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Original Article

Monitoring pond water quality to improve the production of *Labeo rohita* (Hamilton, 1822) in Bannu Fish Hatchery of Bannu district, Khyber Pakhtunkhwa province; An Implications for artificial fish culture

Monitoramento da qualidade da água da lagoa para melhorar a produção de *Labeo rohita* (Hamilton, 1822) na incubadora de peixes Bannu do distrito de Bannu, província de Khyber Pakhtunkhwa; Implicações para a cultura de peixes artificiais

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Abstract

The water quality parameters of a fish pond are essential to be managing properly under control for successful operations of fish culture. Improper management of pond water quality during the juvenile stages can create stressful conditions to produce various harmful diseases, which may decrease the fish quality and results in low profits. The present study was aimed to monitored important water quality parameters of nursery ponds of *Labeo rohita* culturing in Bannu fish hatchery. The study duration was 75 days extends from 10th June to 24th August 2019 for the successful culture of this specie which can play a significant role in breeding season. Furthermore, the concentration of some heavy metals like copper (Cu), nickel (Ni), manganese (Mn), iron (Fe), cadmium (Cd), and zinc (Zn) in pond water and fry stages of this species was also determined. The data obtained from all water quality parameters were analyzed expressed as range, mean and standard deviation using MS Excel 2013. The obtained results of 75 days study revealed that the water pH & temperature, electric conductivity, total dissolved solids (TDS), and total dissolved oxygen (DO) of pond water samples were found within a tolerable limit except salinity and dissolved ammonia concentration were not permissible for fish growth. The average concentration of heavy metals in pond water exhibited descending order Fe>Ni>Mn>Zn>Cd>Ni, which was found in acceptable ranges. Whereas, the average values of heavy metals in fry stages were in the order of Zn>Fe>Ni>Cu>Cd>Mn, and found within the recommended values of WHO/FAO. Thus, it was concluded from this study that good water quality is a precondition, maintaining balanced levels of water quality parameters is fundamental for both the health and growth of fish culture which is quite necessary for assuring increased fish productivity. It is recommended to monitor and assess water quality parameters on a routine basis for promoting healthy fish culture.

Keywords: Bannu fish hatchery, *Labeo rohita*, nursery pond, water quality parameters, heavy metals.

Resumo

Os parâmetros de qualidade da água de um viveiro de peixes são essenciais para um manejo adequado e sob controle para operações bem-sucedidas de piscicultura. O manejo inadequado da qualidade da água do tanque durante os estágios juvenis pode criar condições estressantes para a produção de várias doenças nocivas, o que pode diminuir a qualidade do peixe e resultar em baixos lucros. O presente estudo teve como objetivo monitorar importantes parâmetros de qualidade da água de viveiros de cultivo de *Labeo rohita* em incubadora de peixes Bannu. A duração do estudo foi de 75 dias, estendendo-se de 10 de junho a 24 de agosto de 2019 para o sucesso do cultivo dessa espécie que pode desempenhar papel significativo na época de reprodução. Além disso, a concentração de alguns metais pesados como cobre (Cu), níquel (Ni), manganês (Mn), ferro (Fe), cádmio (Cd) e zinco (Zn) na água do

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tanque e estágios de fritura dessa espécie também foram determinados. Os dados obtidos de todos os parâmetros de qualidade da água foram analisados expressos como faixa, média e desvio padrão usando o MS Excel 2013. Os resultados obtidos de 75 dias de estudo revelaram que o pH e temperatura da água, condutividade elétrica, sólidos totais dissolvidos (TDS) e total de oxigênio dissolvido (OD) das amostras de água do lago foram encontrados dentro de um limite tolerável, exceto salinidade e concentração de amônia dissolvida não eram permitidas para o crescimento dos peixes. A concentração média de metais pesados na água da lagoa apresentou ordem decrescente Fe > Ni > Mn > Zn > Cd > Ni, que foi encontrada em faixas aceitáveis. Já os valores médios dos metais pesados nos estágios de fritura foram da ordem de Zn > Fe > Ni > Cu > Cd > Mn, e encontrados dentro dos valores recomendados pela OMS/FAO. Assim, concluiu-se deste estudo que a boa qualidade da água é uma condição prévia, manter níveis equilibrados dos parâmetros de qualidade da água é fundamental para a saúde e crescimento da piscicultura, o que é bastante necessário para garantir o aumento da produtividade piscícola. Recomendam-se monitorar e avaliar os parâmetros de qualidade da água em uma base rotineira para promover a piscicultura saudável.

Palavras-chave: incubatório de peixes Bannu, *Labeo rohita*, viveiro, parâmetros de qualidade da água, metais pesados.

1. Introduction

Bannu district is found in Khyber Pakhtunkhwa province of Pakistan and is comprised of an area of 7.77 square kilometers. Its freshwater resources include i.e., Kurram river, Gambia or Tochi River, the Tangal River, Sarinde Narai Algada seasonal River, the Razzu Waal River, Makerwal Nus River, Danawaal River, Sarwakai and Mandkai springs, Baran lake and Baran dam that are most familiar for irrigation purpose only. There are about 233 fish species had been reported in the freshwater reservoirs all around Pakistan and most of these species were belonging to the Cyprinidae family (Ullah et al., 2014). Studies on the economically important fish species of the region have been taken in recent years (Ahmad et al., 2020; Khalid et al., 2020; Khan et al., 2021a; Khan et al., 2021b; Khan et al., 2021c; Khan et al., 2021d; Shah et al., 2021; Ullah et al., 2021). Nowadays, carp species including, *Cirrhinus mrigala, Hypophthalamichthys nobilis, Hypophthalamichthys molitrix, Cyprinus carpio,* and *Labeo rohita* are particularly raised in the fish hatcheries of Pakistan (Karim et al., 2016). Rehman et al. (2020) studied and reported the high abundance of carp fishes in the Tochi Rivers of KPK. More recently, with the increasing demands of fish and fisheries products, aquaculture production is now raising more rapidly and constitutes about 50% of the total food fish production of the world (Nyanti et al., 2012; FAO, 2014).

The word 'rohu' is commonly used for a carp species, *Labeo rohita*, which are found in the rivers of South-Asian countries. These are large silver-colored omnivores and polyculture fishes, which are using for aquaculture purposes in Bangladesh, India, Nepal, and Pakistan. It sexual matured between 2 to 5 years (Malik et al., 2018), and generally spawns at an optimal temperature from 22 to 31 °C. *Labeo rohita* accounts for about 15% of the total production of freshwater aquaculture in the whole world (Bakhtiyar et al., 2017). The growth rate of this species becomes higher and can also gain the weight of 700 to 800 grams in a year, while the marketable size attains after 12 to 18 months of its farming. Recently, due to the introduction of extensive carp culture, its production has been raised to the levels of 3 to 5 tons per hector per year. Therefore, due to the implementation of advancement in cultural practices and water quality management, the production level of fish can be increased up to 70 to 80%. *Labeo rohita* normally constitutes 70% of the total production of other carp species in aquaculture. As this species is the cheap resources of protein for human consumption and its cost of production is about USD 0.5 to 0.6 per kg in the case of a semi-intensive culture system (Mirza, 2003; Ramakrishna et al., 2013). Various carp species have been reported abundantly in the inland waters of Pakistan and are also most popular in aquaculture practices as food fishes for the local population (Khan et al., 2016).

The word 'water quality' is now being used to describe all physio-chemical and biological features of water, which can play a significant role in the growth and survival of any aquatic organism lives in it. Therefore, water used for aquaculture cannot give the desired production unless its quality parameters become optimal for the growth and survival of organisms used for aquaculture purposes (Rajkumar et al., 2018; Malik et al., 2020). Observations of water parameters and its monitoring in a fishpond on daily basis would be necessary to ensure the supply of good quality water as well its appropriateness for fish culture (Ngueku, 2014; Malik et al., 2018). As poor water quality can produce low-quality fish products, which can increase the human health risks particularly in the local population of the country. The present study was first attempt to observe water quality parameters of the nursery ponds of *Labeo rohita* that would provide valuable data that could increase the maximum production and survival rate of this species at Bannu fish hatchery.

2. Materials and Methods

2.1. Fish hatchery samples collection

The present study was conducted in Bannu fish hatchery, located in Shahbaz Azmat-Khel (Shaboze Kala) town of Bannu district of the Khyber Pakhtunkhwa province of Pakistan. Water and fish samples were collected from the nursery ponds of *Labeo rohita* for 75 days period of an experimental study, which extends from 10th June to 24th August 2019. The size of nursery ponds in this hatchery ranged from 70x120 square feet and filled with groundwater by tube well up to 3.0 to 4.0 meters depth. About 1500 to 3000 fish seeds/fry stages were stocked in each nursery pond.

2.2. Analysis of water quality parameters

In this study, water parameters like pH, electrical conductivity, temperature, dissolved oxygen, dissolved ammonia, and salinity of the nursery ponds of *Labeo rohita* were observed for 75 days study period by following the methodology of Abdel-Khalek et al. (2016), Rajkumar et al. (2018), Akter et al. (2019) and Rehman et al. (2020). Temperature, pH, and electric conductivity of water samples were determined by using Digital Jenway 3505 pH/mV/Temperature meter. The dissolved oxygen (DO) concentration of water samples was estimated by using the Jenway Model 9500 DO_2 Meter (950001). HI-3824 Ammonia test kit was used for testing the dissolve ammonia concentration, and Brix/Specific Gravity Refractometer w/ATC was utilized for calculating the salinity of water samples.

Water samples were also classified as follows;

WS1= pond water sampling after 3-4 days hatching of fry stage

WS2 = pond water sampling after 15-20 days hatching of fry stage

WS3 = pond water sampling after 75 days of hatching of the fry stage

2.3. Fish samples collection and preservations

Collected fish samples were temporarily preserved in ice, length and weight were calculated immediately after collection and preservation samples were transferred into the laboratory for further analysis in the Department of Environmental Science of Sardar Bahadur Khan Women's University Quetta. Fry stages rearing samples were randomly distributed into three experimental groups according to their size after hatching i.e.,

(1) S1= size group range from 4.0 to 7.5 mm (hatchlings after 3 to 4 days of hatching),

(2) S2= size group range from 20.0 to 25.0 mm (fry stage after 15 to 20 days of hatching), and

(3) S3=size group range from 28.0 to 65.0 mm (fry/ fingerling stage after 75 days of hatching), as presented in Table 1, respectively.

2.4. Heavy metals analysis

In this study, the concentration of six heavy metals i.e., copper (Cu), nickel (Ni), manganese (Mn), iron (Fe), cadmium (Cd), and zinc (Zn) in pond water and fry stage of this species was determined by measured in mg/L by using Atomic Absorption Spectrophotometer (AAS), model Analyat 700 USA with methods followed by Weber et al. (2013) and Radulescu et al. (2014) as per standard protocols given by APHA (2005). Water and fish samples were stored in the refrigerator. Ten samples of each size group (S1, S2& S3) of fry stages along with their water samples (WS1, WS2 & WS3) were processed for estimating of metals to observe their nutritional quality according to WHO/FAO permissible limits (Kaur et al., 2018).

2.5. Bioaccumulation Factor (BAF)

The bioaccumulation factor (BAF) of heavy metals in the tissues of three fry stages (S1, S2 & S3) was also calculated in mg/L by following the equation given by Javed and Usmani (2013) and Abdel-Khalek et al. (2016), as follows;

$$BAF = \frac{\text{Conc. of heavy metal in dry fish tissues}}{\text{Conc. of heavy metal in water samples of fish pond}}$$

2.6. Statistical analysis of data

The data obtained from the analysis of all these water quality parameters were expressed as range, mean, standard deviation. The result was analyzed by using one MS Excel 2013 computer software. The Pearson Correlation Coefficients (R^2) between metal concentration in water and tissues of fish was determined by following the method of Abdel-Khalek et al. (2016).

3. Results and Discussion

3.1. Production of fish seeds in the nursery ponds of Labeo rohita

Table 1 presented the pond size, water depth, number of fry stage stocked/pond, and size range of three groups (S1, S2 & S3) of the fry stage stocked in nursery ponds, respectively. The size of rectangular-shaped nursery pond in Bannu fish hatchery was measured as 70x120sq feet and depth as 5.0 feet and stocked approximately 1500 to 3000 fish seeds/fry stages of *Labeo rohita*. Induced breeding of *Labeo rohita* was started in June by using an injection of ovaprim hormone that mature all eggs and sperms in brooders already kept in ratio 1 male: 3 females in circular tanks. The size of brooders was ranged from 1.0 to 1.5 feet and weight was ranging from 1.0 to 1.5 kg. About 0.5 ml/kg of Ovaprim was injected in each female fish and 0.3ml/

Table 1. Number of Fish Seeds/fry stage stocks, the Size range of juvenile/fry stage, and type of feed used in the Nursery ponds of *Labeo rohita* in Bannu Fish Hatchery.

No. of fish seeds/fry stages stocks per nursery pond	1500-3000
Fish feeds used for feeding in the nursery pond	Wheat bran +Rice bran
The size range of hatchlings after 3-4 days (S1)	4.0-7.5mm
The size range of juvenile/fry stage after 15 to 20 days (S2)	20.0-25.0 mm
Size Range of fry stage (75 days of experimental study) (S3)	28.0-65.0 mm
Body-weight Range of fry stage (75 days of experimental study) (BW) in mg	50000-150000 mg

kg of ovaprim was injected into the male brooder and released in a circular tank for courtship and spawning. After injection, each female brooder lay 100000 eggs/kg of its body weight (BW). In the circulatory pond, the fertilized eggs were kept for 72 hours in circulating water for keeping the eggs in the surface water and prevent them from settling down at the bottom. After hatching in 3-4 days, the hatchlings stages ranged from 4.0 to 7.5 mm in total length (TL) were then transferred in nursery ponds. These fry were fed with a mixture of wheat bran and rice bran in 3:1. After 75 days of experimental analysis; these fry stages attain length between 28.0 to 65.0 mm and weight 50 to 150 grams. The maximum water depth of nursery ponds was found to be 3.0 to 4.0 feet. Md Hosen et al. (2019) have been suggested that water depth 3.0m is more beneficial for carp fish culture.

3.2. Water quality parameters

The results of physicochemical parameters of water samples were collected from the nursery ponds during the experimental study were presented in Table 2. The values of pH, electric conductivity (EC), water temperature, total dissolved solids (TDS), salinity, total dissolved oxygen (DO) and ammonia ranged between 7.27 to 8.37, 271 to 409 µS/cm, 25 to 36 °C, 99.6 to 101.2 mg/L, 3.0 to 3.5ppt, 6.0-6.5 mg/L and 2.0 to 2.4 mg/L. The physico-chemical properties of water can impact all activities of aquatic biota, therefore, variations in the physical, chemical, and biological characteristics of water can play an important role in the growth and survival of all aquatic organisms. Hence, the monitoring of water quality parameters during fish culture is a pre-requisite for obtaining the optimal conditions of fish growth and productivity of any fish species (Rajkumar et al., 2018). Some water quality parameters such as, pH, water temperature, salinity, alkalinity, hardness, dissolved oxygen, carbon dioxide, and ammonia gases and dissolved nutrients including nitrates and phosphates have been considered to be most important, which can determine the composition and productivity of all aquatic biota (Suman et al., 2017). Rajkumar et al. (2018) reported that water hardness of 150 mg/L is suitable for the growth of *Labeo rohita* under normal environmental conditions. Water hardness is the measure of calcium and magnesium in an aquatic body and is also highly essential for normal metabolic reactions like the formation of scales and bone in fishes (Rajkumar et al., 2018). All these physico-chemical parameters (except salinity and ammonia) were found in permissible ranges for the growth and wellbeing of *Labeo rohita* as previously suggested by some workers including Boyd (1982), Bhatnagar & Devi (2013), Sinha et al. (2015), Rajkumar et al. (2018), Md Hosen et al. (2019) and Malik et al. (2020).

3.2.1. Water resources

Due to less annual rainfall, most of freshwater sources are facing water shortage throughout Pakistan, therefore, fish culture is quite difficult for fish farmers in hatcheries. As poor water quality of a fish pond can not only decrease fish production but also shows an observable impact on fish growth, particularly during its juvenile stage. Moreover, groundwater with high magnesium sulfate content that produces respiratory defects in humans can be beneficial for fish farming (Malik et al., 2020). Therefore, some workers like Ngueku (2014) had given the top priority to groundwater as the best water resource than surface water for fish culture because it is the constant temperature at same sites (might vary latitudinal or depth of well) and chemical composition, transparency, alkalinity (pH>7.0), less dissolve ammonia concentration (>2.0mg/L), free from aquatic pollutants and certain disease-causing organisms temperature than surface water resources. In the present study, the open tube well water was used as a source

Table 2. Water quality parameters of the nursery ponds of Bannu fish hatchery during the period from 10th June to 24th August 2019 and their standard values.

Parameters	Range		Mean±S.D	Median	Standard values	Reference
pH	7.27	8.37	7.7±0.43	7.56	6.0-9.0	Boyd, 1982; Rajkumar et al., 2018; Md Hosen et al., 2019
Electrical Conductivity (µS/cm)	271	409	369.2±42.1	351	150-500 µS/cm;	Bhatnagar & Devi, 2013; Sinha et al., 2015; Rehman et al., 2020
Temperature ˚C	25	36	22.7±12.01	23	28-32 ˚C	Boyd, 1982, Devi et al., 2017; Md Hosen et al., 2019; Rehman et al., 2020
Total Dissolved Solid(TDS) mg/L	96.0	101.0	99.04±1.89	99.3	400-500 mg/L	Sinha et al., 2015
Dissolved Oxygen(DO) mg/L	6.0	4.5	6.22±0.19	6.2	3.0-5.0mg/L, Preferable range 4.0-5.0mg/L,	Boyd, 1982; Rajkumar et al., 2018; Md Hosen et al., 2019; Malik et al., 2020
Dissolved Ammonia mg/L	2.0	2.4	2.2±0.15	2.2	>0.05 or 0.1 to 0.3mg/L;	Boyd, 1982; Malik et al., 2020
Salinity ppt	3.0	3.5	3.03±0.177	3.0	>3.0ppt	Boyd, 1982; Malik et al., 2018

of water for carp fish culturing in the nursery ponds of Bannu fish hatchery. As groundwater resources had been found more suitable for carp fish growth as suggested by Ngueku (2014); however, groundwater sometimes contains very high levels of dissolved carbon dioxide (CO_2) (>50mg/L), salinity (brackish water 10‰), water hardness (>150 mg/L) and less amount of total dissolved oxygen (DO) concentration (<2.0 mg/L) than the acceptable ranges for fish growth (Malik et al., 2020). Therefore, all these water parameters were monitored during the experimental study of this species.

3.2.2. Water temperature

In the present study, a gradual increase in water temperature of fish ponds reported during the whole study period might be due to changes in weather conditions of the Bannu district. Bhatnagar and Devi (2013), and Md Hosen et al. (2019) had been suggested that water temperature ranged from 24 to 32 °C is considered good for tropical carp fish spawning. In the present study, the recorded values of water temperature of nursery ponds were found tolerable and in favorable limits for the growth of the fry stage of *Labeo rohita* (Table 2).

3.2.3. Dissolve Oxygen (DO) concentration

In the present study, the reported values of dissolved oxygen (DO) concentration was fall in the standard range as suggested by Rajkumar et al. (2018), Md Hosen et al. (2019), and Malik et al. (2020), therefore favorable for the growth of fry stage of *Labeo rohita* in Bannu fish hatchery (Table 2).

3.2.4. Hydrogen ion concentration (pH)

In the present study, the recorded pH values of water samples collected from nursery ponds was ranged from 7.27 to 8.37 as shown in Table 2. The desirable range of pH appropriate for fish culture is from 6.0 to 9.0, as described by Rehman et al. (2015) and Junaid et al. (2018). Hence, based on water quality standards for fish culture, the pH values of water in nursery ponds were found to be suitable for fish growth. The monitoring of pH level in a safe range is very important in a fish pond because its fluctuations can directly impact metabolism and another physiological process of culture fish species, which finally leading the death of fish (Bhatnagar and Devi, 2013).

3.2.5. Electrical Conductivity (EC)

The present results revealed that electric conductivity was found in ranged from 271 to 409 µS/cm, as shown in Table 2. According to the WHO standards, the permissible range of EC for water is 150 to 500 µS/cm, which is an admissible and sufficient range for survival and growth of fish and can also influence its productivity (Bhatnagar and Devi, 2013; Sinha et al., 2015; Rehman et al., 2020). Therefore, the present study indicated that the electrical conductivity of water samples was favorable for fish growth.

3.2.6. Total Dissolved Solids (TDS)

In the present study, the TDS was ranged from 99.6 to 101.2 mg/L, hence found in the favorable range for fish growth and survival. Light penetration and the rate of photosynthesis also depend on it. According to WHO standards, the applicable range of TDS suitable for fish pond water sample is 400 to 500 mg/L (Sinha et al., 2015).

3.2.7. Dissolve ammonia concentration (NH_3)

The present study revealed that the concentration of ammonia was very highly toxic and ranged from 2.0 to 2.4 mg/L as shown in Table 2 and was not reliable for fish growth. According to WHO standards, the concentration of ammonia must be permissible below 0.05 mg/L (Malik et al., 2020). The most preferred recommended level of dissolved ammonia is > 0.02 and considered safe for the survival of tropical pond fishery; however, short exposer of fish to range 0.6 to 2.0mg/L is highly toxic for pond fish (Bhatnagar and Devi, 2013).

3.2.8. Salinity

The results of the present study indicated that the salinity of water samples ranged between 3.0 to 3.5 ppt, which was not found in the desirable range for fish growth (Table 2). According to WHO standards, 2.0ppt is desirable for carp fishes (Bhatnagar and Devi, 2013; Iffat et al., 2020).

3.2.9. Heavy metals concentration in water samples of nursery ponds

The heavy metals such as copper (Cu), nickel (Ni), manganese (Mn), iron (Fe), cadmium (Cd), and zinc (Zn) in three pond water samples (WS1, WS2 & WS3) were presented in Table 3, respectively. The reported values of all these metals were compared with WHO/FAO standard values. The average values of heavy metals exhibited the following decreasing order: Fe>Ni>Mn>Zn>Cd>Ni in the three pond water samples (WS1, WS2 & WS3) collected during the experimental study period. Furthermore, when the average concentration of these metals was compared with the recommended values of WHO/FAO and also with other investigated values of Javed and Usmani (2013), Abdel-Khalek et al. (2016), and Zafar et al. (2017), it was found that the average values of all these metals (Fe, Ni, Mn, Zn, Cd, and Ni) found in water samples collected from nursery ponds of Bannu fish hatchery were found within tolerable limit; hence consider safe and favorable for fish growth and survival.

3.2.10. Heavy metals concentration in the tissues of fish samples of nursery ponds

The concentration of heavy metals such as copper (Cu), nickel (Ni), manganese (Mn), iron (Fe), cadmium (Cd), and zinc (Zn) found in the fry fish samples (S1, S2 & S3) were presented in Table 4 and Figure 1. The reported values of all these metals were compared with WHO/FAO standard values. The average values of these heavy metals were found in the following decreasing order; Zn>Fe>Ni>Cu>Cd>Mn,. Furthermore, when the average concentration was compared with the recommended values of WHO/FAO and also with other investigated values of Javed and Usmani (2013), Ullah et al. (2016), and Kaur et al. (2018), and it was observed that all

Table 3. The heavy metal content of water samples collected from the nursery ponds of Bannu fish hatchery during the period from 10th June to 24th August 2019 compared with Water Quality Guidelines and their Standards values of WHO/FAO.

Metals	WS1	WS2	WS3	Range		Mean±S.D	Standard values/ WHO/FAO	References
Cu 324.8	-0.012	-0.013	-0.0125	-0.013	-0.012	-0.012±0.0005	1.0-2.0/0.2mg/L	WHO, 1984; Javed & Usmani, 2013
Ni 232.0	-0.151	-0.096	-0.1235	-0.151	-0.096	-0.123±0.0275	0.02-0.2mg/L	WHO, 1984; Javed & Usmani, 2013
Mn 279.8	-0.078	-0.082	-0.08	-0.082	-0.078	-0.08±0.002	0.05-0.5/ 0.1mg/L 0.01 to 1.0 mg/L	WHO, 1984; Javed & Usmani, 2013
Fe 248.3	-0.157	-0.548	-0.3525	-0.548	-0.157	-0.352±0.1955	0.30/ 5.0 mg/L, while 0.1 mg/L for fry stage	WHO, 1984; Javed & Usmani, 2013
Cd 228.8	-0.017	-0.019	-0.018	-0.019	-0.017	-0.018±0.001	0.00025-0.005/ 0.01mg/L	WHO, 1984; Ranbhare & Bakare, 2012
Zn 213.9	-0.048	-0.044	-0.046	-0.048	-0.044	-0.046±0.002	3.0-5.0/2.0 mg/L	WHO, 1984; Javed & Usmani, 2013

All values of heavy metals concentration are in mg/L, WS1= pond water samples collected after 3-4 days of hatching of fry stage; WS2= pond water samples collected after 15 to 20 days of hatching of fry stage; WS3= pond water samples collected after 75 days of hatching of fry stage, S.D=Standard deviation.

Table 4. The heavy metal content of fish samples collected from the nursery ponds of Bannu fish hatchery during the period from 10th June to 24th August 2019 compared with fish quality guidelines and their standards values of WHO/FAO.

Metals	S1	S2	S3	Range		Mean±S.D	Standard values/WHO/ FAO	References
Cu **324.8**	0.794	0.847	0.244	0.244	0.847	0.628±0.334	10.0-30.0/ (100 mg/L in Canadian standard)	FAO/WHO, 1983; Ullah, et al., 2016; Kaur et al., 2018
Ni **232.0**	0.942	1.244	0.884	0.884	1.244	1.023±0.193	70.0-80.0	Javed & Usmani, 2013; Kaur et al., 2018
Mn **279.8**	0.014	0.004	0.041	0.004	0.041	0.020±0.019	1.0-5.0	FAO/WHO, 1989; Kaur et al., 2018
Fe **248.3**	0.214	2.312	2.233	0.214	2.312	1.586±1.189	100	FAO/WHO, 1989; Javed & Usmani, 2013
Cd **228.8**	0.030	0.030	0.028	0.028	0.030	0.029±0.001	1.00	Kaur et al., 2018
Zn **213.9**	3.114	18.53	1.562	1.562	18.530	7.735±1.381	100	FAO/WHO, 1989

All values of heavy metals concentration are in mg/L, S1= fish samples collected after 3-4 days of hatching of fry stage; S2= fish samples collected after 15 to 20 days of hatching of fry stage; S3= fish samples collected after 75 days of hatching of fry stage, S.D=Standard deviation.

these metals were found in permissible range; hence consumption of this species is safe for human and have high nutritional benefits for the local population of Bannu district.

3.2.11. Bioaccumulation of heavy metals in the tissues of three fry stages of Labeo rohita

In the present study, the bioaccumulation factor was also calculated for measuring the accumulation of heavy metals in fry stages related to their concentrations in pond water habitat. The bioaccumulation of heavy metals in the tissues of three fry stages (S1, S2 & S3) were presented in Table 5, and was found in the following ranking: Zn>Cu>Ni>Fe>Cd>Mn, respectively. Moreover, the overall obtained results of heavy metals (i.e., Cu, Ni, Mn, Fe, Zn) found in pond water and fish samples as presented in the Table 3 and 4 were showing very less concentration or accumulation of heavy metals, which revealed that this hatchery location is fit for carp fish productivity, as in agreement with the studies of Sawere and Oghenekowhoyan (2019). Moiseenko and Kudryavtseva (2001), and Uluturhan and Kucuksezgin (2007) had observed that the accumulation of heavy metals in fish mostly depends on physicochemical characteristics of water, type of species, time exposure to these metals, age of fish and its physiological status and their role as an enzymatic activator for numerous metabolic reactions in fish. Monroy et al. (2014) reported less level of bioaccumulation of heavy metals in fish also reflected their less amount in the aquatic habitat and therefore, less uptake of these metals from the surrounding where this species lives and hence shows a perfect image of the interaction between fish and its external environment; as fish mostly accumulate these metals in its body tissues either directly from its surrounding water bodies or bottom sediments or through

Figure 1. Google male shows the location of Shahbaz-Azmat-Khel town of Bannu district, Pakistan

Table 5. Bioaccumulation Factor (BAF) for analyzed metals calculated in mg/L in the tissues of three fry stages (S1, S2 & S3) of *Labeo rohita* collected from Bannu hatchery during the period from 10th June to 24th August 2019.

Metals	**Fry stage (3-4 days)**	**Fry stage (15-20 days)**	**Fry stage (75-days)**	**Mean±S.D**	**Pearson correlation coefficients (R^2) at p>0.05 between bio-accumulated metals in fish samples *vs.* water samples**
	BAF (S1)	**BAF (S2)**	**BAF(S3)**		**R^2**
Cu	-66.16^{a}	-65.15^{b}	-19.52^{c}	-50.28±26.64	0.01^{*}
Ni	-6.24^{c}	-12.95^{a}	-7.16^{b}	-8.78±3.64	0.61^{**}
Mn	-0.18^{b}	-0.05^{a}	-0.51^{c}	-0.24±0.24	0.07^{*}
Fe	-1.36^{c}	-4.22^{b}	-6.33^{c}	-3.97±2.50	0.77^{***}
Cd	-1.76^{a}	-1.57^{b}	-1.55^{c}	-1.63±0.11	-2.26N
Zn	-64.87^{b}	-421.12^{a}	-33.95^{c}	-173.3±215.2	0.68^{**}

All values of heavy metals concentration are in mg/L; N value represents correlation (R^2) is negative. Superscripts indicating the accumulation of heavy metals in three different fry stages, (S1, S2 & S3). if R^2 value >0.70 than correlation is strong, if $R^2 \geq 0.60$ than correlation is moderate, if R^2 <0.50 than correlation is weak, N value represents correlation (R^2) is negative.

the food chain. According to Haq (2005), the amount of heavy metal contents mostly depends on soil texture, groundwater table depth, anthropogenic activities, and ecological conditions of habitat. As these toxic metals are mostly non-biodegradable pollutants, therefore their accumulation in pond water and uptake along with food in bodies of aquatic organisms may not only disturbed the aquatic food chain but also alters the morphological and physiological changes in aquatic biota and could produce worldwide serious health issues for humans upon their consumption (Chatta et al., 2017).

3.2.12. Correlation analysis

In this study, the relationship between the bio-accumulated heavy metal concentrations in water vs, fish samples were represented by Pearson correlation coefficients (R^2), as presented in Table 5, respectively. This study revealed that all heavy metals (Cu, Ni, Mn, Fe, Zn) of water samples showed positive correlations with bio-accumulated metals in the tissues of three fry stages (S1, S2 & S3), except cadmium (Cd) that shows negative correlation (R^2= -2.26), respectively. Thus, present study has been proved that all these metals had common sources, equal distribution, related dependency, and the same performance in their aquatic environment as previously suggested by Abdel-Khalek et al. (2016), who also had been observed that the bio-accumulated metals in fish were greatly influenced by the concentration of metals present in its aquatic environment. Moreover, it had been observed that the relationship between metals found in fish, water, and soil sediments is mostly depending upon the physicochemical and biological activities of aquatic habitats, the release of toxic pollutants, or any other anthropogenic activities that can produce strong influences on metals distribution in aquatic habitats (Abdel-Khalek et al., 2016). The values of correlation coefficients (R^2) were also estimated for detecting the concentrations of heavy metals in water with related to bio-accumulated metals found in the tissues of fry stages rise in it, the correlation analysis provide only a degree of relationships, which however could not finally prove inter-relationship between metals in water and fish, as in agreement with Vasić et al. (2012). Therefore, the main purpose of calculating the bio-accumulation of metals in the tissues of fish was to observe their influence on the fish particularly affected by the total quantity of metals found in the water (Diop et al., 2015).

4. Conclusions

It is concluded that Bannu fish hatchery is a suitable place for the development and growth of juvenile stages of this species. The results suggests that good water quality is a precondition, which is quite necessary for assuring to increased fish productivity. Maintaining balanced levels of water quality parameters is fundamental for both the health and growth of culture organisms. It is recommended to monitor and assess water quality parameters on a routine basis for better and profitable fish culture.

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