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Evaluation of Heavy Metals Toxicity of Pharmaceuticals Industrial Wastewater by Pollution Indexing and Chemometric Approaches

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Abstract : Water is the most important resource for life. Water quality and quantity is the main global issue. Water scarcity dueto increased demand of water by different sectors for business, industrial uses and agricultural activities has pressurized the sources of water. Industrialization has created the most challenging issues of water pollution by different types of organic, inorganic and heavy metals which discharged into the water- bodies. Pharmaceutical also contain different types of chemical constituents which released directly into the water-bodies without processing. Out of the inorganic and organic pollutants, heavy metals are the most important toxicant which severely affects the water quality. In this study, water samples of two pharmaceutical industries (A and B) were subjected to physico-chemical and heavy metal investigations. The obtained mean values of parameters were further processed for Chemometric statistical assessment viz. Principal Component Analysis (PCA)/ Factor Analysis (FA). The heavy metal toxicity was assessed by the indexing method such as Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI). The PCA/FA showed that two factors F1and F2 values in the case of industry A and B were capable to explain 100% of total variances. The HPI values for industry A and B were108.78 and 52.14 respectively and HEI values were 4.15 and 8.67 respectively. The result revealed that the industry A falls in the category of high metal pollution category and low water quality and industry B showed low heavy metal pollution and low water quality.
Keywords: Physico-chemical, chemometric, cluster analysis, heavy metal pollution index, heavy metal evaluation.

Introduction

Recently, the increased demand of the pharmaceuticals has generated a large number of its manufacturing units all over the globe and hence, the increased in pharmaceutical wastewater. Most of the drugs are manufactured by the chemical synthetic routes, which involves a series of complex chemical reactions which release pharmaceutical wastewater. It is evaluated that about half of the global wastewater generated from pharmaceutical industries are discharged as such from the outlets without its further required processing, which contain different types of chemical ingredients in the form of inorganic and organic constituents, spent solvents, catalysts, total solids including heavy metals such as Cobalt, Iron, Cadmium, Nickel, Chromium etc.(Ramola and Singh[1],Rohit and Ponmurugan[2],Rao et al.[3],Mayabhate et al.[4], Vanerkar et al.[5], Sirtori et al.[6])are potential ingredients having toxic characteristics to affect the soil, surface and ground water,which have adverse effects on the human health and living biotas(Oktem et al.[7], Foess and Ericson[8], Rashed 2010[9], Chotpantarat et al.[10], Chotpantarat and Sutthirat[11], Taboada et al.[12]). Water quality monitoring is a complicated process in which a large number of datasets are generated and interpretations of the results become a tedious work. The datasets contain rich hidden information. Water quality assessment of the indexing approach isthe method to study the composite effects of the various water quality parameters by organising the data in simple and easiest way.For this purpose, various investigators have proposed different type of water quality indexing methods and also pollution indices were developed for the specific purposes such as heavy metal pollution study (Prasad and Jaiprakas[13], Prasad and Bose [14]).FA and PCA are the important methods for the study of the relationships between samplevariables, distribution of data, reduction of data and finding out the patterns, origin characteristic of parameters and the data representation, data interpretation and facilitation(Tariq et al.[15,16],Bhuiyan et al.[17], Liu et al.[18], Ozbay et al.[19],Horton[20],Joung et al.[21], Landwehr[22], Nishidia et al.[23], Tiwary and Mishra[24], Franco et al.[25]).Specific pollution indices also have been used to evaluate the extent of pollution with respect to certain metals.In recent years heavy metal toxicity becomes most prominent issues in surface and groundwater. Due to this reason, HPI a new method for evaluation of heavy metal pollution was developed (Prakash and Dagaonkar [26],Hui et al. [27]).

Material and Methods

Study area

Lucknow district is a part of the central Gangetic plain of Uttar Pradesh covering an area of 2528 sq.km. It lies between 26°30´;27°10´N and 80° 30´;81°13´E. Two industries viz. Industry A Sarojaninagar at and Industry B at Chinhat of Lucknow city was selected for the study (Figure 1).

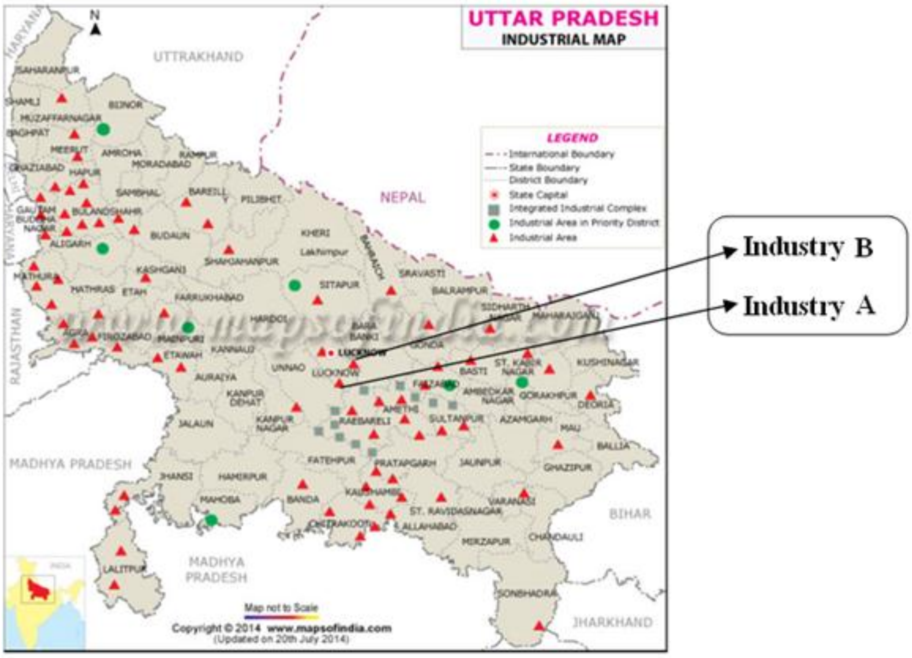

Figure: 1 Location map of the study sites

(Websource = www.mapsofindia.com)

Physico-chemical and heavy metal assessment of wastewater

Total six samples were collected, three from each pharmaceutical industry, which are located at the industrial area of Lucknow, Uttar Pradesh (India).

The samples were collected in 2 liter sterile plastic containers which were preserved by acidifying to pH 2.0 with nitric acid and kept at 4°C until the analysis were carried out. The collected water samples were filtered with Whatman filter paper no. 1.The collected wastewater samples as per the guidelines of the

American Public Health Association (APHA, 2005). The various methods and instruments were used for the parameter were such as pH was measured with a digital pH meter (Metrohm, USA), Electrical conductivity (EC), Total dissolved solids (TDS) were determined by a conductivity meter (Thermo Orion, model 162A, USA) and Turbidity was estimated by nephelometer method. Biochemical oxygen demands (BOD), chemical oxygen demand (COD) were determined by the titration method. Heavy metals were determined with an atomic absorption spectroscopy (AAS) (GBC, Avanta Sigma, Australia).

Multivariate and pollution index assessment of pharmaceuticals industrial wastewater

The Factor Analysis (FA) and Principal Component Analysis (PC) were performed using XLSTAT 2016 statistical software.

Factor Analysis/Principal Component Analysis (FA/PCA)

FA/PCA is an important technique used for the pattern identification from a variety of large number of datasets which is intercorrelated parameter and that is converted into a small number of sets of independent variables (Principal components). Factor analysis is the method of reduction of the unimportant variables acquired from the PCA analysis and the extraction of the new group of variables is carried out by rotating the axis from PCA, which is called as factors (Prakash and Dagaonkar[26],Kunwaret al.[31]). There are three steps in the factor (Boyacioglu and Boyacioglu [32],Alam et al.[33]).The Kaiser Method involves the retaining of those factors having eigen values greater than 1 and in the Scree Plot Method the formation of the cliff on the basis of higher eigen values determine the retaining of the factor.Eigen values is the most significant and important aspect of FA/PCA (Basu and Lokesh[34], Costello and Osborne [35]). Factor loadings fall under categories as “strong,” “moderate” and “weak,” referred to absolute loading values of >0.75, 0.75–0.50 and 0.50–0.30, respectively (Liu et al.[18]).

Water pollutants evaluation by indexing technique

The indexing techniques were proposed by the mathematical method after processing the samples of heavy metals.The indices used in this study, where heavy metal pollution index (HPI) and heavy metal evaluation index (HEI), which provides an overall quality of the water with regard to heavy metals.

(i) Heavy metal pollution index (HPI)

HPI is a technique of water quality rating and procedure of evaluation of composite effects on the quality of water affected by even a single heavy metal.HPI is the weight of the desired individual parameter which is inversely proportional to the standard permissible limit(Si) with respect to desired chosen parameter (Mohan et al. [36], Prasad and Kumari [37], Prasad and Mondal [38]).Computation of HPIis accomplished by the guidelines, given by the Central Pollution Control Board (CPCB [39]), for the discharge of industrial effluents into inland surface water.The calculation comprises of three steps, which are as follows:

- In the first step, the relative weight (Wi)of individual parameter was computed the eq.1.

$W_i = K/S_i$ eq.1

Where the Wiis the unit weightage and Si the recommended standard for ith parameter (i = 1-n), k is the constant of proportionality.

- In the second step, an individual quality rating (Qi) was computed for each parameter using eq.2.

$Q_i = 100\ V_i/S_i$eq.2

Where, Qiis the sub index of ith parameter, Viis the monitored value of the ith parameter in µg/L and Si is the standard or permissible limit for theith parameter

- In the third and final step, summation of, these sub-indices resulted in the overall Index, as in eq. 3.

$$HPI= \sum_{i=1}^{n} (Qi\ Wi)/ \sum_{i=1}^{n} Wi$$

Where, Qi is the sub index of ith parameter, $W_i$is the unit weightage for ith parameter and n is the number of parameters considered. Normally, the critical pollution index value is 100.

(ii) Heavy metal evaluation index (HEI)

HEI is also the process of assessment of water quality with respect to heavy metals by providing assigned values(Edet and Offiong [40]). HEI is computed as follows:

$$HEI = \sum_{i=1}^{n} Hci/Hmaci$$

Where Hcis the monitored value and Hmac is the maximum admissible concentration (MAC) of the ith parameter.

Results and Discussion

Physico-chemical and heavy metal analysis

Various physico-chemicals and heavy metal concentration determined and some basic descriptive statistics of wastewater of both the pharmaceutical industries are shown in Table1.After comparing with CPCB standard, the result shows that pH, electrical conductivity, BOD and COD were above the permissible limit in case of both the industries, whereas in the case of heavy metals, the concentration was almost within the limit except the Pb and As. In both the industries, Pb was above the permissible limit as prescribed by CPCB,while As was greater than the prescribed limit (0.1ppm) in the case of industry B.Based on the concentration range and abundance of heavy metals in both the industries ranking order are as follows:

Industry- A: Zn>Cu>Fe>Cr>Pb>Mn>Co>Cd>As>Ni >Hg
Industry –B:Zn.>Fe> Cu>As >Mn >Cr>Co> Pb>Ni>Cd>Hg

Table 1: Physico-chemical analysis and basic descriptive statistics of Pharmaceutical Industry Aand B

Parameters	Unit	Industry A Mean ± SD	Industry B Mean ± SD	Standard permissible value (CPCB,[39])	Method
pH	NS*	5.6±0.20	6.24±0.30	6.0-8.5	pH-meter
Conductivity	µs/cm	1563.34±305	1336.67±81	1000	Conductivity-meter
TDS	mg/L	920.34±238	741±42	21000	Conductivity-meter
Salinity	ppt	2.7±1.0	1.72±1.0	NS*	Conductivity-meter
BOD	mg/L	7253.34±1770	3693.34±885	100	Winkler azide
COD	mg/L	756.67±1948	7031.67±847	250	Dichromate method
Turbidity	ntu	67.3±10	57.12±10	NS*	Nephelometer
Cu	mg/L	2.21±0.31	1.61±0.12	3	FAAS
Co	mg/L	0.39±0.13	0.27±0.11	NS*	FAAS
Cd	mg/L	0.27±0.20	0.06±0.01	2	FAAS
Ni	mg/L	0.05±0.01	0.07±0.02	3	FAAS
Pb	mg/L	0.85±0.32	0.20±0.10	0.1	FAAS
Mn	mg/L	0.84±0.23	0.56±0.15	2	FAAS
Cr	mg/L	1.50±0.02	0.42±0.15	2	FAAS
Zn	mg/L	3.11±0.45	2.39±0.51	5	FAAS
Fe	mg/L	1.90±0.20	1.68±0.36	3	FAAS
As	mg/L	0.07±0.02	0.82±0.18	0.2	AAS-VGA
Hg	mg/L	0.004±0.001	0.002±0.001	0.01	VGA-Flameless AAS

Note: NS* abbreviate as: Not Specified

Factor/PCA analysis

For the evaluation of multivariate Factor/PCA analysis the raw data of sampling stations were subjected into the Microsoft excel 2003 based statistical software packages known as Xlstat which generates the Pearson correlation matrix shown in Table 2 and 3 of industries A and B respectively. Eigen analysis of the Pearson correlation coefficient matrix was applied to perform the factors/ principal components. Which, were reproduced by the use of the statistical software Xlstat, which involves the centroid methods and varimax rotation (Ahmed et al.[43]). The result of FA/PCA (Table 4) indicated two components, showing the characteristics of pharmaceutical industry wastewater A and B respectively. Only those factors were selected for the analysis, which have eigenvalue greater than one. The Fig. 4 and 6 shows the Scree plot of the eigenvalue for each component in which two Principal Component was obtained with eigenvalues >1 summing 100% of the total variance in the water dataset of Industry A and Industry B respectively. Figure 4 and 5, 6 and 7.0 respectively, represents the factor analysis result of industry A and B in which two significant factors were generated which explain the eigen values, cumulative variability and factor loading variation of data set 100%. The factors are discussed below as in Table 4.0.

Factor Analysis of Pharma Industry A

F1 explained the 61.95% of variances with strong positive loadings with salinity (0.999), Pb (0.993),COD(0.971), TDS (0.959), BOD (0.921), and conductivity (0.843) and negative strong loadings with Mn (-0.981), Zn (-0.896), Hg (-0.887), Fe (-0.862) and moderate positive loading with Cr (0.751) and moderate negative loading with Co (-0.742) and Cu (-0.716).First factor shows the pollution of organic and inorganic ingredients, which was used as raw materials for the chemical synthesis of pharmaceutical.Hence it generates a higher amount of organic and inorganic toxicants, TDS, BOD, COD (Kavitha et al., [44]) and trace heavy metals in pharmaceutical wastewater (Rana et al., [45]).

Table 2: Correlation analysis of water of pharma industry -A

Variables	pH	EC	TDS	Salinity	BOD	COD	Turbidity	Cu	Co	Cd	Ni	Pb	Mn	Cr	Zn	Fe	A	Hg
pH1		0.655	0.422	0.189	0.520	0.377	0.962	-0.795	0.554	0.721	0.655	0.265	-0.335	-0.544	0.308	-0.627	**1.000**	0.327
EC1			0.961	0.866	0.986	0.947	0.424	-0.979	-0.266	-0.052	-0.143	0.903	-0.931	0.278	-0.518	**-0.999**	0.655	-0.50
TDS1				0.970	0.994	**0.999**	0.159	-0.885	-0.521	-0.325	-0.410	0.986	-0.996	0.532	-0.733	-0.971	0.422	-0.719
Salinity1					0.937	0.981	-0.086	-0.746	-0.712	-0.545	-0.619	**0.997**	-0.989	0.721	-0.876	-0.884	0.189	-0.866
BOD1						0.987	0.267	-0.931	-0.423	-0.218	-0.306	0.962	-0.979	0.435	-0.653	-0.991	0.520	-0.637
COD1							0.111	-0.861	-0.562	-0.370	-0.453	0.993	**-0.999**	0.572	-0.765	-0.958	0.377	-0.752
Turbidity1								-0.600	0.760	0.882	0.836	-0.007	-0.065	-0.752	0.555	-0.391	0.962	0.572
Cu1									0.063	-0.153	-0.062	-0.795	0.837	-0.076	0.332	0.971	-0.795	0.312
Co1										0.977	0.992	-0.655	0.599	**-1.000**	0.962	0.301	0.554	0.968
Cd1											0.996	-0.477	0.412	-0.974	0.881	0.089	0.721	0.891
Ni1												-0.555	0.493	-0.990	0.921	0.179	0.655	0.929
Pb1													**-0.997**	0.665	-0.836	-0.918	0.265	-0.824
Mn1														-0.609	0.794	0.944	-0.335	0.781
Cr1															-0.966	-0.313	-0.544	-0.971
Zn1																0.549	0.308	**1.000**
Fe1																	-0.627	0.531
As1																		0.327
Hg1																		

Table. 3: Correlation analysis of water of pharma industry B

Variables	pH	EC	TDS	Salinity	BOD	COD	Turbidity	Cu	Co	Cd	Ni	Pb	Mn	Cr	Zn	Fe	As	Hg
pH1	0.831	0.846	0.869	0.973	0.924	0.868	-0.690	-0.207	0.327	0.737	-0.976	0.458	- 0.960	-0.981	-0.637	0.663	-0.500	
EC1		**1.000**	**0.977**	0.937	0.980	**0.998**	-0.170	-0.717	-0.798	0.236	-0.931	-0.114	-0.953	-0.923	-0.958	0.968	-0.898	
TDS1			**0.999**	0.946	0.986	**0.999**	-0.199	-0.696	-0.780	0.264	-0.941	-0.085	-0.961	-0.934	-0.950	0.960	-0.884	
Salinity1				0.960	0.992	**1.000**	-0.242	-0.664	-0.752	0.306	-0.955	-0.041	-0.973	-0.949	-0.935	0.946	-0.863	
BOD1					0.987	0.959	-0.505	-0.427	-0.536	0.561	-1.000	0.241	**-0.999**	**-0.999**	-0.798	0.818	-0.686	
COD1						0.992	-0.361	-0.566	-0.664	0.422	-0.985	0.084	-0.994	-0.981	-0.884	0.899	0.793	
Turbidity1							0.239	0.666	0.754	0.304	-0.955	-0.044	-0.972	-0.948	-0.936	0.947	-0.864	
Cu1								0.565	-0.458	**-0.998**	0.518	-0.959	0.461	0.536	-0.118	0.084	-0.282	
Co1									0.992	0.509	0.413	0.775	0.472	0.393	0.886	-0.870	0.951	
Cd1										0.397	0.524	0.690	0.578	0.505	0.937	-0.924	0.982	
Ni1											-0.574	0.939	-0.519	-0.592	0.051	-0.017	0.217	
Pb1												-0.256	**0.998**	**1.000**	0.789	-0.809	0.675	
Mn1													-0.192	-0.277	0.393	-0.362	0.540	
Cr1														-0.966	0.827	-0.846	0.722	
Zn1															0.775	-0.796	0.659	
Fe1																**-0.999**	0.986	
As1																	0.980	
Hg1																		

Table 4: The factor loadings and % variance of industry- A and B

Total variance and factor loading of Industry A			Total variance and factor loading of Industry B	
Principal components			**Principal components**	
Parameters	**F1**	**F2**	**F1**	**F2**
pH	0.146	**0.989**	**0.865**	0.502
Conductivity	**0.843**	**0.537**	**0.998**	-0.065
TDS	**0.959**	0.285	**0.999**	-0.036
Salinity	**0.999**	0.044	**1.000**	0.008
BOD	**0.921**	0.389	**0.957**	0.289
COD	**0.971**	0.238	**0.991**	0.133
Turbidity	-0.129	**0.992**	**1.000**	0.006
Cu	**-0.716**	-0.698	-0.234	**-0.972**
Co	**-0.742**	0.670	-0.670	**0.742**
Cd	-0.581	**0.814**	**-0.757**	0.653
Ni	-0.652	**0.758**	0.298	**0.954**
Pb	**0.993**	0.122	**-0.953**	-0.303
Mn	**-0.981**	-0.194	-0.050	**0.999**
Cr	**0.751**	-0.660	**-0.971**	-0.240
Zn	**-0.896**	0.443	**-0.946**	-0.324
Fe	**-0.862**	-0.506	**-0.938**	0.347
As	0.146	**0.989**	**0.949**	-0.315
Hg	**-0.887**	0.462	**-0.867**	0.498
Eigen value	**11.151**	**6.849**	**3.088**	**4.912**
Variability (%)	**61.959**	**38.409**	**72.710**	**27.290**
Cumulative (%)	**61.959**	**100.00**	**72.710**	**100.00**

Note: Significant values are in bold typeface

F2 explained the 38.05% of variances with strong positive loading with turbidity (0.992), pH, As (0.989), Cd (0.814) and moderate positive loading with Ni (0.758),Co(0.670) and conductivity and moderate negative loadings with Cu(-0.698),Cr(-0.660) and Fe (-0.506), which shows that the turbidity and pH are the most important physico-chemical parameters of pharmaceutical wastewater, it can promote redox reaction between other chemical species come in contact with the effluents containing different chemical species and can increase the toxicity of water. Other metals which are significant toxicants and dominating species are As and Cd. Arsenic comes from formulation of medicines, which contains metal salts and can increase the aquatic toxicity.

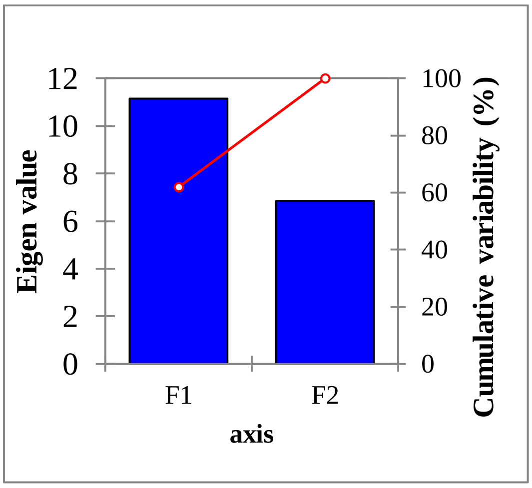

Figure: 4Scree plot of Industry A

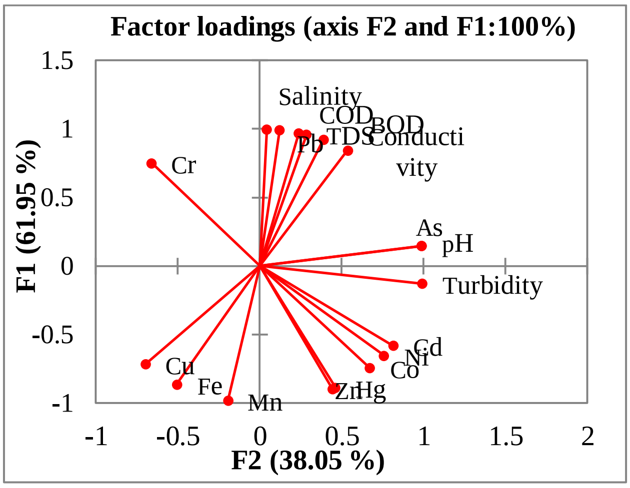

Figure: 5 Factor loading of Industry A

Factor analysis of Industry B

F1 explained 72.71% of variances with strong positive loading with salinity (1.000), TDS (0.999), conductivity (0.998), COD (0.991), BOD (0.957), As (0.949), pH (0.865) and strong negative loading with Cr (-0.971), Pb (-0.953), Zn (-0.946), Fe (-0.938), Hg (-0.867), Cd (0.757). Increase in salinity may be due to the dissolution of salts of metals and other inorganic and organic chemicals, which release different types of mobile elements resulting in the higher TDS and conductivity. The factor also shows that the heavy metals, which are released in pharmaceutical wastewater, can cause the toxicity to the living.

F2 explained 27.290% of variance with strong positive loading with Mn(0.999)and Ni(0.954) and moderate positive loading with Co(0.742),Cd (0.652) and pH(0.502) and positive loading with Hg(0.498).The result shows that the main components are Mn and Ni which are generated from the salts of Mn and Ni that were used either as raw materials or by the processing of the wastes. Other metals, such as Co and Cd are also having the same nature of origin in wastewater as in the case of Mn and Ni. pH were also shown the moderate loading, governed by the interaction of the chemicals, which can promote different types of the redox processes if released into the environment without proper processing it can cause the toxicity of surface as well as groundwater.

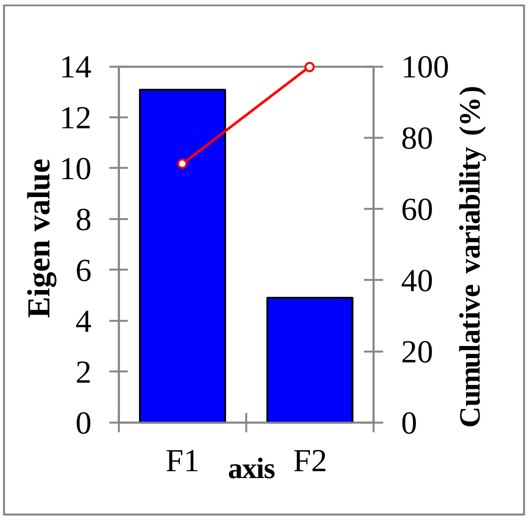

Figure: 6Scree plot of Industry B

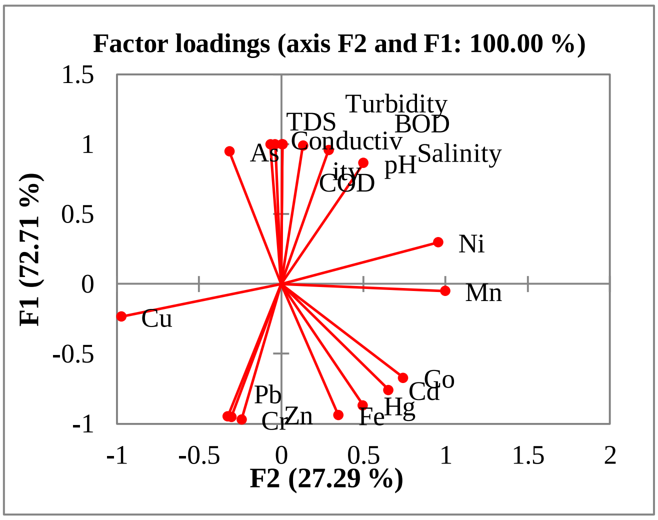

Figure: 7 Factor loading of Industry B

Pollution Index Analysis

HPI of pharmaceuticals wastewater of both the industries were calculated by individually using the international standards (Edet and Offiong [40]). The range values of HPI for industry A and B are 108.78 and 52.14 respectively(Table 5). The result shows the industry A falls in the category of high metal pollution and industry B shows lowheavy metal pollution (Table6) HEI computation range value for industry A and B are 4.15 and 8.67 respectively, which falls into thecategory of low water quality (Edet et al. [46]).

Table 5: Heavy metal pollution index and heavy metal evaluation index of Industry A

Heavy metal (mg/L)	Monitored Mean value Mi (mg/L)	Standard permissible value Si (mg/L)	Unit weightage (Wi)	Sub index (Qi)	QiWi	Mi/Si
Cu	2.21	3.0	0.334	73.7	24.61	0.737
Cd	0.27	2.0	0.5	13.5	6.75	0.135
Ni	0.05	3.0	0.334	16.7	5.58	0.017
Pb	0.85	0.1	10	850	8500	0.085
Mn	0.84	2.0	0.5	42	21	0.42
Cr	1.49	2.0	0.5	74.5	37.25	0.745
Zn	3.11	5.0	0.2	622	12.44	0.622
Fe	1.9	3.0	0.334	63.3	21.15	0.634
As	0.07	0.2	5	35	175	0.35
Hg	0.004	0.01	100	40	4000	0.4
∑Mi/Si=4.15			∑Wi=117.71		∑QiWi=12803.78	

- **Industry A:HPI = 108.78 and HEI = 4.15**
- The calculation steps for the HPI and HEI for the industry B were also same as shown in table 5.0above and the value of HPI and HEI for **Industry B** are **HPI= 52.14**and **HEI=8.67**

Table: 6 HPI & HEI scaling range for heavy metal pollution and water quality categorization(Edet et al. [46]).

HPI	Scaling	HEI	Scaling
< 100	Low heavy metal pollution (LP)	< 400	Low water quality (LWQ)
= 100	Heavy metal pollution on the threshold risk (PTR)	400 < HEI < 800	Moderate water quality (MWQ)
> 100	High heavy metal pollution (HP)	> 800	High water quality (HWQ

Conclusion

Water is the most requisite and valuable resource.The quality of most of the surface water and groundwater is globally affected by different types of water toxicants.Now-a-days, the increased demands of medicines have triggered setup a large number of pharmaceutical industries globally, which consumes a large quantity of water, thus resulting in large quantity of pharmaceutical wastewater. Discharged wastewater contains higher amount of organic and inorganic chemicals and heavy metals, which either were a part of raw materials used for the preparation of medicine, or processing of the expired leftover, medicines that discharged in water. It can bio-concentrate or bio-accumulate in the living beings and if released in environment without proper treatment, shall disturb the homeostasis of the aquatic and other ecosystems. Hence, there is great need to develop monitoring units and methods which can reduce the volume and quantity of toxicants from the raw materials used for final byproduct processing of the medicine synthesis. There is a need to develop an integrated biological, chemical, photochemical and other methods as well which can enhance the removal of heavy metal concentration in discharged wastewater of the pharmaceutical industry.

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