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Original Article

Assessments of some trace metals in water samples of nursery pond of Grass Carp (*Ctenopharyngodon idella*, Valenciennes, 1844) in Bannu Fish Hatchery of Khyber Pakhtunkhwa, Pakistan

Avaliações de alguns vestígios de metais em amostras de água de viveiro de carpa-capim (*Ctenopharyngodon idella*, Valenciennes, 1844) em Bannu Fish Hatchery de Khyber Pakhtunkhwa, Paquistão

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Abstract

The present investigation was aimed to examine the concentrations of trace metals including e.g copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) in water samples collected from nursery pond of grass carp (*Ctenopharyngodon idella)* in Bannu Hatchery of Khyber Pakhtunkhwa during the period from April 2018 to January 2019. The temperature and pH of each water sample were measured for the whole study duration. The concentration of Copper (Cu), Manganese (Mn), Nickel (Ni), and Zinc (Zn) in collected water samples were measured in mg/liter by using Atomic Absorption Spectrophotometer. The blank and standard solutions for device calibration Standard solutions i.e., 2.0 mg, 4.0 mg, and 6.0 were used to measure the concentration of these metals in water samples to verify the measurements. The data was statistically analyzed on descriptive statistics (estimation of proportions and standard deviation) used to summarize mean concentration. The results obtained of both temperature and pH of water samples were found in ranged 10 to 36 ^{0}C and 7.0 to 8.44; whereas the size of fry stages was ranged from 4.0 to 56.0 mm in total length. The results of investigated metals found in pond water samples are in order of Zn>Mn>Ni>Cu, respectively. As optimum temperature and pH for grass carp were mostly between 15 ^{0}C and 30 ^{0}C and pH 6.5 to 8.0. It was concluded from obtained results that temperature, pH, and trace metals were found appropriate for the growth of *Ctenopharyngodon idella* from fry to fingerling stages, but the highest amount of zinc can cause its mortality. It is a preliminary study on grass carp culturing in Bannu fish hatchery so, it would provide useful information for model fish seed production unit in a hatchery.

Keywords: trace metals, water, nursery pond, grass carp, Bannu fish hatchery.

Resumo

A presente investigação teve como objetivo examinar as concentrações de metais traço, incluindo cobre (Cu), manganês (Mn), níquel (Ni) e zinco (Zn) em amostras de água coletadas de viveiro de carpa-capim (Ctenopharyngodon idella) em Incubatório de Bannu de Khyber Pakhtunkhwa durante o período de abril de 2018 a janeiro de 2019. A temperatura e o pH de cada amostra de água foram medidos durante todo o período do estudo. A concentração de cobre (Cu), manganês (Mn), níquel (Ni) e zinco (Zn) nas amostras de água coletadas foi medida em mg / litro usando espectrofotômetro de absorção atômica. As soluções em branco e padrão para as soluções padrão de calibração do dispositivo, ou seja, 2,0 mg, 4,0 mg e 6,0, foram usadas para medir a concentração desses metais em amostras de água para verificar as medições. Os dados foram analisados estatisticamente em estatística descritiva (estimativa de proporções e desvio padrão) usada para resumir a concentração média. Os resultados obtidos tanto de temperatura quanto de pH das amostras de água variaram de 10 a 36 ^{0}C e 7,0 a 8,44, enquanto o tamanho dos estágios de alevinos variou de 4,0 a 56,0 mm no comprimento total. Os resultados dos metais investigados encontrados em amostras de água de lagoas são da ordem de Zn > Mn > Ni > Cu, respectivamente. A temperatura e o pH ótimos para a carpa-capim foram principalmente entre 15 ^{0}C e 30 ^{0}C e pH 6,5 a 8,0. Concluiu-se, a partir dos

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resultados obtidos, que temperatura, pH e traços de metais foram considerados adequados para o crescimento de *Ctenopharyngodon idella* desde os estágios de alevinos até alevinos, mas a maior quantidade de zinco pode causar sua mortalidade. É um estudo preliminar sobre a cultura da carpa-capim em incubatório de peixes Bannu, portanto forneceria informações úteis para modelo de unidade de produção de sementes de peixes em um incubatório.

Palavras-chave: traços de metais, água, viveiro de viveiro, carpa-capim, incubatório de peixes Bannu.

1. Introduction

Bannu district is located in the southern region of Khyber Pakhtunkhwa (KPK) Province of Pakistan and is surrounded by a stony and charred Kho-e-Suleiman mountain. Kurram River and its marshland make it an attractive physique in its southern region. Its annual average temperature is 23.6 °C and rainfall is also very less throughout the year (Petrie et al., 2010). About 54 fish species had yet been reported in the river Kabul and its streams (Yousafzai et al., 2010). The most abundant carp species of Khyber Pakhtunkhwa province includes i.e., *Labeo rohita, Ctenopharyngodon idella Cirrrhinus mrigala, Hypothalmicthyes molitrix,* and *Cyprinus carpio*, and are also culturing in Kohat, Karak, Dera Ismail Khan, Kohat, Mardan, Swabi and Abbottabad regions (Asmat et al., 2014; Hasan et al., 2015). Though, due to the introduction of these carp fish cultures in the last few years, fisheries and aquaculture practices had now become a part of economics that contribute efficiently to fish food production in both government and private sectors (Yousafzai et al., 2010; Cudmore et al., 2017). Some of the economically important fish species of the region have been studied recently (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).

Fish quality can also be defined as a grouping of certain external features e.g., its freshness, healthiness, and reliability. Both growth and nutritional values of fish are mostly influenced by intrinsic factors i.e., type of species, sizes, and sexual maturity and extrinsic factors such as a source of nutrients, seasonal changes, salinity, the temperature of its aquatic environment (Zhao et al., 2018); therefore, the nutritional values of fish could also be affected especially by all these parameters in culture conditions. Thus, the production of high-quality culturable fish species in hatcheries is a major task for aquaculturists (Cheng et al., 2014). As pond culture was the most significant model of aquaculture in China, but various pollutants found in aquatic environments of the fish pond could have deleterious impacts on fish quality that could limit the sustainable development of fish in pond culture systems. The improvement of water quality, different aquaculturist used various techniques Zhang et al., (2019) have used floating-bed planted with *Ipomoea aquatica* as an aquatic macrophyte in ponds, which can decrease the concentrations of various nutrients as nitrite and dissolve ammonia (NH_3^+), phosphorus and certain metals like cadmium and chromium from the water, as well as total suspended solids, and also declined the chemical oxygen demand (COD) and biological oxygen demand (BOD) in fish ponds (Chanu and Gupta, 2016).

Afshan et al. (2015) studied the impact of physicochemical properties of pond water samples collected from the Sibi district of Balochistan on fish growth and their survival rates. Hina et al. (2015) had observed the various dams in Khyber Pukhtunkhwa (KPK) and Federally Administered Tribal Areas (FATA) that can be used for promoting the various freshwater aquaculture in Pakistan.

Grass carp feed mainly on aquatic plants, but sometimes they also feed upon insects and other invertebrates under culture conditions, and are also most frequently been used to control the growth of aquatic weeds in fish ponds. During fry stages, they first feed mostly on zooplanktons i.e., rotifers, cladocerans, copepods, and protozoans. The intensity of feed intake is mostly affected by diet types. On the other hand, Ni and Wang (1999) found that hybrid grass carp produced by a cross between female *Ctenopharyngodon idellus* versus male *Hypophthalmichthys nobilis* show a distinct preference for feeding the aquatic plants e.g., *Chara* sp., *Lemna gibba, Potamogeton peciinatus*, and *Najas guadalupensis* (Opuszynski and Shireman, 2019). Furthermore, Cai et al. (2005) had observed the significant impact of diet processing and its composition on the body composition, phosphorus availability, and growth performance of the juvenile stage of *Ctenopharyngodon idellus*. Wild grass carp normally can survive up to 5 to 11 years; however, in North Dakota, it was observed up to 33-years of age. Likewise, the growth and development of grass carp are also dependent upon its age and various other factors like food, density, temperature, and oxygen in water (Cudmore and Mandrak, 2004).

Heavy metals have been observed as toxic contaminations in the fish culturing system, as they can produce a great disturbance in metabolic rates, physiological activities, and supporting frameworks of aquatic fauna and flora. Accumulations of all these toxic metals in any aquatic organism can produce lethal effects on its biological system. Sewage water, agricultural, and industrial wastes loaded with contain high concentrations of these toxic metals without any treatments are generally discharge in aquatic bodies are absorbed in various tissues of the fish body including particularly in gills, skin and digestive system through water and food. These edible fish species with these toxic metals are found harmful for human health (Javed, 2015). Some factors like temperature, pH precipitation, decayed solids, alkalinity, and acidity are major sources of water contamination. These factors can create a hazardous impact on fish growth, size, and physiological condition of fish as reported by Hussain et al. (2011) and Lawson (2011). Therefore, the current study was designed to examine the concentrations of some trace metals such as copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) in water samples collected from nursery pond of grass carp, *Ctenopharyngodon idella* in Bannu fish Hatchery of KhyberPakhtunkhuwa to observe an impact on the well-being of carp species culturing in a controlled environment.

2. Materials and Methods

2.1. Pond water sampling

In this study, water samples were collected from the available nursery pond (70 x 120 square feet) in Bannu fish hatchery that contains fry stages of grass carp, *Ctenopharyngodon idella* during the periods from April 2018 to January 2019, as shown in Figure 1 respectively. The breeding season of grass carp was started from April till May, the fry stage of grass fishes was produced during these months and reached to fingerlings and mature stage from November to January. Due to monsoon or heavy rainfall during this period from July to September, the physicochemical properties of water samples of fish ponds were found altered during these months. The water samples were collected in two phases **S1** (from April to July) and **S2** (from August to January). The collected samples were brought in the laboratory for the analysis of the water parameters i.e., temperature, pH, and concentrations of some trace metals i.e., copper, manganese, nickel, and zinc in mg/L were measured by Atomic Absorption Spectrophotometer (AAS), model Analyat 700 made the USA with methods as followed by Afshan et al. (2015), and Zubia et al. (2015). The mean concentration and composition of trace metals found in water samples were calculated and measured for each metal in both **S1 and S2** phases.

2.2. Water temperature and pH

Temperature and pH of each water sample were measured by using a digital pH meter (model BANTE 2010) with the same procedures as followed by Chakraborty et al. (2014) and Zubia et al. (2015)

2.3. Size of Fry stage of fish

The hatched eggs in circular tanks, at fry stages, were kept for 72 hours till movement against the water current then they transfer to the nursery pond. The size of fry stages of grass carp fish *Ctenopharyngodon idella* collected from these nursery ponds during the whole study period was also measured to observe their growth from fry to fingerling stage.

2.4. Trace metals analysis by Atomic Absorption Spectrophotometer (AAS)

The concentration of Copper (Cu), Manganese (Mn), Nickel (Ni), and Zinc (Zn) in collected water samples were measured in mg/liter by using Atomic Absorption Spectrophotometer (AAS), model Analyat 700 USA with procedures as used by Weber et al. (2013), Radulescu et al. (2014), and Salem et al. (2014) with some modification. In the laboratory, these water samples before analysis were stored in a refrigerator. Mean and standard deviations of both **S1** and **S2** water samples were observed and calculated

2.5. Calibration of standard solutions

The blank and standard solutions for device calibration were used to measure the concentration of these metals in water samples to verify the validity of the measurements. Standard solutions i.e., 2.0 mg, 4.0 mg, and 6.0 mg of Zn, Ni, Mn, and Cu were measured on electronic balances, and then dissolved in distilled water of 100ml of the volumetric flask used for calibration of water samples.

2.6. Data analysis

The data was analyzed on descriptive statistics (estimation of proportions and standard deviation) was used to summarize mean concentration. The data was analyzed using Statistics Version 8.1.

3. Results

The size of the rectangular shape nursery pond in Bannu fish hatchery was measured as 70x120sq feet. Nursery pond can stock approximately 3000 fry stages of grass carp fish.

3.1. Temperature and pH of pond water samples and size of grass carp fish

The results of water temperature and pH of the nursery pond of *Ctenopharyngodon idella* in Bannu fish Hatchery were reported as ranged from 10 to 36^{0}C, and 7.00 to 8.44, respectively. The minimum temperature was recorded in January 2019, while the maximum temperature was

Figure 1. Shows nursery ponds of Bannu fish hatchery of KPK.

reported in June 2019. The minimum pH value was also reported in January 2019 and the maximum value was noted during September 2018, (Table 1, and Figure 2 and 3), respectively. While the increase in the size of fry stages of grass carp fish was also noted during the whole study period and found in a range from 4.0 to 56.0mm (Table 1 and Figure 3) respectively.

Table 1. Temperature, pH of the water, and the size of fish in nursery ponds of grass carp during the period from April 2018 to January 2019.

Parameters	Min.	Max.	Mean±S.D
Temperature °C	10	36	23.2±8.32
pH	7.0	8.44	7.59±0.43
Fish body length (TL) in mm	4.0	56.0	32.9±16.9

Note: S.D=standard deviation

3.2. Concentrations of trace metals of water samples of a nursery pond

The results of trace metals concentration of water samples collected from the nursery pond of Bannu Fish Hatchery are found in order of Zn>Mn>Ni>Cu as presented in Tables 2 and 3, respectively. These trace metal concentrations were compared with WHO standards as shown in Table 4.

4. Discussion

4.1. Water temperature and pH and their impact on the growth of grass carp

In the present study, both water temperature and pH of nursery ponds of grass carp were found in the appropriate range for the growth of the fry stage of grass

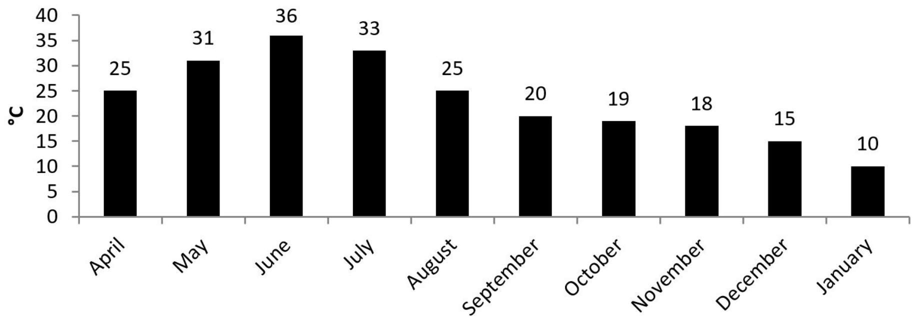

Figure 2. shows temperature of water samples collected from nursery pond of grass carp.

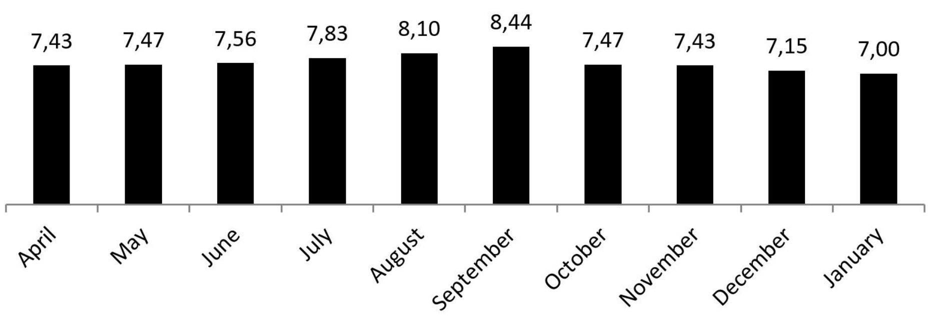

Figure 3. shows pH of water samples in nursery pond of grass carp.

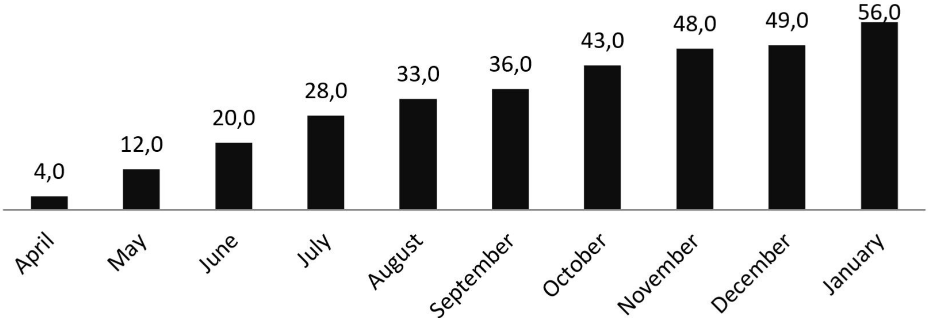

Figure 4. shows the total length of grass carp fish in mm.

Table 2. Mean absorbance of the trace metals at the calibration of standard solutions 2.0, 4.0 & 6.0 mg/L.

Metals	2.0mg/L	4.0mg/L	6.0mg/L
Cu	0.18	0.3539	0.4813
Mn	0.163	0.266	0.4593
Ni	0.0662	0.1131	0.1131
Zn	0.4582	0.7709	0.7709

Table 3. Concentrations of trace metals in water samples of Nursery ponds of Grass carp fish, *Ctenopharyngodon idella* in Bannu fish hatchery during April 2018-January 2019. Concentrations were measured in mg/L.

Water Samplings Phase	Cu	Mn	Ni	Zn
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
April to July (S1)	-0.028 ± 0.013	0.060 ±0.009	-0.028 ± -0.013	-0.000±0.004
August to January(S2)	-0.054 ± 0.149	0.077±0.004	-0.054 ± 0.149	7.134±0.0245

Table 4. Concentrations of trace metals in water samples (mg/L) of Nursery ponds of Grass carp fish, *Ctenopharyngodon idella* in Bannu fish hatchery compared with WHO standards.

Metals	WHO Standards	Nursery pond of Bannu Hatchery
Cu	2.00	-0.012
Ni	0.21	-0.028
Zn	3.10	7.135
Mn	0.51	0.062

carp fish as shown in Table 1 and Figures 2, 3 and 4. Water temperature is a significant controlling factor for all aquatic life because all-natural and artificial procedures in an aquaculture practice are mostly affected by it. Grass carp normally survive in water temperatures range from 0 to 33°C, however, if the temperature increases above 38°C, then become lethal for its growth, and also found lethal for yearlings growth if increases from 35 to 36°C. The lowest temperature range for its eggs is below 18^{0}C. Fingerlings 50 to 70 mm can tolerate if temperature increases from 4.0 to 22°C (Chilton II and Muoneke, 1992). The preferred temperature for grass carp is 10 to 26 °C. While, in contrast, grass carp in the Great Lakes basin can tolerate cold temperatures that occur at 0 to 0.1°C for the fry stage and 1 (one) to 2 °C for older fish (Conover et al., 2007). Authman et al. (2015) had reported the impact of water temperature influencing metabolic rates, movements, growth, and development of carp fish.

The water pH recorded for the nursery ponds of grass carp in Bannu fish Hatchery of this study was also found in a suitable range. Both water pH and temperature may also influence fish growth and sexual maturity. The ideal pH range for the development and growth of carp fishes was found in the range from 7.5 to 8.0 (Heydarnejad, 2012). Allan and Maguire (1992) reported that fish couldn't exist at low pH below 5.0, because the mortality of fishes ensues at this low pH, which has a corrosive effect on their scales and skin, decline their growth and development and also resistance to lethal substances found in water. Holtze and Hutchinson (1989) had determined the lethal impact of low pH ranged from 5.1 to 5.9 on the survival of early cleavage stages of eggs during the prehatch period and fry stages of some freshwater species in Ontario lakes. Moreover, at high pH 9.0 was also intolerable, because maximum ammonium ions (NH^{3+}) would consume dissolve oxygen in bonding with water to form ammonium hydroxide (NH_4OH), and create dissolve oxygen deficiency for fishes in their aquatic environment (Bergheim et al., 2006).

In most tropical regions of the world, cultured grass carp populations mature at earlier ages between 1 to 8 years at small sizes. In temperate areas of the USA, grass carp mature when reached at 4 to 5 years (Cudmore and Mandrak, 2004). Males usually mature one year earlier than females at 500 to 860mm in total length (Bozkurt et al., 2017). Size at maturity is generally 500–860 mm in total length (TL) (Bogutskaya et al., 2017). A water temperature of 15 to 18 °C is required to initiate spawning (Wang et al., 2014; Bogutskaya et al., 2017). Though the onset of spawning occurs at 18 °C (Bogutskaya et al., 2017), however, mass spawning occurs at 26 to 30 °C in China (Chilton II and Muoneke, 1992). Spawning seasons of grass carp had been reported in late April to early June in Oklahoma by Hargrave and Gido (2004), and also in late April to early July in China by Duan et al. (2009) The hatching time of eggs is mostly related to water temperature and may take about 33 to 72 hours (George and Chapman, 2015). The larval stage reaches up to 20 mm in length has rapid growth, averaging 217.6 mm at age 1(one) as reported in the southern Lake Erie by Cudmore et al. (2017).

4.2. The concentration of trace metals in water samples of nursery ponds

The overall results revealed that the concentration of trace metals like zinc (Zn), nickel (Ni), manganese (Mn), and copper (Cu) in water samples collected from nursery ponds were found in desirable amount, hence suitable for fry stage growth except zinc in S2 phase samples that can cause the death of fish at the larval stage before reaching to the fry stage. Due to the presence of a large concentration of heavy metals in water, its pH values could be changed from basic to acidic. Radulescu et al. (2014) had observed that the level of heavy metals in water also depends on the physicochemical parameters of water including, e.g., pH, turbidity, and salinity; because the pH of water can decreases the solubility of metals like iron and zinc also increases, except nickel and manganese. Furthermore, seasonal variations can also produce changes in physicochemical parameters of water that in turn affect the levels of some metals like cadmium and lead in lakes. Huang et al. (2015) had observed the impact of zinc concentration on the levels of other metals found in the liver, muscle, bone, and as well as on growth of tilapia fish. Yaqub and Javed (2012) reported that Cu, Cd, and Zn can affect the growth and longevity of fish. Most metals are considered essential micronutrients for fish growth, but they become toxic if found at higher concentrations (Jabeen et al., 2012). Shaukat and Javed (2013), Kousar and Javed (2014) had observed the acute and lethal impact of chromium on *Ctenopharyngodon idella* that enters the fish body from its aquatic environment or along with their diets and also produce prominent effects not only on fish growth but also on its other physiological functions (Naz et al., 2013). As growth in fish was considerably affected by some metals includes Cd, Zn, and Cu, also depends on fish tolerance limits, but their impact on fish growth was found in order, Cu>Cd>Zn, respectively. Unlike waterborne metals, the impact of dietary metals on fish well-being is still unclear; however, various studies revealed that Cu accumulation in fish has produced significant impacts on fish growth. Furthermore, the effects of all these metals were found to vary due to types of fish species, types of the body organ, salinities, diets, and time exposure to these toxic metals in various carp fish species as reported by Javed (2015). Liu et al. (2012) had observed the mining of Purple Mountain for more than 30 years are the main source of bioaccumulation of certain metals e.g., Cr, Ni, Mn, Cu, Zn, As, Cd, Hg, and Pb in the skin, scales, gills, liver, kidney muscles, liver, heart, stomach, and intestine of *Ctenopharyngodon idellus* collected from Tingjiang river. Hosseini-Alhashemi et al. (2012) had also observed the distributions of some metals i.e., Cu, Cd, Mn, Cr, Ni, Pb, V, and Zn in both water and six fish species in the largest wetland in the southwest of Iran and found that the accumulation of these metals in carnivore fishes was lower than omnivore fishes because showed high consumption of these toxic metals in their diets. Yousafzai & Shakoori (2008) had observed the bioaccumulation of some metals i.e, chromium, lead, mercury, zinc, copper, and nickel has a destructively effect on the body tissues e.g., liver, muscle, and kidney of *Clarias gariepinus.* Early embryonic developmental stages of fishes are particularly affected by the metals found in water, because if these metals gather in their gonads during spawning season and unfavorably influence gamete productions. The eggshell rapture due to metal entering, especially during the growth stage; and in this way, metals can accumulate in the eggs (Jezierska et al., 2009). Welsh et al. (1996) had demonstrated the lethal impact of Cu on larval stages fathead minnow, *Pimephales promelas* in Ontario Lake. Nickel may also cause some morphological changes in various fish blood, cells, tissues and produces chromosomal abnormalities (Vinodhini and Narayanan, 2009).

Javed (2013) had found the prolonged impacts of cobalt and nickel on the growth of Fish. Wiener and Giesy Junior (1979) had also observed the lethal impact of Cadmium, lead, copper, manganese, and zinc on stocked bluegill, *Lepomis macrochirus* found in acidic ponds on the Southeastern coastal plain of the United States. Vilizzi and Tarkan (2016) studied the concentration of some metals in *Cyprinus carpio* in Anatolia of Turkey and observed its lethal effect on human consumption. Love (2016) investigates the lethal impact on osmoregulation and swimming in juvenile *Oncorhynchus mykiss* upon exposure to the copper.

5. Conclusions

It was concluded from the results that the physiological conditions of water resources like the Baran dam used in culturing of carp fishes in Bannu fish hatchery were found appropriate for fish growth and survival rate. Moreover, the present investigation is also very much important to be fulfilled for the upgrading of aquaculture sector performance in Khyber Pakhtunkhwa province of Pakistan.

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