a coliform bacteria in some other samples that collected from spring water affected by earth quick (16/100ml) in most probable number (M.P.N) of bacteriological examination of water so that called the results were unsatisfactory.

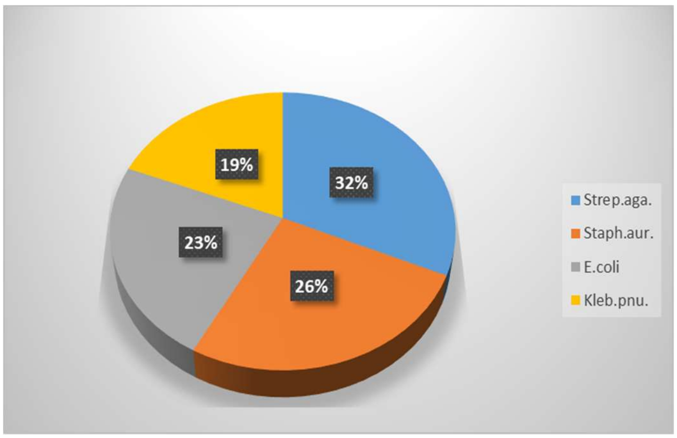

Figure 5: All isolated bacteria as a percentage according to the CFU/litter

The analytical results of physicochemical parameters of all examined spring water samples during the study periods was listed in Tables (3-4). Results of examined water samples has been comparable with WHO standard (WHO, 2006).

Turbidity: is a measure of the relative clarity or cloudiness of water (Allen et al, 2008). Turbidity is observed as an important parameter for drinking water. Turbidity caused by the presence in water of particulate matter such as clay, colloidal particles and planktons and may be other organisms (Katz, 1985). The turbidity values as shown in (tables 3, 4) of spring water were ranged from 15.7 NTU in spring water (affected) to 1.5 NTU in spring water (Unaffected) with a difference mean value 14.2 NTU. The results revealed that all the spring waters were exceeded and were within the permissible limit recommended by WHO standards and not safe for drinking purposes.

pH: The pH scale determines whether a solution or body of water is acidic or basic. pH is an important marker that may be used to evaluate the quality of the water and the level of contamination in water bodies (Ameen, 2019). The minimum pH value was 7.2 in spring water that (affected) by earth quick. The results showed that all spring water samples fall below the allowable limit set by WHO standards and safe for drinking purposes.

Electrical conductivity (EC): is the ability of water to conduct electric current signifying chemical purity of a low electrical conductance (Benain et.al., 1993). The EC value of spring water affected by earthquick 586 µS/cm in spring water unaffected 54.6µS/cm in spring water unaffected with a difference mean value of 532.6 µS/cm. Accordingly, none of the spring water samples exceeding the threshold EC limit of 1000 µS/cm and remain safe for drinking use.

Total Dissolved Solids (TDS): is a measure of all the chemical constituents dissolved in water, it is mostly influenced by the concentration of major ions: calcium, bicarbonate, magnesium, sulfate and chloride and it is closely linked to the EC (Shareef and Kafia, 2008). The TDS values of spring water affected by earth quick 298 µS/cm , in spring water unaffected 28.9µS/cm with a difference mean value of 269.9 µS/cm . According to WHO standard for TDS of 500 mg/l, none of the spring sites showing higher values and remain suitable for drinking purposes.

Chloride (Cl^-): is widely distributed in nature, generally in the form of (NaCl), (KCl) and ($CaCl_2$) salts. It is constituents approximately 0.05% of the lithosphere (WHO, 1984). The chloride values 5.5 mg/l in spring water affected , and 6.3 mg/l in spring water unaffected with a difference mean value 0.8 mg/l.). So all spring water samples were within the allowable limit for chloride of 250 mg/l and still safe for drinking purposes.

Sulfate (SO_4^{2-}): is an abundant ion in the earth's crust and its concentration in water can range from few milligrams to several thousand milligrams per liter (Bartram and Balance, 1996) and it is derived from most sedimentary rocks in many natural water (Lind, 1970). Sulfates occur naturally in ground water combined with calcium, magnesium and sodium as sulfates salts (Kendall, 2007). The Sulfate values 37.00 mg/l in spring water affected, and 45.00 mg/l in spring water with a mean value 8.00 mg/l. The permissible limit for SO_4^{2-} is 250 mg/l, so accordingly, all spring water samples bellow the permissible limit and suitable for drinking.

Total alkalinity (TA): is a measure of the buffering capacity of water. According to (WHO, 1997) the high desirable level of alkalinity concentration is (125 mg $CaCO_3$/l), and the high permissible level is (200 mg $CaCO_3$/l). The alkalinity values 212 mg$CaCO_3$/l in spring water affected, and 215.3mg $CaCO_3$/l in spring water unaffected with a difference mean value of 3.3 mg $CaCO_3$/l. almost all the rest spring water samples during the study periods recorded high levels of alkalinity in compliance with WHO standards. High alkalinity in water is due to the bicarbonates, carbonates, and hydroxides (Toma, 2006). Accordingly, the spring water was unfit for drinking purposes.

Total hardness (TH): is one of the parameters which are generally used for monitoring water quality in the different water systems in the world (WHO, 2006). It is primarily caused by the presence of the high amount of Ca^{2+} and Mg^{2+} ions in water. The total hardness values 432 mg

$CaCO_3$/l in spring water affected , and 462 mg $CaCO_3$/l in spring water unaffected with a difference mean value of 30 mg $CaCO_3$/l. all spring water exceeded the threshold limit of 200 mg $CaCO_3$/l recommended by WHO standards, thereby indicating hard water character of the springs. The higher values of total hardness may be due to the geological formation of the catchment area and human activity (Hassan, 1998).

Calcium (Ca^{2+}): is one of the major inorganic cations, or positive ions, in saltwater and freshwater. It can originate from the dissociation of salts, such as calcium chloride or calcium sulfate, in water (Saoud et al, 2003). The Ca^{2+} values 109 mg/l in the spring water affected , and 115.6 mg/l in spring water unaffected with a difference mean value of 6.6 mg/l. These high values of Ca^{2+} due to the abundant of carbonate rocks in the catchment area (Bui and Loudhi, 2020).

Magnesium (Mg^{2+}): Magnesium is also an important parameter for assessing water quality because of its direct relationship with the development of water hardness. The concentrations of this element in natural water depend upon the type of rocks. (Drinking Water Standard, 2001). The Mg^{2+} values 38.8 mg/l in spring water affected , and 40.6 mg/l in spring water unaffected with a difference mean value of 1.8 mg/l. The high concentration of Mg^{2+} may be attributed to the limestone, gypsum and dolomite rocks in the catchment area (Hameed et al, 2019).

Sodium (Na^+): Salts Na^+ are highly soluble in water their ratio is generally (200 mg/L), whereas according to (USEPA, 2004) the health-based value is 20.00 mg/L according to this ratio the concentration for drinking purposes. The Na^+ values 6.4 mg/l in spring water affected, and 5.3 mg/l in spring water unaffected with a difference mean value of 1.1 mg/l. Results revealed that all spring water samples were within the permissible limit of 200 mg/l set by WHO standards and fit for drinking purposes.

Potassium (K^+): is an essential element in humans and is seldom, if ever, found in drinking water at levels that could be a concern for healthy humans. It occurs widely in the environment, including all natural waters. It can also occur in drinking water as a consequence of the use of potassium permanganate as an oxidant in water treatment (Gennari, (2002). The K^+ values 0.7 mg/l in spring water affected , and 0.8 mg/l in spring water unaffected with a difference mean value of 0.1 mg/l. The K^+ concentrations of all spring water samples were in compliance with the WHO standard of 10.00 mg/l and still suitable for drinking purposes.

Nitrate (NO_3^-): Nitrate causes blue baby syndrome in infants, one of the most significant disease-causing factors of water quality (Meride and Ayenew, 2016). The runoff from fertilized land use, leaching from septic tanks, sewage, and erosion of natural deposits are the main sources of nitrate in water (Shareef et al., 2009). The nitrate concentrations 5.7 mg/l in spring water affected, and 3.6 mg/l in spring water unaffected with a difference mean value of 2.1 mg/l. Results revealed that all spring waters were within the allowable limit of 50.00 mg/l for NO_3^- and remain safe for drinking purposes.

Table (3):Results of physicochemical parameters of spring water affected and spring water unaffected within Akre region.

Sites	Units	S.Affected	S.Unaffected	WHO
Turbidity	NTU	15.7	1.5	5
pH	-	7.2	7.3	6.5-7.5
EC	µS/cm	586	54.6	1000
TDS	mg/l	298	28.9	500
Cl^-	mg/l	5.5	6.3	250
SO_4^{2-}	mg/l	37	45	250
T. Alkalinty	mg $CaCO_3$/l	212	215.3	200
T. Hardness	mg $CaCO_3$/l	432	462	200
Ca^{2+}	mg/l	109	115.6	100
Mg^{2+}	mg/l	38.8	40.6	30
Na^+	mg/l	6.4	5.3	200
K^+	mg/l	0.7	0.8	10
NO_3^-	mg/l	5.7	3.6	50

Table (4): Calculated water quality index WQI of springs water affected and spring water unaffected in Akre region.

parameters	Sample Affected		Sample Unaffected	
	WQI	WQ status	WQI	WQ status
Turbidity	15.7	Not Good	1.5	Good
pH	7.2	Excellent	7.3	Excellent
EC	586	Excellent	54.6	Good
TDS	298	Excellent	28.9	Good
Cl^-	5.5	Good	6.3	Good
SO_4^{2-}	37	Good	45	Good
T. Alkalinty	212	Not Good	215.3	Not Good
T. Hardness	432	Not Good	462	Not Good

Ca^{2+}	**109**	**Not Good**	**115.6**	**Not Good**
Mg^{2+}	**38.8**	**Not Good**	**40.6**	**Not Good**
Na^{+}	**6.4**	**Excellent**	**5.3**	**Good**
K^{+}	**0.7**	**Excellent**	**0.8**	**Good**
NO_3^{-}	**5.7**	**Excellent**	**3.6**	**Good**

Conclusion

Based on the results of this study, it was observed that the levels of Ca^{2+}, Mg^{2+}, Total hardness, T. Alkalinty, and Turbidity in the water samples exceeded the WHO standard limits with exception of pH and temperature. It was also observed that the levels of all the metals in the water samples exceeded the WHO limits. However, the concentrations of all the metals in the sediment samples exceeded the recommended values stipulated by WHO, the suitability for drinking purposes of springs water within Akre district- Iraqi Kurdistan region was investigated. It can be concluded:

The analysis revealed that all physicochemical parameters are almost all below the permissible limit based on WHO standards, with the exception of total Alkalinity, Total.hardness,Ca^{+2}, Mg^{2+} showed higher levels. This may be attributed to the geological formation and the nature of the rock of the area.

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