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Kalsoom Shaikh
a) Department of Zoology, University of Sindh, Jamshoro 76080, Pakistan
b) Institute of Advanced Research in chemical sciences, University of Sindh, Jamshoro 76080, Pakistan

Gulam Sarwar Gachal
Department of Zoology, University of Sindh, Jamshoro 76080, Pakistan

Saima Qayoom Memon
Institute of Chemistry, University of Sindh, Jamshoro 76080, Pakistan

Muhammad Yusuf Shaikh
Department of Zoology, University of Sindh, Jamshoro 76080, Pakistan

Correspondence:
Kalsoom Shaikh
a) Department of Zoology, University of Sindh, Jamshoro 76080, Pakistan
b) Institute of Advanced Research in chemical sciences, University of Sindh, Jamshoro 76080, Pakistan

Evaluation of Water Quality and Seasonal Variation in Aquatic Environment of Amphibians in District Hyderabad, Sindh, Pakistan

Kalsoom Shaikh, Gulam Sarwar Gachal, Saima Qayoom Memon, Muhammad Yusuf Shaikh

Abstract
Amphibians decline rapidly in the areas where pollution is high. Contamination in water reservoirs affects amphibians very badly as their physical as well as physiological development depends on the quality of their aquatic ambient. Present study that concerns with analysis of water quality and seasonal changes was conducted to know the status of amphibian environment in District Hyderabad. This investigation was carried out during 2013 during which water samples were collected from permanent amphibian dwelling in agricultural ponds. Water samples were analyzed via ultraviolet-visible spectroscopy. The study revealed water quality polluted due to high values of Sulphate (443.7±133.2), Phosphate (432.6±123.8), Nitrite (4.6±1.7) and Nitrate (7.4±3.2) during entire study period. This analysis discovered that the amphibians are exposed to high contamination in District Hyderabad; hence concerned authorities must take quick efforts to save amphibian fauna from declination.

Keywords: Amphibian environment, non-metallic contaminants, District Hyderabad, Sindh, Pakistan

Introduction
Present study is consecutive investigation of previous investigation that analyzed District Hyderabad (3,198 Km^2) to identify physic-chemical nature of amphibian ambient in relation to values of pH, electric conductivity and total dissolved solids and discovered whole study area unsuitable for the survival of amphibians [1].

Frogs are valued in several economic fields of human benefit such as they play vital role in maintenance of food web, agriculture, anatomy experiments, Pharmacology and they are also utilized as food in several countries of the world. Therefore amphibians need to be protected from various threats which cause them decline.

Amphibians are very sensitive to the contamination of their environment. Their decline in wild suggests the problematic condition of natural water reservoirs that need to be responded attentively. The deterioration of amphibian diversity is an issue of great concern worldwide. Amphibians are estimated to decline significantly since 1950, presenting more than one third threatened species and over one hundred and twenty declined species [2-3]. Water pollution is one of the main reasons that have led amphibians to mortality by destroying their eggs and larvae before flourishing into adults [3].

The increasing amounts of nonmetallic elements i.e. Sulphate (SO_4), Phosphate (PO_4), Nitrite (NO_2) and Nitrate (NO_3) have proved to be highly toxic causing harmful effects on their aquatic life by damaging their internal organs [4-6].

The global loss of amphibians was first recognized in 1989 and became worldwide important. The US National Research Council Workshop in 1990 monitored the diversity and population declines of amphibian fauna. By 1993 more than 500 species of amphibians were listed as declining or of conservation concern [7].

According to IUCN assessment in 2008 Latin American countries such as Colombia, Ecuador and Mexico have largest number of threatened amphibian species and also in Haiti and Caribbean where about 92% and 80% amphibian species are at the risk of extinction respectively. A comprehensive assessment of IUCN based on conservation status of amphibian fauna indicates upsurge in number of threatened amphibian species from 1996 to 2014 in 60 different countries of the world (Table 1.1). The IUCN, 2014 reports total percentage of threatened species (88%), lower estimate of threatened species (31%), best estimate of threatened species (41%), and Upper estimate of threatened species including number of threatened and Data Deficient of extant evaluated species (56%). It has also highlighted the

changes in number of threatened species from 1996 to 2014.

The SO_4, PO_4 and NO_3 are potentially harmful pollutants with a destructive role by supporting eutrophication. Eutrophication affects the water quality and makes reservoirs unsuitable for the survival of amphibians [7].

NO_2 equally creates alarming problems by disrupting metamorphosis along with multiple physiological effects such as body of amphibians swell and becomes transparent, tadpoles exhibit slow swimming and less feeding; overdeveloped head, digestive deformities, edema and paralysis may also develops before eventual death [8- 9].

Due to deplorable survival and sensitive nature of amphibians some international organizations such as IUCN (International Union for the Conservation of Nature), ASA (Amphibian Survival Alliance), ISSCA (International Society for the Study and Conservation of Amphibians) and WWF (World Wide Funds for conservation of nature) are actively working to protect amphibians in several parts of the world.

In consequence present study was carried out to record more comprehensive knowledge regarding the status of amphibians in District Hyderabad through analysis of some important parameters that were not evaluated and recorded by the previous study [1].

Material and Method

Present research was focused on the determination of amphibian survival. In this regard field ssurveys were conducted for the observation of amphibian eggs, larvae and adults to confirm their permanent location in agricultural fields of District Hyderabad.

All study sites were bearing different types of crops such as wheat was cultivated at study sites Samanpur and Shadabpur (S-1 and 2). The fields of Khan pur (S-3) produced rice, whereas Khushhal Khan (S-4) and Sahib khan (S-5) consisted of vegetables crops. Rabi Dero and Khuman (S-6 and 7) were full with the cultivation of different types of fruits. The agricultural field of Mohammad Alam ((S-8) was prominently rich with agronomy of sorghum.

The ponds of each field were selected for regular and systematic water sampling from March to October during 2013. The size of agricultural ponds was measured using measuring tape and confirmed as 10-15 feet. The depth of ponds was ranging between 5 to 8 feet duly measured via Secchi disk. The water sampling was conducted between 10 am to 06 pm in Van Dorn plastic bottles from different places of same pond to make a gross water sample for evaluating water quality of complete pond. Water samples were kept in stoppered polyethylene plastic bottles prior to use later soaked in 10 % HNO_3 for 24 hours and rinsed with ultrapure water obtained from ELGA Lab water system. All the water samples were stored in insulated cooler containing ice and delivered to the laboratory of Institute of Advanced Research in chemical Sciences (IARCS) University of Sindh, Jamshoro Pakistan. All samples were analyzed using Ultra-Violet Spectrophotometer (Hitachi 200). The specific wavelength through which absorbance of certain parameter was recorded is mentioned in Table 1.

Table 1: Specific wave-length of UV-visible light for analysis of each non-metallic parameter

Parameters	UV Wavelength (nm)
Sulphate	420
Phosphate	880
Nitrite	540
Nitrate	410

The analytical method and procedure for the evaluation of each parameter [10-11] is described below:

Sulphate analysis

Conditioning reagent was prepared by mixing 50 ml glycerol with a solution containing 30 ml conc: HCl, 300 ml distilled water, 100 ml 95 % isopropyl alcohol and 75 g NaCl.

Barium chloride ($BaCl_2$) Crystals (E. Merck) was also used.

Standard sulphate solution (100 µg SO_4/ml) was prepared by dissolving 147 mg anhydrous Na_2SO_4 in distilled water and diluted to 1,000 ml.

Procedure: In a conical flask containing 25 ml water sample was added 1.25 ml conditioning reagent and contents were mixed with magnetic stirrer. While the solution was being stirred, $BaCl_2$ (0.05 g) crystals were added and stop watch was immediately started. This mixture was stirred for exactly 15 seconds at a constant speed. The mixture was transferred to cuvette (1 cm) and absorbance was measured at interval of 30 seconds for four minutes on UV Spectrometer at specific wavelength (Table 1). The calibration curve was prepared with 5-40 mg L^{-1} sulphate. The sulphate concentration in the sample was calculated by comparing the turbidity reading with a calibration curve prepared by carrying sulphate standards through the entire procedure.

Phosphate analysis

Phosphate in water reacts with molybdate, under acidic condition, to form a Phosphomolybdic acid which is reduced to molybdenum blue by ascorbic acid.

Ammonium molybdate $(NH_4)_6$ solution: $Mo_7. O_{24}. 4H_2O$ (20 g) was dissolved in 500 ml distilled water, and stored in a bottle at 4ºC.

Potassium antimony potassium tartrate (K (SBO) $C_4H_4.O_6.1/2.H_2O$) solution: 1.3715 g of this chemical was dissolved in 400 ml distilled water and diluted up to 500 ml in volumetric flask.

Ascorbic acid solution: 1.76 g of ascorbic acid was dissolved in 100 ml of distilled water and stored in an amber bottle.

Combined reagent: 50 ml salfuric acid (5N) solution was mixed with 5.0 ml antimony potassium tartrate solution. 15 ml ammonium molybdate and 3 ml ascorbic acid solution was further added.

Phosphate-phosphorous (5.0 u g PO_4-P/ 100 ml): Potassium-dihydrogen-phosphate (0.21 g) was dissolved in 1 Liter of distilled water.

Procedure: To a sample of water (42 ml) was added 8.0 ml of combined reagent and was make up the volume of 50 ml. the solution was mixed and absorbance was measured against blank at certain wavelength of UV Spectrophotometer (Table 1). The calibration graph was plotted in the range of 0.15 to 1.30 mg L^{-1} and amount of phosphate was calculated from the calibration graph.

Nitrite analysis

The most widely used method for nitrite determination is based upon the diazotization reaction to produce diazonium ions which then complex with N (1-naphthyl) ethylene-diamine-dihydrochloride (NED) to produce stable pink azo dye.

Sulfanilamide solution: 1 g sulfanilamide dissolved in HCL and solution volume was made up to 100 ml with distilled water.

NED solution: 0.1 g NED was dissolved in 100 ml distilled water

Procedure: To a 50 ml volumetric flask was added 48 ml water sample and 1.0 ml sulfanilamide solution. After 2.0 to 8.0 minutes, 1.0 ml NED solution was added and volume was adjusted 50.0 ml. Absorbance was measured using Ultra-violet Spectrophotometer. The calibration graph was plotted in the range of 1.0-25 u g/L NO_2-N and the amount of nitrite in water samples was evaluated from calibration curve.

Nitrate analysis

Nitrate was evaluated using Brucine as derivatizing reagent. The reaction between nitrate and brucine sulphate produces yellow color, which could be used for the determination of nitrate.

Anhydrous potassium nitrate (0.1 mg L^{-1}) solution: Anhydrous potassium nitrate 72.1 mg L^{-1} was dissolved in distilled water and volume was adjusted to 100 ml. Further solutions were prepared by appropriate dilutions.

Brucine Sulphate solution: Brucine Sulphate (1.0 g) and sulfanilic acid (0.1 g) were dissolved in 70 ml of hot distilled water and was added 3.0 ml of HCL (37 %), cooled and volume was adjusted up to 100 ml.

Sulphuric acid solution: Concentrated sulphuric acid (500) was added to 125 ml distilled water, which was cooled to room temperature before using.

Sodium chloride solution: Sodium chloride (30 g) was slowly dissolved in a beaker using glass rod and was diluted to 100 ml with distilled water.

Procedure: 10 ml water sample was added 2.0 ml NaCl solution and 10.0 ml H_2SO_4 solution. This solution was mixed well and was added 0.5 ml Brucine-sulfanilic acid reagent. This mixture was heated at 100 C for 20 minutes on water bath and was cooled to room temperature. The absorbance was measured using UV Spectrophotometer.

Results and Discussion

Water is crucial for survival of all organisms but particularly for the aquatics which respire through the water, thus physical and chemical components of water have great impact on them. The research on amphibian conservation has been carried out in several regions of the world by many researchers and organizations (already described in introduction of this research paper) but wild environment of Sindh province has not been improved to sustain wild animals including amphibian fauna.

District Hyderabad gave shelter to three amphibian species at different locations as indicated in Figure 1. The environment of this area was reported to be unsuitable for the amphibians due to high level of EC (electric conductivity) and TDS (total dissolved solids) at the same stations [1].

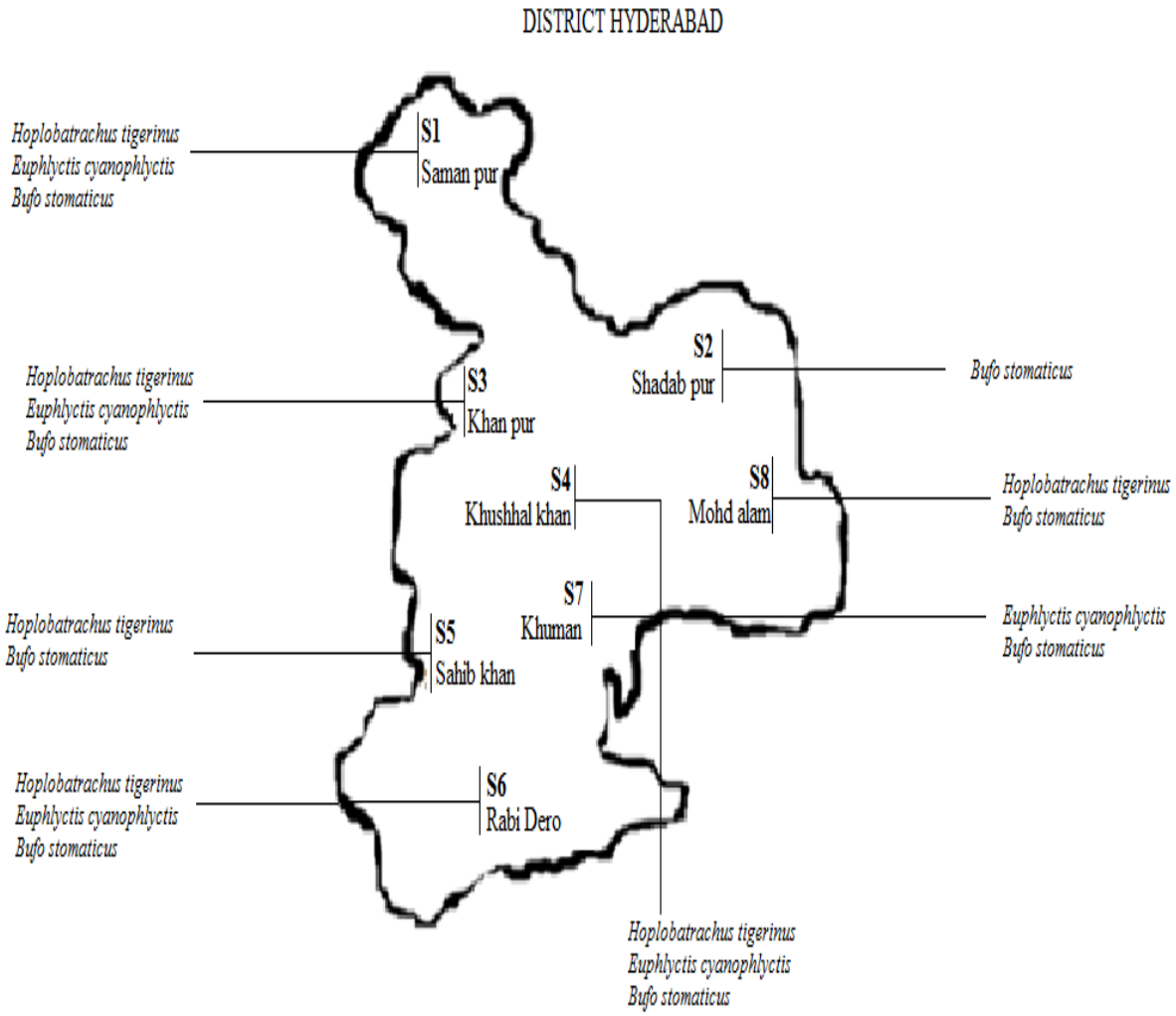


Fig 1: Amphibian diversity in District Hyderabad, Sindh-Pakistan

The water quality of all study sites varied from each other in relation to concentration of analyzed parameters (Figure 2).

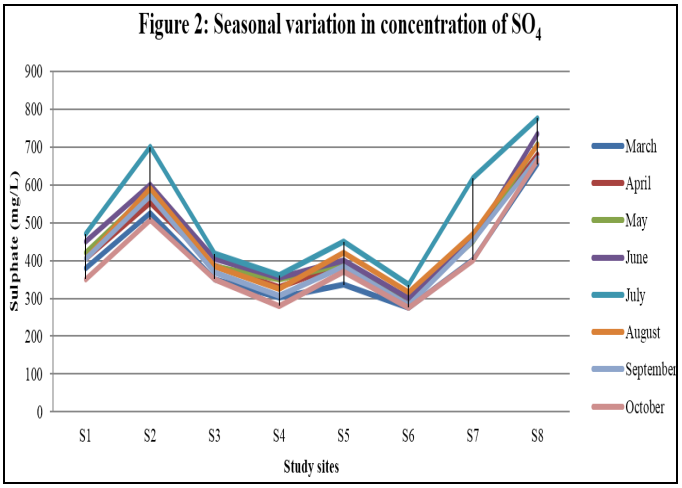
Table 2: Concentration of analyzed parameters in amphibian habitats of District Hyderabad

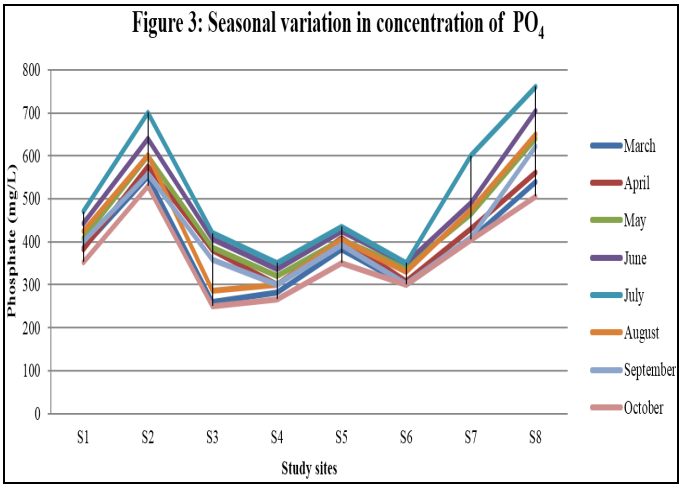
Parameters mg^{-L}	S1	S2	S3	S4	S5	S6	S7	S8	Mean±Stdev
March									
Sulphate	380.2	525.1	350.5	302.8	335.5	275.5	402.5	655.2	403.4±126.8
Phosphate	385.4	552.2	260.4	282.6	382.8	300.0	405.7	540.2	388.7±110.6
Nitrite	2.8	5.2	2.9	1.2	4.4	3.3	4.0	4.5	3.5±1.3
Nitrate	5.1	7.5	4.7	3.6	7.2	5.9	7.2	10.1	6.4±2.0
April									
Sulphate	405.9	552.1	385.9	330.8	390.2	282.5	460.8	680.2	436.1±127.5
Phosphate	382.2	575.4	380.4	300.3	409.2	308.2	430.5	562.0	418.5±103.1
Nitrite	3.0	5.8	4.0	2.2	5.3	3.6	5.5	6.0	4.4±1.4
Nitrate	5.5	8.2	5.4	4.2	7.0	5.1	7.2	15.0	7.2±3.4
May									
Sulphate	420.5	582.8	383.5	350.7	390.2	300.5	450.1	708.5	448.4±133.9
Phosphate	408.2	600.0	385.5	320.2	405.2	340.8	465.3	640.5	445.7±116.8
Nitrite	3.2	7.8	3.8	2.7	5.9	4.8	5.0	6.5	5.0±1.7
Nitrate	5.7	8.8	5.2	4.5	7.5	5.6	7.5	15.0	7.5±3.4
June									
Sulphate	450.0	600.0	405.3	355.5	400.2	300.8	455.2	735.7	462.8±140.7
Phosphate	442.2	640.0	407.2	335.5	425.2	350.6	490.2	705.2	474.5±132.8
Nitrite	3.9	8.5	4.2	3.5	4.8	3.5	5.5	7.1	5.1±1.8
Nitrate	6.1	9.2	5.5	4.8	8.2	5.8	8.5	16.9	8.1±4.0
July									
Sulphate	470.2	700.5	418.2	360.5	450.2	335.5	618.1	776.5	516.2±162.6
Phosphate	470.0	700.7	420.3	350.8	435.3	350.9	600.8	760.9	511.2±157.4
Nitrite	4.2	8.5	5.2	4.0	5.5	4.2	6.87	8.0	5.8±1.8
Nitrate	6.5	10.2	6.0	5.7	8.5	6.5	10.5	17.2	8.9±3.8
August									
Sulphate	405.8	592.2	383.5	325.5	420.1	315.5	470.5	705.2	452.3±134.5
Phosphate	425.2	600.8	285.2	300.5	405.2	330.8	475.1	650.0	434.1±135.1
Nitrite	3.7	7.8	3.8	2.0	5.4	4.2	5.6	6.7	4.9±1.8
Nitrate	5.9	9.2	5.3	4.4	8.1	6.2	7.7	16.2	7.9±3.7
September									
Sulphate	405.0	570.8	368.5	307.1	385.0	282.5	452.5	670.2	430.2±131.7
Phosphate	400.5	557.8	358.4	300.7	392.6	300.8	410.5	622.8	418.0±115.6
Nitrite	3.2	6.5	3.2	1.5	5.0	3.5	4.6	6.2	4.2±1.7
Nitrate	6.1	8.8	4.8	3.5	6.5	4.9	7.5	13.5	7.0±3.1
October									
Sulphate	350.5	508.2	350.5	280.5	370.7	275.5	400.5	665.2	400.2±129.6
Phosphate	352.4	530.0	250.2	265.8	350.1	300.0	405.2	505.8	370.0±104.3
Nitrite	2.5	5.0	2.7	1.57	4.6	2.7	4.5	5.5	3.6±1.4
Nitrate	4.0	7.7	4.2	3.0	6.0	5.0	7.2	10.3	5.9±2.4

Stdev= Standard deviation

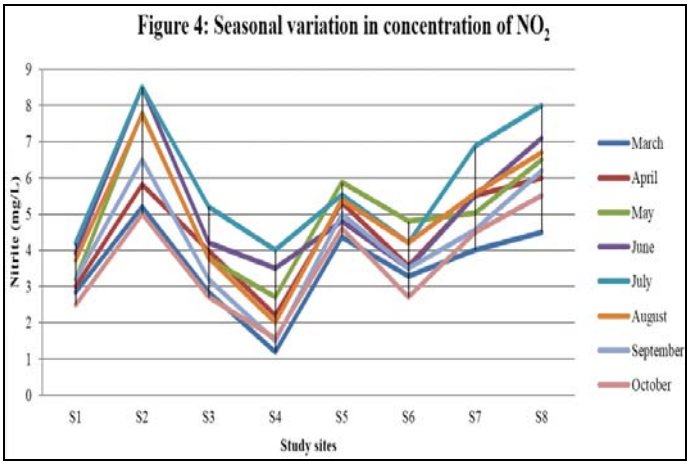
The level of SO_4 in entire study area was extremely high and beyond the recommended level (50-100 mg^{-L}) [11] as its concentration was recorded between 275.5 to 776.5 mg^{-L} (Figure 2). Highest quantity of this parameter was found at study site 8 (Mohammad alam), whereas study site 6 (Rabi Dero) was concentrated with comparatively lowest value of SO_4 (Figure 2).

Phosphate concentration (250.2-760.88 mg^{-L}) was also up to threatening level (Figure 3) as it is preferred when under 0.05 mg^{-L} into lakes or other reservoirs where aquatic life exists to control algal growth [13]. The concentration of PO_4 was recorded highest alternatively at two study sites. Monthly variation in value of PO_4 was similar to fluctuation in level of SO_4 (Figure 3).

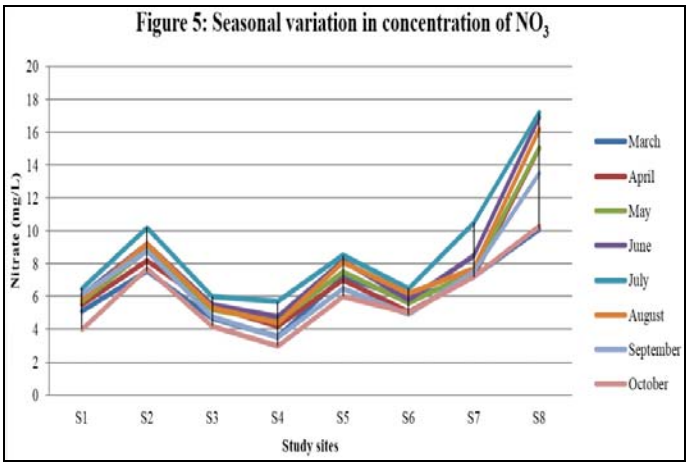




Nitrite concentration as indicated in Figure 4 was also extremely high (8.5 mg^{-L}). This parameter is reported to affect negatively at the concentration up to 2.1 mg^{-L} specially during hatching time [14]. Its high level was recorded to persistent at study site 2 (Shadab pur), wherein study site 4 (Khushhal khan) low value of the parameter was recorded. Most elevated value of NO_2 was recorded from all amphibian dwellings in July, wherein March its concentration reduced to least level.



Nitrate starting to affect lethally and sub lethally from the concentration of 2.5 to 100 mg^{-L} [15]. For that concern the NO_3 concentration at all study sites of District Hyderabad may not support amphibian life. Study site 8 was consisting of extreme value of NO_3 (10.1 to 17.2 mg^{-L}). The maximum and minimum value of this parameter was also comparable to variation in other parameters (Figure 5).



Present study indicated unfavorable environment at all the amphibian localities of District Hyderabad. Extreme level of all parameters from May to August may make environment tougher for the survival of eggs and larvae as breeding and hatching take place during this period. This environmental condition may ultimately affect amphibians negatively and may lead them towards massive mortality. This environmental status implies the careless and negligent role of local people and concerned authorities towards aquatic life as these pollutants may enter the water reservoirs (inhabited by amphibian) as a result of anthropogenic discharges and negligent usage of chemical fertilizers. This environmental status may be the reason behind deplorable diversity of only three amphibian species (*Hoplobatrachus tigerinus, Euphlyctis cyanophlyctis* and *Bufo stomaticus*) in District Hyderabad [9] out of 24 species discovered from other areas of Pakistan [16-18].

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