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A comprehensive assessment of habitats for amphibian fauna in Taluka Larkana, district Larkana, Sindh-Pakistan

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Abstract
The present study aimed to analyze water quality of amphibian habitats via physico-chemical parameters in Taluka Larkana of Sindh Province from March to October in year: 2011, 2012 and 2013. All 26 permanent habitats of amphibian fauna were selected for analytical study using material and methods of analytical grade. Value of analyzed parameters was recorded as followed; pH: 7.8±0.8, EC µS cm^{-1}: 2563.9±935.2, TDS mg L^{-1}: 1770.7±511.0, T-Hard mg L^{-1}: 555.8±123.8, T-Alk mg L^{-1}: 284.4±65.9, Cl mg L^{-1}: 446.3±79.2, SO_4 mg L^{-1}: 74.1±97.2, PO_4 mg L^{-1}: 476.8±82.0, NO_2 mg L^{-1}: 4.4±1.8, NO_3 mg L^{-1}: 6.7±2.1, CO_2 mg L^{-1}: 17.0±3.5 and K mg L^{-1}: 73.8±10.0. All the water quality parameters were concentrated above the dreadful level in entire study area especially during breeding and hatching period. This unsuitable environmental condition may deteriorate amphibian population at large; therefore wild habitats in study area require urgent execution of management plans for the conservation of amphibian fauna.

Keywords: Amphibian habitations, physico-chemical parameters, Taluka Larkana, Sindh, Pakistan

Introduction

Amphibians being water dependent are highly sensitive to environmental contamination mainly due to their permeable skin that allows toxins of their aquatic habitats enter inside the body [1]. Deterioration of amphibian diversity is an issue of great concern for which water quality of their habitats is blamed as amphibian's problematic extinction and decline is recorded in many countries of the world due to water contamination [2-5]. It is appraised that all amphibians are threatened and declining more rapidly than overall species of birds and mammals combine [6].

Amphibians possess numerous economic values in various fields such as pharmaceutics. Skin of ranid species (*Rana tigerina*) is analyzed to retain lipid components which are able to develop therapeutic and pharmaceutical potential that leads wounds to be healed successfully by enhancing the proliferation of epidermal and dermal cells which are involved in healing the wounds naturally [7-8]. Skin collagen of *Rana tigerina* is also widely appreciated for its contribution in wound healing by causing proliferation, migration, and differentiation of epithelial cells that regenerate skin cells [9]. Frog skin also possesses specific peptides which have anti-microbial activity against harmful attacks of bacteria, virus and fungi on human body [10-13]. Many frog species are investigated for their skin properties and determined to possess antihistaminic capacity also [14]. It is also determined that the skin secretions of amphibians are a rich source to develop defensive mechanism as their skin is opulent with peptides able for bioactivity such as "vasorelaxin" which is a muscle relaxing peptide that help vascular tissues of human body to relax normally is extracted from some particular frogs [15]. The recent studies on skin of some ranid frogs i.e. *Rana arvalis* and *Rana ridibunda* have evaluated and isolated three peptides each consisting of more than 20 different amino acids that are of medicinal use to human beings as these peptides retain the properties that inhibit the proliferation of prostate cancer cells, affect antagonistically on artery smooth muscles and reduces the undesirable side effects on different physiological processes and also may retain capacity to cure inflammation, cardiovascular diseases and may also be useful in anticancer treatment [16].

Considering the high economic values, the studies relating amphibian decline have achieved worldwide interest [17] and many environmental factors are studied to confirm which factor effect amphibians most. Habitat degradation due to water quality issues are ubiquitous and found one of the most effective destructive factors [18-21].

Water quality controls the distribution of amphibians and this might be the reason behind poor diversity of amphibians in Taluka Larkana [22]. In this perspective, present study was based on some important features i.e. searching amphibian's permanent habitations, evaluation of physico-chemical parameters of their habitats and comparison of monthly as well as yearly variation in water quality of habitats.

Material and Method

Present investigation consisted of field surveys conducted from year 2011-2013 during which amphibian habitations were marked for monthly (March-October) water sampling. Gross water sampling method was executed in order to fully evaluate the quality of whole pond and water sample was collected into Van Dorn plastic bottles and labelled distinctively so that study of each pond can be differentiated from other ponds of same area. Water samples were soaked in 10% HNO_3 for 24 hours and were kept at 4 °C until processing and analysis at the laboratory of Institute of Advanced Research in chemical Sciences (IARCS) University of Sindh Jamshoro, Pakistan. This entire investigation was carried out following the distinct scientific guideline [23-24]. Laboratory equipment used during analysis of physico-chemical parameters is mentioned in Table 1.

Table 1: Equipment for physico-chemical analysis.

Parameters	Abbreviations	Units	Analytical equipment
pH	Ph	pH	pH meter (Model: Orion,420)
Electric conductivity	EC	$\mu S\ cm^{-1}$	Conductivity meter (Model: Orion. 115)
Total dissolved solids	TDS	$mg\ L^{-1}$	Conductivity meter (Model: Orion. 115)
Total hardness	T-Hard	$mg\ L^{-1}$	Titration
Total alkalinity	T-Alk	$mg\ L^{-1}$	Titration
Chloride	Cl	$mg\ L^{-1}$	Titration
Sulphate	SO_4	$mg\ L^{-1}$	UV-Vis Spectrophotometer (Model: Hitachi 200) (420nm wavelength)
Phosphate	PO_4	$mg\ L^{-1}$	UV-Vis Spectrophotometer (Model: Hitachi 200) (880nm wavelength)
Nitrite	NO_2	$mg\ L^{-1}$	UV-Vis Spectrophotometer (Model: Hitachi 200) (540nm wavelength)
Nitrate	NO_3	$mg\ L^{-1}$	UV-Vis Spectrophotometer (Model: Hitachi 200) (410nm wavelength)
Carbon dioxide	CO_2	$mg\ L^{-1}$	Titration
Potassium	K	$mg\ L^{-1}$	Atomic absorption Spectrophotometer (Model: Perkin Elemer Analyst 800)

All the physico-chemical parameters were evaluated by following the analytical procedures of [25-27], whereas water quality was identified following the scientific water quality criteria [28-29] and scientific literature [30-38]. The statistical study of scientific data of present research work was carried out using Microsoft Excel 2010.

Results

Concentration of parameters varied every month in a cyclic and synchronizing manner during every year: 2011, 2012 and 2013 as mentioned in Table 2-4 through total range, mean value and standard deviation (stdev). Present study recorded yearly variation in water quality as noticeable (Table 5).

Table 2: Value of physico-chemical parameters during year-2011.

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	7.2-8.6	7.5-8.8	7.7-8.9	7.7-9.0	8.0-9.0	7.2-8.8	7.0-8.2	6.8-8.0
	Mean	7.6	7.9	8.1	8.2	8.4	7.9	7.9	7.4
	Stdev	0.5	0.6	0.5	0.6	0.5	0.6	0.5	0.5
EC $\mu S\ cm^{-1}$	Range	1780.5-3809.0	1845.7-4000.0	1870.3-6223.0	1945.0-7441.0	2000.8-7472.6	1850.5-3750.2	1809.5-3690.6	1650.7-3648.5
	Mean	2405.1	2498.4	2915.3	3162.1	3264.2	2496.7	2442.2	2304.9
	Stdev	742.6	701.0	1650.2	2122.3	2108.6	781.1	697.8	730.9
TDS $mg\ L^{-1}$	Range	1300.7-2530.39	1430.0-3110.0	1468.3-3420.0	1500.6-4220.0	1578.1-4309.2	1500.0-2400.8	1338.2-2348.2	1200.0-2309.1
	Mean	1654.2	1782.1	1948.3	2130.6	2177.8	1831.1	1657.6	1528.6
	Stdev	457.8	348.4	743.7	1049.2	1069.1	635.7	393.4	403.3
T-Hard $mg\ L^{-1}$	Range	380.3-800.12	400.5-890.0	420.5-950.2	450.8-963.5	500.6-972.5	450.2-759.2	428.5-700.4	400.0-680.5
	Mean	515.3	558.0	597.6	644.4	679.9	572.8	531.8	501.3
	Stdev	159.2	181.8	199.2	193.4	176.7	112.9	100.9	107.2
T-Alk $mg\ L^{-1}$	Range	240.7-350.99	250.8-358.7	255.1-410.8	262.7-450.2	280.7-450.8	250.5-338.4	200.5-305.8	180.9-280.0
	Mean	266.4	294.8	320.2	332.4	348.7	295.8	283.2	243.1
	Stdev	37.2	28.9	56.5	67.1	61.1	39.1	39.1	40.4
Cl $mg\ L^{-1}$	Range	300.9-525.8	317.5-600.0	332.7-662.6	350.0-700.8	400.2-745.1	350.0-502.8	335.8-480.8	300.0-465.0
	Mean	400.0	427.4	454.0	491.6	541.5	449.1	427.4	395.6
	Stdev	64.4	102.7	122.1	129.1	115.9	73.1	66.3	86.2
SO_4 $mg\ L^{-1}$	Range	365.8-695.6	390.0-750.2	400.7-762.3	430.9-780.7	450.7-800.8	400.8-650.7	370.7-535.7	250.8-500.8
	Mean	449.8	507.0	531.7	555.8	583.0	508.4	465.5	402.9
	Stdev	61.3	133.7	126.8	121.2	120.6	86.0	123.1	86.1
PO_4 $mg\ L^{-1}$	Range	368.5-550.91	380.5-580.1	410.8-585.8	426.8-690.0	450.1-750.5	350.2-540.1	370.9-550.2	305.8-530.5
	Mean	429.3	459.7	483.9	522.2	550.5	471.5	454.7	426.0
	Stdev	66.4	64.8	58.8	92.0	110.2	65.4	69.7	80.2
NO_2 $mg\ L^{-1}$	Range	1.9-6.0	2.2-6.5	2.6-7.0	3.1-7.7	3.8-9.2	3.0-6.0	2.5-5.7	1.5-5.0
	Mean	3.1	3.7	4.4	4.9	5.7	4.1	3.5	2.9
	Stdev	1.2	1.6	1.6	1.6	2.1	1.1	1.2	1.6
NO_3 $mg\ L^{-1}$	Range	4.5-9.6	5.0-11.9	5.0-14.5	4.9-15.6	5.0-15.8	5.0-14.2	4.8-8.0	2.5-8.0
	Mean	6.0	7.4	8.6	8.8	20.0	8.1	6.6	5.2
	Stdev	1.2	2.6	3.8	3.7	32.5	3.5	2.0	1.8
CO_2 $mg\ L^{-1}$	Range	13.5-20.0	10.3-19.5	12.2-19.0	12.0-16.0	10.0-15.0	13.2-20.2	14.0-24.0	15.8-25.5
	Mean	18.3	16.2	14.5	13.4	12.6	14.6	16.5	19.5
	Stdev	4.1	2.9	2.7	1.5	1.7	3.3	2.4	4.5
K $mg\ L^{-1}$	Range	62.8-84.5	65.0-87.2	72.0-88.5	70.0-90.0	73.7-95.7	65.5-81.5	60.7-77.9	57.8-75.2
	Mean	69.1	72.5	80.3	80.3	85.4	75.1	72.3	65.3
	Stdev	6.5	6.9	5.9	7.1	7.59	9.1	8.4	6.4

Table 3: Value of physico-chemical parameters during year-2012.

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	6.8-9.0	6.8-9.0	7.0-9.0	7.0-9.2	7.0-9.3	6.8-9.0	6.8-8.8	6.5-8.8
	Mean	7.5	7.6	7.7	8.0	8.2	7.8	7.6	7.2
	Stdev	0.8	0.9	0.9	0.8	0.8	0.8	0.8	0.8
EC µS cm^{-1}	Range	1748.8-3730.7	1782.5-3780.4	1800.3-3890.5	1850.3-3957.2	1908.5-4074.2	1809.4-3900.4	1759.4-3850.5	1680.3-3700.7
	Mean	2391.7	2454.9	2528.0	2592.8	2690.4	2540.8	2492.6	2346.8
	Stdev	728.3	732.0	763.2	764.0	789.7	759.2	755.7	737.4
TDS mg L^{-1}	Range	1310.8-2400.3	1350.2-2472.5	1366.4-2557.2	1400.9-2608.4	1480.8-2800.4	1378.5-2580.9	1320.8-2500.2	1280.6-2365.9
	Mean	1615.4	1683.6	1768.3	1813.9	1926.8	1774.6	1720.2	1584.2
	Stdev	402.3	409.3	430.8	429.3	482.4	430.7	420.8	400.4
T-Hard mg L^{-1}	Range	438.5-688.2	450.6-690.5	458.2-718.5	470.2-750.2	486.2-820.9	450.0-700.8	400.7-680.2	400.0-650.8
	Mean	514.2	523.1	542.3	577.8	614.3	561.2	540.7	493.8
	Stdev	103.5	96.0	97.2	107.4	128.6	101.0	90.4	96.4
T-Alk mg L^{-1}	Range	200.0-275.9	215.1-285.2	235.7-330.5	255.8-350.6	258.2-400.8	200.0-325.7	21.5-300.8	180.5-259.8
	Mean	218.8	250.1	268.8	292.1	311.9	280.0	261.1	217.2
	Stdev	31.0	29.2	44.5	35.8	53.1	33.0	25.6	101.8
Cl mg L^{-1}	Range	350.8-472.8	365.7-480.9	370.0-528.5	400.0-550.2	405.8-670.2	380.5-508.5	350.7-480.5	300.9-450.3
	Mean	421.3	421.9	448.5	472.0	510.6	454.5	434.8	395.0
	Stdev	51.9	52.4	49.9	52.0	92.0	55.2	46.1	62.3
SO_4 mg L^{-1}	Range	265.8-520.5	300.2-550.8	335.5-580.5	408.7-600.2	443.7-700.8	380.5-590.7	375.2-572.5	250.1-500.2
	Mean	414.4	441.9	468.6	496.8	534.2	470.6	453.2	400.2
	Stdev	87.1	85.0	84.2	66.8	91.0	76.0	68.9	89.5
PO_4 mg L^{-1}	Range	338.5-550.0	350.8-577.2	362.8-600.2	400.0-635.1	435.8-700.0	400.0-600.7	400.0-557.9	306.9-529.1
	Mean	448.7	460.8	478.7	508.7	542.6	489.6	464.2	418.0
	Stdev	75.9	83.0	86.3	83.2	93.2	74.0	61.0	79.1
NO_2 mg L^{-1}	Range	2.0-5.1	2.5-5.5	2.8-6.0	3.5-8.0	3.9-8.8	3.0-7.8	2.8-7.5	2.0-5.0
	Mean	3.3	3.5	4.8	5.3	6.0	5.0	4.0	3.1
	Stdev	1.1	1.1	1.8	1.8	2.1	1.8	1.2	1.1
NO_3 mg L^{-1}	Range	3.0-7.9	3.8-8.0	4.2-8.6	6.0-9.2	6.2-10.0	5.5-9.0	5.0-8.5	3.0-7.5
	Mean	5.5	5.8	6.6	7.3	7.9	7.0	6.4	5.1
	Stdev	1.6	1.4	1.4	1.3	1.4	1.4	1.4	1.5
CO_2 mg L^{-1}	Range	14.0-22.7	14.0-19.7	13.8-20.0	13.0-18.5	12.0-18.0	14.4-22.8	15.0-23.8	15.0-25.0
	Mean	19.5	17.7	16.8	15.7	14.9	16.5	18.0	20.3
	Stdev	3.4	3.2	2.5	2.2	2.4	2.4	3.3	3.8
K mg L^{-1}	Range	59.7-77.8	60.5-80.2	65.0-85.5	68.4-90.8	70.0-98.5	65.0-90.0	60.9-85.0	55.0-75.0
	Mean	65.3	68.8	74.8	78.6	83.2	76.1	72.5	62.0
	Stdev	8.0	7.8	8.2	8.5	10.6	9.1	8.9	8.5

Table 4: Value of physico-chemical parameters during year-2013.

Parameters	Value	March	April	May	June	July	August	September	October
pH	Range	6.5-8.5	6.8-9.0	7.0-9.0	7.0-9.0	7.2-9.4	6.5-8.9	6.8-8.8	6.7-8.5
	Mean	7.4	7.7	7.6	7.9	8.3	7.8	7.5	7.2
	Stdev	0.8	0.9	1.0	0.8	0.8	0.8	0.8	0.7
EC µS cm^{-1}	Range	1600.8-3579.2	1766.8-3750.3	1800.0-3820.5	1807.8-3905.8	1950.3-4082.8	1850.2-3943.2	1775.3-3892.2	1750.2-3850.3
	Mean	2450.1	2495.8	2515.9	2568.3	2710.6	2555.2	2424.5	2286.3
	Stdev	756.4	732.7	767.4	762.6	779.8	763.4	729.3	716.2
TDS mg L^{-1}	Range	1250.6-2209.3	1350.2-2438.0	1390.5-2500.8	1375.3-2580.2	1507.5-2835.9	1385.5-2587.2	1350.2-2536.5	1302.5-2500.0
	Mean	1685.3	1732.3	1747.9	1796.0	1956.4	1790.2	1650.1	1540.3
	Stdev	427.0	403.3	426.7	424.3	485.7	431.6	404.8	348.9
T-Hard mg L^{-1}	Range	380.2-600.2	450.1-700.0	458.2-700.3	460.7-735.8	490.1-850.7	461.4-715.2	430.2-689.3	400.7-650.2
	Mean	509.3	540.5	555.1	571.1	634.6	555.4	532.7	473.1
	Stdev	92.0	97.3	93.9	105.2	137.2	97.0	97.4	85.7
T-Alk mg L^{-1}	Range	180.0-250.0	240.7-280.5	245.2-300.5	242.8-337.8	265.5-425.3	200.0-330.1	200.3-300.0	180.6-290.7
	Mean	238.1	264.8	270.9	283.9	327.2	272.5	249.0	213.5
	Stdev	40.5	16.9	45.7	33.6	60.6	19.6	41.1	25.1
Cl mg L^{-1}	Range	300.0-449.1	363.2-485.5	387.1-495.8	378.5-540.7	420.2-685.2	385.4-530.3	355.7-500.2	335.5-500.0
	Mean	414.2	438.4	446.0	461.4	526.9	461.0	428.9	389.4
	Stdev	64.2	54.2	43.4	55.6	92.9	52.1	54.4	60.2
SO_4 mg L^{-1}	Range	300.0-500.0	275.3-550.0	350.9-570.2	340.9-588.2	450.6-700.0	392.5-600.0	380.1-590.2	350.8-550.0
	Mean	431.0	460.5	461.3	485.2	544.6	477.0	430.8	404.0
	Stdev	68.7	76.7	74.0	71.6	87.4	82.8	92.3	72.9
PO_4 mg L^{-1}	Range	300.7-500.0	350.0-550.0	370.9-580.6	370.0-610.5	450.0-750.1	400.8-608.2	400.8-575.5	380.5-550.8
	Mean	456.4	475.5	477.3	501.6	566.8	491.2	458.2	408.6
	Stdev	66.6	70.7	68.1	72.5	106.5	80.9	72.5	73.4
NO_2 mg L^{-1}	Range	2.0-4.8	2.0-5.2	2.8-6.0	3.0-8.0	3.8-9.0	3.5-8.0	2.5-8.0	2.0-7.8
	Mean	4.8	4.1	5.0	5.3	6.5	4.8	3.6	3.0
	Stdev	2.1	1.2	2.0	1.8	1.9	1.9	1.2	1.2
NO_3 mg L^{-1}	Range	3.0-7.0	3.0-7.5	5.0-8.9	4.8-9.0	6.2-10.2	5.5-9.0	4.8-8.5	4.2-8.2
	Mean	6.4	6.5	6.8	7.2	8.18	6.9	5.7	4.9
	Stdev	1.5	1.4	1.5	1.4	1.5	1.5	1.5	1.3
CO_2 mg L^{-1}	Range	15.5-25.0	13.0-20.0	13.0-20.0	13.5-19.0	12.0-17.8	14.2-20.7	16.0-24.0	17.4-24.5
	Mean	21.0	17.8	16.4	16.1	14.1	16.3	20.1	20.7
	Stdev	2.8	2.6	2.5	2.6	2.4	2.1	2.8	3.7
K mg L^{-1}	Range	50.9-70.8	60.0-80.5	65.0-85.3	68.9-88.5	72.0-98.9	66.7-92.2	65.0-85.0	60.0-80.0
	Mean	68.2	73.8	74.8	78.2	85.9	77.3	70.4	59.8
	Stdev	8.6	7.5	7.2	9.0	9.6	7.5	7.2	7.5

Table 5: Year-wise variation in values of physico-chemical parameters.

Parameters	Year-2011	Year-2012	Year-2013
Ph	7.9±0.6	7.7±0.8	7.7±0.8
EC µS cm^{-1}	2686.1±1284.5	2504.8±703.2	2500.8±702.8
TDS mg L^{-1}	1838.8±676.6	1735.9±407.1	1737.3±404.6
T-Hard mg L^{-1}	575.2±157.8	545.9±101.8	546.5±103.9
T-Alk mg L^{-1}	298.1±55.1	262.5±55.9	265.0±47.3
Cl mg L^{-1}	448.3±101.5	444.8±64.2	445.8±68.3
SO_4 mg L^{-1}	500.5±115.8	460.0±85.6	461.8±83.2
PO_4 mg L^{-1}	474.7±82.5	476.4±82.1	479.4±83.2
NO_2 mg L^{-1}	4.1±1.7	4.4±1.7	4.6±1.9
NO_3 mg L^{-1}	8.8±11.7	6.4±1.6	6.6±1.6
CO_2 mg L^{-1}	15.7±3.6	17.4±3.2	17.8±3.5
K mg L^{-1}	75.0±9.2	72.6±10.4	73.6±10.4

A combined study of three years (2011-2013) showed minimum and maximum value of each parameter as followed: pH: 6.5-9.4, EC µS cm^{-1}: 1600.8-7472.6, TDS mg L^{-1}: 1200.0-4309.2, T-Hard mg L^{-1}: 380.2-972.5, T-Alk mg L^{-1}: 150.2-477.5, Cl mg L^{-1}: 300.0-745.1, SO_4 mg L^{-1}: 250.1-800.8, PO_4 mg L^{-1}: 300.7-750.5, NO_2 mg L^{-1}: 1.5-9.2, NO_3 mg L^{-1}: 2.5-15.8, CO_2 mg L^{-1}: 10.0-25.5 and K mg L^{-1}: 50.9-98.9.

Discussion

Comprehensive study of three years recorded value of most parameters too high to maintain natural quality of ponds wherefrom three amphibian species (*Hoplobatrachus tigerinus, Euphlyctis cyanophlystis* and *Bufo stomaticus*) are previously recorded [39-40].

According to results of present study, pH value in amphibian habitats persisted within 6.5-9.4. This value of pH may be considered as slightly higher than the normal limit (6.0 - 9.0), but may not be affectual for amphibians because of existing for short time. Meanwhile electric conductivity parameter was recorded highly effective as value of EC is directly proportional to the amount of salts dissolved in the water which may not be tolerated by amphibians [32]. Inorganic dissolved salts such as chloride, nitrite, nitrate, sulfate, phosphate (negatively charged inorganic solids) and calcium, magnesium, sodium, iron (positively charged inorganic solids) are efficient conductors of heat measured via EC. Increased level of heat conductivity across water has lethally harmful impact on its inhabitants especially on their eggs and larvae that can be severally damaged. In the area of present study, the value of EC was tremendously high as it was measured from 1600.8 µS/cm to 7472.6 µS/cm, however normal value of EC is 150.0 – 500.0 µS/cm [28-32]. Value of EC remained high for entire study period without any major variation and so was the status of TDS value as well (Table 2-4). Concentration of TDS was recorded within 1200.0 to 4309.2 mg L^{-1} making amphibian survival difficult particularly of their eggs and larvae which can tolerate TDS value only within 50.0-250.0 mg/L [36-38]. TDS act as an aggregate indicator of the presence of a broad array of chemical contaminants of which calcium, phosphate, nitrates, sodium, potassium, chloride are some of the most common constituents. High concentration of TDS constrains organism productivity. Spawns and juveniles being very sensitive to high TDS levels may undergo desiccation or dehydration when amphibians are exposed to excess salts [34]. Total hardness refers mainly to concentration of calcium and magnesium which are the most common sources for making water hard and may cause many physiological problems as hardness has a major effect on pH and pH stability thus it's too high or too low concentration can make aquatic environment stressful for amphibians [36]. Amphibian habitats in Taluka Larkana were concentrated with hardness from 380.2 to 972.5 mg L^{-1} that is much higher than that of its normal level (75.0 - 200.0 mg/L) [36]. Alkalinity was also recorded up to harmful level as its entire range persevered between 180.0-450.8 mg L^{-1}, however normal value of the parameter in question extends between 50.0 - 150.0 mg/L. Alkalinity may cause severe effects on amphibians as it is the base neutralizing or "buffering" capacity of water i.e., measures the total amount of base present and indicates a water's ability to resist pH changes and fluctuations in values of alkalinity may cause stress, poor growth and death of eggs and larvae [36]. Harmful fluctuations in pH may also cause mortality as it inhabits the activity of enzymes that control hatching process thus it may traps fully developed embryo inside the egg capsule and arrests the metamorphosis [36]. Concentration of Cl was also out of favorable level as it remained constantly higher all through three years: 2011, 2012 and 2013 (Table 5). High concentration of Cl can be toxic to amphibians along with many other forms of aquatic life including fish, macro-invertebrates and aquatic arthropods [37-41]. Elevated chloride levels can threaten the health to species survival, growth, and reproduction and may damage species diversity. Chloride concentrations beyond 50.0 - 150.0 mg/L can harm amphibians by interfering with their osmoregulation and poor osmoregulation can hinder their developmental cycle, growth and reproduction [42]. Concentration of SO_4 (250.1 to 800.8 mg L^{-1}) was entirely out of favorable limit (50-100 mg L^{-1}) during whole study period, during which, PO_4 concentration was also recorded higher than compatible level (0.03 - 0.05 mg/L). Both SO_4 and PO_4 are influential as these parameters deplete dissolved oxygen levels by causing algal blooms through Eutrophication [43]. Eggs and larvae of amphibians remain confined into water bodies till completion of their development into adult; hence water containing too much quantity of SO_4 and PO_4 may cause them die even before developing into adults [44]. It was recorded that the value of PO_4 did not varied insignificantly during 2011, 2012 and 2013, but the concentration of SO_4 varied in an obvious manner. Range of NO_2 value was evaluated as high as 9.2 mg L^{-1} from amphibian habitations of study area. All the habitats exhibited concentration of NO_2 approximately same during year 2011, 2012 and 2013 with slight difference. This nitrogenous parameter is highly water soluble contaminant disrupting the thyroid axis; hence it may adversely affect the metamorphosis in amphibians [45-46]. Nitrite causes the body of amphibians to swell and become transparent and induces behavioral and morphologic changes in them [39]. The results measured concentration of NO_3 as 2.5-15.8 mg L^{-1}, contributing more stress to the amphibian environment [25, 31]. High concentration of nitrate is highly severe for survival of amphibians as it potentially causes their death mainly during early stage of their life [47-48]. High level of nitrate is main cause of eutrophication and algal blooms in stagnant water bodies [43] and meanwhile it may directly impair the immune system and inhibits the growth and development of amphibian fauna [41]. Carbon dioxide is a water soluble gas and most common source of acidity in water bodies. The excesses of dissolved CO_2 in water will negatively affect the health of amphibians and it was found suspended in appropriate level in the study area. Value of CO_2 remained slightly high than normal range (12.0 - 25.0 mg/L) during few months but this slight unconstructiveness may not contribute to adversity. In the case of hypercapnia, amphibians could face several kinds of physiological problems including metabolic deformities and breathlessness [47]. As showed by present study, the concentration of potassium was highly above than the normal limit (25.0-50.0 mg L^{-1}), therefore Potassium in water may influence the developmental and physiological process of amphibians of study area [38].

The entire study area may not support amphibian survival as out of 12 parameters, only two (pH and CO_2) were within normal level, however other all parameters (EC, TDS, T-Hard, T-Alk, Cl, SO_4, PO_4, NO_2, NO_3 and K) were found up to deleterious level (Table 2-5). Monthly variation in values of parameters (except CO_2 that varied antagonistically to other parameters) was recorded even and akin during the year 2011, 2012 and 2013, suggesting presence of destructive factors for amphibians during entire length of study. Though pollution rate was high in year-2012, but water quality didn't vary significantly during year 2011 and 2012, exhibiting overall status of amphibian habitats as unsuitable.

Conclusion

Study area "Taluka Larkana" provided unsuitable environment to amphibian populations. Extreme level of chemical contaminants throughout the study period of three years implied the careless role of people and concerned authorities towards conservation of amphibians and this negligence may result massive decline in their diversity and population in future.

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