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Research Article

Study of Physico-chemical parameters in amphibian habitats of District Jamshoro, Sindh-Pakistan

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Abstract: Present study was conducted in District Jamshoro (11,517 Km²) for the determination of water quality of amphibian habitations in agricultural ponds for an extensive period of three years (2011-2013). Investigation comprised of regular surveys of 18 permanent habitations for the collection of water samples and laboratory analytical study was also carried out for the evaluation of some main physico-chemical parameters (pH, EC, TDS, T-Hard, T-Alk, Cl, SO₄, PO₄, NO₂, NO₃, K and CO₂). Specific scientific methodology and distinct analytical instrumentation was applied discretely for the study of each parameter. Results of this study showed that the values of pH and CO₂ were normal; however concentration of all other parameters was very high making entire study sites highly contaminated and unfavorable for the delicate creatures in question. Tremendously inauspicious level of studied physico-chemical parameters persisted for the entire study period with seasonal variation.

Keywords: Amphibian habitats, agricultural ponds, Physico-chemical parameters, District Jamshoro, Sindh, Pakistan

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INTRODUCTION

Class Amphibia consists of a group of unique vertebrates of about 7000 species of which nearly 32% population has declined within last two decades ¹. It is also reported that about one third of amphibian species have declined massively, whereas remaining are susceptible to decline fast due to various factors occurring differently in different parts of the world, but environmental instability is a major ubiquitous issue faced by amphibians widely ². For amphibians, the biggest predicament arises when there is large a number of physical, chemical and biological properties accumulating concurrently into their habitats ². Aamphibians are very sensitive creatures as they have semi-permeable skin which remains exposed to variety of dreadful water pollutants which may affect them very badly especially during breeding season ³. Rate of susceptibility becomes too high when amphibians undergo metamorphosis during which water contaminants influence their growth and development and make them prone to mortality as they live in confinement until fully developed ^{3,4}.

Amphibian diversity of District Jamshoro is extremely poor embracing only four species i.e. *Hoplobatrachus tigerinus, Euphlyctis cyanophlyctis, Allopa hazarensis and Bufo stomaticus* alike other areas of Sindh province such as Jamshoro and Kashmor ⁵, however research in other areas of Pakistan has discovered vast range of anuran species⁶⁻⁷. Voluminous discoveries have been documented in many parts of Sindh Province where environmental volatility is well-known to maltreat the amphibian fauna ⁸⁻¹⁴.

Agricultural ponds which are main occupancies of amphibian fauna rank first at the pollution rate ¹⁵. Contamination contributes to amphibian decline which has become 211 times greater than the past extinction rate ¹⁶. This deterioration of amphibian population is time and again related with a number of problems which are considered to be the major threat to them. Amphibian developmental unsteadiness is potentially associated with environmental contaminants which make them susceptible to the variety of abnormalities and infections ¹⁷. Water may appear clean but there are millions of microscopic elements suspended in it which when increase in high amounts are termed to be the pollutants.

Parameters selected for present study have potential impact over quality of water, therefore they were analyzed in order to be familiar with survival of amphibian in District Jamshoro. pH is a crucial parameter for detection of hydrogen ion's concentration which may be maintained within moderate level as amphibians are unable to endure it's too high or too low occurrence that is responsible for basicity or acidity into water ¹⁸. The membranes surrounding amphibian eggs wane due to acidic water, in this condition eggs undergo mortality even before reaching adulthood ¹⁹. It is equally important for water not to let too much heat to travel across itself and these are the electrolytes responsible for increasing electric conductivity (EC). Total dissolved solids (TDS) specify cumulative dissolution of all the inorganic and organic components that may gradually kill eggs by desiccating them ¹⁹.

Analysis of total hardness (T-Hard) exhibits concentration of overall divalent salts making water intolerable for amphibians when increases in large quantity and so occurs in case of high level of alkalinity as well ²⁰. Both of the above parameters are main factors instigating stress, poor growth and death of eggs and larvae of amphibian fauna ^{21, 22}. High chloride concentrations in freshwater can similarly harm the health of amphibians by interfering with their osmoregulation and as a consequence proper regulation of solutes fails inside bodies of amphibians ²¹. Stagnant water is mainly contaminated by the high concentration of non-metallic parameters such as sulphate (SO₄) and phosphate (PO₄), both are liable for massive eutrophication into water bodies inhabited by amphibians ^{21, 22}. Eutrophication is a

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major source of algae production which consumes large amount of oxygen and let other aquatic creatures like amphibians suffer from shortage of this important respiratory gas ²¹. Nitrogenous parameters such as nitrite and nitrate are also potential factors interfering with various physiological processes of amphibians, for instance high concentration of nitrite and nitrate disrupts the thyroid and result the stunted metamorphosis ²³. It is also revealed that the development of amphibians is also influenced by the high quantity of Potassium ²⁴. Dissolved CO₂ can also have a negative impact on the aquatic organisms as its elevated value creates hypercapnia and may cause various defects into metabolic functions, growth and reproduction of amphibians ²⁵.

High levels of these elements hinder growth and development and induce physical and physiological abnormalities and make amphibians susceptible to the predators, parasites and other infectious organisms ²⁶. Water condition worsens when all the above parameters increase simultaneously, therefore their regular measure, check and balance is mandatory in order to keep water bodies pollution-free for amphibians.

EXPERIMENTAL

Present study was carried out from March to October during year 2011, 2012 and 2013. At first, field surveys were conducted in District Jamshoro for confirming the location of aquatic habitats in agricultural area where interviews from local community helped in finding their perpetual habitations and altogether 18 locals were marked for present investigation (Figure 1).



Figure 1: Map of District Jamshoro with study sites

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Inch tap was used to measure the size of each pond and meanwhile water depth was also measured using sechi disc. In order to determine water quality of complete pond at once the gross water samples were collected and stored in stoppered polyethylene plastic bottles. All the water samples were delivered to laboratory for the physico-chemical analysis using standard methods and analytical instrumentation as followed: pH meter of Model Orion, 420 was used to measure hydrogen ion concentration, however conductivity meter (Orion 115) was used to record level of electric conductivity (EC) and total dissolved solids (TDS). T-Hard (T-Hard) was analyzed through titration procedure ²⁷, while value of non-metallic elements such as sulphate, Phosphate, nitrite and nitrate (SO₄, PO₄, NO₂ and NO₃ respectively) was recorded using ultra-violet spectrophotometer (Hitachi 200). Besides application of specific reagents, specific wavelength of ultraviolet visible light was also applied distinctively through which concentration of each non-metallic parameter was absorbed. For the analysis of SO₄ 420 nm wavelength of ultraviolet visible light was applied for the detection of phosphate concentration. NO₂ and NO₃ values were analyzed respectively through 540nm and 410nm wavelength ²⁸. Concentration of potassium (K) was analyzed using atomic absorption spectrophotometer (Model: Perkin Elemer Analyst 800). Status of amphibian habitats was identified via scientifically approved water quality criteria ²⁹⁻³¹.

Scientific literature provided favorable range of each physico-chemical parameter as followed: pH value (6.0-9.0), whereas normal level of EC and TDS lies respectively within 150.0-500.0 μ S cm⁻¹ and 50.0-250.0 mg L^{-1 31, 32}. Favorable value of T-Hard extends between 75.0-200.0 mg L⁻¹, whereas normal value of Cl is recommended from 50.0 mg/L to 150.0 mg/L ^{29, 33}. Standard value of SO₄ extends within 50.0-100.0 mg L^{-1 33, 34}, however 0.03-0.05 mg L⁻¹ quantity of PO₄ is suitable for amphibian ponds ³⁵. Auspicious value of NO₂ and NO₃ is verified respectively as 1.0-2.0 mg/L ²² and 1.0-2.5 mg/L of NO₃ is verified to be auspicious ^{22, 29, 36}. K is also an important factor that impacts amphibians negatively when beyond the concentration of 25.0-50.0 mg/L ^{24, 31, 37}, while value of CO₂ within 12.0-25.0 mg/L is normal for the steady survival of amphibians ²².

RESULTS AND DISCUSSION

Size of amphibian ponds extended from 04 to 25 feet, while their depth was 05-10 feet. Water quality of these amphibian habitats consisted of distinct values of all the physico-chemical parameters each year as explicated by the average value and standard deviation (**Table 1**), measuring unit of per parameter is also specified.

Entire study of three years prominently indicated normal range of pH i.e. 6.5-9.4. It was the only parameter found within auspicious limit for most of the time except June and July of each year of present investigation. Value of EC (electric conductivity) remained all the time extremely high ranging from 945.8 μ S cm⁻¹ to 5130.0 μ S cm⁻¹ and so was the position of TDS (total dissolved solids) that was recorded from 580.6 mg L⁻¹ to 3437.1 mg L⁻¹. Maximum quantity of both parameters was noted in July; however their minimum value was recorded in October each year. Range of T-Hard (130.0-950.9 mg L⁻¹), T-Alk (145.5-537.0 mg L⁻¹) and Cl (177.5-685.15 mg L⁻¹) was also exceptionally high into all amphibian ponds during entire study period. Concentration of SO₄ stuck from 200.0 mg L⁻¹ to 800.0 mg L⁻¹, meantime PO₄ enormousness was recorded between 150.8 mg L⁻¹ to 800.0 mg L⁻¹. Quantity of NO₂ (0.1-14.6 mg L⁻¹), NO₃ (1.0-15.75 mg L⁻¹) and K (46.8-105.7 mg L⁻¹) was also concentrated copiously, although level of CO₂ was moderate as it was noted as 12.0 mg L⁻¹ to 26.0 mg L⁻¹.

⁹² J. Chem. Bio. Phy. Sci. Sec. D; August 2017 – October, 2017, Vol. 7, No. 4; 889-897. DOI:10.24214/jcbps.D.7.4.88997.]

It was distinguished that the monthly value of pH, EC, TDS, T-Hard, T-Alk, Cl, SO₄, PO₄, NO₂, NO₃ and K was maximum in July and minimum in October except CO₂ that exhibited antagonistic fluctuation in its monthly values (its upmost value was recorded in October, while down most value was recorded in July). Monthly values of physico-chemical parameters varied in comparable manner each year. On the basis of three years extensive study in District Jamshoro, it was obvious that pollution rate was higher into amphibian locals during the year-2012, while comparative analysis indicated utmost pollution at study sites S13-S18, wherein habitats S1-S6 least contamination was recorded (**Figure 1**).

Parameters	Year-2011	Year-2012	Year-2013
рН	7.9±0.6	8.1±0.6	8.1±0.6
EC µS cm ⁻¹	2456.1±1111.3	2510.8±1087.0	2449.2±1045.1
TDS mg L ⁻¹	1624.3±724.2	1662.5±668.4	1640.0±656.3
T-Hard mg L ⁻¹	386.7±180.4	417.7±167.4	411.8±169.0
T-Alk mg L ⁻¹	330.8±80.8	351.0±67.8	329.2±61.4
Cl mg L ⁻¹	349.6±124.1	359.4±93.8	344.4±93.8
SO ₄ mg L ⁻¹	432.5±149.4	455.3±129.8	438.0±112.5
PO ₄ mg L ⁻¹	400.5±136.7	417.5±94.0	378.2±92.2
NO ₂ mg L ⁻¹	3.0±2.7	4.1±2.6	4.1±2.5
NO ₃ mg L ⁻¹	6.0±3.5	7.1±3.4	5.9±3.0
$CO_2 mg L^{-1}$	18.0±3.5	18.1±3.5	18.2±3.6
K mg L ⁻¹	69.1±10.5	72.6±10.5	71.6±9.6

Table 2: V	Value of para	meters in amphibiar	n habitats at	District Jamshoro
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Physico-chemical analysis of 18 amphibian habitats in District Jamshoro revealed dreadful status overwhelming for three year study period (Table 1). pH and CO₂ values (8.1 ± 0.6 and 18.1 ± 3.5 respectively) were normal in whole District Jamshoro but the concentration of others including EC (2472.0±1079.3), TDS (1642.4±682.0), T-Hard (405.4±172.5), T-Alk (337.0±71.0), Cl (351.1±104.8), SO₄ (441.9±131.5), PO₄ (398.8±110.5) and K (71.1±10.3) was extremely high above the suitable criteria. Concentration of NO₂ (3.7±2.6) and NO₃ (6.3±3.4) was highly variable, ranging from satisfactorily low (for diminutive period of time) to disparagingly high existing for most of the time.

Present study showed the range of pH approximately same in two consecutive years i.e. 2012 and 2013 exhibiting values as followed 6.5-9.2 mg/L and 6.5-9.4 mg/L respectively; however its lowest level i.e. 6.7-9.0 mg/L was recorded from March-October in year-2011. This slight escalation may not be influential as that persisted very briefly. Value of EC was all the time higher in whole study area especially during the year-2012 when it extended from 1009.5 uS/cm to 4730.8 uS/cm, though it did not vary significantly in years 2011 and 2013 but during year-2013 it's comparatively lowest range (1000.0-4577.5 uS/cm) was noted. Overall level of TDS was determined highest during year-2012 when its 700.0 mg/L - 3250.7 mg/L range was verified, wherein year-2011 this parameter persisted in comparatively low range (580.6-3437.1 mg/L). Values of EC and TDS were found interdependent and in fact it is the

concentration of TDS that makes water conduct heat (measured by EC). Total hardness also remained out of auspicious limit, but it was the year-2012 when the concentration of this parameter surpassed very highly (200.0 to 950.9 mg/L); however in year-2011 its value remained diminished to the level of 130.0-920.0 mg/L which is slightly low but still unfavorable for any of the anuran species existing in study area. Concentration of SO₄ was indeed unavoidable as it prevailed from 250.0 to 800.0 mg/L and PO₄ level varied between 180.9-650.7 mg/L. Both SO₄ and PO₄ were concentrated very high during year-2012. The lowest value of SO₄ (200.0-780.8 mg/L) was recorded in 2011 and in year-2013 its concentration was noted as 237.2-685.5 mg/L, while lowest value of p PO₄ was evaluated within 159.4-566.5 mg/L in year-2013. It is very important to note that the both parameters increased tremendously high concomitantly into all amphibian habitats where they persistently troubled the amphibians especially during their breeding season.

Range of NO₂ did not exhibit major variation in its value from year 2011-2013 recorded as 0.1-14.6 mg/L (year 2011), 0.2-11.9 mg/L (year 2012) and 0.5-11.1 mg/L (year 2013) and so was the range of NO₃ (1.3-15.8 mg/L in year-2011, 1.8-14.0 mg/L in year-2012 and 1.0-12.8 mg/L in year-2013). Even little amount of NO₂ and NO₃ is very hazardous for amphibian eggs and larvae and it was prominent that their value remained pejorative for the most of the time. Value of NO₂ and NO₃ fluctuated greatly and varied from normal to high quantity. NO₂ started ranging from 0.1 mg L⁻¹ (normal) to 14.6 mg L⁻¹ (high), whereas concentration of NO₃ was recorded to persist between 1.0 mg L⁻¹ (normal) to 15.75 mg L⁻¹ (high). Both parameters retained their normality for short time and persisted high for the most of the study period. Therefore significantly higher concentration of these parameters may definitely be harmful to amphibians of the study area. Concentration of K was highest in year 2011 ((50.0-105.7 mg/L), while in year 2013 its lowest range (46.8-95.0 mg/L) was still indeed harmful for amphibian fauna. Range of CO₂ was exactly similar with difference of only 0.5 mg/L in year 2011 (12.0-25.0 mg/L) and 2013 (12.0-25.5 mg/L) and not too much of it persevered even in year 2012 (12.0-26.0 mg/L).

Variation in values of parameters suggested uneven level of dissolved substances available for amphibians during different seasons and even years. Extreme upsurge in concentration of all the factors during breeding and hatching time (March to August) may cause noteworthy impact on development of eggs and even larvae which are exclusively aquatic with transparent skin and are bond to respire utterly through the water of their habitat. Thus the condition of all amphibian habitations was severely polluted. Herein these area amphibian populations may fail to interact and interconnect successfully with their daily ecological tasks as these creatures are considered as "environmental sponges" which absorb toxins of their habitats (especially aquatic) through their semi-permeable skin. Therefore at all stages of their life cycle, amphibians remain extremely vulnerable to toxins and acidity of their ambient.

CONCLUSION

Massive concentration of major physico-chemical parameters in all amphibian ponds indicated deleterious environmental conditions of whole District Jamshoro for the amphibian fauna. Monthly variations in water quality were prominent, whereas yearly variations were insignificant. High rate of pollutants especially from March to September shows alarming threat to eggs and larvae of amphibian fauna as their breeding season fall usually during that period. High level of studied parameters in agricultural ponds implied that there is an urgent need of conservation actions for saving amphibians.

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REFERENCES

- 1. L.S. Ford, D. Cannatella. The major clades of frogs. Herpetol. Monotgraphs. 1993, 7: 94-117.
- I. Das. Folivory and seasonal changes in diet in *Rana hexadactyla* (Anura, Ranidae). Jour. Zool. 1996, 238: 785-794.
- 3. Y.M. Issa, A.A. Elewa, M.S. Rizk, A.A. Hassouna. Distribution of heavy metals in Qaroun lake and river nile Egypt, menofiya. J. Agric. Res. 1996, 213: 733-746.
- A.R. Blaustein, D.B. Wake, W.P. Sousa. Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. Biol. Conserv. 1994, 8: 60-71.
- 5. K. Shaikh, G.S. Gachal, S.M. Yusuf, G. Nabi, A.H. Qadri, A. Afghan. Checklist and Distribution of Amphibian fauna in Sindh, Pakistan. Sindh Univ. Res. J. 2014, 46: 159-162.
- 6. M.S. Khan. Checklist of Amphibians of Pakistan. Pak. J. Wildlife. 2010, 1: 37-42.
- K. Shaikh, G.S. Gachal, S.Q. Memon, N.A. Sodho, M.Y. Shaikh, A.H. Qadri. Analysis of non-metallic contaminants from amphibian environment in Sindh, Pakistan. J. Fauna. Biol. Stud. 2014a, 1: 54-58.
- K. Shaikh, G.S. Gachal, S.Q. Memon, R.Z. Brohi, M.Y. Shaikh. Assessment of Physicochemical parameters in the amphibian environment in District Hyderabad Sindh, Pakistan. J. Entomol. Zool. Stud. 2014b, 2: 241-245.
- K. Shaikh, G.S. Gachal, S.Q. Memon, R.Z. N.A. Sodho, M.Y. Shaikh. Assessment of amphibian environment through Physico-chemical analysis in Pakistan. Bio. Envi. Sci. 2014c, 5: 255-261.
- K. Shaikh, G.S. Gachal, S.Q. Memon, M.Y. Shaikh. Evaluation of water quality and seasonal variation in aquatic environment of amphibians in District Hyderabad Sindh, Pakistan. J. Entomol. Zool. Stud. 2015a, 3: 331-335.
- 11. K. Shaikh, G.S. Gachal, S.Q. Memon, M.Y. Shaikh. Hardness and alkalinity in amphibian environment at District Hyderabad Sindh, Pakistan. Flora-Fauna. 2015b, 21: 3-8.
- K. Shaikh, G.S. Gachal, S.Q. Memon, M.Y. Shaikh, A.H. Qadri. Diversity, Morphology, Distribution and Population of Amphibian Fauna in District Jamshoro and Larkana, Sindh-Pakistan. J. Entomol. Zool. Stud. 2015c, 3: 475-479.
- K. Shaikh, G.S. Gachal, S.Q. Memon, N.A. Sodho, M.Y. Shaikh. Assessment of environmental issues of amphibian fauna in Taluka Thano Bula Khan (district jamshoro) Sindh-Pakistan. Int. J. Conserv. Sci. 2016, 7: 579-588.
- M.Z. Khan, M. Nazia. Impact of habitat destruction on the population of amphibians: current status of frogs and toads in Karachi and Thatta. Germany. ISBN-13: 978-3-8473-3684-6, 104. LAP Lambert Academic Publishers, 2012.
- 15. R.A. Alford, S.J. Richards. Global amphibian declines: A problem in applied ecology. Annu.

⁸⁹⁵ J. Chem. Bio. Phy. Sci. Sec. D; August 2017 – October, 2017, Vol. 7, No. 4; 889-897. DOI:10.24214/jcbps.D.7.4.88997.]

Rev. Ecol. Syst. 1999, 30: 133-165.

- 16. M.L. McCallum. Amphibian Decline or Extinction? Current Declines Dwarf Background Extinction Rate. J. Herpetol. 2007, 41: 483–491.
- J.G. Burkhart, A. Gerald, B. Heidi, C. Hillary, F. Douglas, G. David, G. Henry, H. Robert, C.H. Judy, P. Jepson, D. Johnson, L. Michael, L. David, L. Joseph, M. Joseph, M., D. Michael, L. George. Strategies for assessing the implications of malformed frogs for environmental health. Environ. Health Perspect. 2000, 108: 83–90.
- P.A. Krenkel, V. Novotney. Water Quality Management. 3rd Edition. New York: Academic Press; 1980.
- C.E. Boyd. Water Quality: An Introduction. The Netherland: Kluwer Academic Publishers Group, <u>ISBN 0-7923-7853-9.</u> 1999, 38-105.
- W.A. Wurts, M.P. Masser. Southern Regional Aquaculture Center, Publication No. 4100, 2004, 59-70.
- 21. W.A. Wurts, R.M. Durborow. Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center Publication No. 464. 1992, 60-83.
- 22. Environmental Protection Agency (EPA) Quality Criteria for Water. 1976, 534.
- 23. H. Ashley, M.E. Thea, J. Louis, J. Guillette, C.H. Caren. Influence of nitrate and nitrite on thyroid hormone responsive and stress-associated gene expression in cultured *Rana catesbeiana* tadpole tail fin tissue. Front. Genet. 2012, 4: 32-50.
- 24. E.S. Shirley, F.H. Shaw, B. Susanne, M. Margaret. The Relationship between Sodium, Potassium and Chloride in amphibian Muscles. J. Gen. Physiol. 1956, 23: 753-777.
- 25. C.E. Crocker, J.J. Cech. Effects of hypercapnia on the growth of juvenile white sturgeon. *Acipenser transmotanum*. Aquaculture. 1996, 147: 293-299.
- 26. C.M. Bridges. Tadpole swimming performance and activity affected by acute exposure to sub lethal levels of carbaryl. Environ. Toxicol. Chem. 1997, 16: 1935-1939.
- 27. R. Sunita. Experiments in Applied Chemistry. 2nd Edition. 2002, 77-93.
- 28. C.H. Denial. Quantitative chemical analysis. 3rd edition. 1948, 52-57.
- 29. Environmental Protection Agency (EPA). Quality Criteria for Water (Gold Book). Washington D.C. 1986.
- 30. B.A. Pierce. Acid tolerance in amphibians. Biosc. 1985, 35: 239-243.
- American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 18th Edition. 1992, 35-50.
- 32. Boyer, Robin, E.G. Christian. The Need for Water Quality Criteria for Frogs. Environ. Health Perspect. 1995, 103: 352–357.
- 33. N.E. Karrakar. Impacts of road deicing salts on amphibians and their habitats. Urban Herpetol. 2008, 20: 183-196.
 - 896 J. Chem. Bio. Phy. Sci. Sec. D; August 2017 October, 2017, Vol. 7, No. 4; 889-897. DOI:10.24214/jcbps.D.7.4.88997.]

- 34. Environmental protection division (EPD). Ambient Water Quality Guidelines for Sulphate. Overview Report. Environment Management, 2000.
- 35. J. Rouse, C. Bishop, J. Struger. Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival. Environ. Health Perspect. 1999, 107: 77-89.
- 36. P. Raven, G. Johnson. Biology (4th edition) Iowa: W C. Brown Publishers. 1990, 68-112.
- I.G. Warkentin, D. Bickford, N.S. Sodhi, J.A. Corey. Eating frogs to extinction. Biol. Conserv. 2009, 23: 1056–1059.

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