Chapter III

Seasonal variability study of acid mine drainage formation in Ledo high-sulphur coal mine area, Northeastern coalfield

3.0 Introduction:

Assam and other states of Northeast India, namely Meghalaya, Arunachal-Pradesh, and Nagaland also have good reserves of coal. These coals have high sulphur content (2-7 %), high volatile matter (30-50 %), and low ash content (Mukherjee, 2003; Singh et al., 2012; Singh et al., 2013a; Singh et al., 2013b). In the process of open cast coal mining, various physical and chemical properties of soil and water changed for mining and storage of coal in different seasons (Mukhopadhyay et al., 2014; Quadros et al., 2016). Wetting and drying seasons (monsoon and non-monsoon seasons) in any of the mine workings including coal mine can affect the character of acid mine drainage (AMD) produced. Continuous raining has a tendency to generate a more constant volume of AMD and containing other contaminants like heavy metals as water moves through and blushes oxidation products out of the sulphide bearing materials. The accumulation of contaminants in these materials is proportional to the duration of the monsoon season (Ferguson and Erickson 1988; Doepker 1993). As the length of the non-monsoon season increases, oxidation products tend to accumulate and a high magnitude raining will wash accumulated contaminants out of the sulphide material. Thus, this is a typical relationship of the increase in contaminant load detected for the mining areas during non-monsoon season (Technical document acid mine drainage prediction, 1994). According to Hem (1970), AMD formation was found to be more severe in moist area with a moderate rainfall where fast oxidation and dissolution of exposed sulphide bearing minerals can occur. From physiological experiments it was found that microorganisms can impact on the rates of sulphide oxidation during the dissolution of pyrite, arsenopyrite, marcasite, and Sphalerite. Oxidation of sulphide minerals to sulfate takes place through a series of intermediate sulfur-bearing compounds where microbial utilization of sulphide and intermediate sulphur compounds can significantly affect the acidification along with the pyrite dissolution rates (Boon and Heijnen, 1993; Edward et al., 2000). Thus, despite the high acidity, heat, and maximum concentrations of sulfate and heavy metals, a number of microorganisms are responsible for increasing AMD environments. Microbial activity increases the rate of AMD formation and may be responsible for the bulk of AMD generated (Baker and Banfield, 2003). Also, Edward (2000) suggested that microbial activity is responsible for about 75% of the AMD production. Thus, overall actions of microbes which is directly affected by sulphide oxidation rate along with the increased concentrations of the heavy metals is responsible for the highest formation of AMD during monsoon season.

The seasonal variation of the specific area can alter these parameters as well. Singh (1987) revealed that the rate of flow of AMD produced in the Ledo open cast mining in the monsoon season is 181.7m^3/h, which is quite high when compared to non-monsoon season rate of 45.43m^3/h. Thus, in this chapter, the focus is on the analysis of seasonal variations of the physico-chemical characteristics and metal concentrations of the coal, overburden, soil and water in the monsoon and the non-monsoon seasons, and their relationship with the amount of AMD formation in the different seasons. So, this study will help in identifying the influences of different physico-chemical parameters including pH, Electrical Conductivity (EC), Total Dissolved Solid (TDS), mineral structures and heavy metal concentration on the environment of the mining and surrounding areas in different seasons.

3.1 Methods and materials

3.1.1 Sampling and sampling area

Freshly mined coal and overburden were collected from the Ledo colliery. The soil and sediment samples were collected from the mine and nearby areas and streams. The solid samples were air dried and ground into 72 BS (0.211mm), preserved in a desiccator to prevent the absorption of moisture and subjected to different physico-chemical analytical processes. Mine water samples were also collected from the AMD source at different locations within the 5km radius from the mining source of the Ledo colliery and were kept in plastic containers for further analyses. The samples were collected four times in two different seasons, monsoon (July to September) and non-monsoon seasons (December to March) to study the effect of variation of seasons on physico-chemical properties of mine samples.

3.2 Analyses of mine water/mine affected water samples/other solid samples

The different analytical processes including physico-chemical analysis like pH, TDS, and EC of mine water/water, forms of sulphur in coal by sulphur analyzer, elemental concentrations of mine water/water by using inductively coupled plasma optical emission spectrometric analysis (ICP-OES) , and cationic and anionic concentrations in mine and mine affected water by ion-chromatographic analysis were done for complete characterization of mine water samples.

The details of these methods used are described in Chapter II

3.3. Cytotoxicity assays in AMD affected water

The cytotoxicity study that was done at CSIR-NEIST to study the effect of mine affected water on different cells including normal rat muscle (L6), human carcinoma viz., Pancreatic (MIAPaCa2), Lung (A459) and Liver (HepG2) cell by studying the percentage of cell viability with the concentration of mine water samples. The methods and materials used in this study were as follows:

Cell lines obtained from NCCS, Pune were cultured in respective complete media supplemented with 10% Foetus Bovine Serum (FBS), 10% Penstrep, 1% Gentamycin (Sigma-Aldrich Co. LLC, St Louis, MO, USA) and incubated under standard conditions at 37°C and 5% CO_2 atmosphere. Cytotoxicity of various water samples collected from the mining sites were tested against L6 and MIAPaCa2 cells cultured in Dulbecco's Modified Eagle's medium (DMEM), A549 cells in Ham's F12K medium and HepG2 cells in Minimum Essential Medium Eagle (MEM) respectively, following the method of Mosmann 1983 as described by Saikia et al.(2016a). 1×10^6 cells were seeded in tissue culture grade 96 well plates (Nunc, Thermo Fisher Scientific Inc., Waltham, MA, USA) for invitro MTT assay and kept for overnight incubation. One day (24 hrs) prior to treatment, the complete medium was replaced with incomplete medium (FBS free) and incubated. The cells were then treated with 10 mine water samples (17-1, 17-2, 17-3, 17-4, 17-5, 17-6, 17-7, 17-8, 17-9 and 17-10) which were designated as Tube-well water (17-1), Ledopani (17-2), Seepage-1 (17-3), AMD-1(17-4), Seepage-2 (17-5), Kachanalla (17-6), AMD-2 (17-

7), Seepage-3 (17-8), Deep-well (17-9), and drain water (17-10) respectively, in different concentrations (2, 5, 10, 20, 50, 100, 200, 250 and 300 µg/ml) into each well and incubated for 24hrs. Untreated (Sample free) cells were taken as control. Subsequently, 10 µl of MTT (5 mg/ml) was added to each well containing control and treated cells, mixed gently and incubated for another 4 hrs. All experiments were performed in triplicate. The cell proliferation was expressed as the viability percentage calculated as Absorbance of treated cells/Absorbance of control cells × 100.

3.4 Polycyclic aromatic hydrocarbon (PAH) analyses of AMD water

Three representative AMD water samples (sample code: 17-3, 17-5, and 17-8) from Ledo coalfield site was analysed for presence of PAHs by using high-performance liquid chromatography (HPLC) technique (Shimadzu HPLC–PDA system) follwing the extraction methods reporetd elsewhere (Essumang, 2009; Saikia et al., 2016b; Hou et al., 2013). 16 PAHs were examined in the AMD samples which are known to be carcinogenic (USEPA, 2009). The variable concentrations of EPA 610 PAHs mix standards (Sigma Aldrich) were used for calibration (Saikia et al., 2016b).

3.5 Results and discussions

3.5.1 Physico-chemical parameters of acid mine water collected from different sites

The mine water samples were collected four times during monsoon and non-monsoon seasons and the sets of samples were designated as S1, S2, S3, and S4 respectively. The sample sets S1 and S2 were collected in the monsoon season and S3 and S4 were for non-monsoon season. The samples were collected from different sites within the 5 km radius from the Ledo OCP. The variations of different physico-chemical properties of the samples with seasons are depicted in Figure 3.1, Figure 3.2, and Figure 3.3 which are also tabulated in Table 3.1. Since strongly acidic (or strongly basic) i.e. low pH (or high pH) solution will have high electrical conductivity. From the analysis it is clear that pH of the mine water samples collected in the non-monsoon season is higher than those collected in the monsoon season because of the low rate of pyrite oxidation in the winter due to low temperature (Sgambet et al,1980; Baruah et al., 2016) where pH is a major contributing factor for pyrite formation (Ryan and Ledda, 2010). The

SO_4^{2-} present in the coal undergoes reduction to H_2S at pH values of 7- 8.5 which, in combination with ferrous oxide (FeO) and elemental sulphur, forms iron pyrite (FeS) and water. The Figure 3.2 shows that the value of the electrical conductivity increases with low and high values of pH, but for approximately normal value of pH (6.1 in monsoon season) a low value of electrical conductivity was observed. Due to very low values of pH (3.3 and 3.5), the seepage water (in the monsoon season) had high values of electrical conductivity (2.49 and 2.29 ms cm^{-1}). This indicates that the metal and non-metals can dissolve easily in mine water at very low and high pH by contamination of the ground and surface water resources. Water with a pH less than 6.5 can leach metal ions, including iron, manganese, copper, lead, zinc, aluminium, lead and mercury from the coal. Among these, aluminium, lead and mercury are potentially hazardous in nature. When the pH of the water source is low, these elements can have extremely detrimental impact on aquatic life. According to MoEF (2000), the pH level of industrial effluent should be 5.5-9.0. In the present analysis it was found that the mine water samples (seepage 1 and 2) having highest value of EC (1.30-2.49 ms cm^{-1}) had the highest TDS value (1068-1339 ppm). TDS is a measure of inorganic salts and small amounts of organic matter dissolved in water. It can cause toxicity to aquatic life through an increase in salinity, changes in the ionic composition of the water, and the toxicity of individual ions. The principal constituents of TDS are anions like nitrates, sulphates, chlorides etc. and cations like calcium, magnesium, sodium, and potassium. According to the Bureau of Indian Standards (BIS) the desirable limit of TDS in potable water is 500 ppm. From the present investigation it was found that most of the mine water samples had TDS values higher than the desirable limit, especially during the monsoon season. From Figure 3.1 and Figure 3.2, it is clear that the EC and TDS values of the mine water samples have the highest value in the monsoon season (S1 and S2). This is an implication of severe contamination of the water resources in the monsoon season compared to that found for the non-monsoon season.

Table 3.1: Physico-chemical parameters for sample set S1, S2, S3, and S4*

Sample type	**Distance from AMD source (km)**	**pH**				**Electrical conductivity (ms cm^{-1})**				**Total dissolved solid (ppm)**			
		S1	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
AMD(1)	**0**	**4.0**	**6.1**	**8.1**	**8.0**	**1.23**	**0.90**	**1.07**	**2.02**	**843**	**812**	**535**	**657**
AMD(2)	**0.3**	**4.1**	**5.6**	**5.3**	**5.02**	**1.53**	**1.20**	**0.95**	**2.02**	**947**	**973**	**507**	**621**
Seepage water(1)	**0.5**	**3.5**	**3.9**	**5.8**	**5.05**	**2.29**	**1.60**	**1.02**	**1.07**	**1127**	**1339**	**654**	**531**
Seepage water(2)	**1**	**3.3**	**4.0**	**5.1**	**5.92**	**2.49**	**1.30**	**1.24**	**1.03**	**1068**	**1135**	**620**	**414**
Ledopani	**2**	**4.4**	**5.2**	**8.4**	**8.04**	**0.94**	**0.60**	**0.86**	**0.521**	**606**	**572**	**430**	**262**
Kachanallah	**5**	**5.0**	**4.9**	**8.4**	**7.98**	**0.66**	**0.60**	**0.90**	**0.658**	**497**	**507**	**452**	**329**

*S_1 and S_2 = sample sets for monsoon season

S_3 and S_4 = sample sets for non-monsoon season

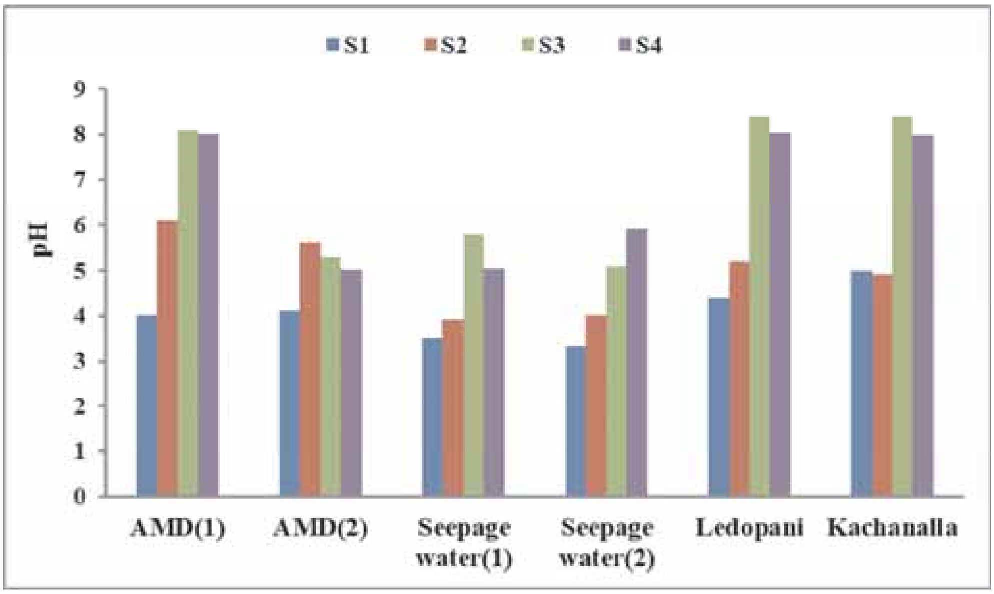

Figure 3.1: Seasonal variation of pH of mine and mine affected water

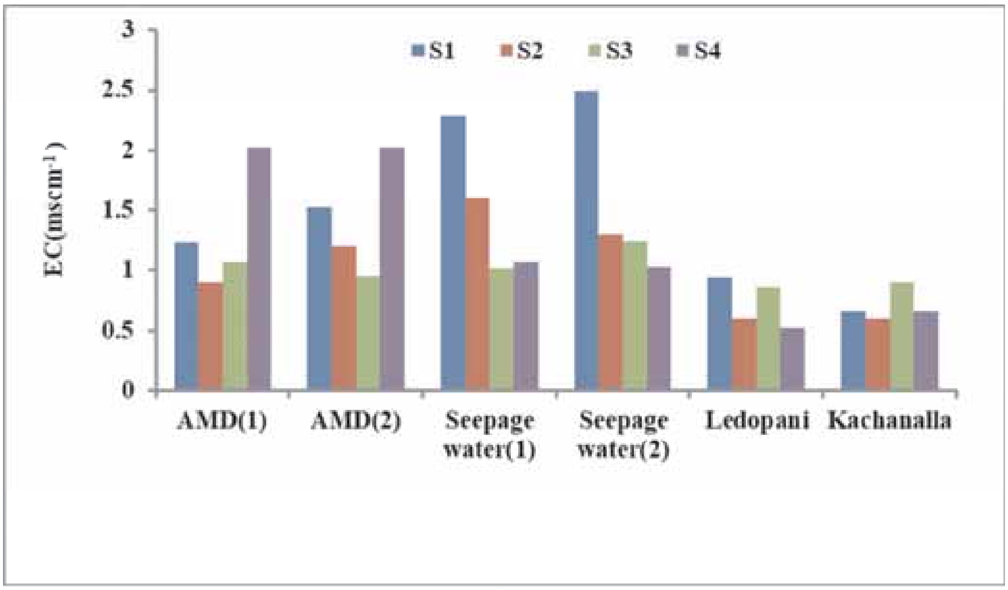

Figure 3.2: Seasonal variation of EC of mine and mine affected water

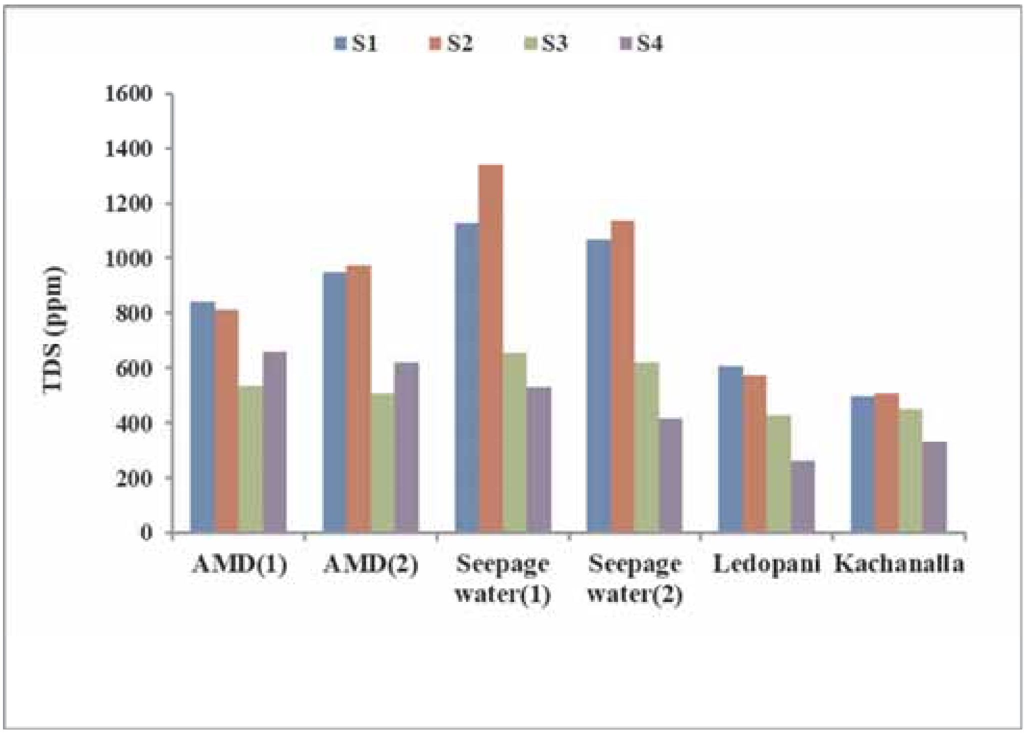

Figure 3.3: Seasonal variation of TDS of mine and mine affected water

Table 3.2: Co-relation among physico-chemical parameters of mine water in different seasons

	S1-PH	S2-PH	S3-PH	S4-PH	S1-EC	S2-EC	S3-EC	S4-EC	S1-TDS	S2-TDS	S3-TDS	S4-TDS
S1-PH	1											
S2-PH	0.485	1										
S3-PH	0.760	0.499	1									
S4-PH	0.655	0.480	0.943	1								
S1-EC	-0.954	- 0.673	-0.867	-0.780	1							
S2-EC	-0.854	-0.556	-0.873	-0.902	0.918	1						
S3-EC	-0.805	-0.384	-0.585	-0.348	0.781	0.582	1					
S4-EC	-0.275	0.571	-0.324	-0.318	0.152	0.331	0.270	1				
S1-TDS	-0.942	-0.442	-0.866	-0.834	0.943	0.966	0.703	0.434	1			
S2-TDS	-0.902	-0.567	-0.851	-0.861	0.945	0.994	0.634	0.310	0.980	1		
S3-TDS	-0.894	-0.629	-0.725	-0.689	0.932	0.925	0.767	0.217	0.919	0.948	1	
S4-TDS	-0.407	0.369	-0.395	-0.439	0.310	0.531	0.332	0.949	0.588	0.510	0.445	1

The relationship (Pearson correlation) among the physico-chemical properties can be explained by using the co-relation diagram (Table 3.2). From the co-relation diagram, it is found that there is a poor relationship of pH with the EC and TDS in mine water collected during the non-monsoon season as well as for the samples collected during the monsoon season. But in both the season, EC (S1-EC) has high positive correlation with TDS (S1-TDS, r = 0.943) and TDS (S3-TDS, r = 0.932).

3.5.2 Types of sulphur analyses in raw coals

The sulphur content in the coal is a major determinant factor for the AMD formation in the Ledo mine. It was found that the seasonal variation can alter the sulphur content in coal. Table 3.3 shows the alteration of the amount of sulphur in coal in the monsoon and non-monsoon seasons, which are also indicated in Figure 3.5. From literature(Baruah et al., 2006) it was found that out of the total sulphur, the Ledo coal of Assam has 21.5-28.2% inorganic and 71.7-78.5% organic sulphur. From the present analysis it is clear that the inorganic sulphur in the Ledo coal has increased to the concentration of 30.5-40.9%. Only the inorganic form of sulphur is responsible for increasing the acidity of the mine water by oxidizing pyrite (Tiwary, 2001; Baruah et al., 2005, 2006; Baruah, 2009; Baruah and Khare, 2007a), because the organic forms of sulphur are found mainly in macromolecular structures and are not easily separated without breaking of the macromolecular network (Calkins, 1994). The higher the percentage of inorganic sulphur, the higher would be the AMD formation. In India the sulphur content is highest in the samples collected during monsoon season, compared to that in non-monsoon season (Guttikunda et al., 2001), which is shown in Figure 3.4.

Table 3.3: Forms of sulphur in coal in monsoon and in non-monsoon seasons (wt%)

Sample type	Sample code	Total Sulphur	Pyrite sulphur	Sulphate sulphur	Organic sulphur
Coal	S1	4.35	0.70	0.877	2.77
	S2	5.54	1.25	0.490	3.80
	S3	2.74	0.747	0.377	1.61
	S4	1.94	0.368	0.118	1.46

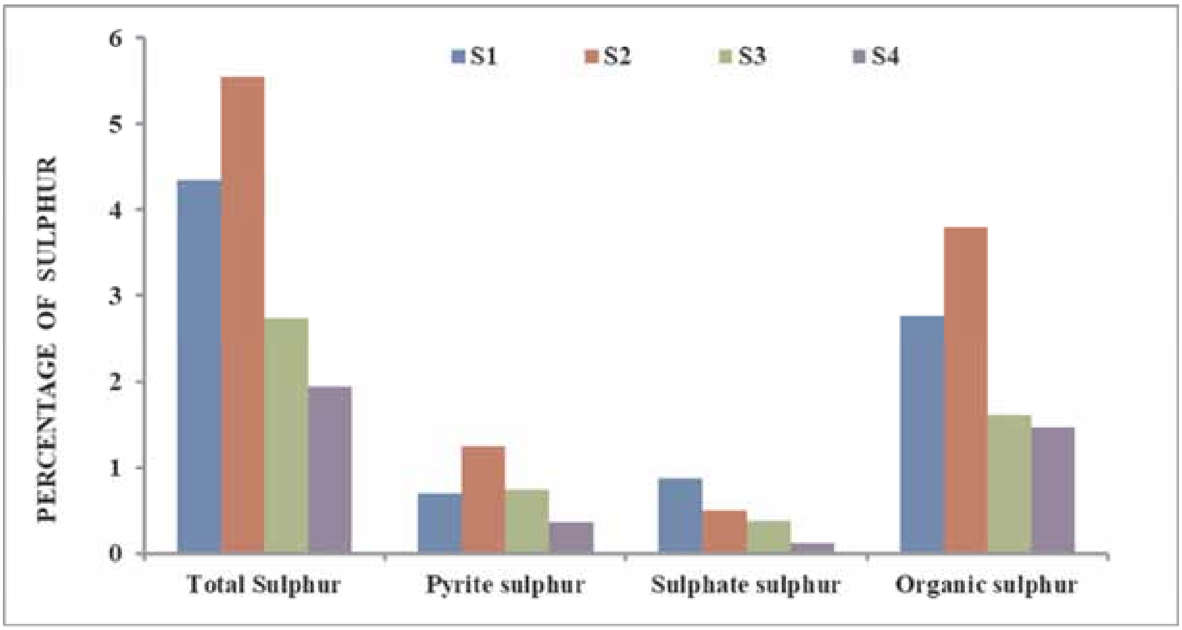

Figure 3.4: Forms of sulphur in coal in monsoon and in non-monsoon seasons

3.5.3 Potentially hazardous elements (PHEs) in mine water samples

This analytical method is used to determine the concentrations of trace and hazardous elements present in the collected mine water samples. Different mine water samples from the mine source as well as from the nearby areas within 5 km radius from the source were collected.

This analysis has been done to detect whether the seasonal variation can alter the concentrations of these elements or not. Table 3.4 gives the detail information about the concentration of elements in the coal mine water in the monsoon season for sample sets S1 and S2 which is reflected in Figure 3.5 and Figure 3.6.

Table 3.4 : ICP-OES analysis of mine water samples (S1, S2) in monsoon season

Sample	**S1**										**S2**									
type	**Al**	**Co**	**Cd**	**Cu**	**Zn**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**	**Al**	**Co**	**Cd**	**Cu**	**Zn**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**
AMD(1)	**3.71**	**0.25**	**bdl**	**0.05**	**0.55**	**0.42**	**8.78**	**1.09**	**0.01**	**0.01**	**2.56**	**0.31**	**bdl**	**0.03**	**0.46**	**0.67**	**2.54**	**0.039**	**0.005**	**0.31**
AMD(2)	**3.19**	**0.23**	**bdl**	**0.05**	**0.51**	**0.42**	**8.07**	**1.01**	**0.02**	**0.07**	**0.067**	**0.53**	**0.001**	**0.02**	**0.125**	**0.84**	**3.33**	**0.007**	**0.009**	**0.53**
Seepage(1)	**13.88**	**0.31**	**bdl**	**0.02**	**0.75**	**66.56**	**11.89**	**1.53**	**0.01**	**0.02**	**8.10**	**0.331**	**bdl**	**0.03**	**0.605**	**60.52**	**6.68**	**0.447**	**0.16**	**0.331**
Seepage(2)	**24.89**	**0.41**	**bdl**	**0.10**	**1.39**	**40.12**	**7.44**	**2.06**	**0.17**	**0.05**	**13.88**	**0.425**	**bdl**	**0.13**	**0.962**	**39.01**	**8.38**	**0.087**	**0.18**	**0.425**
Ledopani	**7.82**	**0.10**	**bdl**	**0.02**	**0.21**	**13.26**	**4.56**	**0.53**	**0.19**	**0.05**	**5.21**	**0.13**	**bdl**	**0.01**	**0.11**	**12.72**	**3.21**	**0.004**	**0.13**	**0.13**
Kachanalla	**6.55**	**0.07**	**bdl**	**0.02**	**0.16**	**8.36**	**3.35**	**0.36**	**0.03**	**0.03**	**3.75**	**0.04**	**0.001**	**0.009**	**0.65**	**9.21**	**2.03**	**0.002**	**0.11**	**0.04**

bdl: below detection limit

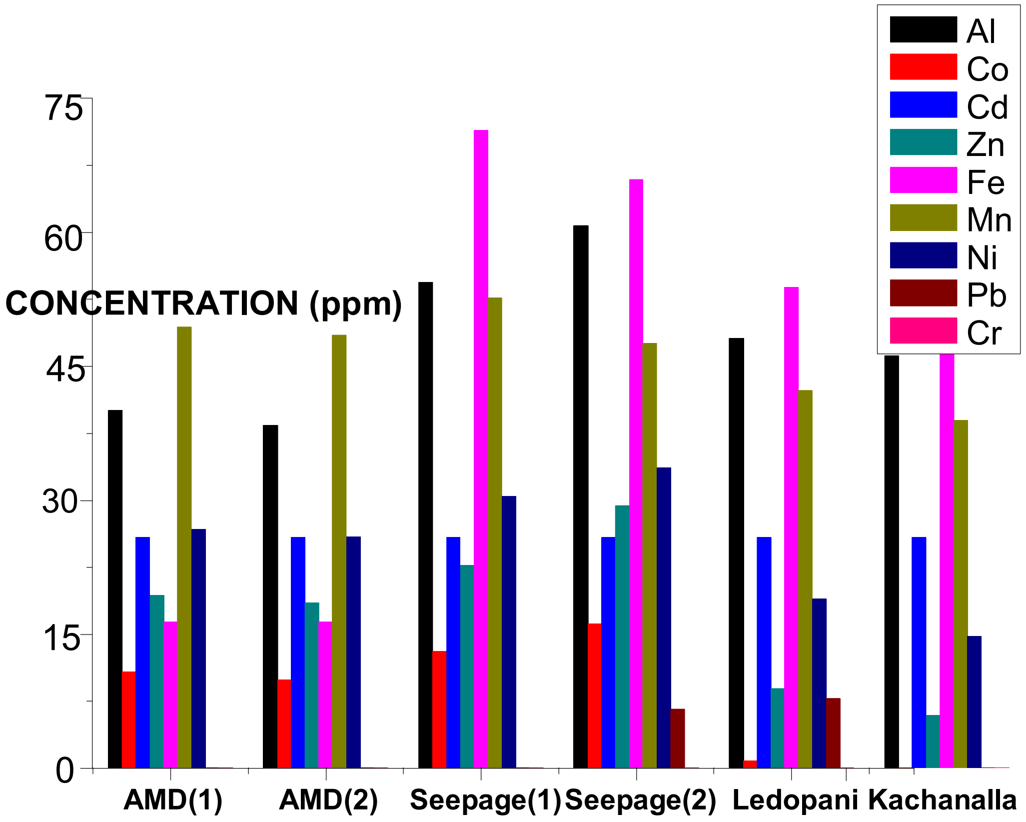

Figure 3.5 : Elemental concentration of mine water samples in monsoon season (S1)

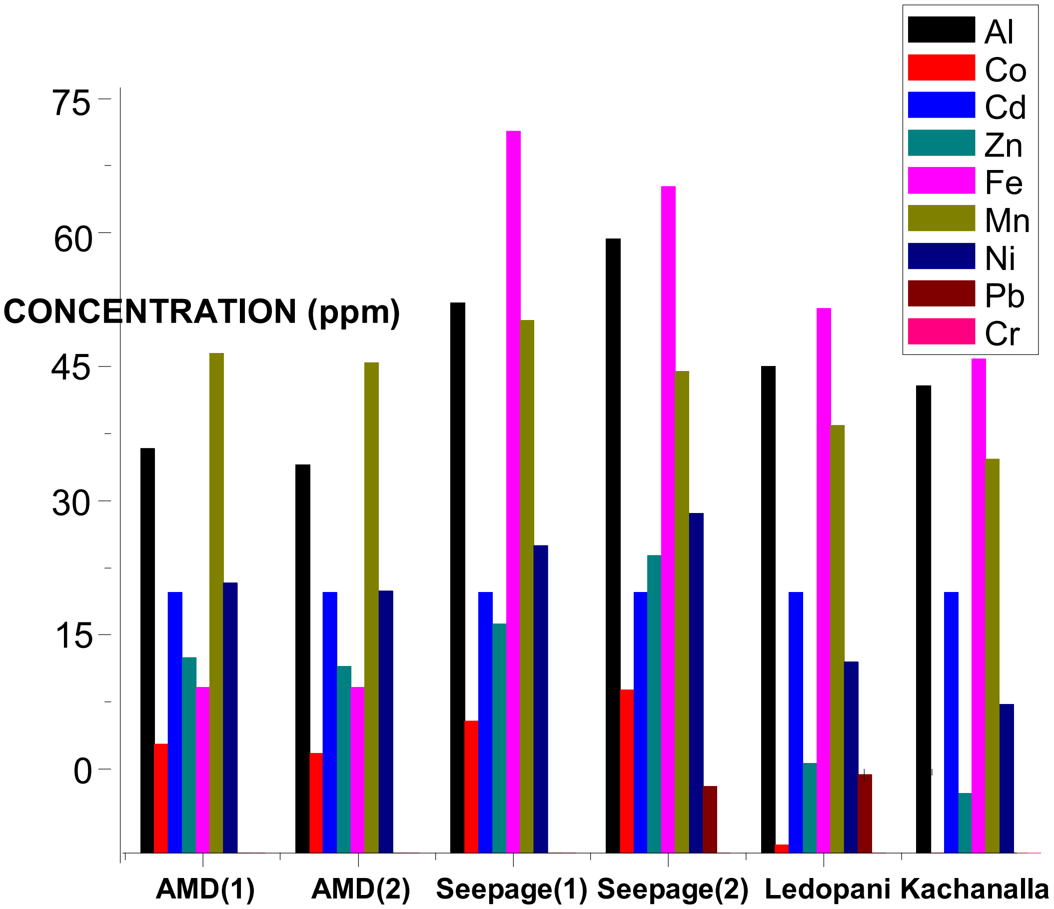

Figure 3.6: Elemental concentration of mine water samples in monsoon season (S2)

Table 3.5: ICP-OES analysis of mine water samples (S3, S4) in non-monsoon season

Sample type	**S3**										**S4**									
	Al	**Co**	**Cd**	**Cu**	**Zn**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**	**Al**	**Co**	**Cd**	**Cu**	**Zn**	**Fe**	**Mn**	**Ni**	**Pb**	**Cr**
AMD(1)	**0.527**	**bdl**	**bdl**	**bdl**	**bdl**	**0.075**	**0.019**	**0.006**	**bdl**	**bdl**	**bdl**	**0.015**	**bdl**	**0.003**	**0.422**	**bdl**	**10.30**	**0.167**	**bdl**	**bdl**
AMD(2)	**0.654**	**0.011**	**bdl**	**bdl**	**bdl**	**0.056**	**0.024**	**0.018**	**bdl**	**0.002**	**bdl**	**0.448**	**bdl**	**0.007**	**0.957**	**0.250**	**16.60**	**1.820**	**0.015**	**0.001**
Seepage(1)	**0.051**	**0.015**	**bdl**	**bdl**	**0.024**	**bdl**	**0.424**	**0.066**	**bdl**	**0.001**	**bdl**	**bdl**	**bdl**	**0.002**	**0.408**	**bdl**	**6.02**	**0.262**	**bdl**	**bdl**
Seepage(2)	**0.298**	**0.019**	**bdl**	**0.003**	**0.002**	**0.079**	**0.094**	**0.008**	**bdl**	**bdl**	**bdl**	**0.108**	**bdl**	**0.003**	**0.428**	**bdl**	**8.80**	**0.395**	**0.006**	**bdl**
Ledopani	**0.611**	**bdl**	**bdl**	**bdl**	**bdl**	**0.371**	**0.123**	**0.015**	**bdl**	**bdl**	**bdl**	**0.067**	**bdl**	**0.004**	**0.585**	**0.002**	**10.20**	**0.337**	**0.036**	**bdl**
Kachanalla	**0.221**	**0.005**	**bdl**	**bdl**	**bdl**	**0.353**	**0.201**	**0.024**	**bdl**	**bdl**	**bdl**	**bdl**	**bdl**	**0.003**	**0.437**	**bdl**	**0.22**	**0.082**	**bdl**	**bdl**

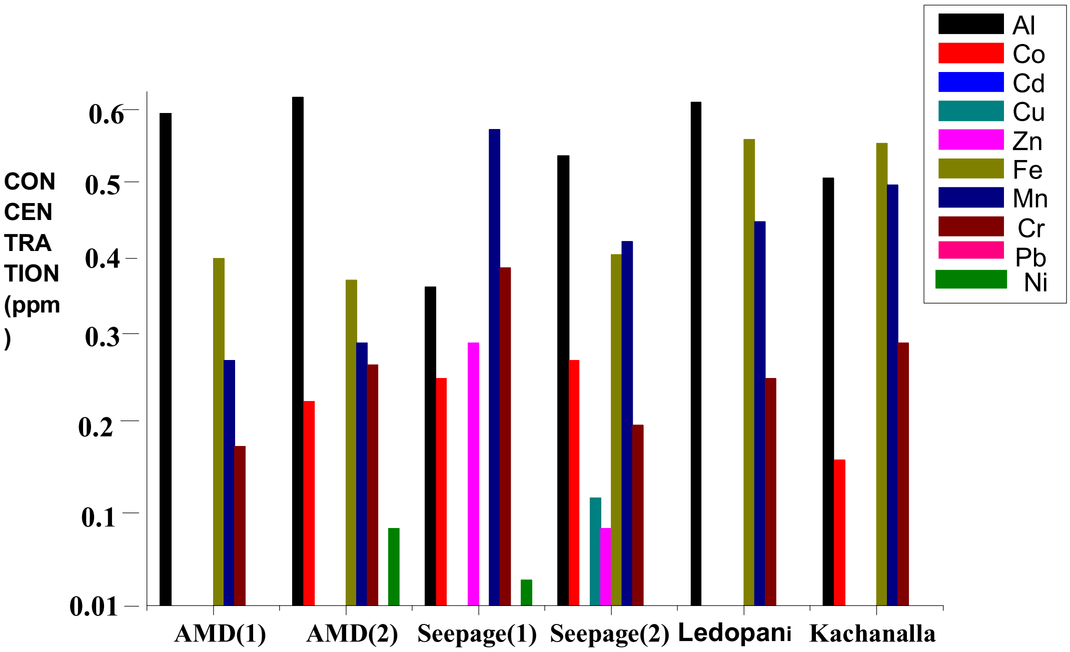

Figure 3.7: Elemental concentration of mine water samples in non-monsoon season (S3)

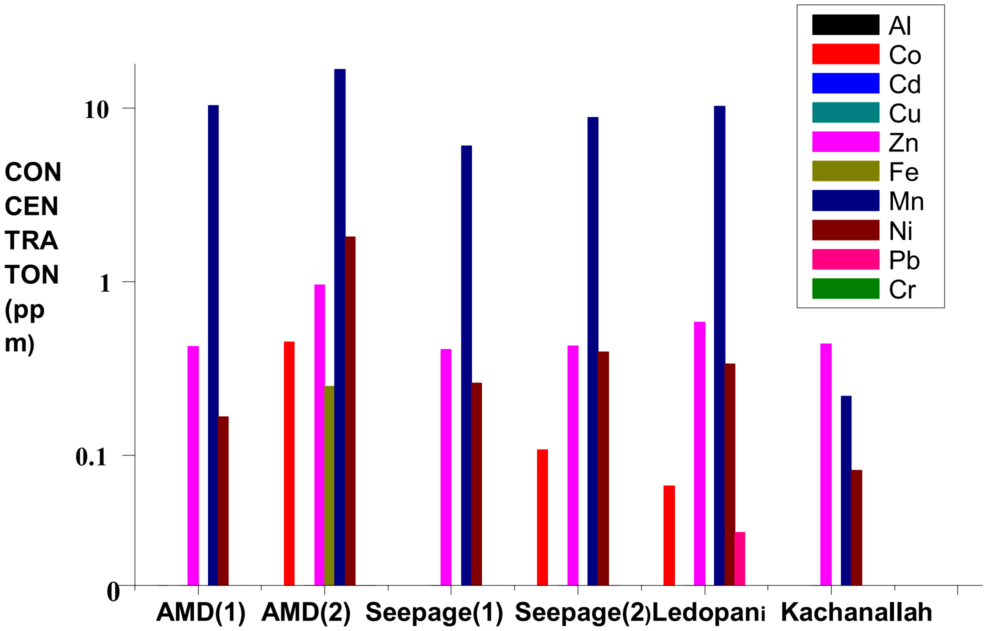

Figure 3.8: Elemental concentration of mine water samples in non-monsoon season (S4)

On the other hand, the Figure 3.7 and Figure 3.8 show the elemental concentrations in mine water for sample sets S3 and S4 collected during non-monsoon season. The change in elemental concentrations in non-monsoon season from monsoon season is also indicated by Table 3.5.

The mine water samples were collected in two seasons, monsoon and non-monsoon seasons and from ICP-OES analysis it is found that the concentration of metals like Al, Fe, Mn, Ni, and Pb is higher in the mine water collected during monsoon. The maximum Al content (24.89 ppm) is found in seepage water and the minimum concentration is found in the AMD sources (0.067-3.71ppm). The Ledopani and Kachanalla rivers are the nearby streams in the mining area which have a higher Al level (3.75-7.82 ppm) in the monsoon season than mine water collected from the source. This level of Al is higher than the regulatory level (5ppm) according to EPA, 2002. These streams have also higher Fe (8.36 - 13.86 ppm), and Pb (0.03-0.19ppm) levels than the mine source water. This is an indication of contamination of the water resources by the mine water (AMD and seepage) through rain water or by other anthropogenic activities like coal washings. The analytical data shows that major elements have concentration levels, higher than the permissible limit (permissible limits for Pb, Fe, Cr, Co, Mn, Zn, Cu, and Ni are 0.015, 0.3, 0.1, 0.005, 0.05, 2, 0.5, 0.1 ppm respectively; EPA, 2002) in most of the water samples collected during the monsoon season than for those collected during the non-monsoon season. Equeenuddin (2010) found that the concentrations of metals like Al, Fe, Mn, Ni, Zn, Pb, and Cu were very high in AMD of Makum coal field which are almost similar to the results obtained in the present investigation. Low pH is the major contributing factor for dissolving metals in mine water. Since pH of the water samples is high in dry season due to low rate of pyrite oxidation, leaching of a number of metals from coal or OB during non-monsoon season is not possible.

3.6 Ions present in AMD water

This analytical method is used to detect the concentrations of major cations, especially Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+ ions and major anions including F^-, Cl^-, Br^-, and SO_4^{2-} in mine water samples. The Ion-Chromatographic analysis of the mine water and the samples from mine affected areas collected during the two different seasons show a large variation in the concentration of cations and anions. Table 3.6 provides information about the concentration of cations in mine water samples in monsoon and non-monsoon seasons respectively which are graphically summarized in the Figure 3.9, Figure 3.10, Figure 3.11, and Figure 3.12.

Table 3.6: Ion Chromatographic analyses of cations in mine water during monsoon and non- monsoon seasons

Sample type	Monsoon season										Non-monsoon season									
	S1					S2					S3					S4				
	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+
AMD(1)	**3.559**	**0.103**	**bdl**	**44.91**	**0.162**	**5.790**	**0.711**	**bdl**	**182.6**	**0.173**	**0.301**	**bdl**	**bdl**	**bdl**	**bdl**	**0.119**	**bdl**	**bdl**	**bdl**	**bdl**
AMD(2)	**2.508**	**0.262**	**bdl**	**110.26**	**0.116**	**2.611**	**0.464**	**0.012**	**272.3**	**0.119**	**0.249**	**bdl**	**bdl**	**bdl**	**bdl**	**0.273**	**0.032**	**bdl**	**bdl**	**bdl**
Ledopani	**1.661**	**1.19**	**bdl**	**49.78**	**0.023**	**2.052**	**0.448**	**bdl**	**50.91**	**0.013**	**0.069**	**bdl**	**bdl**	**bdl**	**bdl**	**0.731**	**0.012**	**bdl**	**bdl**	**0.101**
Kachanalla	**0.724**	**2.14**	**bdl**	**60.78**	**0.025**	**1.961**	**4.08**	**bdl**	**48.72**	**0.021**	**bdl**	**bdl**	**bdl**	**bdl**	**bdl**	**0.997**	**0.053**	**bdl**	**bdl**	**0.069**
Seepage(1)	**0.682**	**0.301**	**bdl**	**29.29**	**0.103**	**0.964**	**0.377**	**bdl**	**32.19**	**0.142**	**0.386**	**bdl**	**bdl**	**bdl**	**bdl**	**0.521**	**0.062**	**4.89**	**0.370**	**0.003**
Seepage(2)	**0.931**	**0.350**	**bdl**	**35.02**	**0.201**	**1.142**	**0.592**	**bdl**	**41.32**	**0.194**	**1.23**	**bdl**	**bdl**	**bdl**	**bdl**	**0.592**	**0.057**	**5.40**	**1.152**	**0.089**

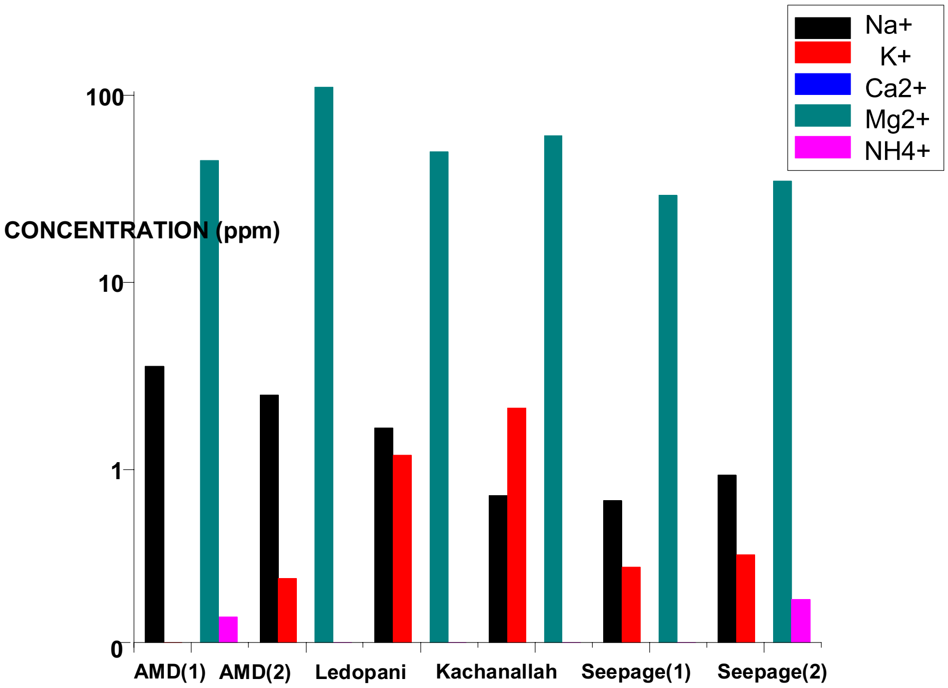

Figure 3.9: Cationic concentration of mine water in monsoon season (S1)

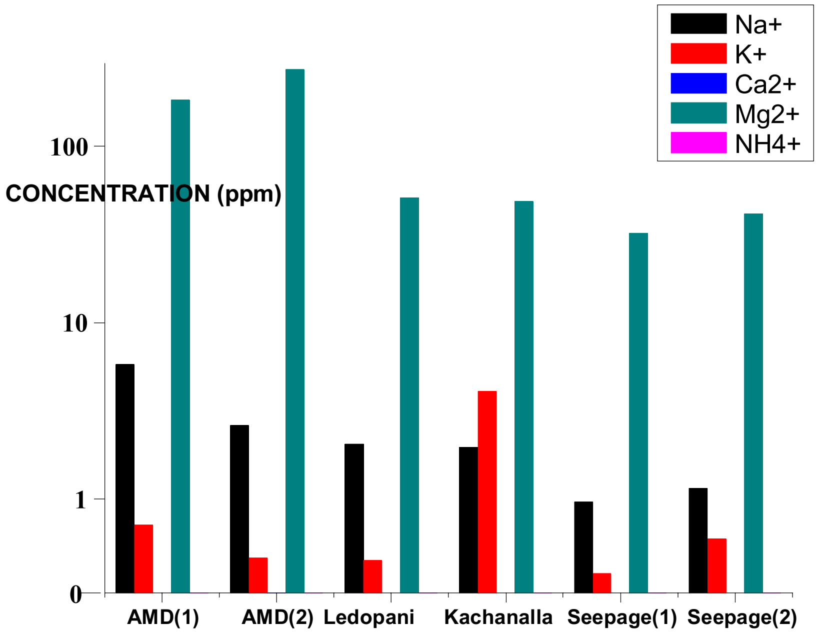

Figure 3.10: Cationic concentration of mine water in monsoon season (S2}

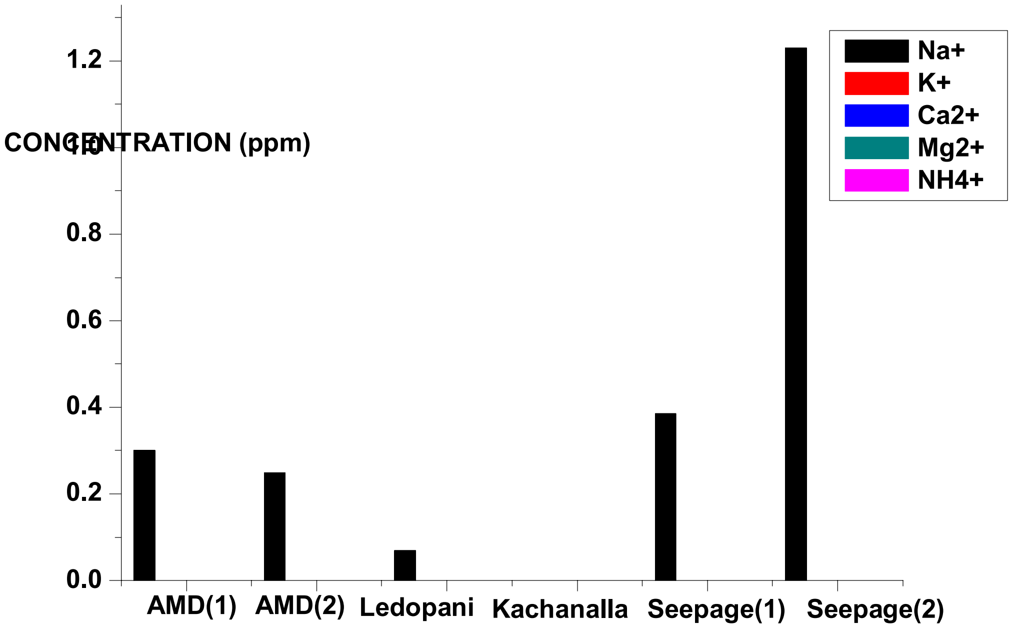

Figure 3.11: Cationic concentration of mine water in non-monsoon season (S3)

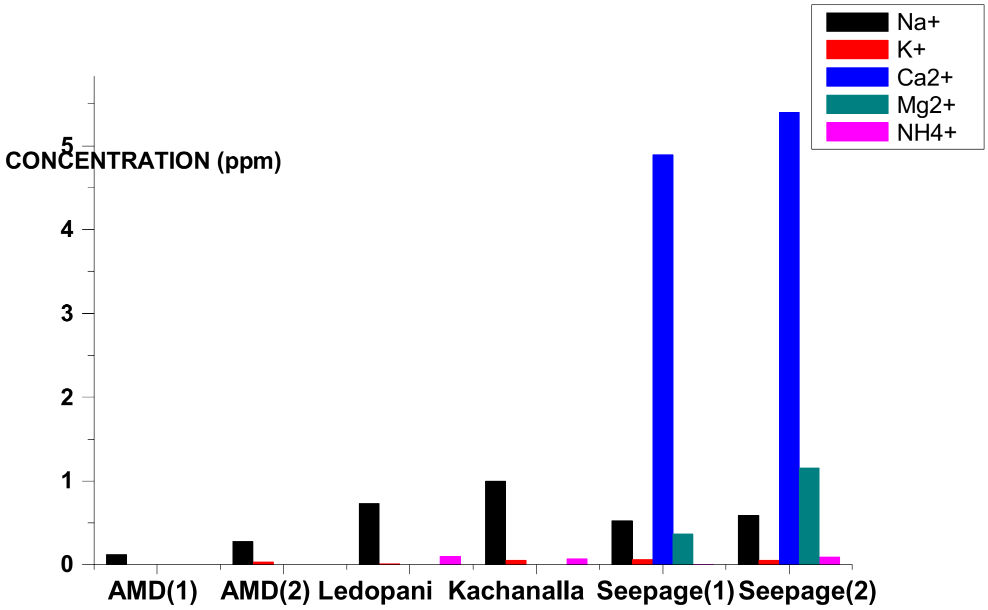

Figure 3.12: Cationic concentration of mine water in non-monsoon season (S4)

On the same way, Figure 3.13 and Figure 3.14 provide information on the variation of concentration of the anions in mine and mine affected water samples collected during the monsoon season for sample sets S1 and S2 from the non-monsoon season for sample sets S3 and S4 which is clearly indicated by the Figure 3.15 and Figure 3.16.

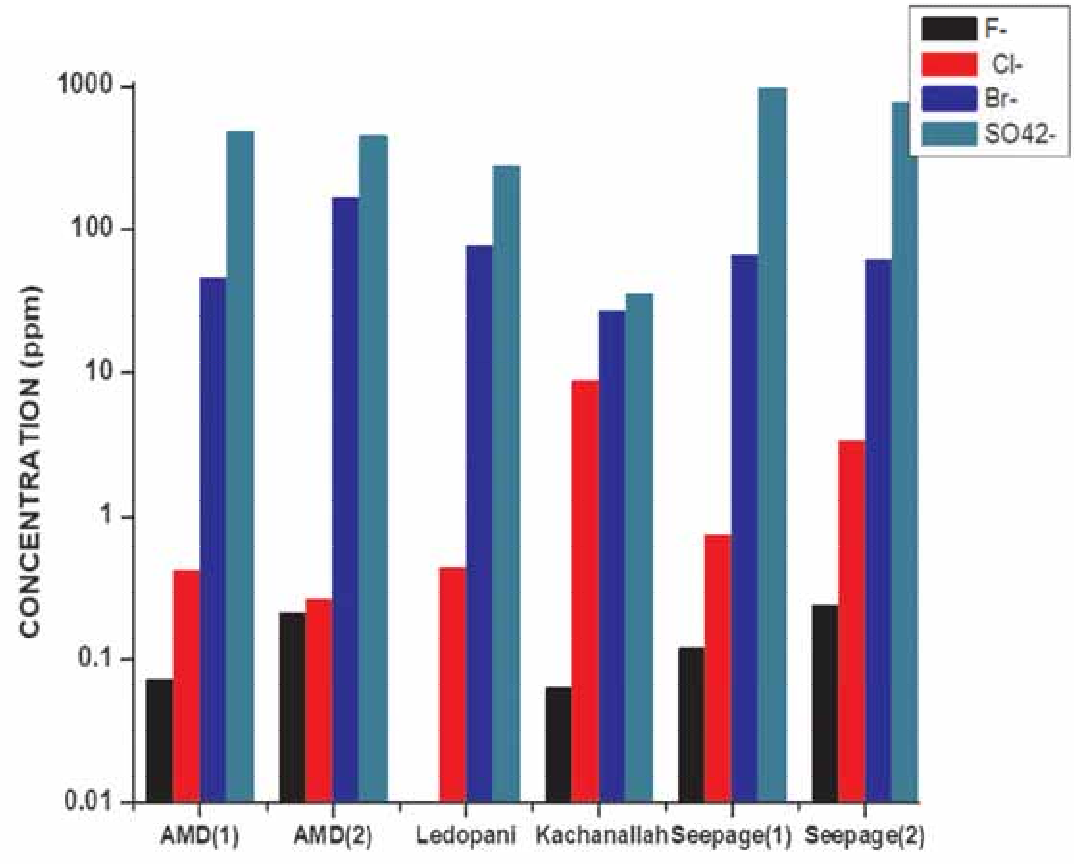

Figure 3.13: Anionic concentration of mine water in monsoon season (S1)

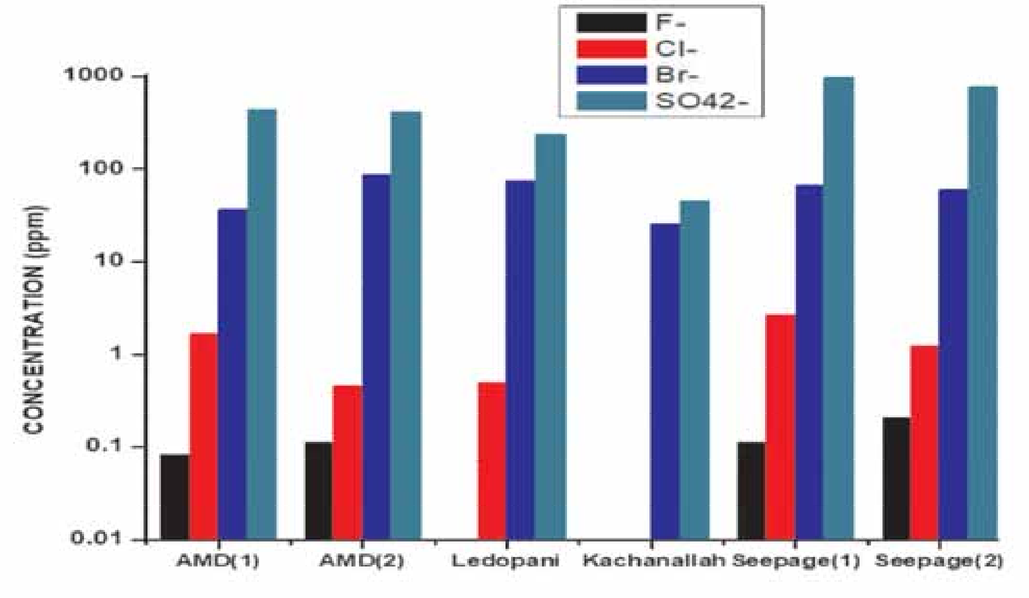

Figure 3.14: Anionic concentration of mine water in monsoon season (S2)

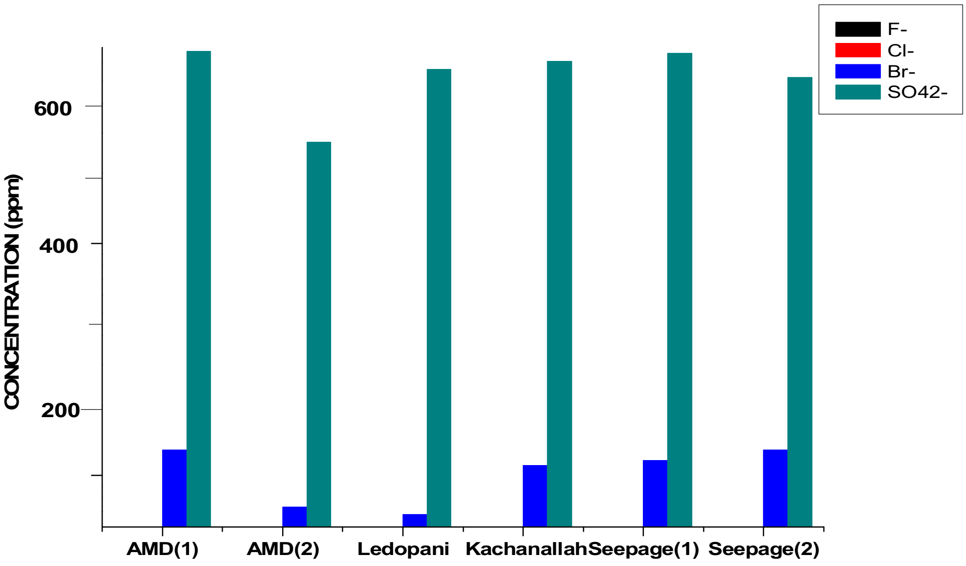

Figure 3.15: Anionic concentration of mine water in non- monsoon season (S3)

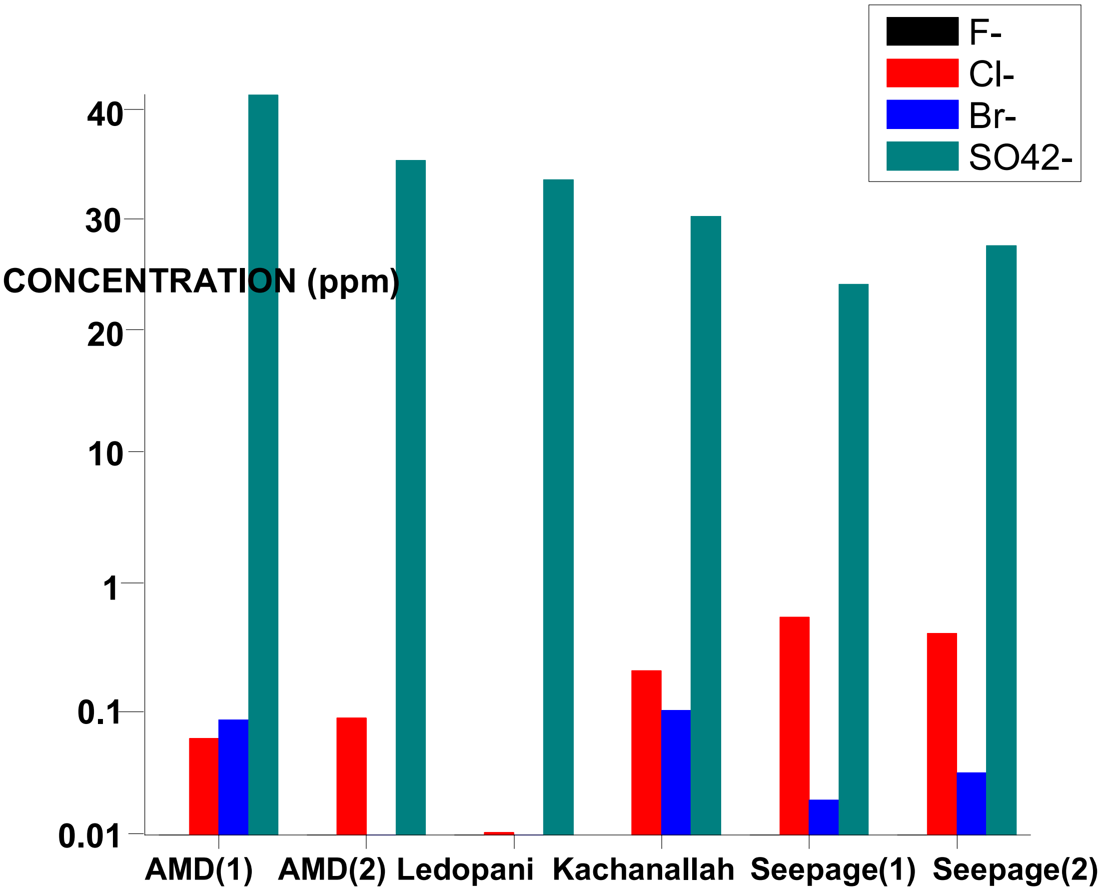

Figure 3.16: Anionic concentration of mine water in non- monsoon season (S4)

The overall data for monsoon and non-monsoon seasons are furnished in Table 3.7.

Table 3.7: Ion Chromatographic analyses of anions in mine water during monsoon and non-monsoon seasons

Sample	**Monsoon season**								**Non-monsoon season**							
	S_1				**S_2**				**S_3**				**S_4**			
	F^-	**Cl^-**	**Br^-**	**SO_4^{2-}**	**F^-**	**Cl^-**	**Br^-**	**SO_4^{2-}**	**F^-**	**Cl^-**	**Br^-**	**SO_4^{2-}**	**F^-**	**Cl^-**	**Br^-**	**SO_4^{2-}**
AMD(1)	**0.072**	**0.422**	**46.202**	**483.69**	**0.082**	**1.689**	**36.76**	**438.82**	**bdl**	**bdl**	**12.86**	**670.8**	**0.003**	**0.103**	**0.123**	**41.773**
AMD(2)	**0.213**	**0.268**	**170.1**	**458.92**	**0.112**	**0.454**	**87.34**	**414.15**	**bdl**	**bdl**	**7.28**	**272.53**	**0.002**	**0.125**	**0.022**	**22.75**
Ledopani	**bdl**	**0.437**	**78.314**	**279.24**	**bdl**	**0.495**	**75.21**	**233.91**	**bdl**	**bdl**	**6.77**	**561.15**	**0.002**	**0.043**	**0.028**	**18.94**
Kachanalla	**0.064**	**8.846**	**27.172**	**35.89**	**bdl**	**bdl**	**25.641**	**45.408**	**bdl**	**bdl**	**11**	**612.24**	**bdl**	**0.194**	**0.134**	**13.445**
Seepage(1)	**0.121**	**0.741**	**66.869**	**977.32**	**0.113**	**2.695**	**67.23**	**965.5**	**bdl**	**bdl**	**11.6**	**660.69**	**0.003**	**0.321**	**0.058**	**7.127**
Seepage(2)	**0.243**	**3.384**	**61.41**	**776.15**	**0.211**	**1.234**	**60.74**	**769**	**bdl**	**bdl**	**12.89**	**520.02**	**0.002**	**0.275**	**0.075**	**10.278**

From Figure 3.9, and Figure 3.10, it can be concluded that the concentration of Mg^{2+} in the mine water samples was highest (29.29-282.60 ppm), Na^+ and K^+ ion has a moderate concentration (0.682-5.790 and 0.103-4.08 ppm) in the water samples of monsoon season (in sample sets S1 and S2). The magnesium concentration is comparable to the desirable limit (30 ppm) according to the WHO (1997). The Na^+ and K^+ ion concentrations were found to be very low than the desirable limit (50 and 100 ppm respectively). The concentration of Ca^{2+} ion is below the detection limit and the NH_4^+ ion was present in very low concentration (0.023-0.194 ppm) in all the samples collected during the monsoon season. The order of cation abundance in the mine water sample collected in the monsoon season was $Mg^{2+} > Na^+ > K^+ > NH_4^+ > Ca^{2+}$. Again, from Figure 3.11, and Figure 3.12, it is clear that the concentrations of Mg^{2+}, Ca^{2+}, K^+, and NH_4^+ ions in the water collected in the non-monsoon season were below the detection limit or low in all samples, except the concentration of Ca^{2+} in seepage (1) and seepage (2), which was exceptionally higher (4.89 and 5.40 ppm respectively) than its overall concentration in other samples. Figure 3.13 and Figure 3.14 show the anionic concentrations of water samples collected from mine source and from the mine affected areas (Ledopani and Kachanalla rivers) in monsoon season as furnished in Table 3.7.Two sets of samples were collected in the monsoon season namely S1 and S2 and both sample sets have the highest SO_4^{2-} ion concentration (35.89-977.32 ppm). In these water samples, SO_4^{2-} was the dominant anion among all the major anions. According to the WHO, the concentration of SO_4^{2-} ions is higher than the maximum desirable and highest permissible limit (200 and 600 ppm respectively). This may be responsible for the health problem of the people in these areas. The F^- ion had a concentration limit between 0.064 and 0.243 ppm which is still below the desirable limit (0.6-0.9 ppm). The highest concentration value of the Br^- ion (25.64-170.10 ppm) was found in water samples collected in the monsoon season. The Br^- content in coal is 0.5- 90 ppm (Davision, 1996 ; Swaine, 1990) and the maximum desirable and permissible limit of Br^- in drinking water is 1.0 and 1.5 ppm respectively. Two sources namely Ledopani and Kachanalla are used by the local habitant for their domestic use and for drinking purpose. High level of Br^- ion in Ledopani (75.21-78.314ppm) and Kachanalla (25.641-27172ppm) may be responsible for water contamination. The high level of bromine in plasma can produce bromism which is related to various health problems including disorder in nervous system and gastrointestinal tract (Dutta et al., 2017). The anionic concentrations of different ions in non-monsoon season are shown in Figure 3.15 and Figure 3.16. From these figures it can be revealed that only the SO_4^{2-} ion is the most prominent ion in water samples collected during non-monsoon season. Other anions especially F^- and Cl^- have very low concentration in the mine water during this season.

3.7 Study of cytotoxicity assay of AMD water

Cytotoxicity is the quality which is responsible for toxicity in cells. In this study the toxic effect of mine affected water in different human cell and rat cells were studied by using four cell lines including normal rat muscular cells (L6), human pancreatic carcinoma (MIAPaCa2), human lung carcinoma (A549) and human hepatocellular carcinoma (HepG2), which are widely accepted parameters for such studies. Cytotoxicity of the mine water samples was measured against these cells by MTT [(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay for 24hrs time. These normal and immortal cell lines provide an insight in respect to toxicity in non-mutated and mutated cells in the pancreas, Lung and liver (Koivusalo et al., 1995; Burczynski et al., 2000; Wilkening et al., 2003; Signorelli et al., 2010; Jia et al., 2008). The control cells were 100% viable. The cell viability assay demonstrated an overall dose-dependent decrease in percentage viability. It was observed that an increase in the dose of water samples from the mining sites led to a decrease in the percentage viability in all four cell lines with maximum effect on HepG2 cell lines. L6 cells showed a viability of 54-67% when treated with 300 µg/ml dose of water samples with minimum in 17-7 sample treated and maximum in 17-5 sample treated cells. In MIAPaCa2 cells 39-59% viability was seen with minimum viability in 17-7 sample treated and maximum in 17-3. A549 cells shows decrease in viability of 34-65% with least viability in 17-4 treated cells and maximum viability in 17-8 treated cells. However, HepG2 cells marked the significant decline in viability of 31-46% with the same dose at 24 h where 17-6 sample treated cells were least viable and 17-10 sample treated cells were most viable. It can be concluded that water samples from mining sites depict more toxicity in HepG2 followed by A549, MIAPaCa2 and L6 cell lines at higher concentrations. The cell morphology also supports the result wherein changes in HepG2 cells are more prominent at the dose of 300µg/ml than A549, MIAPaCa2 and L6 cells at 300 µg/ml dose (Figure 3.17).

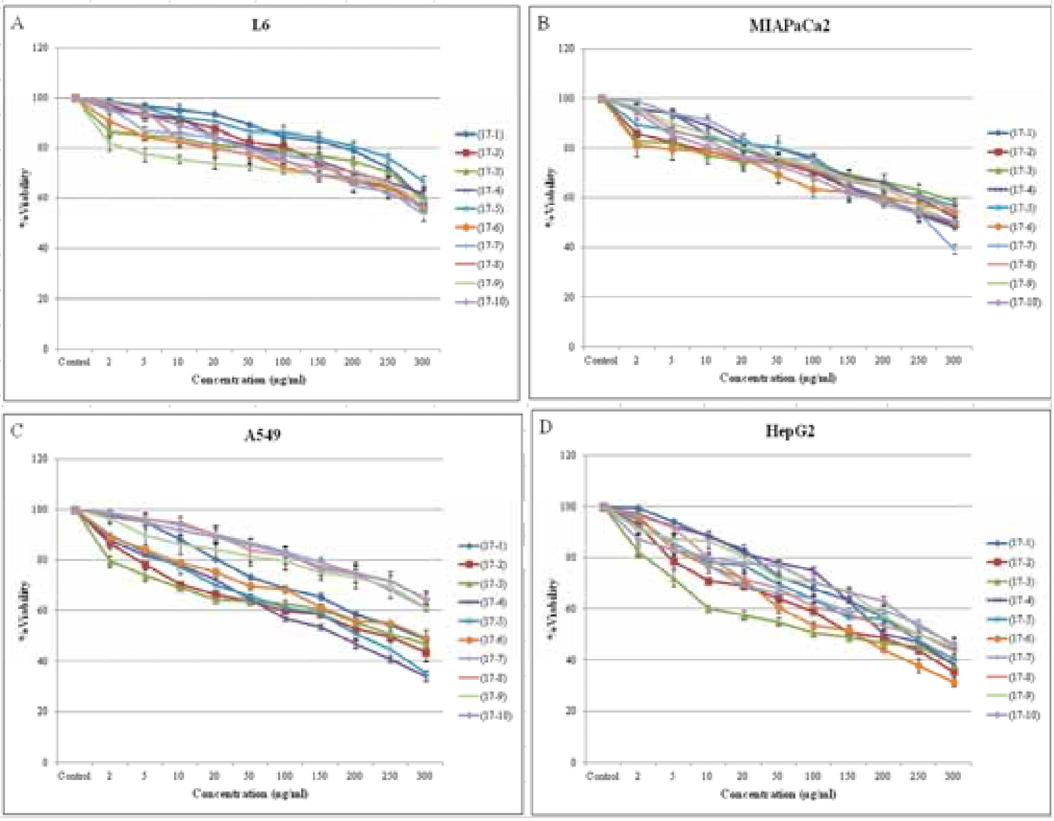

Figure 3.17 : Water samples from mining sites induces decline in percentage viability of (A)Normal rat muscle (L6), human carcinoma viz. (B) Pancreatic (MIAPaCa2), (C) Lung (A459) and (D) Liver (HepG2) cell lines

3.8 PAHs contents in AMD water

The concentrations of 16 PAHs in the AMD water samples of the study area are summarized in Table 3.8. Among all the three samples, sample-17-3 was recorded to be the highest total average PAHs concentration with 17.33±4.10 µg/l followed by sample17-8 and sample17-5with the mean concentrations of 14.82±4.34, 11.54±1.34 µg/l, respectively. These samples were collected from nearby point sources of AMD in the coalmine area, which generally leached into the nearby water bodies. Out of 16 PAHs, Naphthalene, Acenaphthylene, and Fluoranthrene were detected in all the samples. The high Phenanthrene concentration (84.962 µg/l) in sample-17-3 may be also due to the spill oil and other petroleum fuels from engine used in the mining operation which also lead to the discharge of polycyclic aromatic hydrocarbons in runoffs (Mahvi and Mardani, 2005) Mostly carcinogenic PAHs detected at the sites were Benzo(a)anthracene, dibenzo(a)anthracene, Benzo(b)fluoranthrene (Essumang, 2009) with a concentration of 7.038 µg/l, 1.114 µg/l, and 0.169 µg/l are found to be present in sample 17-3. This is to be mentioned that all the total concentrations identified at the study sites exceeded the WHO's limit of 0.05µg/l (WHO,1998) revealing a high level of PAHs contamination in the AMD water samples. In previous studies, the PAHs are reported to be one of the major threaten to aquatic ecological system (Hou et al., 2013) The presence of these carcinogenic PAHs in the samples also strongly supports the cytotoxicity assay as observed. Thus the cytotoxicity study of the water samples from mining sites depict more toxicity in HepG2 followed by A549, MIAPaCa2 and L6 cell lines at higher concentrations.

Table 3.8: PAHs contents in AMD Water from Ledo Colliery (µg/l)

PAHs	17-3	17-5	17-8
Napthalene	39.938	27.839	36.530
Acenapthylene	0.470	0.151	0.140
Acenaphthene	bdl	bdl	bdl
Fluorene	0.374	bdl	bdl
Phenanthrene	84.962	bdl	bdl
Anthracene	bdl	bdl	bdl
Fluoranthrene	4.587	6.644	7.795
Pyrene	bdl	bdl	bdl
Chrysene	bdl	bdl	bdl
Benzo(a) anthracene	7.038	bdl	bdl
Benzo (b) fluoranthrene	0.169	bdl	bdl
Benzo(k) fluoranthrene	bdl	bdl	bdl
Benzo (g) pyrene	bdl	bdl	bdl
Di benzo (a)anthracene	1.114	bdl	bdl
Benzo (ghi) perylene	bdl	bdl	bdl
Indenopyrene	bdl	bdl	bdl
Total PAHs (Mean±Stdv)	138.65 17.33±4.10	34.63 11.54±1.34	44.46 14.82±4.34

(bdl: below Detection Limit)

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