



A Study on Distribution of Major ions and Heavy Metals in Drinking Water of Govt. Kallar. Hr. Sec. Schools in Theni, Tamil Nadu, Season wise

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ABSTRACT

Tamil Nadu is one of the water starved states in India. Nearly 73 per cent of the geographical area of the state is occupied by a variety of hard and fissured crystalline rock formations of the Archaean age like Charnockites, granites and associated rocks. The study was undertaken to assess the status of drinking water quality in the rural areas. A total number of eight groundwater samples were taken from Govt. Kallar Hr.Sec Schools in and around Theni District with necessary precaution. Water quality parameters such as Major ions like Calcium, Magnesium, Sodium, potassium, Fluoride, Nitrate, Sulphate, Chloride, Bicarbonate, pH, Total hardness (TH), Temperature, and Some heavy metals such as Cu, Zn, Fe, Ni and Cr have been analyzed during post and premonsoon season of year 2010-2012. Multivariate statistical tools like Box and Whisker plots, cluster analysis and principal component analysis were applied to evaluate the quality of drinking water. There is a relationship between chronic diseases and geologic environment. Many school students and the people consume the water for drinking purpose is affected by dental and skeletal sclerosis on account of the fluoride contamination. Ground water acquires fluoride from natural rock and excess dissolution of minerals during rainy seasons. Seasonal effect in geochemical environment is indeed a significant factor in the serious health problems of school students. This research revealed that drinking water quality is highly affected during post moon than premonsoon on account of dissolution of minerals into ground water during rainy season.

Keywords: Major ions, Heavy metals, Box and Whisker plots, cluster analysis, principal component analysis.

INTRODUCTION

Of all the natural resources, water is unarguably the most essential and precious. Life began in water and life is nurtured with water. Ninety seven percent of the world's water is found in oceans. Only 2.5% of the world's water is non-saline fresh water. There are organisms, such as anaerobes, which can survive without oxygen. But no organism can survive for any length of time without water. It is a universal solvent and as a solvent it provides the ionic balance and nutrients, which support all forms of life. In India the

major source of water used to meet the domestic, agricultural and industrial needs is the ground water. The ground water is defined as water that is found underground in cracks and spaces in soil, sand and rocks. This source has two distinct functions; firstly, it is a significant source of both urban and rural population's water supply and secondly it sustains many wet land ecosystems.

Besides the shortage, drinking water may be contaminated by different contaminants which have an impact on the health and economic status of the consumers [1-3]. Contaminants such as bacteria, viruses, heavy metals, nitrates and salt have found their way into water supplies due to inadequate treatment and disposal of waste (human and livestock), industrial discharges, and over-use of limited water resources [4-5]. Even if no sources of anthropogenic contamination exist, natural sources are also equally potential to contribute higher levels of metals and other chemicals that can harm human health. This is highlighted recently in Bangladesh where natural levels of arsenic in groundwater were found to be causing harmful effects on the population [8-10].

In India, the dominant source of drinking water used to supply major urban and rural communities is from wells and springs. There are increasing indications of water contamination problems in some parts of the country. The major causes of this contamination could be seasonal effect, soil erosion, and domestic waste from urban and rural areas and industrial wastes. So far, no sufficient study has been conducted on heavy metal contamination of drinking water of the Govt. Kallar Hr.Sec Schools in Tamil Nadu. For this reason, due emphasis is given to the analysis of these contaminants. Heavy metals normally occurring in nature are not harmful to our environment because they are only present in very small amounts [11-12] However, if the levels of these metals are higher than the recommended limits, their roles change to a negative dimension. Human beings can be exposed to heavy metal ions through direct and indirect sources like food, drinking water, exposure to industrial activities and traffic. Drinking water is one of the important sources for heavy metals for humans. Concentration of the heavy metal ions in drinking water are generally at $\mu\text{g L}^{-1}$. The main goal of this paper is to determine the levels/concentration of some of the physicochemical parameters and heavy metals (Cu, Cr, Fe, Mn, Ni, and Zn) in drinking water in different parts of the Theni region, Tamil Nadu, India.



Fig.1. Drinking water source of Govt. Kallar Hr.Sec School- Uthamapuram.

The aim of the present study was to analyze the water quality parameters and compare with the WHO standards during pre and post monsoon seasons. The contaminated drinking water schools have been identified and implement the proper controlling measures to supply the quality drinking water to school children.

MATERIALS AND METHODS

A total of eight samples were collected from Govt. Kallar Hr.Sec. Schools located in Theni. The drinking water samples were collected during post and premonsoon seasons of year 2010-2012 in prewashed (with detergent, dilute HNO₃, doubly de-ionized distilled water respectively) double capped polyethylene bottles. In the field, the sampling bottles and caps were rinsed three times with the water to be sampled prior to sampling. Most of the samples were obtained directly from the tap after allowing the water to run for at least 5 min so as to stabilise the variation in EC and Temperature. Heavy metals (Cu, Cr, Fe, Ni, and Zn) analysis was done at analytical laboratory of Elico SL-173 AAS(14-15). All the other physico-chemical parameters testing of samples was done according to the procedure prescribed by APHA(1995). Electrical conductivity values were measured by using Elico digital conductivity meter. (Model No.L1 CM 180)¹¹ Exactly 25 mL of filtered water sample was taken separately, in a previously weighed (W₁), clean, dry china dish (100 mL capacity). Water samples were heated slowly to evaporate on a water bath until dryness. Further, the residues were dried in an air-oven at 105-110 °C for an hour and cooled in desiccators until the concordant weight (W₂) was obtained. Total dissolved solids present in water samples was calculated using the formula:

$$\text{Total Dissolved Solids} = [(W_1 - W_2) \times 10^3] / 25 \quad \text{----- 1}$$

where, W₁ = Weight of the empty china dish (g) W₂ = Weight of the china dish with solid residue Total hardness (TH) values were determined by using EDTA (Sodium salt of Ethylenediaminetetraacetic acid, Na₂H₂Y) complexometric titration method.

Permanent Hardness: Exactly 25 mL of water sample was taken separately in a clean, dry 100 mL beaker and placed on a hot plate to reduce the samples to half the volume [6]. After the reduction of volume the solutions were filtered and made up to 25 mL using DD water. The contents were transferred in to a clean conical flask, and 1 mL buffer solution (pH 10 buffer; ammonia and ammonium chloride) and two drops of Eriochrome black-T indicator were added. Further this mixture solution was titrated against 0.02N EDTA solution until the end point (wine red to distinct blue) was reached. From the observed volume of EDTA solutions, the permanent hardness (PHA) was calculated as CaCO₃ in mg/L or ppm. Standard error is ± 0.01 ppm and unit is ppm.

Magnesium (Mg²⁺) content present in water sample was determined by the complexometric method using EDTA as a Complexing agent. Sulphate -Exactly, 25 mL of water sample was taken separately in a clean, dry 100 mL beaker and 1 mL of (1:1) HCl solution was added. After boiling for about one minute, 5 mL of 5 % (w/v) BaCl₂ solution was slowly added with constant stirring. The precipitate was allowed to settle down for 12 h. Further, the precipitate was filtered through the previously weighed sintered glass crucibles (G-4), washed with hot water and the sintered glass crucible was dried in air-oven at 105 °C, cooled in a desiccator and weighed until the concordant value of weight was obtained. From the weight difference, the sulphate (SO₄²⁻) content was calculated by the following equation, in ppm.

$$\text{Sulphate, in ppm} = W_1 / V_1 \times 411.5 \quad \text{----- 2}$$

where, W₁ = Weight of the precipitate in mg/L; V₁ = Volume of samples; the standard error is ± 0.1 ppm and unit is ppm. Exactly, 25 mL of water sample was taken separately, in a clean, dry 250 mL conical flask and 1 mL of 20 % (w/v) KOH solution was added[] to bring the pH of the water to 12. Then, 0.5 g of on Pattern-Reeder's [2-hydroxy-1-(2-hydroxy-4-sulphonaphthylazonaphthoic acid)] indicator was added. The solution was titrated against standard EDTA (0.02N) until the end point (wine red to blue color). From the titer value, calcium (Ca²⁺) concentration was calculated, in ppm by using the following equation:

$$\text{Total calcium as CaCO}_3 \text{ (in ppm)} = [A \times B \times 400.8] / V \quad \text{----- 3}$$

where, A = Volume of EDTA consumed by the samples (mL); V = Volume of sample of water taken (mL); B = mg of CaCO₃ equivalent to 1 mL of 0.02N EDTA titrant at the calcium endpoint. Total

Alkalinity Alkalinity is the quantitative capacity of aqueous media to react with hydrogen ions. The alkalinity of natural or treated water is normally due to the presence of bicarbonate, Carbonate and hydroxide compounds of calcium, magnesium, sodium and potassium. Alkalinity can be determined by titrating the strong alkali (such as carbonate, free NaOH) to pH 8.3. At this pH all the free CO₂ is converted into bicarbonate. Exactly 25 mL of the sample¹⁴ of water was taken separately in a clean dry conical flask. Two drops of mixed indicator (Methyl red and Bromocresol green) solution were added. Pink color appeared and then the solutions were titrated against sulphuric acid (0.02N) solution until the end point (pink to colorless). From the observed titre value, the Total alkalinity (TA) was calculated as ppm of CaCO₃.

$$\text{Total alkalinity} = [V_2 \times 1 \times 100] / V_1 \quad \text{-----4}$$

where, V₁ volume of water sample (mL); V₂ Volume of (titer value) sulphuric acid (0.02N). Sodium and potassium were calculated by using flame photometer. The statistical tools were applied to draw meaningful conclusions from raw data set by using XLSTAT 2013-5. Box and whisker plot, cluster analysis, principal component analysis are used to evaluate the drinking water quality. Box and whisker plots are the indicator for seasonal and spatial variations in the water quality. It has 5 statistical summary such as the size and shape of the box which interpret the same or different range of parameters in 8 schools, upper and lower whisker interpret the highest and lowest value within 8 schools, first and third quartiles explain the mean values from highest and the lowest values, outliers explain extreme values during post and premonsoon seasons in 8 schools. Cluster analysis interpret the similar and dissimilar nature of water quality parameters, principal component and factor analysis explain the contaminated level of each parameters by their loading contribution as >.0.75 high, >0.5 medium and <0.5 minimum contribution in factor loadings. There are eight Govt. Kallar Hr.Sec schools in Theni District. People belonging to Piramalaikallar community are being given preference to study in Kallar Reclamation Schools which comes under the direct control of the district revenue officer, Department of Kallar Reclamation, Madurai.

Table 1. Name of the schools located in Theni District and their codes

School Code	
S1	Government Kallar Hr.Sec School at Karunakkamuthanpatti
S2	Government Kallar Hr.Sec School at Vellaiammalpuram
S3	Government Kallar Hr.Sec School at Uthamapuram
S4	Government Kallar Hr.Sec School at Melagudalur
S5	Government Kallar Hr.Sec School at MuthaiyanChettipatti
S6	Government Kallar Hr.Sec School at PuthuPatti
S7	Government Kallar Hr.Sec School at RajaDhani
S8	Government Kallar Hr.Sec School at Ethakovil

RESULTS AND DISCUSSION

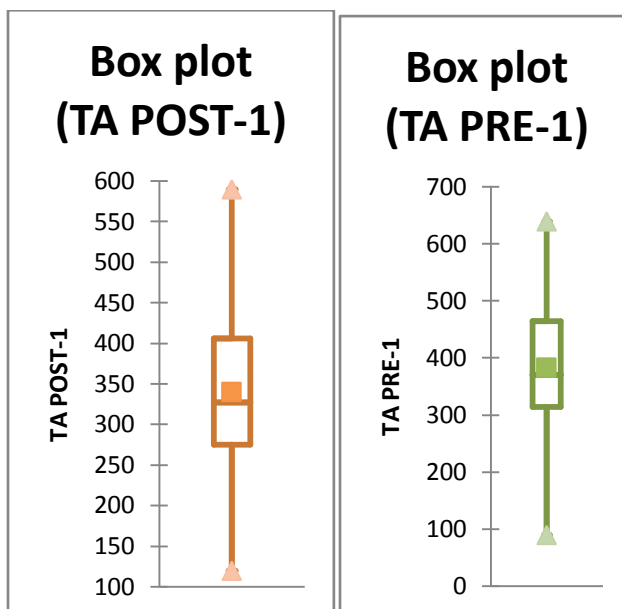
The analyzed results are summarized in the table 1 and subjected to statistical approach to draw meaningful conclusions. EC, TDS, TA, TH, F⁻, HCO₃, SO₄, Chloride, Ca, Mg and Na were higher than the permissible limits. So the drinking water is not potable and hence further treatment is needed before usage.

Table 2. Summarized water quality parameters of Theni district schools during post and premonsoon seasons of year 2010-2011

WQPs	Post-Monsoon 2010-11			Pre-Monsoon 2010-11		
	Min.	Max.	Avg.	Min.	Max.	Avg.
pH	7.2	8.2	7.937	6.7	8	7.575
EC	280	3040	1087.5	230	3410	1368.75
TDS	136	1365	517.625	118	1984	764.125
TA	120	590	340	90	640	383.125
TH	135	1080	413.125	115	1060	456.25
Cl⁻	11	496	131.625	14	454	155.625
SO₄²⁻	3.0	125	25.375	6.0	86	29.375
F⁻	0.42	1.8	1.08	0.4	1.9	1.168
HCO₃²⁻	146	720	414.875	110	781	467.375
NO₃	16	123	57.125	27	353	126.375
Ca²⁺	32	88	58.5	24	120	62.75
Mg²⁺	13	209	87.5	13	185	72.875
Na⁺	10	210	90.625	21	295	126
K⁺	1.0	20	4.25	1.0	19	4.625
DO	5.6	8.1	6.3375	5.6	8.1	6.85
COD	3.0	6.0	4.125	3.0	6.0	4.0
BOD	0.6	1.8	1.25	0.4	3.2	1.1875

*All the values are mg L⁻¹ except pH and EC

Box and whisker plots explain the seasonal and spatial variations in all the 8 schools in Theni district by its 5 statistical summaries such as mean, first quartile, third quartile, upper and lower whiskers, and outliers. The figures 1-4 reveal the Total alkalinity for all seasons of 8 schools show different statistical summaries. Like that all the other parameters are also different for both the seasons and 8 different schools.



Figs 2 and 3. Box and whisker plots for Total alkalinity of 8 schools in Theni District during post and premonsoon seasons of year 2010-2011.

Cluster analysis: As shown in figure-1 and 2, the cluster analysis reduced the 32 parameters into 5 and 6 clusters on the basis of their similarities and dissimilarities of their chemical characteristics of drinking water of all the 8 schools in Theni district during post and pre monsoon seasons of the year 2010-2011.

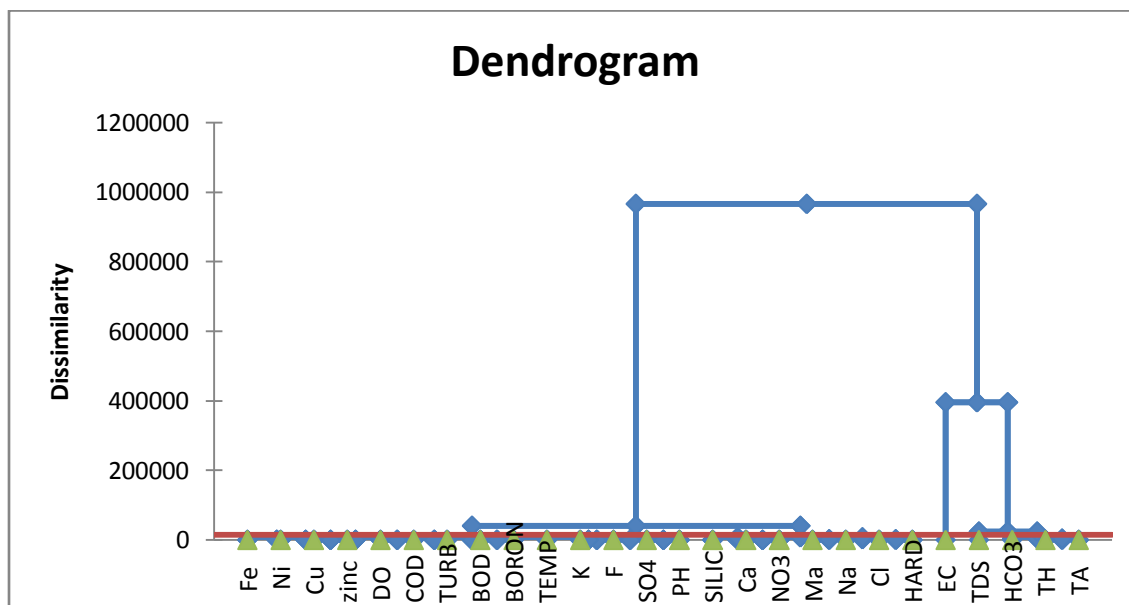


Fig-4 Dendrogram of water quality parameters of Theni district during post monsoon season of year 2010-2011

Table 3. Total clusters with water quality parameters during post monsoon season of year 2010-2011

Cluster-1	cluster-2	cluster-3	cluster-4	cluster-5
TH	Cl-	SO4 2-	EC	TDS
TA	Ca2+	K+		
HCO32-	Mg2+	F-		
	Na+	PH		
	NO3	Temperature		
	Hardness(Obs16		
	Silicate	DO		
		COD		
		BOD		
		Borane		
		COPPER		
		ZINC		
		IRON		
		NIKEL		

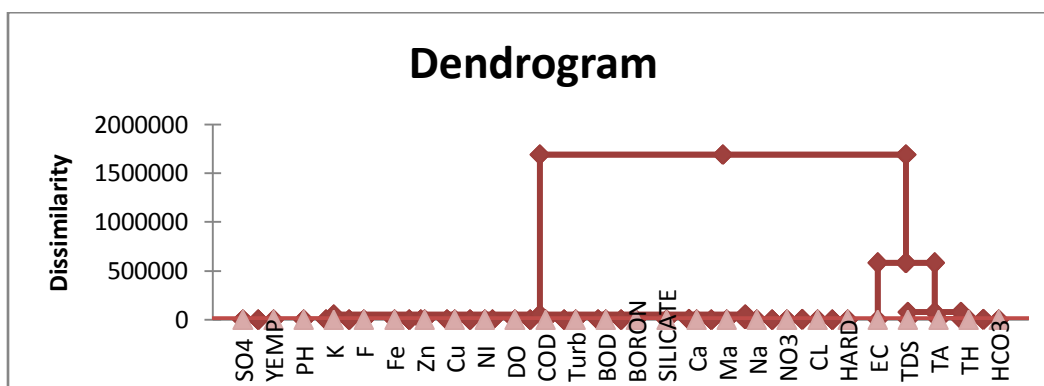


Fig 5. Dendrogram of water quality parameters for Theni District during pre monsoon season of year 2010-2011

Table 4. Total clusters with water quality parameters during pre monsoon season of year 2010-2011

clus-1	clus-2	clus-3	clus-4	clus-5	clus-6
TH	Cl	Ca	SO4	EC	TDS
TA	Na	Mg	K		
HCO3	NO3	SILICA	F-		
	HARD		PH		
			Temperature		
			Turbidity		
			DO		
			COD		
			BOD		
			Borane		
			COPPER		
			ZINC		
			IRON		
			NIKEL		

During post monsoon season, Cluster-1 comprises TH of the drinking water, cluster-2 comprises Cl-, Mg2+, Na, NO3- hardness (Ca, Mg), silicate which infers the high chlorination of water related with Na-Cl, Mg-Cl, and abundant earth crust silica, cluster-3 infers the total alkalinity with bicarbonate, cluster-3

comprises, large number of parameters which show large information about the water quality such as the critical fluoridation from KF, CaF₂, with favorable alkaline condition of optimum pH, and temperature, Heavy metals and biological properties explain the anthropogenic factors, agricultural run-off, and dissolution of minerals from the rock-water interaction [18-22]. Cluster-4 comprises EC which is the key indicator of all the analyzed parameters. Cluster-5 consists of TDS and infers the total dissolved chemicals and non-chemical substances and inform the salinity, brackish type and fresh type of water. The polluting range of clusters are in the increasing order as cluster -1 < cluster-2 < cluster -3 < cluster-4. EC and TDS are connected with all the other parameters and accounting the high polluting range of clusters. The alkalinity, salinity and the risk of fluoridation of water sources are explained by using cluster analysis.

Post monsoon vs Pre monsoon: During premonsoon the number of clusters is increased into six. These variations occurred only in the clustering of major ions but not in the EC, TDS, biological properties, heavy metals and favorable relation of fluoridation into water. Total alkalinity decreased in premonsoon than post monsoon. It infers that during post monsoon the ground water is recharge with rain water, the dissolution of minerals from rock-water interaction, and leachate of other degradable materials into ground water. Thus the cluster analyses show the new frontier knowledge about the characters of the drinking water during post and premonsoon seasons of the study period.

Table-5 and 6. Factor scores and % of contribution of water quality parameters for post Monsoon.

	Contribution of the observations (%):	
	F1	F2
TH	0.879	0.121
Cl-	0.528	0.472
TA	0.933	0.067
Ca ²⁺	0.255	0.745
Mg ²⁺	0.418	0.582
SO ₄ ²⁻	0.095	0.905
Na ⁺	0.394	0.606
K ⁺	0.021	0.979
HCO ₃ ²⁻	0.919	0.081
F ⁻	0.006	0.994
PH	0.045	0.955
Temperat	0.119	0.881
EC	0.516	0.484
TDS	0.460	0.540
NO ₃	0.956	0.044
Turbidity	0.943	0.057
Hardness(0.354	0.646
DO	0.996	0.004
COD	0.976	0.024
BOD	0.951	0.049
Borane	0.923	0.077
Silicate	0.711	0.289
COPPER	0.864	0.136
ZINC	0.839	0.161
IRON	0.806	0.194
NIKEL	0.798	0.202
	F1	F2
Eigenvalu	1.257	0.743
Variability	62.846	37.154
Cumulativ	62.846	100.000

	Contribution of the observations (%):	
	F1	F2
TH	9.052	2.101
Cl-	3.802	5.748
TA	7.460	0.907
Ca ²⁺	1.339	6.606
Mg ²⁺	1.656	3.892
SO ₄ ²⁻	0.368	5.898
Na ⁺	0.916	2.384
K ⁺	0.053	4.248
HCO ₃ ²⁻	4.451	0.662
F ⁻	0.010	2.654
PH	0.052	1.877
Temperat	0.083	1.037
EC	26.189	41.489
TDS	3.827	7.611
NO ₃	0.397	0.031
Turbidity	1.194	0.122
Hardness(0.241	0.744
DO	1.937	0.014
COD	2.460	0.102
BOD	3.057	0.265
Borane	3.681	0.522
Silicate	2.934	2.013
COPPER	5.032	1.341
ZINC	5.807	1.878
IRON	6.479	2.634
NIKEL	7.525	3.221

From the above trends it is clear that the water samples collected contains more amounts of salinity, alkalinity, and also temporary hardness. The high loading of bicarbonate, chloride, heavy metals, nitrate and biological pollutions are due to improper maintenances of water tanks, improper usages of agricultural fertilizers and anthropogenic sources. The major ions, pH, and temperature accounts for the permanent hardness of drinking water and the possibility of abnormal fluoridation. The same trend has also been observed for both post and pre monsoon seasons.

Principal component analysis: As shown in table 5 to 8 the principal component/ Factor analysis explained the dominant parameters comprised in factor-1 and moderately loaded parameters are in factor-2. For the post monsoon season, factor-1 explains 63.4% of total variance of the following water quality parameters in descending order as DO > COD > BOD > Turb > boron > TA > HCO_3^- > TH > NO_3^- > Cu > Zn > Fe > TA > Cl > EC > . Factor -2 explains 37.5% of the total variance of the following the water quality parameters in descending order as F > K > PH > SO_4^{2-} > Na > Ca²⁺ > Mg > Temp > hardness > TDS [23-24].

For the pre monsoon season factor 1 explains 62.4% of total variance of the following the water quality parameters in descending order as DO > COD > BOD > NO_3^- > HCO_3^- > TH > Turb > boron > TA > Cu > Zn > Fe > Cl > EC > . Factor -2 explains 37.1% of the total variance of the following the water quality parameters in descending order as F > PH > K > Temp > SO_4 > Ca > Mg > Na > TDS.

Table-7 and 8. Factor scores and % of contribution of water quality parameters for pre monsoon

Squared cosines of the observations:			Contribution of the observations (%):		
	F1	F2		F1	F2
TH	0.944	0.056	TH	11.303	1.051
Cl-	0.464	0.536	Cl-	3.429	6.260
TA	0.774	0.226	TA	5.196	2.387
Ca ²⁺	0.215	0.785	Ca ²⁺	1.191	6.852
Mg ²⁺	0.283	0.717	Mg ²⁺	1.201	4.804
SO ₄ ²⁻	0.197	0.803	SO ₄ ²⁻	0.691	4.430
Na ⁺	0.383	0.617	Na ⁺	0.918	2.327
K ⁺	0.029	0.971	K ⁺	0.072	3.885
HCO ₃ ²⁻	1.000	0.000	HCO ₃ ²⁻	2.286	0.000
F-	0.004	0.996	F-	0.006	2.441
PH	0.038	0.962	PH	0.043	1.718
Temperat	0.114	0.886	Temperat	0.079	0.967
EC	0.517	0.483	EC	25.641	37.817
TDS	0.470	0.530	TDS	7.075	12.605
NO ₃	0.631	0.369	NO ₃	0.081	0.074
Turbidity	0.951	0.049	Turbidity	1.167	0.094
Hardness(0.735	0.265	Hardness(0.534	0.304
DO	0.993	0.007	DO	1.916	0.023
COD	0.970	0.030	COD	2.446	0.119
BOD	0.943	0.057	BOD	3.047	0.288
Borane	0.913	0.087	Borane	3.674	0.549
Silicate	0.707	0.293	Silicate	2.988	1.952
COPPER	0.855	0.145	COPPER	5.060	1.353
ZINC	0.829	0.171	ZINC	5.830	1.892
IRON	0.798	0.202	IRON	6.546	2.606
NIKEL	0.789	0.211	NIKEL	7.581	3.202
	F1	F2			
Eigenvalu	1.224	0.776			
Variability	61.199	38.801			
Cumulativ	61.199	100.000			

In figure [6], the scattered diagram derived from the above two factors and get the clear idea about the grouping and loading influence of the parameters. First quarter represents HCO_3^- EC and TDS, which support the temporary hardness of water. Second quarter represents the major ions which support the alkalinity, permanent harness of water. Third quarter represents the heavy metals, soil characters, biological properties. Fourth quarter explains pH, F^- , temperature, NO_3^- and turbidity. It supports the high load of F^- , NO_3^- which depend pH and temperature. Alkaline nature of water increases the dissolution of fluoride into water [25-29].

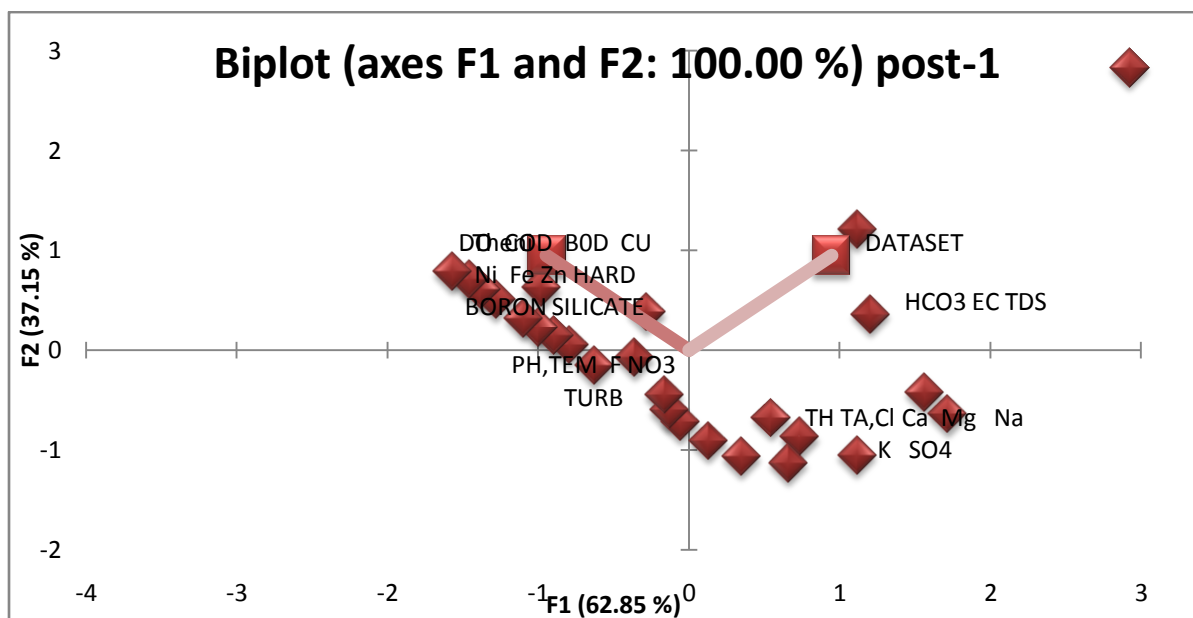


Fig-6 Rotated factor analysis for water quality parameters.

Heavy metals are dangerous because they tend to bio accumulate. Heavy metals are the major category of toxic pollutants, globally distributed in water bodies, which have been extracted from earth’s crust [30-36]. Heavy metal can enter a water supply by industrial and consumer waste, or even from acidic rain breaking soils and releasing heavy metals into stream, lakes and ground. Its abundance, distribution, sources, varies with ground water town ship in Theni district, Tamil Nadu are of this study. Seasonal variations of heavy metals in ground water of different sampling sites are shown in fig 7.

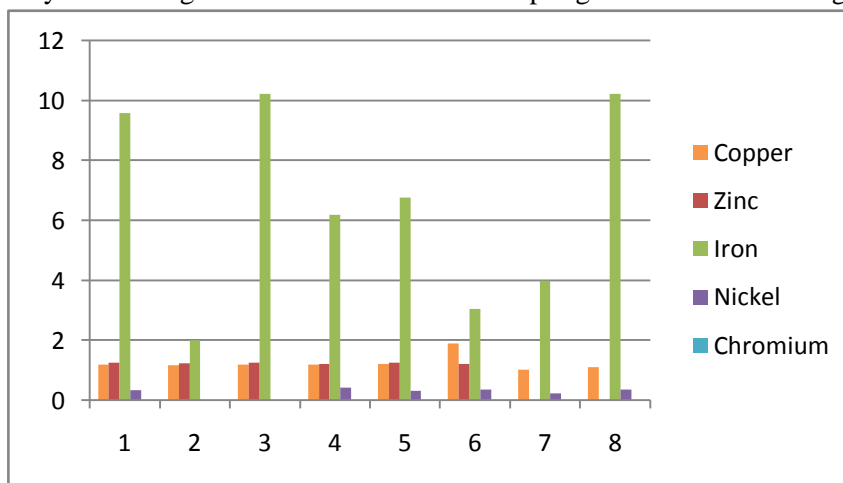


Fig-7 Distribution of Heavy Metals of Theni District schools during 2010-2011 Post Monsoon

APPLICATIONS

These Multivariate statistical methods can be applied to find the spatial variation in 8 schools. All the schools are applied as dependent variables for each parameter in post and premonsoon periods. Seasons is applied as independent variables. This might be applied to draw meaningful conclusion about the seasonal effect in water quality.

CONCLUSIONS

The main goal of this paper is to assess the status of drinking water quality in Government Kallar Hr.Sec. Schools located in rural pockets of Theni District, Tamil Nadu, with special emphasis on fluoride contamination and heavy metals. These results show that the concentration of major ions particularly fluoride exceeds the permissible limit during post monsoon season mainly because of mineral dissolution from the geogenic rock in ground water. During rainy season, the water table rise is accompanied by high concentrations of minerals. The above results are supported by Multivariate statistical tools such as rotated factor analysis, principal component and cluster analysis. Box and whiskers plots, by means of five statistical summaries, show the seasonal and spatial variations for each parameter. Moreover, the quality of water during post monsoon is not potable as against pre monsoon due mainly to the dissolutions of minerals. Cluster analysis with its cluster pattern of the analyzed parameters confirmed the quality of water. Principal component analysis has also supported the contribution of individual parameter to determine the hardness, high salinity, and high concentration of fluoride which is favored by alkaline nature of the drinking water. Thus the statistical tools are used to draw the meaningful conclusion about the quality of water. All the samples were analyzed for five heavy metals (Cu, Cr, Fe, Ni, and Zn) using standard procedures and the amount of heavy metals is found to exist within the permissible limits. The order of concentration of heavy metals during the both post monsoon and pre monsoon seasons of the year 2010-2011 follows the order : Iron > Zinc > Copper and Nickel = Chromium. Of the 8 schools, only 3 schools at Uthamapuram, Melagudalur and Muthayanchettipatty are highly affected by high level of fluoride concentration and almost in all schools the water meant for drinking purpose is not fit to drink because of the hardness that results from more amount of major ions. To rectify this problem some innovative techniques are needed and we have planned to do that work in the near future in our further studies.

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