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Status of Amphibian Habitats and their Conservation in Agricultural Fields of Sindh, Pakistan

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Abstract
Suitable environmental conditions are necessary for stable survival of amphibians as they have transparent skin that remains susceptible to dreadful pollutants which may affect their development and survival and may also make them vulnerable to mortality. In this context, we proposed to explore the agricultural fields in one of the main provinces of Pakistan "Sindh" to record the status of amphibian's habitats as well as their diverse forms. Field surveys were conducted and water quality of habitats was analyzed through parameters: electric conductivity (EC), total dissolved solids (TDS), total hardness (T. Hard), total alkalinity (T. Alk), sulphate (SO_4) and phosphate (PO_4) using equipment of analytical grade. The agricultural fields were recorded to embrace species: *H. tigerinus, E. cyanophlyctis, A. hazarensis* and *B. Stomaticus*. The value of water quality parameters was recorded as extremely high as permissible limit of environmental protection agency. Water quality changes were prominent every month and values of parameters remained higher during spawning, hatching and metamorphosing. Such water quality of habitats especially during breeding season may deteriorate amphibian populations at large; therefore, conservation efforts may be taken to save amphibians from possible decline in future.
Keywords: Amphibian fauna, Ecology, Water quality, Physico-chemical parameters

Introduction
The worldwide studies indicate that agricultural ponds which are main occupancies of amphibian fauna rank first at the pollution rate due to anthropogenic contamination (Alford and Richards, 1999). Amphibian eggs and

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larvae are more prone to non-optimal water quality as they remain confined there where they were spawned till developing into adulthood. Thus, spawns and tadpoles fail resist against contamination of their habitats by moving away and hence they eventually die even before being adult (Alford and Richards, 1999). The human encroachment is a main reason behind deterioration of water reservoirs which serve as shelter to several aquatic wild animal species (Davies, 2007). Biological degradation of the organic matter (Wetzel 2001) and nitrogenous fertilizers have ability to accumulate in aquatic systems and interfere with physiological systems of aquatic animals (Miller, et al., 2002).

The parameters selected for present study have great impact on amphibian populations worldwide (Wetzel 2001). The electric conductivity (EC) shows concentration of electrolytes which make water conduct heat, whereas measurement of total dissolved solids (TDS) indicates cumulative concentration of all the inorganic and organic substances contained in water that may gradually kill eggs by desiccating them (Boyer, et al., 1995 and Boyd, 1999). Total hardness represents the overall concentration of divalent salts such as calcium, magnesium, iron and zinc etc, but mainly calcium and magnesium make water hard and hard may cause many physiological problems into amphibians (Wurts and Masser, 2004).

Total alkalinity is a base neutralizing or "buffering" capacity of water and its measurement indicates that how much able water is to resist pH changes (Wurts and Durborow, 1992; Kaushal, et al., 2013). When Alkalinity fails to work properly, changes in pH may cause stress, poor growth and even death of tadpoles (Wurts and Durborow, 1992; EPA., 1976). The sulphate (SO_4) and phosphate (PO_4) are also greatly considered as major threat to amphibians as their high concentration may cause respiratory problems by supporting eutrophication and also damaging internal organs of permeable skinned animals (Wurts and Durborow, 1992). High concentration of physico-chemical parameters may hinder growth and development of amphibians and may induce into them variety of physical and physiological abnormalities (Wurts and Durborow, 1992; Kaushal, et al., 2013).

Considering the dreadful impact of water pollution, present study aimed to highlighting issues related with conservation of amphibians and their habitats and therefore four districts of Sindh province were explored to confirm whether amphibian habitats suffer from degradation or not.

Material and Methods

Present investigation was carried out in Larkana, Khairpur, Sukkur and Jamshoro districts of Sindh that encompasses geographical area of 140,914 km², situated in southeastern area of Pakistan, located at 25.8943° N, 68.5247° E. The field surveys were conducted and interviews from local people were also achieved for confirming locations of amphibians in agricultural fields. The views of study sites are show in Figure 1.

Water samples were collected in sterilized Van Dorn plastic bottles of 1.5 liter capacity from 09 am to 05 pm and delivered to the laboratory where samples were kept at 4 °*C* until analyzed. Conductivity meter (Orion 115) was used to record value of EC and TDS, whereas T. Hard and T. Alk were analyzed through titration procedures by following instructions of Sunita, 2002. The concentration

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DIALOGUE SOCIAL SCIENCE REVIEW
SLOGAN HERE

Vol. 3 No. 1 (January) (2025)

of non-metallic parameters: SO_4 and PO_4 was recorded using spectrophotometer (Hitachi 200) as instructed by Denial, 1948. Water quality of habitats was identified using scientific literature (Boyer, et al. 1995; EPA, 1976 and 1986; EPD, 2000; Wurts, and Durborow, 1992).

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DIALOGUE SOCIAL SCIENCE REVIEW
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Vol. 3 No. 1 (January) (2025)

Figure 1: Amphibian habitats in agricultural zones of Sindh province.

Results and Discussion

Water may appear clean and clear but the fact is there are millions of microscopic elements suspended in it which when increase in dangerous amounts are termed to be the pollutants and these pollutants are able to harm aquatic animals badly (Raven and Johnson, 1990). Thus aquatic environment with its water quality is considered to be the main factor influencing the health and survival of aquatic animals (Raven and Johnson, 1990).

Climate of Sindh is less optimal for amphibian populations to thrive abundantly due to its arid climate that is not preferred by most of amphibian species especially those which are highly aquatic like species of family Ranidae. Arid environmental conditions fail to attract majority of amphibian species and therefore, Anura (frogs and toads) is the only order of class amphibia that exists here (Muhammad, et al., 2016).

Thorough analysis of physico-chemical parameters revealed water quality of amphibian habitats as detailed in figure 2-7, while table 1 shows status of habitats during each year distinctly with mean value, standard deviation and total range (minimum and maximum values) of water quality parameters.

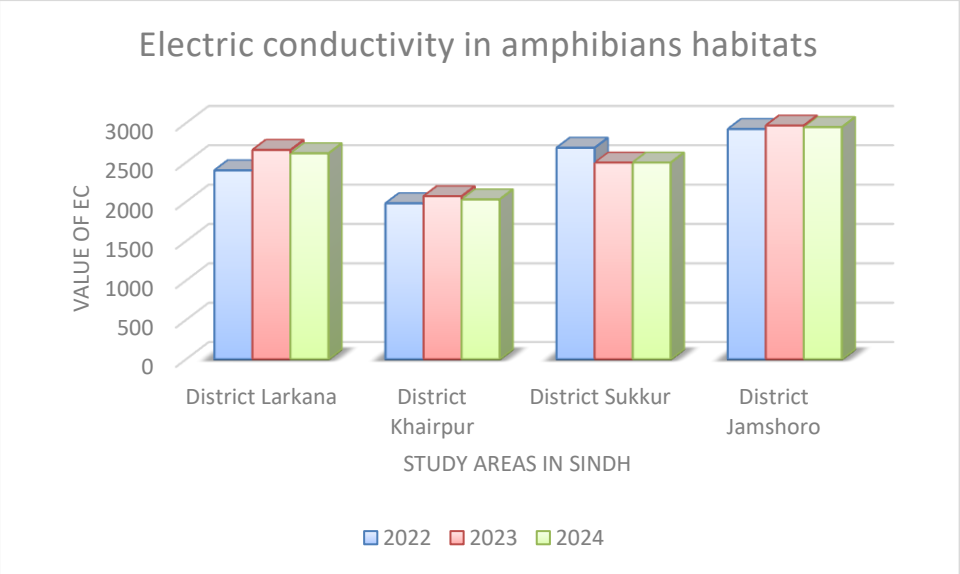

Figure 2: Value of electric conductivity (EC) in amphibian habitats

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Vol. 3 No. 1 (January) (2025)

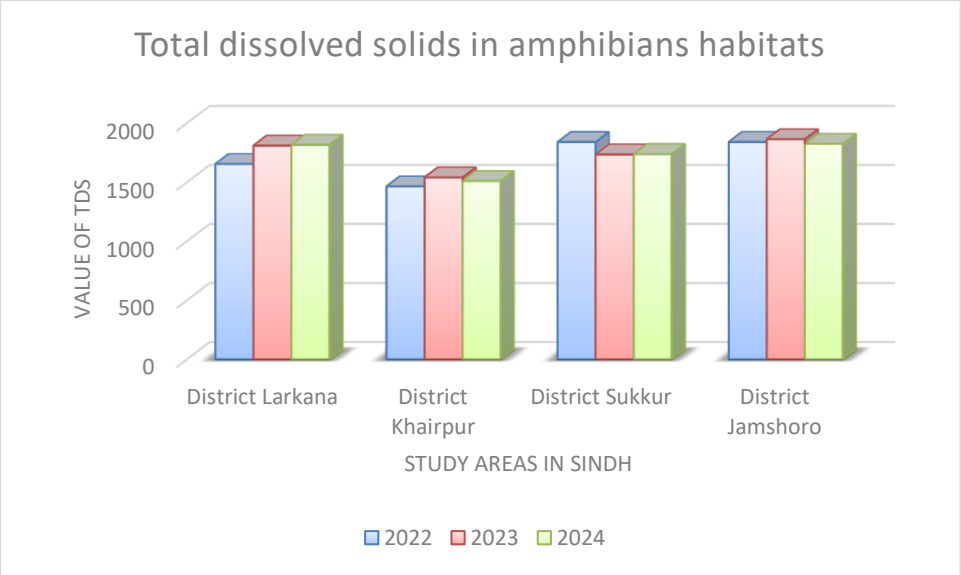

Figure 3: Value of total dissolved solids (TDS) in amphibian habitats

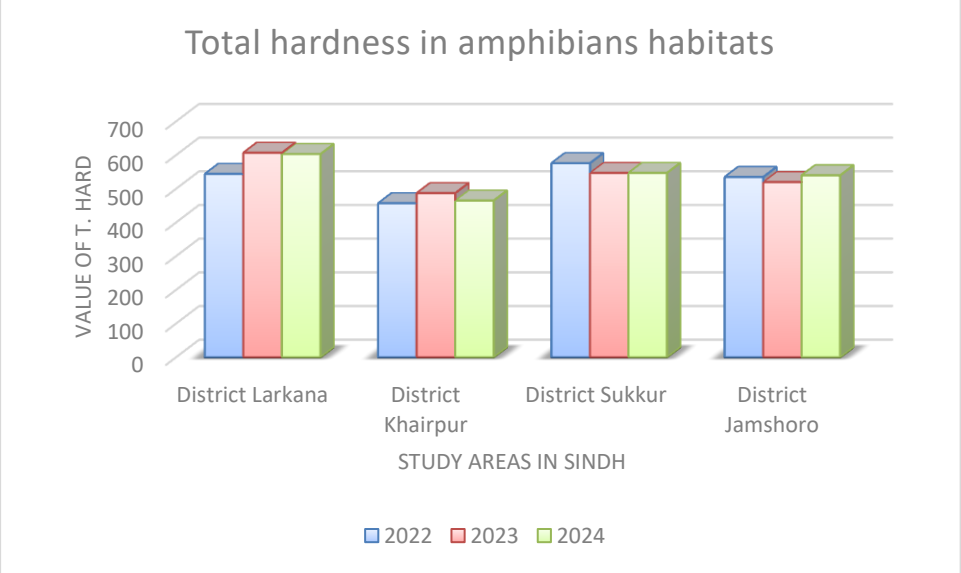

Figure 4: Value of total hardness (T. Hard) in amphibian habitats

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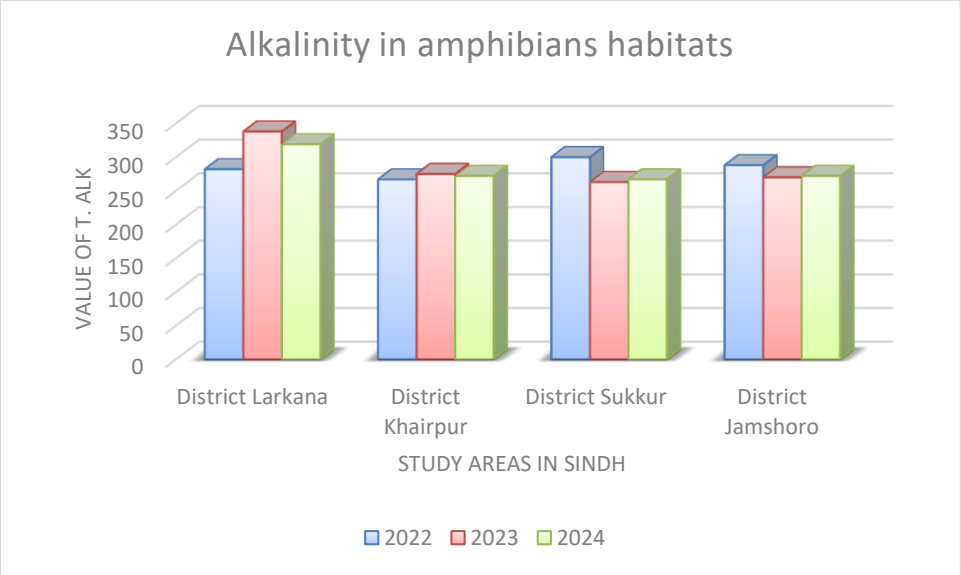

Figure 5: Value of total alkalinity (T. Alk) in amphibian habitats

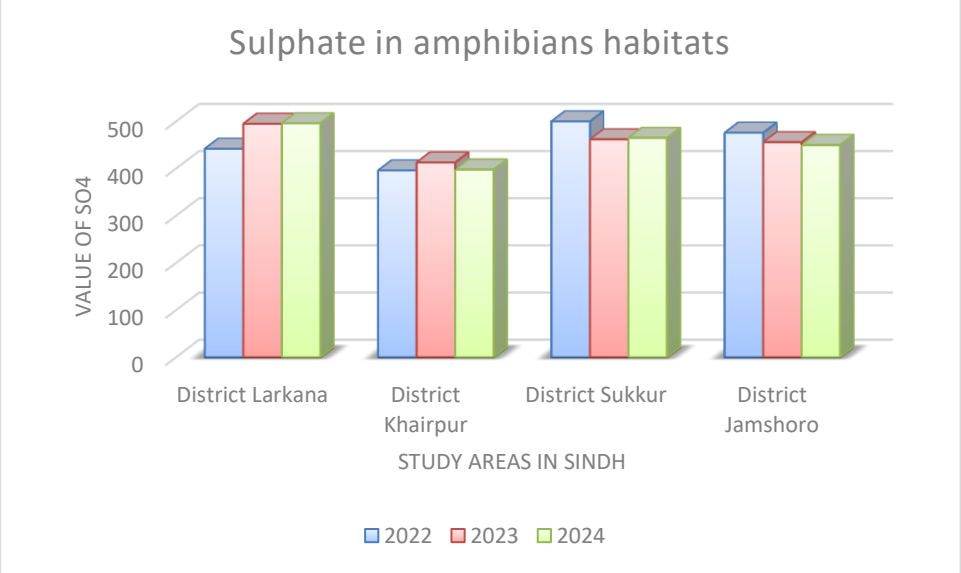

Figure 6: Value of sulphate (SO_4) in amphibian habitats

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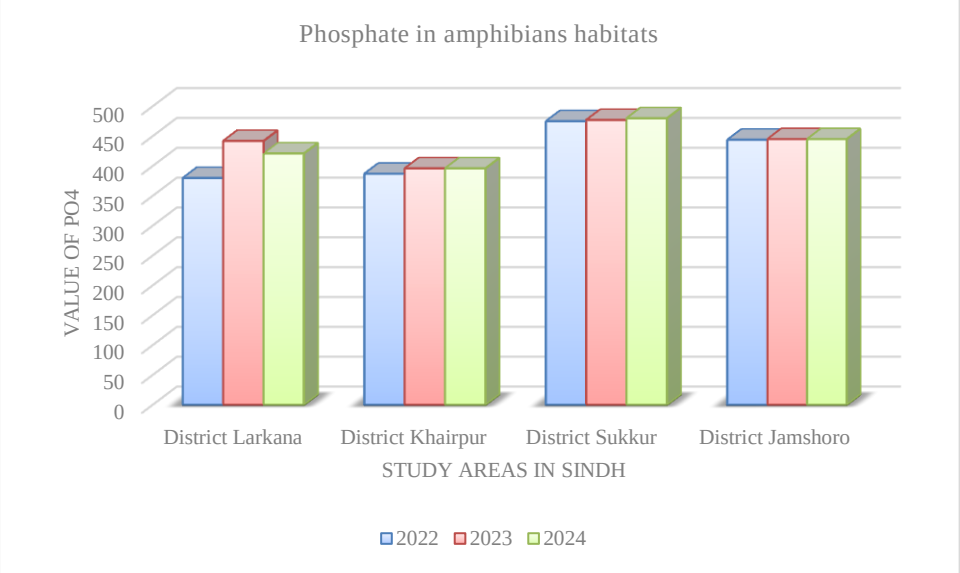

Figure 7. Value of phosphate (PO_4) in amphibian habitats

In year-2022, the study of all habitats recorded minimum and maximum values of physico-chemical parameters as followed: EC: 1180.5-7472.6 µS cm^{-1} (2473.9±1198.2), TDS: 788.8-4309.2 mg L^{-1} (1688.8±622.4), T-Hard: 200.5-980.7 mg L^{-1} (525.0±176.2), T-Alk: 150.2-477.5 mg L^{-1} (282.4±63.8), SO_4: 200.8-817.9 mg L^{-1} and PO_4: 200.0-780.8 mg L^{-1} (417.9±95.4).
Habitats analysis in year-2023 showed the value of value of parameters as EC µS cm^{-1}: 1270.5-6639.8 (2536.1±1112.5), TDS mg L^{-1}: 880.2-3508.5 (1731.7±563.8), T. Hard mg L^{-1}: 300.0-960.3 (540.0±168.0), T-Alk mg L^{-1}: 221.5-470.0 (287.1±73.0), SO_4 mg L^{-1}: 250.1-817.9 (454.9±122.2), PO_4 mg L^{-1}: 228.5-760.7 (437.3±93.8). Whereas in year 2024, the physico-chemical parameters were evaluated from amphibian habitats in Larkana, Khairpur, Sukkur and Jamshoro and their value was recorded comparatively different from previous studies. Value of EC µS cm^{-1} and TDS mg L^{-1}were recorded as 1250.2-6610.2 (2508.9±1109.1) and 900.5-3475.2 (1715.9±557.3) respectively. Value of T-Hard mg L^{-1}: 265.9-950.8 (537.7±167.6), T-Alk mg L^{-1}: 157.5-477.5 (282.8±63.1), SO_4 mg L^{-1}: (255.3-780.2 (449.8±114.5) and PO_4 mg L^{-1}: 258.0-750.1 (432.9±93.1) were also recorded to vary as compared to previous analysis.
Amphibian diversity of Sindh province is known to be extremely poor, whereas Jamshoro and Kashmore had richer diversity as compared to other areas of Sindh (Shaikh, et al., 2014). Research conducted outside Sindh discovered enormous range of 24 amphibian species contained in four families including Bufonidae, Megophryidae, Microhylidae, and Ranidae (Khan, 2010), while some studies show that amphibians of pakistan are represented by 21 species within Bufonidae, Dicroglossidae, Megophryidae and Microhylidae families of order Anura (Muhammad, et al., 2016). Amphibian species of Himalayan highlands are dissimilar from the species of central and eastern areas (Muhammad, et al., 2016).
Though amphibians of Pakistan have conservation status of least concern on the data list of IUCN (International Union for the Conservation of Nature) as they are not vulnerable to grave threats, but some species of family Ranidae including

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DIALOGUE SOCIAL SCIENCE REVIEW
SLOGAN HERE

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Hoplobatrachus tigerinus and *Euphlyctis cyanophlyctis* are facing various ecological problems including habitat loss and water contamination (Muhammad, et al., 2016). In this perspective, existence of poor amphibian diversity may be due to unsuitable habitats.

Some studies in Sindh have analyzed amphibian habitats in relation to physico-chemical parameter and revealed contaminated status being responsible for miserable diversity (Kalsoom et al. 2013). Present investigation also heighted the issues of water quality in all 26 habitations of amphibian fauna and revealed unprotected status of amphibian populations due to high rate of contamination caused by high values of studied parameters (Figure 2-7).

Value of EC and TDS was recorded higher in amphibian dwellings of study areas (Figure 1). These parameters remained out of permissible limit of EPA, 1976; 1986 and EPD, 2000 that emphasize on keeping EC in habitats within 150.0-500.0 uS/cm, while value: 50.0-250.0 mg/L is recommended for TDS. Concentration of total hardness was also found massive and beyond the standard criteria (75.0-200.0 mg/L), along with total alkalinity that is recommended to exist within 50.0 mg/L to 150.0 mg/L (Wurts, and Durborow, 1992). Value of T. Hard and T. Alk remained persistently highest in all habitations.

Value of SO_4 was also observed as unsatisfactory and very high than 50.0-100.0 mg/L which is considered as suitable range of sulfate in aquatic habitats to prevent loss of oxygen for aquatic animals in water bodies (EPD, 2000 and Wurts, and Durborow, 1992). Meanwhile concentration of PO_4 also persisted beyond normal value 0.03-0.05 mg/L as studies of Boyer, et al. 1995 and EPD, 2000 call it alerting condition when phosphate value increases. Concentration of SO_4 and PO_4 persisted beyond the favourable limit.

All the habitats remained unfavorable to amphibians constantly from 2022 to 2024, but rate of pollution was observed higher during year 2023. Entire study of three years exhibited value of parameters as followed: EC (2506.3±1139.1), TDS (1712.1±581.2), T-Hard (534.2±170.5), T-Alk (284.4±65.9), SO_4 (451.8±122.1) and PO_4 (429.4±94.3). Value of all physico-chemical parameters was above the normal level level for the steady survival of amphibians.

Monthly as well as annual Fluctuations in water quality of habitats were synchronizing: extreme concentration of parameters prevailed during July, while their minimum value was recorded during October of each year of investigation. These fluctuations may contribute in making environment harsher for eggs and larvae to flourish successfully in their exclusively aquatic primary habitats.

Conclusion

We recorded the prevalence of unfavorable environmental conditions that Sindh province provided to amphibian fauna. High concentration of physico-chemical parameters was the indicative of pollution that may create conservation issues and may threaten amphibian populations of study area. Therefore conservation actions may urgently be implemented to save these neglected wild animals which play important role in maintaining ecosystem within balanced state.

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