Statistical analysis on physical and chemical parameters andheavy metals in pond water

Dr.Yaser Qureshi
Asstt. Professor
Govt. College KherthaDistt. Balod (Chhattisgarh)

Abstract. The study attempts to determine the distribution of heavy metal pollution and to identify the physical and chemical water analysis parameters concerning water quality status along Budhasagar Pond of Rajnandgaon from 2015-16 to 2016-17. There are several freshwater parameters considered which are temperature, total hardness, chloride, pH and dissolved Oxygen (DO), and heavy metals (mercury, lead, chromium, cadmium, and iron) which affect the quality of fresh water. Kendall's tau correlation and Friedman'sANOVA test were employed to analyze statistical results. Significant correlations were seen between different freshwater parameters and heavy metals. wecan conclude that at least one of the five types of investigated metal has a statistically significant effect under seasons. Also, the median response for Iron is substantially higher.
Keywords: Heavy metals, Freshwater, Pond, Statistical analysis

1. Introduction: Heavy metals are considered pollutants of concern in freshwater bodies due to their cancer-causing effect, mutagenic activity, and noxious impact. They are found in every ecosystem of the world. The most threatening effect of them is their ability to bioaccumulation and bioconcentration in living systems. They are also called trace metals because of their lower quantity requirement by different flora and fauna.

Heavy metals can be categorized into two main groups – essential and non-essential heavy metals. Some heavy metals like iron, zinc, chromium, copper, and manganese play a vital role in the proper functioning of body organs because of their role in metabolism. If their concentration exceeds an optimum level, they pose health risks to the body (Unger, 2002).
Although all Heavy metals are toxic above a certain level, mercury, cadmium, and lead posea greater risk and are considered lethal toxicants (Baskaran et al., 1990).Heavy metal pollution is one of the highly discussed environmental problems for its harmful effects on different living beings. One of the biggest threats due to heavy metals is their tendency to bioaccumulate/bioconcentrate. Once they enter the food chain, they are transferred to the upper trophic level with higher concentration, so their effect of bioaccumulation is seen easily in the food chain at the topmost trophic levels (Kalay, 2000).
The incorporation of these Heavy metals in flora and fauna depends on their concentration, the different physiological effects of metal, and the physiochemical nature of the water (Gharib et al., 2003).
Developed countries have pulled up their socks and restricted the discharge of industrial and agricultural wastes into water bodies, but in developing countries like ours, there is a lack of regulation as well as the implementation of the few rules that do exist regarding the discharge of harmful waste into water bodies. On top of this, the pressure of fast industrialization and rapid urbanization comes at the cost of ignorance of the environmental cost of this rapid development. Urban water bodies are at greater risk of contamination, and it is the urban water bodies that pose greater health risks to human lives as they are used for activities such as bathing, cleaning dishes, laundry, and sometimes, as drinking water for pets and humans. Out of the various types of obnoxious wastes that contaminate urban water bodies, heavy metals released from industrial sources are the most toxic of all.

The term "heavy metals" denotes elements with an atomic density of more than 4 g./c.m.^{3}. Some metals and metalloids come under this category (Nriagu, 1988). The term is generally used to refer to metals like mercury (Hg), Cadmium (Cd), Silver (Ag), Iron (Fe), Chromium (Cr), Lead (Pb), and Copper (Cu).
On the opposite end of the spectrum, the international union of Pure and applied chemistry calls 'heavy metals' a meaningless term (Duffus, 2002).

2. Materials and methods :

2.1 Study area :

BudhasagarPond is situated in Latitude: 21.093269N Longitude: 81.028455E at Rajnandgaon town of Chhattisgarh State. Chhattisgarh is the 26th State of India, it was carved out of Madhya Pradesh on 1st November 2000. Located in central India, it covers an area of 135,133 sq km. Raipur is the capital of Chhattisgarh. The state shares borders with Bihar, Jharkhand, and Uttar Pradesh in the north, Andhra Pradesh and Telangana in the south, Orissa in the east, and Madhya Pradesh in the west. The state lies at 17°46' N to 24°5'North latitude and 80°15' E to 84°20' East longitude.
Rajnandgaon district is a part of the Durg Commissionaire Division of Chhattisgarh State. Rajnandgaon District comprises nine blocks namely Chhuikhadan, Khairagarh, Dongargarh, Rajnandgaon, AmbagarhChowki, Manpur, Dongargaon, and Mohla. Rajnandgoan City is situated between Durg and Dongargarh railway stations. Rajnandgaon City is situated on the Mumbai Howrah railway line. National highway no. 53 passes across it. BudhasagarPond is situated in the municipal area of Rajnandgaon town. It is the district headquarter of Rajnandgaon district and is situated on the east border of Durg District and lies at a latitude 21° 06' to 21° 29' North and 81°23' to 81°29' longitude East. Rajnandgaon district has 8022.55 sq. km. of Geographical area. Rajnandgaon town stands at 330.78 meters above sea level.

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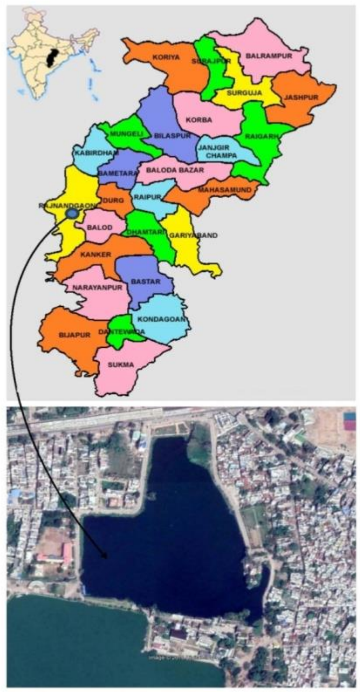
Map 1: Budhasagar Pond, Rajnandgaon, Chhattisgarh, India



2.2 Data preparation

2.2.1. Physicochemical analysis of water

For the physicochemical analysis, standard methods as suggested by Trivedy and Goel (1986) and APHA (2005) were followed. The Physicochemical parameters analyzed for the present study include water temperature, pH, dissolved oxygen, chloride, and total hardness.

a. Water Temperature: The temperature of surface water was recorded on the spot by using a graduated Celsius Thermometer. The bulb of the thermometer was dipped in water for at least two minutes for obtaining the water temperature. The results were expressed in ºC.

b. pH: The pH of the water sample was determined using a composite digital device, a digital pH meter.

c. Dissolved Oxygen: The dissolved oxygen was determined by Winkler's method (Winkler, 1888).

d. Total Hardness: it was determined by Buffer solution, Eriochrome Black T, and Standard EDTA solution

e. Chloride:it was determined by Potassium chromate indicator and Silver nitrate solution

2.2.2. Heavy metal analysis

Heavy metals analysis of fish samples was done by Inductively coupled plasma-optical emission spectrometry (ICP-OES). Heavy metals such as lead, cadmium, chromium, iron, and mercury were selected for the study. Samples were digested and analyzed using inductively ICP-OES (Perkin Elmer, Optima 4100DV) according to the method described in the American Public Health Association (2005) and the United States Department of Agriculture (2008).

Statistical Analysis

Descriptive statistics such as average, range, standard deviation, and standard error values were calculated. Friedman test was applied as a nonparametric alternative to repeated measure Analysis of variance (ANOVA) to compare three mated groups. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$).

The nonparametric Kendall tau correlations were used to examine relationships among tested parameters. The Kendall Tau rank correlation coefficient (or simply the Kendall Tau coefficient, Kendall's τ or Tau test is used to measure the strength of the relationship (or the degree of correlation) between two variables. It is feasible to carry out the hypothesis tests (assessing the significance) with the help of Kendall's tau.

Interpretation of output of Kendall's tau (τ):

The interpretation of the strength of the correlation of Kendall's tau was based on the following:

- 0.00 correlation indicates no correlation i.e. there is no relationship between the two variables
- 0.00 - 0.20 Very Weak
- 0.21 - 0.40 Weak
- 0.41 - 0.60 Moderate
- 0.61 - 0.80 Strong
- 0.81 - 1.00 Very Strong

Kendall's tau is a measure of correlation that is used to quantify the strength and direction of the association between two variables. It is a non-parametric measure, which means that it does not assume any particular form for the distribution of the data.

Kendall's tau is often used when the data being analyzed consists of ranked or ordinal variables. The basic idea behind Kendall's tau is to compare the number of concordant and discordant pairs of observations in the two variables being compared.

A concordant pair is one in which the values of both variables increase or decrease together. A discordant pair is one in which the values of one variable increase while the values of the other variable decrease, or vice versa.

Kendall's tau takes on values between -1 and 1. A value of 1 indicates a perfect positive correlation (i.e., as one variable increases, the other variable increases), while a value of -1 indicates a perfect negative correlation (i.e., as one variable increases, the other variable decreases). A value of 0 indicates no correlation between the variables.

Kendall's tau is a useful measure of correlation when the data being analyzed is not normally distributed, or when the relationship between the variables being analyzed is not linear. It is also robust to outliers and can be used with small sample sizes

All statistical calculations were performed with Statistical Package for the Social Sciences (SPSS) 16.0, Minitab 18. MS Excel Toolpak (2007) was used for descriptive statistics. MS Excel (2007) was used to plot graphs.

3: Results and Discussion

Statistical Analysis

Correlation analysis

Correlation analysis was performed to show the relationship between different variables. It was done by SPSS 16.0 and Minitab 18 software. The results are presented in the following tables.

In the year 2015-16 summer season very strong and significant correlation ($p < 0.01$) was found between temperature and chloride, temperature and chromium total hardness and pH, and chromium and chloride (τ

= 1), the strong correlation was found between dissolved oxygen and temperature, dissolved oxygen and chloride, iron and dissolved oxygen, chromium, and dissolved oxygen (τ = 0.81) and weak correlation found between other variables.

Table 1: Correlation matrix among physio-chemical parameters and metal concentration during summer, the year 2015-16

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.33	1.00								
DO	0.81	0.00	1.00							
TH	0.33	1.0**	0.00	1.00						
CL	1.0**	0.33	0.81	0.33	1.00					
Hg	-	-	-	-	-	1.00				
Pb	-	-	-	-	-	-	-			
Fe	0.33	0.33	0.81	0.33	0.33	-	-	1.00		
Cd	-	-	-	-	-	-	-	-	-	
Cr	1**	0.33	0.81	0.33	1.0**	-	-	0.33	-	1.00

**Correlation is significant at the< 0.01 level (2 -tailed)

In the year 2015-16, post-monsoon season very strong and significant correlation ($p < 0.01$) was found between temperature and chloride, cadmium and pH, dissolved oxygen and iron, chromium, and dissolved oxygen, total hardness, and mercury and iron and chromium (τ = 1), the strong correlation found between total hardness and temperature, mercury and temperature, dissolved oxygen and pH, iron and pH, chromium and dissolved oxygen (τ = 0.81) moderate to weak correlation found between other variables.

Table 2: Correlation matrix among physio-chemical parameter and metal concentration during the post-monsoon year 2015 -16

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.00	1.00								
DO	0.33	0.81	1.00							
TH	0.81	0.50	0.00	1.00						
CL	1.0**	0.00	0.33	0.81	1.00					
Hg	0.81	0.50	0.00	1.0**	0.81	1.00				
Pb	-	-	-	-	-	-	1.00			
Fe	0.33	0.81	1.0**	0.00	0.33	0.00	-	1.00		
Cd	0.00	1.0**	0.81	0.50	0.00	0.50	-	0.81	1.00	
Cr	0.33	0.81	1.0**	0.00	0.33	0.00	-	1.0**	0.81	1.00

**Correlation is significant at the< 0.01 level (2 –tailed)

In the year 2015-16 in the winter season very strong and significant correlation ($p < 0.01$) was found between temperature and chloride, pH and dissolved oxygen, total hardness and pH, iron and pH, total hardness and dissolved oxygen, iron, and dissolved oxygen, and iron and total hardness ($\tau = 1$), other variable shows weak to moderate correlation.

Table 3: Correlation matrix among Physio-chemical parameters and metal concentration during the winter year 2015 -16

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.33	1.00								
DO	0.33	1.0**	1.00							
TH	0.33	1.0**	1.0**	1.00						
CL	1.0**	0.33	0.33	0.33	1.00					
Hg	-	-	-	-	-	1.00				
Pb	-	-	-	-	-	-	1.00			
Fe	0.33	1.0**	1.0**	1.0**	0.33	-	-	1.00		
Cd	-	-	-	-	-	-	-	-	1.00	
iCr	-	-	-	-	-	-	-	-	-	1.00

**Correlation is significant at the< 0.01 level (2 -tailed)

In the year 2015-16 in gill tissue,a very strong and significant correlation ($p < 0.01$) was found between temperature and pH, temperature and chloride, pH and chloride, dissolved oxygen and total hardness, iron, and dissolved oxygen, and iron and total hardness. ($\tau = 1$), other variable shows weak to moderate correlation.

Table 4: Correlation matrix among Physio-chemical parameter and metal concentration Year 2016 -17 in Summer

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.00	1.00								
DO	0.81	0.50	1.00							
TH	0.33	0.81	0.00	1.00						
CL	1.0**	0.00	0.81	0.33	1.00					
Hg	-	-	-	-	-	1				
Pb	0.33	0.81	0.81	0.33	0.33	-	1.00			
Fe	0.33	0.81	0.81	0.33	0.33		1.0**	1.00		
Cd	1.0**	0.00	0.81	0.33	1.0**		0.33	0.33	1.00	
Cr	0.33	0.81	0.81	0.33	0.33		1.0**	1**	0.33	1.00

**Correlation is significant at the< 0.01 level (2 -tailed)

In the year 2016-17, in the post-monsoon season, very strong and significant correlation ($p < 0.01$) was found between temperature and chloride, cadmium and dissolved oxygen, iron and lead and chromium, and total hardness, ($\tau = 1$), other variable shows weak to moderate correlation.

Table 5: Correlation matrix among Physio-chemical parameters and metal concentration Year 2016 -17 in Post-monsoon

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.81	1.00								
DO	0.00	0.50	1.00							
TH	0.33	0.81	0.81	1.00						
CL	1.0**	0.81	0.00	0.33	1.00					
Hg	-	-	-	-	-	1				
Pb	0.33	0.00	0.81	0.33	0.33	-	1.00			
Fe	0.33	0.00	0.81	0.33	0.33	-	1.0**	1.00		
Cd	0.00	0.50	1.0**	0.81	0.00	-	0.81	0.81	1.00	
Cr	0.33	0.81	0.81	1.0**	0.33	-	0.33	0.33	0.81	1.00

**Correlation is significant at the< 0.01 level (2 -tailed)

In the year 2016-17, in the winter season very strong and significant correlation ($p < 0.01$) was found between temperature and total hardness, chloride and temperature, chloride and total hardness, lead and dissolved oxygen, iron and mercury, cadmium, and pH, chromium and dissolved oxygen and chromium and, ($\tau = 1$), other variable shows weak to moderate correlation.

Table 6: Correlation matrix among Physio-chemical parameter and metal concentration Year 2016 - 17 in Winter

Vari.	Tem	pH	DO	TH	CL	Hg	Pb	Fe	Cd	Cr
Tem	1.00									
pH	0.00	1.00								
DO	0.33	0.81	1.00							
TH	1.0**	0.00	0.33	1.00						
CL	1.0**	0.00	0.33	1.0**	1.00					
Hg	0.33	0.81	0.33	0.33	0.33	1.00				
Pb	0.33	0.81	1.0**	0.33	0.33	0.33	1.00			
Fe	0.33	0.81	0.33	0.33	0.33	1.0**	0.33	1.00		
Cd	0.00	1.0**	0.81	0.00	0.00	0.81	0.81	0.81	1.00	
Cr	0.33	0.81	1.0**	0.33	0.33	0.33	1.0**	0.33	0.81	1.00

**Correlation is significant at the< 0.01 level (2 -tailed)

In the year 2016-17, in gill tissue very strong and significant correlation ($p < 0.01$) was found between temperature and pH, dissolved oxygen and temperature, dissolved oxygen and pH, chloride and temperature, chloride and pH, chloride, and dissolved oxygen, lead and temperature, lead, and pH, lead and dissolved oxygen, lead and chlorine, chloride, and total hardness, lead and dissolved oxygen, chromium and temperature, chromium and pH, chromium and dissolved oxygen, chromium and chloride, and chromium and lead, ($\tau = 1$), other variable shows weak to moderate correlation.

Repeated measure ANOVA (Friedman's test):

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Friedman's test is a non-parametric alternative to repeated measures ANOVA. It does not assume normality or equal variances and is more robust to outliers and non-normality. It tests the null hypothesis that there is no significant difference between the median values of the repeated measures across the different conditions. If the null hypothesis is rejected, posthoc tests can be conducted to determine which specific conditions are significantly different from each other.

For the year 2015-16

1) Response = summer, treatment: metal type, Block = (Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.0	6.0
Chromium	3	2.2	12.0
Iron	3	390.9	15.0
Lead	3	0.0	6.0
Mercury	3	0.0	6.0

Grand Median = 78.6

S = 9.60 DF = 4 P = 0.048

S = 12.00 DF = 4 P = 0.017 (adjusted for ties)

Here p-value = 0.048 (0.017 under adjusted for ties) is less than the significance level of 0.05, So we conclude that at least one of the 5 types of metal has a statistically significant effect for the summer season under the 2015-16 years. Also, the median response for Iron is substantially higher.

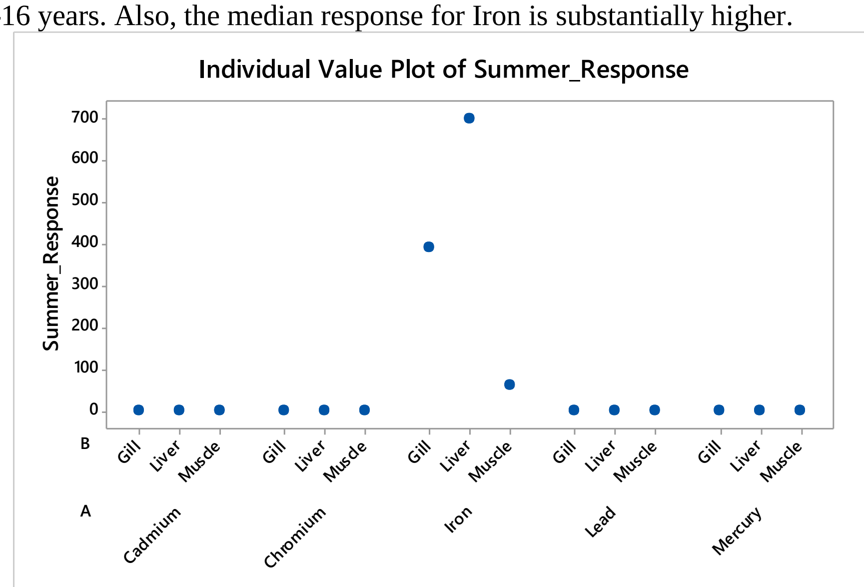

Figure 1 (The year 2015-16 ANOVA Friedman test during summer)

2) Response = post-monsoon, treatment metal type, Block = (Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.0	6.5
Chromium	3	2.6	12.0
Iron	3	312.0	15.0
Lead	3	0.0	5.0
Mercury	3	0.0	6.5

Grand Median = 62.9
S = 9.80 DF = 4 P = 0.044
S = 10.89 DF = 4 P = 0.028 (adjusted for ties) Here p-value =0.044 (0.028 under adjusted for ties) is less than the significance level of 0.05, So we conclude that at least one of the 5 types of metal has a statistically significant effect for the post-monsoon season under the 2015-16 year. Also, the median response for Iron is substantially higher.

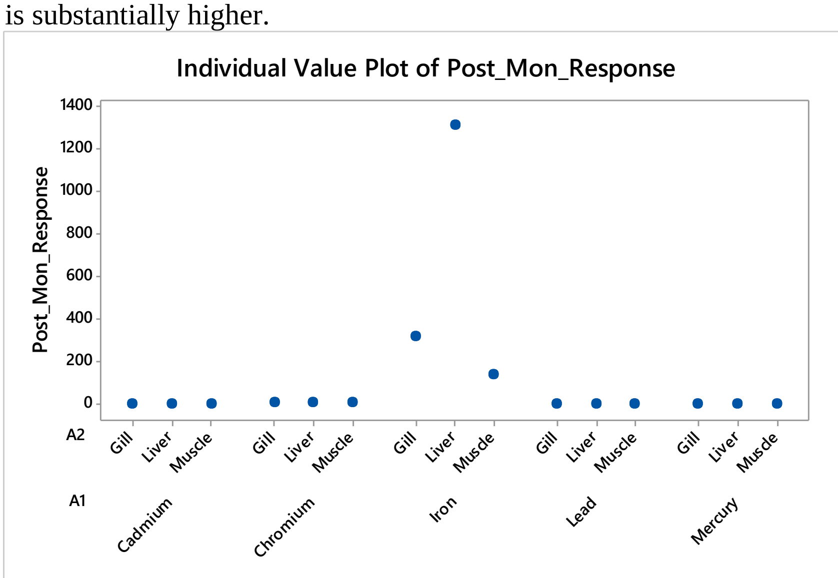

Figure 2 (The year 2015-16 ANOVA Friedman test during post-monsoon)

3) Response = winter, treatment: metal type, Block = (Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.0	7.5
Chromium	3	0.0	7.5

Iron	3	53.80	15.0
Lead	3	0.0	7.5
Mercury	3	0.0	7.5

Grand Median = 10.76
S = 6.00 DF = 4 P = 0.199, S = 12.00 DF = 4 P = 0.017 (adjusted for ties)
Here p-value = 0.199 is more than the significance level of 0.05, But here P-value is 0.017 under adjusted for ties Which is less than the significance level of 0.05. So we conclude that at least one of the 5 types of metal has a statistically significant effect for the winter season under the 2015-16 year under adjusted for ties and all the 5 types of metals have the same effect under non-adjusted ties. Also, the median response for Iron is substantially higher.

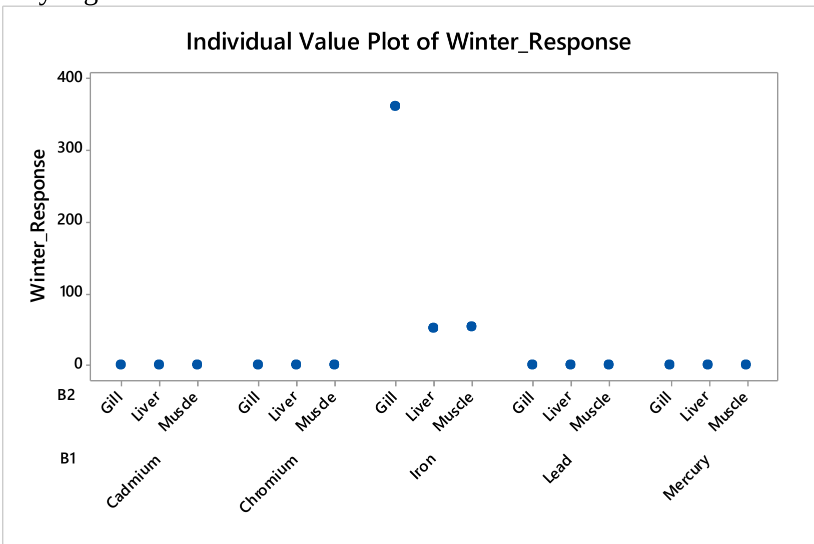

Figure 3 (The year 2015-16 ANOVA Friedman test during winter)

For the year 2016-17

1) Response = Summer, Treatment: Metal types, Block=(Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.4	5.5
Chromium	3	1.3	10.0
Iron	3	336.0	15.0
Lead	3	3.8	11.0
Mercury	3	0.1	3.5

Grand Median = 68.3
S = 11.32 DF = 4 p = 0.025

S = 11.32 DF = 4 p = 0.023 (adjusted for ties) Here p values = 0.025 (0.023 under adjusted for ties) is less than the significance level of 0.05. So we conclude that at least one of the 5 types of metal has a statistically significant effect on the summer season under the 2016-17 year. Also, the median response for Iron is substantially higher.

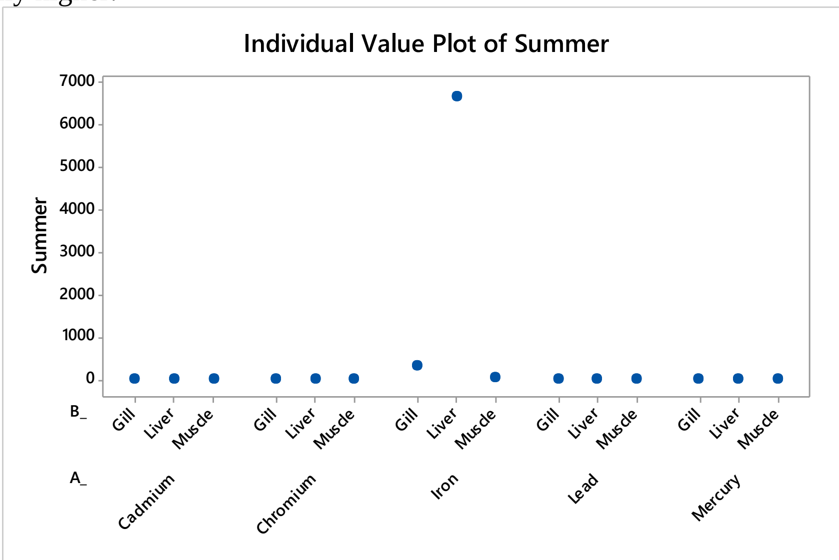

Figure 4 (The year 2016-17 ANOVA Friedman test during summer)

2) Response= Post-monsoon, Treatment: Metal types, Block= (Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.1	5.0
Chromium	3	2.3	10.0
Iron	3	347.0	15.0
Lead	3	2.9	11.0
Mercury	3	0.0	4.0

Grand median = 70.5
S = 10.93 DF = 4 P = 0.027
S = 11.31 DF = 4 P = 0.023 (adjusted for ties)
Here P-values=0.027 (0.023 under adjusted for ties) is less than the significance level of 0.05. So we conclude that at least one of the 5 types of metal has a statistically significant effect on the Post-monsoon season during the 2016-17 years. Also, the median response for Iron is substantially higher.

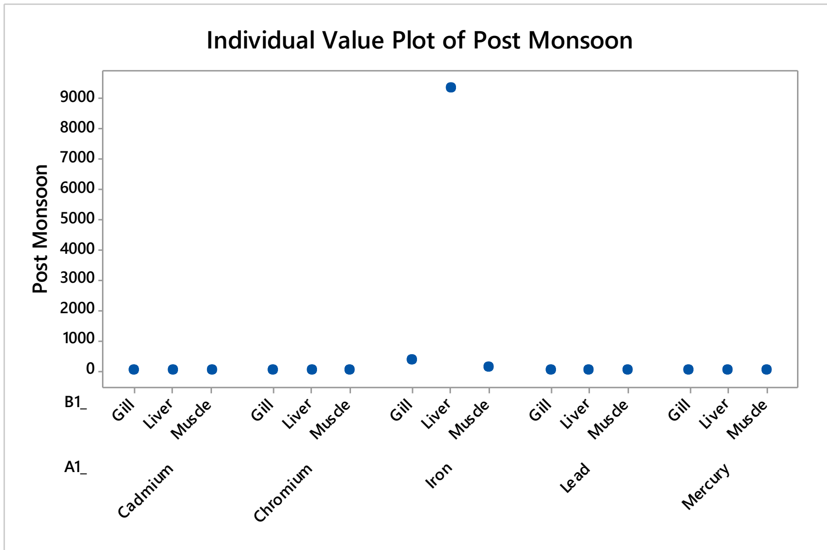

Figure 5 (The year 2016-17 ANOVA Friedman test during post-monsoon)

3) Response= winter season, Treatment: Metal types, Block= (Gill, Liver, Muscle)

A	N	Est. Median	Sum of Ranks
Cadmium	3	0.0	3.0
Chromium	3	3.7	12.0
Iron	3	286.0	15.0
Lead	3	2.9	8.0
Mercury	3	0.1	7.0

Grand Median = 58.8

S = 11.47 DF = 4 p = 0.022

Here p values=0.022 are less than the significance level of 0.05. So we conclude that at least one of the 5 types of metal has a statistically significant effect on the winter season under the 2016-17 years. Also, the median response for Iron is substantially higher.

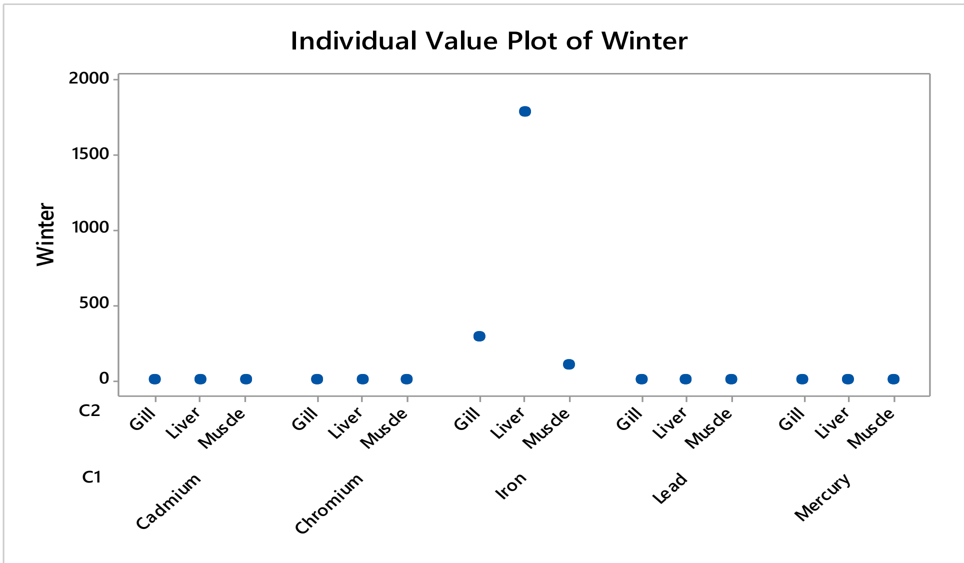

Figure 6 (The year 2016-17 ANOVA Friedman test during winter)

Association between responses for the year 2015-16 and 2016-17 by Kendall's rank correlation tau (τ)

1) For the summer season

z = 3.1227, p-value = 0.001792, tau (τ) = 0.6654578

Conclusion: Here p-value of the test is 0.001792 which is less than the significance level of 0.05. So, we conclude that responses for the summer season for different years are significantly associated. Here Kendall's tau (τ) correlation coefficient is 0.6655 which concludes that both responses for the summer season under different years are significantly associated.

2) For the post-monsoon season

z = 2.7801, p-value = 0.005434, tau (τ) = 0.5821058

Conclusion: Here p-value of the test is 0.005434 which is less than the significance level of 0.05. So, we conclude that responses for the post-monsoon season for different years are significantly associated. Here Kendall's tau (τ) correlation coefficient is 0.5821 which concludes that both responses for the post-monsoon season under different years are significantly associated.

3) For the winter season

z = 2.5045, p-value = 0.01226, tau (τ) = 0.549565

Conclusion: Here p-value of the test is 0.0123 which is less than the significance level of 0.05. So, we conclude that responses for the winter season for different years are significantly associated. Here Kendall's tau (τ) correlation coefficient is 0.5496 which concludes that both responses for the winter season under different years are significantly associated.

4. Conclusion

A significant and strong correlation has been observed between water temperature and pH during the post-monsoon season ($p \leq 0.05$) while a weak correlation during the winter season. A positive correlation between water temperature and pH has also been observed by Kamal et al.(2007), while a very weak correlation between them has been observed by Ogundiran and Afolabi (2008), Shyamala et al.(2008) and Sithik et al.(2009). Water temperature shows a moderate and weak correlation with dissolved oxygen and a significantly strong correlation with total hardness in the post-monsoon season ($p \leq 0.05$) Similar correlation patterns have been observed by Gonzalves and Joshi (1946), Welch (1952), Hutchinson (1957), Reid (1961), Munawar (1970a & b). pH shows no correlation with dissolved oxygen during winter and a weak correlation in summer but a strong and significant correlation (p≤0.05) in the post-monsoon season. Kumaresan et al.(2004) and Joshi et al.(2008) have also recorded similar findings between pH and dissolved oxygen. A strong and significant correlation between pH and total hardness is found in the post-monsoon and summer seasons but a moderate correlation in the winter season. Singh (2005) has also recorded a similar correlation trend during different seasons. Shyamala et al. (2008) on the other hand, have recorded no correlation between these two variables. Dissolved oxygen shows a moderate to weak correlation with total hardness during all the seasons of the study period has also been recorded by Joshi et al.(2009).

In liver tissue, iron showed a strong and significant correlation with lead and cadmium (p≤0.05) correlation with other Heavy metals recorded between moderate to weak.

In gill tissue, a strong and significant correlation was found between iron and mercury (p≤0.05). Other Heavy metals showed moderate to weak correlation.

In muscle no strong and significant correlation was observed in the whole study period, a similar finding has been observed by, Kalay et al., 1999, Kalay and 2000, Nussey et al. 2000, and Canli and Atli 2002.

To assess the environmental quality of fish tissues in terms of heavy metal concentration and water quality, we used a variety of statistical techniques in this study (ANOVA and correlation analysis). The metal concentrations were discovered to be reasonably well differentiated and appropriately identified using discriminant analysis. The ANOVA revealed that the seasons and tissues significantly affect the distribution of heavy metals. The results of the ANOVA in different seasons and different tissues on the accumulation of heavy metals are supported by correlation analysis.

5. References :

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