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ASSESSMENT OF ENVIRONMENTAL ISSUES OF AMPHIBIAN FAUNA IN TALUKA THANO BULA KHAN (DISTRICT JAMSHORO) SINDH-PAKISTAN

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Abstract

The present study was conducted in a subdivision of District Jamshoro "Taluka Thano Bula Khan" to analyze the water quality of amphibian's ponds. The field surveys were carried out from 2011-2013 at six ponds, where the permanent habitation of amphibians was confirmed The water quality of each pond was analyzed using the standard methodology and scientific instrumentation. During this three years study, it was determined that the pH and carbon dioxide (mg L^{-1}) were the only parameters, whose values (8.0±0.6 and 18.2±3.32 respectively) were normal in whole study area, while the values of electric conductivity (µS cm^{-1}) 2821.8±1202.2, total dissolved solids (mg L^{-1}) 1861.8±759.0, total hardness (mg L^{-1}) 367.7±56.0, total alkalinity (mg L^{-1}) 351.7±54.9, chloride (mg L^{-1}) 377.6±135.4, sulphate (mg L^{-1}) 463.8±125.5, phosphate (mg L^{-1}) 439.2±124.9 and potassium (mg L^{-1}) 67.5±10.7 were above the normal limit. It was also determined that the concentration of nitrite (mg L^{-1}) 2.2±1.6 and nitrate (mg L^{-1}) 6.1±4.3) varied from low to high values. Present research recorded the high rate of pollution in the entire environment of amphibians, where no conservation action is ever implemented for the conservation of these delicate animals; even this present study is unique, due to the fact that it highlights the ruined status of amphibian ambient for the first time in Taluka Thano Bula Khan.

Keywords: *Amphibian environment; Water quality issues; Physico-chemical Parameters; Taluka Thano Bula Khan; District Jamshoro; Sindh; Pakistan*

Introduction

Class Amphibia consists of a group of unique vertebrates of about 7000 species which are greatly considered to be threatened worldwide [1]. The assessment of IUCN (International Union for Conservation of Nature) has reported that nearly 32% of the amphibian population has declined significantly, mainly within last two decades [2]. There are many reasons behind the amphibian decline in different parts of the world. The most complex factors that affect amphibian fauna include habitat obliteration, deforestation and disintegration of amphibian habitats by exposing them towards a vast number of potential competitors or predators.

Water contamination by negligent use of toxic fertilizers and garbage throwing in agricultural ponds also contribute to massive pollution. Other factors responsible for amphibian deterioration include climate alteration, infectious maladies and ultraviolet radiations [3]. The

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status of amphibian fauna has mainly been destroyed due to the massive use of toxicants especially in form of agro chemicals, which affect amphibian populations significantly in many parts of the world like Karachi and Thatta of Pakistan [4].

Amphibian decline is a serious issue in some countries of the world where deforestation is responsible for damaging the diversity of not only amphibians, but of all the animal species that occur in wild. Amphibians are protected in several parts of the world from being intentionally killed or harmed. Traditional and economical practices involving deliberate capture and exportation have been banned in some regions [5]. Some international organizations including IUCN, ISSCA (International Society for the Study and Conservation of Amphibians) and WWF (World Wide Funds for conservation of nature) work actively in many countries to save the amphibian fauna.

Present investigation was conducted for the physico-chemical analysis of amphibian ponds, to understand their problems in their primary aquatic environment. The previous studies carried out in other areas of Sindh Province have revealed a highly contaminated environment with negative impact on the amphibian fauna [6-9]. Therefore, Taluka Thano Bula Khan was selected as a study area for recording new fangled information about the amphibian conservation status in one more area of Sindh province.

Material and Methods

The study extended from March to October in the years 2011, 2012 and 2013. The field surveys and water sampling were executed on the same day from 9am-5pm at six amphibian ponds (Fig. 1) by following the standard instruction [10].

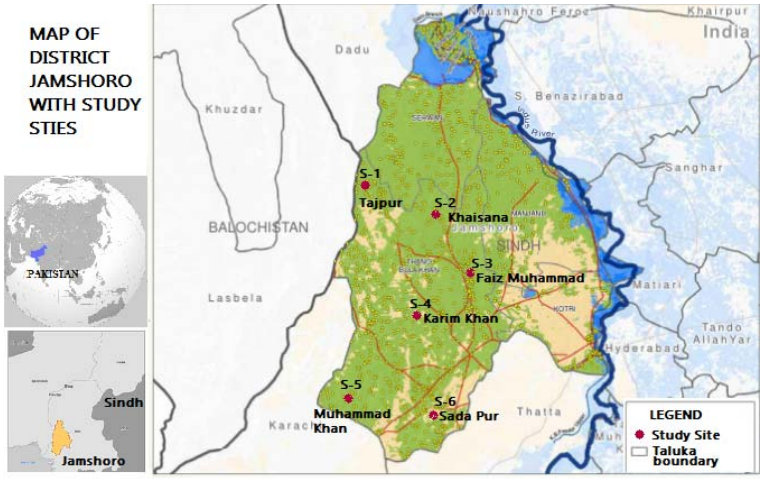

Fig. 1. Map of study area

The gross water sample of each pond was collected into Van Dorn plastic bottle and brought to the laboratory for physico-chemical analysis. The Orion 420 pH-meter was to analyze the concentration of hydrogen ions, whereas a conductivity meter (Model: Orion 115) was used to record the value of both electric conductivity (EC) as well the total dissolved solids (TDS). The values of total hardness (T-Hard), total alkalinity (T-Alk), chloride (Cl^-) and carbon dioxide (CO_2) were analyzed via titration procedures following the analytical methodology [11-12]. The concentration of sulphate, phosphate, nitrite and nitrate in water samples was recorded

using an Ultraviolet visible Spectrophotometer (Model: Hitachi 200). Different wavelength of ultra violet visible light was applied during the analysis of different non-metallic parameters. For example, the sulphate was analyzed at 420nm wavelength of ultra violet visible light, phosphate at 880nm, nitrite at 540nm and nitrate was evaluated at 410nm wavelength. The quantity of potassium was recorded using an Atomic Absorption Spectrophotometer (Model: Perkin Elemer Analyst 800). Scientific literature [13-25] helped in identifying the water quality of the amphibian environment.

Results and Discussion

The study conducted at six amphibian ponds indicated the value of each parameter fluctuating every month as well as every year (Table 1-3). The range of each parameter is given along with the mean value and standard deviation to show the status of amphibian environment throughout the years 2011, 2012 and 2013.

Table 1. Physico-chemical nature of amphibian ponds during year 2011

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	7.2-8.6	7.5-8.8	7.5-8.8	7.7-9.0	8.0-9.0	7.5-8.9	7.2-8.9	7.0-8.5
	Mean	7.9	8.2	8.3	8.4	8.5	8.2	8.0	7.6
	Stdev	0.6	0.5	0.5	0.5	0.4	0.5	0.6	0.6
EC $\mu S \cdot cm^{-1}$	Range	1485.5-4380.9	1552.8-4480.7	1582.7-4535.0	1650.2-4575.2	1762.0-5130.0	1585.7-4489.2	1550.2-4386.0	1450.2-4352.0
	Mean	2751.5	2836.3	2879.9	2934.2	3162.5	2872.1	2773.5	2707.6
	Stdev	1270.7	1313.1	1311.8	1312.0	1500.8	1284.5	1288.1	1284.6
TDS $mg \cdot L^{-1}$	Range	1050.2-2975.0	1098.5-3007.5	1109.2-3048.2	1134.8-3085.0	1180.5-3437.1	1100.5-3000.0	1098.8-2868.0	1000.2-2950.0
	Mean	1801.8	1876.8	1901.9	1942.3	2130.0	1895.6	1827.5	1760.8
	Stdev	84.0	877.3	876.4	886.6	993.3	854.5	803.0	841.1
T-Hard $mg \cdot L^{-1}$	Range	270.2-400.5	285.5-438.2	300.0-450.2	300.2-465.5	333.5-500.0	300.0-450.7	289.5-400.8	250.8-378.5
	Mean	340.5	362.0	378.2	386.0	405.4	377.8	354.3	321.6
	Stdev	50.5	54.3	58.1	64.1	66.8	56.9	40.5	49.3
T-Alk $mg \cdot L^{-1}$	Range	265.5-400.8	280.5-420.5	289.5-444.9	305.5-450.0	320.8-462.8	287.9-445.8	280.5-405.8	250.4-350.2
	Mean	336.9	351.8	365.0	381.1	397.1	363.7	346.3	312.9
	Stdev	58.0	57.4	57.6	57.2	56.7	60.1	48.9	48.6
Cl^{-} $mg \cdot L^{-1}$	Range	270.0-570.5	275.5-584.9	280.2-600.0	289.6-652.5	300.0-685.2	275.5-638.5	266.5-659.5	250.8-480.6
	Mean	384.2	405.0	413.5	429.4	434.4	406.6	396.9	339.2
	Stdev	137.7	176.600	162.1	169.2	163.8	143.6	138.0	99.8
SO_4^{2-} $mg \cdot L^{-1}$	Range	287.7-650.8	300.5-690.4	335.5-710.5	350.8-750.0	372.7-780.8	342.5-741.2	335.2-700.5	260.3-600.0
	Mean	454.2	481.9	503.3	514.8	535.0	494.4	478.4	412.7
	Stdev	158.0	153.8	160.1	164.1	168.8	158.8	162.7	135.4
PO_4^{3-} $mg \cdot L^{-1}$	Range	300.5-600.0	315.8-650.5	340.2-686.5	350.0-729.8	350.0-800.0	338.2-700.5	325.5-682.5	283.5-550.0
	Mean	437.1	475.2	488.4	503.8	530.1	486.0	468.6	411.4
	Stdev	126.2	147.8	150.8	158.0	181.0	145.2	140.8	116.5
NO_2^{-} $mg \cdot L^{-1}$	Range	0.2-4.5	0.2-4.7	0.3-5.0	0.4-5.0	0.2-5.6	0.4-4.9	0.3-4.5	0.1-3.5
	Mean	1.46	1.6	1.8	1.9	2.1	1.8	1.6	1.2
	Stdev	1.7	1.8	1.9	1.9	2.2	1.8	1.7	1.3
NO_3^{-} $mg \cdot L^{-1}$	Range	2.2-13.0	2.2-13.8	3.0-14.5	3.3-15.0	3.3-15.75	3.0-14.2	3.0-14.0	1.5-9.8
	Mean	5.8	6.4	6.8	7.1	7.8	6.7	6.3	4.6
	Stdev	4.8	5.0	5.2	5.4	5.6	5.0	5.2	3.7
CO_2 $mg \cdot L^{-1}$	Range	12.7-24.6	14.0-22.5	13.0-22.0	13.8-22.5	14.5-20.5	12.8-23.8	14.2-22.8	12.0-20.0
	Mean	18.8	18.5	17.8	17.6	15.8	17.8	18.6	19.2
	Stdev	4.2	3.6	4.5	3.3	3.0	2.4	2.9	3.1
K^{+} $mg \cdot L^{-1}$	Range	54.2-78.2	55.5-84.5	58.7-90.0	60.8-95.5	60.1-105.7	56.8-90.6	54.8-85.5	50.0-70.5
	Mean	63.8	66.2	69.7	71.7	76.7	69.4	65.9	60.1
	Stdev	8.7	11.5	11.5	12.8	16.6	12.5	10.5	7.2

Stdev. = Standard deviation

Table 2. Physico-chemical nature of amphibian ponds during year 2012

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	7.0-8.5	7.0-8.5	7.2-8.8	7.5-9.0	7.5-9.2	7.0-9.0	7.0-8.8	6.7-8.5
	Mean	7.7	7.8	8.1	8.4	8.4	8.2	7.9	7.6
	Stdev	0.6	0.8	0.7	0.6	0.6	0.6	0.6	0.7
EC µS·cm^{-1}	Range	1483.5-4380.8	1584.7-4452.9	1638.8-4482.5	1668.2-4527.8	1750.0-4730.8	1682.5-4578.2	1490.5-4479.5	1450.2-4385.1
	Mean	2745.0	2788.5	2874.4	2900.8	2989.0	2896.2	2814.8	2665.4
	Stdev	1309.4	1334.5	1292.0	1300.7	1332.4	1313.0	1294.6	1301.7
TDS mg·L^{-1}	Range	1015.5-2836.1	1103.8-2850.0	1208.5-2878.2	1259.2-2920.5	1175.1-3250.7	1150.9-3200.8	1108.5-3150.8	1085.2-3075.2
	Mean	1784.2	1816.6	1892.7	1961.4	2002.7	1903.7	1866.8	1767.7
	Stdev	809.2	795.8	911.9	926.9	912.5	779.7	782.7	837.1
T-Hard mg·L^{-1}	Range	280.2-382.0	300.0-385.5	325.1-390.7	330.8-410.8	335.5-500.2	300.8-487.5	288.5-475.5	275.5-450.8
	Mean	355.0	353.3	371.5	390.5	405.7	372.6±	363.0	336.9
	Stdev	62.2	32.2	66.5	65.8	60.0	30.6	24.5	40.8
T-Alk mg·L^{-1}	Range	267.5-400.5	280.3-438.5	300.0-450.0	308.9-450.0	350.2-460.5	335.8-450.2	300.6-435.9	280.5-400.7
	Mean	339.7	353.9	368.2	387.7	398.2	378.0	364.4	331.2
	Stdev	48.3	52.1	51.7	44.9	43.6	51.0	49.0	46.8
Cl^- mg·L^{-1}	Range	250.2-510.5	258.2-552.5	260.5-567.5	275.2-570.8	300.0-630.4	275.5-600.2	250.2-580.5	225.5-567.5
	Mean	353.9	370.2	384.8	399.2	419.5	395.6	378.7	350.4
	Stdev	143.2	124.8	129.1	145.0	146.9	131.1	147.9	122.2
SO_4^{2-} mg·L^{-1}	Range	300.9-600.9	310.5-610.8	335.9-620.1	350.2-650.0	365.5-700.0	350.8-670.5	335.4-650.6	300.8-610.5
	Mean	444.7	454.9	469.3	492.9	513.9	484.5	468.4	442.0
	Stdev	124.2	126.9	129.8	134.1	137.1	123.1	121.2	128.7
PO_4^{3-} mg·L^{-1}	Range	300.0-550.2	300.2-568.5	308.5-570.2	320.5-581.5	300.0-650.7	285.8-635.2	270.1-600.2	250.2-578.5
	Mean	418.3	421.7	447.4	472.0	487.2	450.0	433.6	406.0
	Stdev	117.5	131.6	108.7	133.5	135.9	129.0	111.6	111.8
NO_2^- mg·L^{-1}	Range	0.2-4.0	0.8-4.8	1.0-5.0	1.8-5.7	1.0-5.9	1.0-5.5	0.9-5.0	0.5-4.5
	Mean	2.0	2.2	2.6	3.1	3.2	2.9	2.4	1.4
	Stdev	1.5	1.5	1.6	1.5	1.8	1.7	1.5	1.6
NO_3^- mg·L^{-1}	Range	2.5-10.6	3.0-11.0	3.2-12.3	3.9-12.9	3.5-11.0	2.8-13.7	2.5-13.0	1.8-12.5
	Mean	5.6	5.8	6.3	7.1	8.2	6.7	6.3	5.3
	Stdev	4.9	3.9	4.2	5.1	4.3	5.1	5.0	3.9
CO_2 mg·L^{-1}	Range	12.8-22.1	14.8-22.5	13.8-22.0	13.0-20.8	15.2-23.5	13.8-22.0	12.9-22.0	14.8-24.2
	Mean	19.3	18.5	17.8	17.3	16.9	17.6	17.8	20.1
	Stdev	3.2	3.0	3.3	3.7	3.1	3.2	3.6	3.5
K^+ mg·L^{-1}	Range	50.6-75.5	55.0-77.5	57.2-80.2	60.0-82.8	58.7-98.5	55.8-95.2	55.0-80.5	50.8-76.5
	Mean	62.3	64.5	67.5	71.3	74.8	70.8	66.7	62.0
	Stdev	9.4	9.6	9.8	14.3	14.6	9.5	10.5	10.4

Stdev. = Standard deviation

The status of the amphibian environment in years 2011, 2012 and 2013 was studied comparatively, in order to make obvious the differences in conditions which prevailed each year in Taluka Thano Bula Khan (Fig. 2).

The range of each parameter during the entire study period was determined as followed: pH extended between the normal values (6.5 - 9.2), meanwhile the range of CO_2 (12.0 - 25.5mg·L^{-1}) was also normal throughout the present investigation. The value of EC was recorded very high, ranging from 1387.5 to 5130.0µS·cm^{-1}; meanwhile, the concentration of TDS (998.5 - 3437.1mg·L^{-1}), T-Hard (237.5 - 500.2mg·L^{-1}), T-Alk (230.5 - 462.8mg·L^{-1}), Cl^- (180.5 - 685.2mg·L^{-1}), SO_4^{2-} (250.0 - 780.8mg·L^{-1}), PO_4^{3-} (210.5 - 800.0mg·L^{-1}) and K^+ (46.8 - 105.7mg·L^{-1}) was also high up to harmful level. It was evaluated that the values of NO_2^- (0.1 - 5.9mg·L^{-1}) and NO_3^- (1.0 - 15.8mg·L^{-1}) varied abruptly from normal to unfavorable concentration but they remained mostly higher than the favorable limit. It was also noted that the concentration of all the parameters fluctuated in a synchronized manner. Their highest quantity was identified in July and their lowest quantity was recorded in October, except the value of the CO_2 that fluctuated in the opposite manner. It was recorded that the rate of

pollution was higher during 2011 and 2012. However, a comparatively low level of contamination persevered during the year 2013.

Table 3. Physico-chemical nature of amphibian ponds during year 2013

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	6.9-8.0	7.0-8.0	7.0-8.5	7.5-9.0	8.0-9.2	8.0-9.0	6.9-8.5	6.5-8.0
	Mean	7.4	7.6	7.8	8.4	8.6	8.5	7.6	7.2
	Stdev	0.5	0.4	0.6	0.5	0.4	0.4	0.6	0.6
EC $\mu S \cdot cm^{-1}$	Range	1450.2-4255.2	1482.4-4350.0	1500.8-4382.0	1578.2-4450.8	1650.8-4577.5	1608.2-4550.7	1563.2-4370.5	1387.5-4207.8
	Mean	2662.6	2727.8	2771.5	2832.8	2918.6	2869.4	2755.6	2593.3
	Stdev	1277.1	1288.5	1268.8	1285.7	1291.9	1296.9	1288.8	1285.6
TDS $mg \cdot L^{-1}$	Range	1000.8-2680.5	1100.8-2748.5	1147.2-2775.4	1180.5-2800.5	1248.6-2951.8	1180.5-2900.2	1100.2-2782.5	998.5-2612.8
	Mean	1711.2	1786.7	1833.6	1871.7	1959.5	1912.0	1826.5	1648.8
	Stdev	755.9	744.6	735.6	721.7	736.1	742.6	731.8	743.4
T-Hard $mg \cdot L^{-1}$	Range	250.5-400.7	275.5-425.8	300.0-450.0	325.7-465.5	350.0-480.5	330.1-468.3	300.8-450.2	237.5-390.5
	Mean	335.4	355.3	374.3	395.9	415.6	398.0	372.4	303.4
	Stdev	54.0	52.4	50.0	53.0	49.1	54.1	60.4	57.5
T-Alk $mg \cdot L^{-1}$	Range	258.5-380.9	264.9-388.9	270.5-400.1	280.8-417.5	292.5-450.5	280.7-435.5	250.7-400.0	230.5-372.5
	Mean	312.7	321.9	341.1	348.9	366.2	350.9	331.8	291.7
	Stdev	55.0	52.3	48.8	55.7	56.4	49.6	50.3	54.2
Cl^- $mg \cdot L^{-1}$	Range	200.0-518.5	200.8-540.7	225.8-550.0	566.7-242.7	250.0-580.2	228.2-550.4	205.8-550.0	180.5-480.5
	Mean	325.8	338.9	356.3	364.2	383.8	371.1	352.8	307.5
	Stdev	142.5	146.5	148.0	143.9	145.3	144.8	143.2	127.8
SO_4^{2-} $mg \cdot L^{-1}$	Range	280.2-550.0	288.5-565.5	300.8-570.3	320.5-578.5	350.1-580.7	342.8-550.0	315.5-550.0	250.0-500.7
	Mean	410.3	430.4	443.1	455.8	471.9	457.4	434.9	381.8
	Stdev	117.5	112.0	110.1	83.1	91.8	102.0	92.0	101.4
PO_4^{3-} $mg \cdot L^{-1}$	Range	250.7-500.8	255.4-520.5	265.8-535.5	277.5-550.0	280.5-566.5	265.5-552.8	250.4-540.2	210.5-500.2
	Mean	375.6	390.9	406.4	419.1	436.0	420.0	396.6	360.1
	Stdev	105.3	111.3	113.7	117.6	117.8	112.9	113.6	112.0
NO_2^- $mg \cdot L^{-1}$	Range	0.7-4.0	0.9-4.6	1.0-5.0	1.4-5.5	2.0-5.8	1.5-5.5	1.0-5.0	0.5-3.8
	Mean	1.7	2.4	2.7	3.0	3.5	3.1	2.6	1.4
	Stdev	1.3	1.3	1.4	1.5	1.4	1.5	1.6	1.3
NO_3^- $mg \cdot L^{-1}$	Range	1.5-9.5	2.0-10.4	2.5-12.0	2.7-12.7	3.0-12.8	2.3-12.2	1.8-11.5	1.0-9.5
	Mean	4.5	4.9	5.7	5.6	6.4	6.0	5.1	4.2
	Stdev	3.6	3.9	4.3	4.4	4.3	4.5	4.4	3.8
CO_2 $mg \cdot L^{-1}$	Range	12.8-22.5	14.8-22.5	12.8-22.9	12.0-22.4	12.0-20.5	13.5-20.0	15.0-22.4	15.8-25.5
	Mean	19.1	19.0	17.6	18.3	16.4	17.5	18.2	21.1
	Stdev	3.0	2.8	3.9	4.1	3.3	2.7	3.8	3.8
K^+ $mg \cdot L^{-1}$	Range	50.0-70.4	53.8-74.8	55.8-78.9	62.0-80.5	68.8-82.8	65.0-78.8	58.5-75.0	46.8-70.0
	Mean	60.2	64.3	68.1	71.8	75.8	72.1	66.1	57.3
	Stdev	8.3	7.7	8.7	7.3	5.5	5.3	6.3	9.4

Stdev. = Standard deviation

Discussion

Amphibian conservation is an issue of great concern due to their delicate respiratory skin which absorbs chemical elements found in their aquatic environment, thus becoming susceptible to environmental abnormalities. Eggs and larvae of amphibians are more exposed to the degraded water quality of ponds, where they remain confined until the completion of their development into adults [26]. About 168 amphibian species including true frogs have waned and more than 43% of the amphibian population is on the verge of decline [26]. This threatened status of amphibian fauna indicates that the rate of extinction may probably continue to increase in recent times, their decline being already regularly recorded in several regions of the world [26].

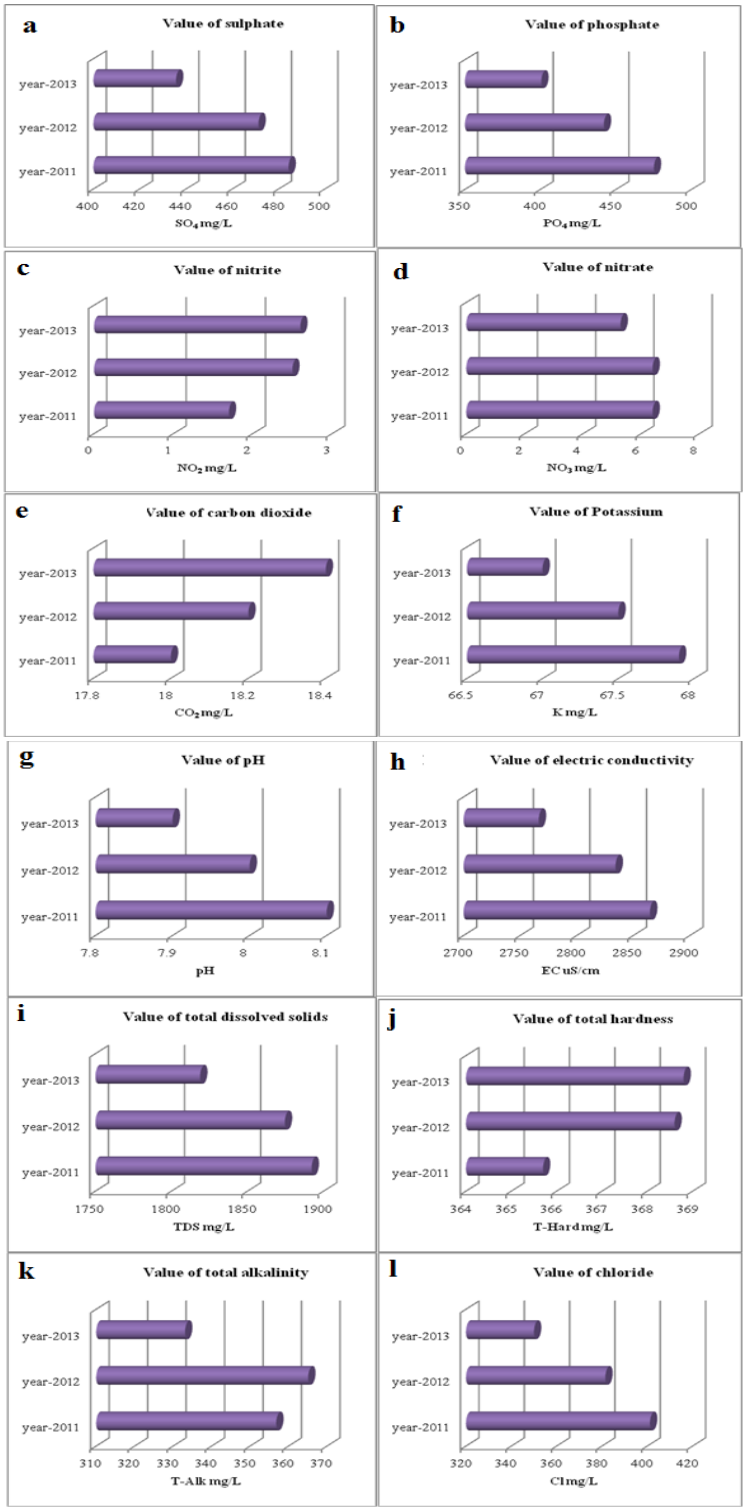

Fig. 2. The status of amphibian environment for various parameters in 2011-2013

The existence of only four species in District Jamshoro has been documented [27-29] and thereafter, present investigation was carried out in Taluka Thano Bula Khan to confirm the exact environmental conditions that may cause great suffering to amphibians and why many species may not prefer to dwell here or have perished due to harsh physico-chemical quality of their aquatic environment. Water quality plays a key role in either developing or damaging amphibian populations by inhibiting their growth and development. In Pakistan, amphibian status is reported from various areas including Punjab and Baluchistan Provinces [30]. The study in various parts of Sindh Province showed the contaminated environmental conditions of the amphibian habitats [31-35]. Though conservation actions are not implemented for saving amphibians in Sindh Province, the amphibian environmental problems were searched, evaluated, highlighted and even reported various times [32-36].

In past, very little information was available on the conservation status of amphibian species but in recent time, amphibian status has seriously been investigated thoroughly and protected through conservation efforts in some areas of world. According to the computational studies, amphibian decline is going 211 times faster than the past extinction rate mainly due to uncaringly role in many parts of the world regarding amphibian conservation [37]. All amphibian species are declining globally, due to different reasons including environmental pollution. Environmental Protection Agency reported that agriculture ranks first as the leading source of water quality problem. The contaminated lakes and rivers affect amphibian populations or lead them towards anomalous conditions [38]. The most important reason that causes amphibians to decline is water contamination that actually occurs due to agricultural, industrial and pharmaceutical chemicals. These chemical pollutants cause lethal or sub lethal effects on amphibians according to their concentration. The sub lethal effects of contaminants include hampered growth, poor development and abnormal behavior in amphibians [39].

Various types of physical and physiological abnormalities become common within amphibian populations which inhabit the polluted environment [40]. The affected amphibians encounter a variety of cancers, endocrine disruption and malfunctioning of reproductive system [41]. Chemical pollutants can also cause sexual malformations such as hermaphroditism into amphibians [41]. Water contamination can induce different types of diseases and developmental deformities [42]. High level of chemical elements weakens the immune system and thus making amphibians susceptible to parasites and other infectious organisms [43]. In many cases the central nervous system of amphibians is altered and amphibians become completely inactive and thus become easily attacked by their predators [41].

The physico-chemical analysis of six amphibian ponds revealed dreadful quality of amphibian life during the study of three years (Table 1-3) in entire Taluka Thano Bula Khan. The pH value was recorded within recommended level [17-18] (Fig. 2a), contrariwise the value of EC was extremely high than standard criteria [16, 18] especially during 2011 the maximum value (2864.7±1227.4) of the parameter was recorded, while lowest level i.e. 92766.4±1190.2 persisted throughout the year 2013 (Fig. 2b). Meanwhile, the concentration of TDS was also awfully higher than propitious limit [18, 23] during the entire study period. Its concentration was determined as high as 1892.1±812.0 during the year 2011, while its lowest concentration (1818.8±688.6) prevailed in year 2013 (Fig. 2c) at all six habitations where high value of T-Hard was also beyond the normal measures [17, 23]. During 2012 and 2013, level of T-Hard remained slightly higher and noticeably similar (368.6±51.0 and 368.8±60.6 respectively) than its value (365.7±57.2) in year 2011 (Fig. 2d). Value of alkalinity was also up to unfavorable level [23] and this status persisted from year 2011 to 2013 (Fig. 2e). Its extreme level was noted as 365.1±49.8 during eight months of year 2012, while comparatively lowest value of same parameter was analyzed as 333.1±53.7 in year 2013. The average concentration of Cl^- was measured as 377.6mg·L^{-1} considered unsuitable for amphibians [17-20]. The analysis during 2013 registered the lowest concentration of Cl^- (350.0±133.9) as compared to its concentration in other study years, while its maximum concentration (401.2±141.8) was noted in 2011 (Fig.

2f). Concentration of SO_4^{2-} was also higher than the normal value [18-19]. Amphibian ambient presented maximum concentration of SO_4^{2-} (484.3±150.1) in year 2011, whereas it's minimum level (435.7±97.8) persisted during year 2013 (Fig. 2g). The concentration of PO_4^{3-} was also registered at a very high value during three years study. The PO_4^{3-} concentration was recorded much higher than standard concentration [14, 17]. The three years study in relation to concentration of PO_4^{3-} indicated its highest concentration in year 2011 (475.1±140.0), however its lowest level (400.6±107.0) was recorded in year 2013 (Fig. 2h). Almost all habitations were registered with a normal concentration of NO_2^- during the months: March and October, but its concentration rose above the normal range from June to August of the years 2011-2013 (Table 1-3). Whole study area was containing high and closely same concentration of NO_2^- in years: 2012 and 2013 (2.5±1.6 and 2.6±1.4 respectively), while in year 2011 comparatively lessened value (1.7±1.7) of this parameter was analyzed (Fig. 2i). NO_3^- quantity was within normal limit for some time, but its value remained elevated from May to August; hence this parameter may also be not be suitable for amphibian population to develop successfully [15,16, 24]. The concentration of this parameter remained rather same during all the study years with slight difference, however comparative study displayed lowest value of NO_3^- in year 2013 (5.3±3.9) and its uppermost concentration i.e. 6.4±4.7 and 6.4±4.3 was recorded respectively in year 2011 and 2012 (Fig. 2j). Whole area displayed normal value of CO_2 during the whole study period in accordance with previous analytical studies [14, 16]. The analysis of the three years indicated the value of CO_2 was highest in year 2013 and lowest in 2011 (Fig. 2k). The range of potassium value was also above the normal level [25]. Its utmost concentration prevailed during the year 2011 and the most diminished quantity was suspended in amphibian ponds during 2013 (Fig. 2l).

High concentration of all the parameters except pH and CO_2 indicated high rate of pollution which affect amphibians badly especially during their early development.

Conclusion

Present study recorded high rate of contamination in amphibian ponds located in Taluka Thano Bula Khan. The variable concentration of analyzed parameters implied the availability of uneven level of dissolved substances for amphibians during different seasons. Extreme upsurge in concentration of physico-chemical parameters during breeding and hatching time (March to August) may induce noteworthy negative effect on development of eggs and larvae and even adult amphibians also remain susceptible to pollution because of their respiratory skin.

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