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Regression Studies of Planktonic Productivity and Fish Yields with Reference to Physico-chemical Parameters of the Ponds Stocked with Sub-Lethal Metal Stressed Fish

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ABSTRACT

Three major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* at fingerling stage were exposed to none and sub-lethal concentrations of individual metals (Fe, Zn, Pb, Ni, Mn) and their mixture in glass aquaria for 90 days. After stress period three fish species were grouped metal/treatment wise and stocked, separately in earthen ponds and reared for one year under semi-intensive culture system. All the physico-chemical variables, except water temperature, showed significant variations among ponds stocked with control and metal stressed fish. However, season exerted significant effect on the characteristics of pond's water. In all the ponds, except that stocked with Ni stressed fish, dissolved oxygen showed direct relationship with planktonic productivity of water. Temperature exerted significant effect on planktonic productivity of the ponds stocked with Fe and Mn stressed fish. Results revealed significant contribution of total alkalinity on the planktonic productivity also. The correlation coefficient between alkalinity of water and planktonic productivity was positive. Step-wise regression computed for each pond revealed K to be a sole water quality variable that exerted substantial influence on fish yield in all the ponds except those of control fish. In all ponds, except those stocked with Zn and Ni stressed fish, total alkalinity showed negative relationship with fish yield. Under all the treatments, fish yield showed direct dependence on the existing planktonic productivity indices of ponds except those stocked with Zn and Ni stressed fish. The regression coefficients of variables in the regression model computed for all the ponds were non-significant except the pond stocked with metal mixture stressed fish. It is concluded that exposure of major carps, at fingerling stage, to sub-lethal levels of different metals have resulted variable fish yields under semi-intensive culture depending upon the planktonic productivity and physico-chemical parameters of ponds.

Key Words: Major carps; Sub-lethal metal stress; Fish yield; Growth

INTRODUCTION

There are several causes of pollutant discharges into the rivers of Pakistan. Swift increase in population, spontaneous urbanization and founding of industries are few to name. The environmentalists have already raised apprehensions about the detrimental effects of un-treated wastewater discharge on the aquatic ecosystems of the rivers (Javed & Hayat, 1999). It is because the presence of heavy metals such as Fe, Zn, Pb, Ni and Mn beyond the permissible limits in the untreated wastewater have adversely affected the indigenous fish fauna especially the major carps (Javed & Mahmood, 2001). Though these trace elements promote physiological processes yet their too high concentration have toxic effects on the aquatic life (Javed, 2003).

Catla catla, *Labeo rohita* and *Cirrhina mrigala* are the most successful species of polyculture in Pakistan due to suitable climate for their culture on one hand and their consumer liking on the other. Therefore, the polyculture of these major carps has assumed much popularity among the private as well as the public sector farms in the recent past. Nevertheless, the density of major carps in natural waters has alarmingly been declined due to un-treated sewage and industrial discharges into the rivers bearing higher concentrations of heavy metal contaminants. Ultimately, the fish production of Pakistani inland waters has reduced due to population depletion and decline in growth potential of these three indigenous fish species (Javed, 2004). In addition, the natural spawning grounds of major carps have also been adversely affected.

Fish seed collected from polluted sites of the rivers has been found to deliver stunted and variable growth. Under the scenario, the farmers are un-able to obtain higher fish yield that exercises negative effect on their socio-economic conditions. This necessitated planning a research project to investigate the effects of sub-lethal heavy metals toxicity on the yield potential of major carps depending upon the planktonic productivity and physico-chemical parameters of pond water under semi-intensive polyculture system being practiced in Pakistan at large scale.

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MATERIALS AND METHODS

The research work was conducted at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. The induced bred two months old fingerling major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were kept for two weeks, under laboratory conditions in cemented tanks for acclimation. After acclimation, the stock of each fish species was divided in seven groups for metal stress experiment.

The wet weights, fork and total lengths of all groups of fish were measured and recorded prior to the start of experiment. One group of each fish species was kept un-stressed (control), while the other six groups of each species were exposed to sub-lethal levels (1/3rd of LC_{50}) of individual metals Fe, Zn, Pb, Ni and Mn as determined by Javed and Abdullah (2003) and mixture of all metals for each species (Table I) At constant temperature (25^{o}C), pH (8) and total hardness (250 mg L^{-1}) of water, metal stress of 90 days was given to each fish species with three replications.

Pure chloride of iron, zinc, lead, nickel and manganese were dissolved in distilled water to prepare stock solutions of 1000 mg L^{-1} for desired dilutions. Continuous air was supplied to all the test media with automatic air compressor through capillary tubing. The exposure media were continuously replenished and partly exchanged to maintain the sub-lethal concentration of metals for fish species, separately, throughout the experimental period of 90 days. The fish were dispensed with the crumbled feed (35% Digestible Protein (DP) and 2.90 kcal g^{-1} Digestible Energy (DE)) to satiation, at 10:00 h daily, throughout the stress period. The concentrations of metals in all test mediums were determined by the methods of A.P.H.A. (1998).

Growth studies of metal stressed fish in ponds. After 90 day metal stress, the three fish species were grouped metal and treatment-wise and stocked into earthen ponds with two replications for each treatment, while the ponds stocked with un-stressed fish were designated as control. Each pond (0.012 ha) was stocked with 66 individuals of *C. catla*, *L. rohita* and *C. mrigala* with the percentage of 30, 50 and 20, respectively. All the ponds were fertilized with poultry droppings on the basis of its nitrogen contents @ 0.16 g N 100^{-1} g of fish weight daily (Hassan, 1996). However, supplementary crumbled feed (35% DP & 2.90 kcal g^{-1} DE) was offered to the fish when water temperature exceeded 22^{o}C, daily (six days a week) at the rate of 2% of the fish biomass (Jobling, 1995). For fish growth/yield studies, test netting of fish was performed on fortnightly basis. Fish were sampled according to Javed (1988) and released back into their respective ponds.

Physico-chemical studies of pond water. Among physico-chemical parameters, temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO) of pond water were recorded on daily basis by using digital meters, while total ammonia, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, total hardness, total alkalinity, NO_3^- and PO_4^{3-} were determined on weekly basis following the methods of A.P.H.A. (1998). Planktonic dry weights mass in the ponds was also measured indirectly from the total solids and total dissolved solids of water by following the method described by Javed (1988).

Statistical analysis. The data obtained from this experiment was statistically analyzed using MICROSTAT and MINITAB packages of the computer by following Steel *et al.* (1996). Correlation and regression analyses were also performed to find-out relationships among various parameters under study.

RESULTS

Planktonic productivity and physico-chemical analysis of pond water. The dependence of planktonic productivity on physico-chemical variables of all the ponds were computed by regression method (Table II). In ponds stocked with Fe stressed fish the regression model explained 77.37% variations in planktonic productivity of these ponds due to total alkalinity, DO, temperature and total hardness of water. However, total hardness in the regression model gave negative but significant ($p<0.01$) partial regression coefficient.

In ponds stocked with Zn stressed fish, the regression model showed combined contribution of DO and K^+ was 85.18% towards planktonic productivity of ponds. The partial regression coefficients for both these variables were significant (Table II). In ponds stocked with Zn stressed fish, the regression equation revealed significant regression of DO and K^+ on planktonic productivity. This relationship explained over 80% variations in planktonic productivity. The high value of R^2 predicted high precision of this regression model. In ponds stocked with Ni stressed fish, 51.38% contribution of both K^+ and total hardness towards planktonic productivity was observed. The partial regression coefficients for both these variables were positive and significant (Table II).

In ponds stocked with Mn stressed fish the regression equation with the R^2 value of 0.8747, predicted the variations in planktonic productivity due to DO, temperature, total alkalinity, total ammonia and total hardness of water. However, the partial regression coefficients for all these variables were positive except ammonia and total hardness, while no regression was evident for total hardness. In ponds stocked with metal mixture stressed fish, the regression equation revealed 90.65% variations in planktonic productivity due to dissolved oxygen, EC, pH and Na^+ of water. However, this relationship revealed negative partial regression coefficient for Na^+, while the same for dissolved oxygen, EC and pH were positive. A high value of R^2 for this regression equation revealed high reliability of this model explaining the variations in planktonic productivity of these ponds.

In control ponds stocked wit un-stressed fish a

Table I. Sublthal doses of individual and mixture of heavy metals used

	Sub-lethal levels (mg L^{-1}) of metals					
Fish species	**Fe**	**Zn**	**Pb**	**Ni**	**Mn**	**Metal Mixture**
Catla catla	34.72	7.77	8.59	4.43	21.56	3.99
Labeo rohita	17.73	9.45	9.07	8.78	23.72	7.29
Cirrhina mrigala	28.99	15.77	13.26	9.21	25.41	8.04

combined contribution of NO_3^-, Na^+, EC and dissolved oxygen towards planktonic productivity was 94.53%. The partial regression coefficients for all these variables were significant except Na^+. High value of R^2 for this equation showed high precision of this regression model (Table II).

Relationship between fish yield and physico-chemical variables of pond water. The dependence of fish yield on physico-chemical variables was determined by using regression method to evaluate relationship between fish yield and physico-chemistry of ponds stocked with metal stressed and control fish. Table III shows regression models for all treatments. In ponds stocked with iron stressed fish ($T_{1)}$, the fish yield showed 96.21% dependence on K^+, total alkalinity, DO and EC of water. However, partial regression coefficients for all the variables, except total alkalinity, were positive. Total alkalinity, DO and EC had significant partial regression coefficients, while the same for potassium was non-significant. In T_2, K^+, DO and NO_3^- were the water quality variables that caused 84.43% variations in fish yield. Partial regression coefficients for all the three variables were significant. However, both dissolved oxygen and nitrates gave negative partial regression coefficients. The ponds designated as T_3 exhibited 96.68% variations in fish yield due to total alkalinity, pH, total hardness and total ammonia.

The partial regression coefficients for all these variables were positive except total alkalinity. However, all the partial regression coefficients were significant. High precision of this regression model is evident from the value of R^2 (0.9668). In T_4, K^+, Na^+ and temperature were the three variables that were responsible for 92.64% variations in fish yield. The partial regression coefficients for K^+ and temperature were positive, while for sodium remained negative ($p<0.01$). The regression model computed for T_5 reveals 89.95% contribution of three variables K^+, total alkalinity and pH of water toward increase in fish yield. The partial regression coefficients for K^+ and pH were positive, while that of total alkalinity was negative. However, pH exhibited non-significant partial regression coefficient in this regression model. In T_6, five variables viz. K^+, total alkalinity, EC, PO_4^- and NO_3^- contributed 93.89% variations in fish yield. The partial regression coefficients for all these variables were significant except NO_3^-. Both K^+ and EC had positive, while total alkalinity, PO_4^- and NO_3^- exhibited negative partial regression coefficients. The high value of R^2 (0.9389) predicted high reliability of this regression model. Fish yield in T_7 showed 82.78% dependence on pH and total alkalinity of water with statistically significant ($p<0.001$) correlation coefficients. However, the partial regression coefficient for pH was positive, while that of total alkalinity remained negative (Table III).

Relationship between planktonic productivity and fish yield of ponds. Table IV shows dependence of fish yield on planktonic productivity of ponds. In all the ponds, fish yield showed direct dependence on the existing planktonic productivity indices of ponds except T_2 and T_4. The regression coefficients of variables in the regression model computed for all the ponds were statistically non-significant except T_6.

DISCUSSION

In all the ponds, except T_4, DO showed significantly direct relationship with planktonic productivity of water. Mahboob and Sheri (2002) reported significant contribution of DO towards increase in planktonic biomass of ponds stocked with major carps (*C. catla*, *L. rohita* & *C. mrigala*) under semi-intensive culture conditions. Zooplankton productivity in fertilized (with broiler droppings) fish ponds showed negative regression on DO of water (Javed, 1996). Temperature appeared to be another water quality variable that exerted significant impact on planktonic productivity in both T_1 and T_5. Garg and Bhatnagar (1999) reported significant effects of different doses of organic fertilizer on planktonic productivity and fish yields in fish ponds, because temperature exerted significant impact on the release of nutrients to augment plankton population and species diversity. Water temperature is an important physico-chemical characteristic of fish ponds causing positive impact on planktonic productivity (Javed *et al*., 1996). This investigation revealed significant contribution of total alkalinity on the planktonic productivity also. The correlation coefficient between alkalinity of water and planktonic productivity was positive. In T_4, planktonic productivity was positively ($p<0.01$) dependent upon K^+ and total harsness of water. Mahboob *et al.* (1988) reported significant ($p<0.05$) correlation of planktonic productivity with K^+ and total hardness in fish ponds. Water quality in fish ponds is an outcome of interaction of various chemical factors viz. carbon dioxide, pH, alkalinity and hardness that are interrelated and caused profound effect on planktonic productivity of fish ponds (William & Robert, 1992). In T_5, ammonia concentration in water showed negative impact on planktonic biomass (Table II).

Table III shows the dependence of fish yield on physico-chemical variables of ponds stocked with metal stressed and control fish. Step-wise regression computed for each pond indicated K^+ as a sole water quality variable that exerted significantly positive impact on fish yield increments in all the ponds except T_7 (control fish ponds). However, Javed *et al*. (1996) reported K^+ as a water quality variable limiting the growth of fish either directly or indirectly through its response towards significantly low zooplankton productivity. In all the ponds, except T_2 and T_4,

Table II. Dependence of planktonic productivity (dry weight in mg L^{-1}) on physico-chemical variables of pond's water

Treatment	y = Regression Equation (y = a + bx)	r/MR	R^2
Ponds stocked with iron stressed fish (T_1)	Plk. Bio. = –9.288 – 0.129 (T. Alk.) + 24.328 (D.O.) + 4.361 (Temp.) – 0.449 (T.H.) $(0.054)^{p<0.05}$ $(4.601)^{p<0.001}$ $(1.093)^{p<0.001}$ $(0.118)^{p<0.01}$	0.8796	0.7737
Ponds stocked with zinc stressed fish (T_2)	Plk. Bio. = –523.408 + 74.751 (D.O.) + 7.559 (K) $(6.646)^{p<0.001}$ $(2.011)^{p<0.01}$	0.9230	0.8518
Ponds stocked with lead stressed fish (T_3)	Plk. Bio. = –346.099 + 55.532 (D.O.) + 6.439 (K) $(5.807)^{p<0.001}$ $(1.891)^{p<0.01}$	0.9052	0.8194
Ponds stocked with nickel stressed fish (T_4)	Plk. Bio. = –140.669 + 4.562 (K) + 0.544 (T.H.) $(1.426)^{p<0.01}$ $(0.220)^{p<0.05}$	0.7168	0.5138
Ponds stocked with manganese stressed fish (T_5)	Plk. Bio. = –786.480+ 65.743 (D.O.) + 16.789 (Temp.) + 0.468 (T.Alk.) – 75.399 (NH_3) – 0.472 (T.H.) $(6.722)^{p<0.001}$ $(2.768)^{p<0.001}$ $(0.126)^{p<0.01}$ $(23.005)^{p<0.01}$ $(0.269)^{N.S.}$	0.9353	0.8747
Ponds stocked with metal mixture stressed fish (T_6)	Plk. bio. = – 908.908 + 40.159 (D.O.) + 193.994 (E.C.) + 43.576 (pH) – 0.232 (Na) $(7.817)^{p<0.001}$ $(26.252)^{p<0.001}$ $(20.536)^{p<0.05}$ $(0.115)^{N.S.}$	0.9521	0.9065
Ponds stocked with control (un-stressed) fish (T_7)	Plk. bio. = – 550.754 + 81.138 (D.O.) + 75.304 (E.C.) – 0.255 (Na)+10.066 (NO_3) $(5.214)^{p<0.001}$ $(32.564)^{p<0.05}$ $(0.096)^{p<0.05}$ $(4.604)^{p<0.05}$	0.9723	0.9453

(r = Correlation coefficient; MR = Multiple r; R^2 = Coefficient of determination; Values within parentheses are the standard errors)
Plk. bio. = Planktonic biomass (mg L^{-1}); T. Alk = Total alkalinity (mg L^{-1}); D.O. = Dissolved oxygen (mg L^{-1}); Temp. = Temperature (°C); T.H. = Total hardness (mg L^{-1}); K = Potassium (mg L^{-1}); NH_3 = Total ammonia (mg L^{-1}); E.C. = Electrical conductivity (mS cm^{-1}); Na = Sodium (mg L^{-1}); NO_3 = Nitrates (mg L^{-1})

Table III. Dependence of fish yield (g) on physico-chemical variables of pond's water

Treatment	Regression Equation (y = a + bx)	r/MR	R^2
Ponds stocked with iron stressed fish (T_1)	Fish yield = –14372.475 + 302.567 (K) – 23.936 (T.Alk.) + 1565.518 (D.O.) + 4516.594 (E.C.) $(176.643)^{NS}$ $(3.027)^{p<0.001}$ $(309.436)^{p<0.001}$ $(1375.570)^{p<0.01}$	0.9808	0.9621
Ponds stocked with zinc stressed fish (T_2)	Fish yield = 38591.351 + 1413.090 (K) – 5042.766 (D.O.) – 4047.489 (NO_3) $(303.252)^{p<0.001}$ $(1028.673)^{p<0.001}$ $(1108.344)^{p<0.01}$	0.9188	0.8443
Ponds stocked with lead stressed fish (T_3)	Fish yield = –154190.678 – 64.332 (T.Alk.) +16784.678 (pH) + 127.868 (T.H.) + 3519.378 (NH_3) $(7.915)^{p<0.001}$ $(2015.055)^{p<0.001}$ $(32.504)^{p<0.001}$ $(1453.122)^{p<0.05}$	0.9833	0.9668
Ponds stocked with nickel stressed fish (T_4)	Fish yield = –11302.988 + 1654.707 (K) – 18.459 (Na) + 416.775 (Temp.) $(238.895)^{p<0.001}$ $(5.955)^{p<0.01}$ $(194.704)^{p<0.05}$	0.9625	0.9264
Ponds stocked with manganese stressed fish (T_5)	Fish yield = –41793.921 + 897.652 (K) – 21.619 (T.Alk.) + 5371.642 (pH) $(314.028)^{p<0.01}$ $(9.016)^{p<0.05}$ $(2781.650)^{NS}$	0.9484	0.8995
Ponds stocked with metal mixture stressed fish (T_6)	Fish yield = –12391.481 + 802.932 (K) – 23.488 (T.Alk.) + 6341.762 (E.C.) – 6295.568 (PO_4) – 945.502 (NO_3) $(160.871)^{p<0.001}$ $(5.179)^{p<0.001}$ $(1896.239)^{p<0.01}$ $(2015.847)^{p<0.01}$ $(478.641)^{NS}$	0.9690	0.9389
Ponds stocked with control (un-stressed) fish (T_7)	Fish yield = –161576.850 + 25647.697 (pH) – 127.987 (T.Alk.) $(4527.262)^{p<0.001}$ $(32.580)^{p<0.001}$	0.9098	0.8278

(r = Correlation coefficient; MR = Multiple r; R^2 = Coefficient of determination; Values within parentheses are the standard errors)
K = Potassium (mg L^{-1}); T. Alk = Total alkalinity (mg L^{-1}); D.O. = Dissolved oxygen (mg L^{-1}); E.C. = Electrical conductivity (mS cm^{-1}); NO_3 = Nitrates (mg L^{-1}); T.H. = Total hardness (mg L^{-1}); Na = Sodium (mg L^{-1}); Temp. = Temperature (°C).

total alkalinity was the variable that showed negative but relationship with fish yield increments in ponds (Table III). Other researchers have also reported significant effect of total alkalinity on the growth of *C. catla, L. rohita* and *C. mrigala* reared in ponds (Singh & Jee, 2000). Fish production showed positive correlation with alkalinity of water in fertilized hatchery ponds as reported by Tave and Anderson (1993). In T_2, DO showed negative impacts, while in T_1 the same remained positive towards fish yield increments. William and Robert (1992) suggested that most of the physico-chemical factors are not constant but affected the fish pond productivity, level of stress, fish health, oxygen availability, toxicity of ammonia and fish growth. Singh and Jee (2000) reported significant effect of DO on fish (major carps) growth in semi-intensive culture system, while Tave and Anderson (1993) reported negative correlation between fish production and DO concentrations of water.

Table IV. Relationship between planktonic productivity (dry weight) and fish yield of ponds

Treatment	Net weight increment (all species) (g)	Fortnightly average weight increment (all species) (y) (g)	Fortnightly average planktonic productivity (x) (mg L^{-1})	Regression equation (y = a + bx)	r
Ponds stocked with iron stressed fish (T_1)	14811.51	617.15 **f**	102.33 ± 38.12 **b**	**y** = 199.89 + 4.077 **(x)** $(2.679)^{NS}$	0.3090
Ponds stocked with zinc stressed fish (T_2)	31500.69	1312.53 **c**	194.38 ± 88.10 **a**	**y** = 1810.01 – 2.559 **(x)** $(3.022)^{NS}$	–0.1780
Ponds stocked with lead stressed fish (T_3)	38654.78	1610.62 **b**	182.81 ± 86.39 **a**	**y** = 791.49 + 4.481 **(x)** $(4.339)^{NS}$	0.2150
Ponds stocked with nickel stressed fish (T_4)	25241.89	1051.75 **d**	83.58 ± 38.61 **c**	**y** = 1053.40 – 0.020 **(x)** $(5.337)^{NS}$	–0.0010
Ponds stocked with manganese stressed fish (T_5)	24990.27	1041.26 **d**	178.28 ± 93.35 **b**	**y** = 511.71 + 2.970 **(x)** $(2.016)^{NS}$	0.3000
Ponds stocked with metal mixture stressed fish (T_6)	18786.82	782.78 **e**	214.26 ± 92.19 **a**	**y** = 95.65 + 3.207 **(x)** $(1.553)^{p<0.05}$	0.4030
Ponds stocked with control (un-stressed) fish (T_7)	52803.60	2200.15 **a**	217.35 ± 113.80 **a**	**y** = 1321.26 + 4.044 **(x)** $(3.960)^{NS}$	0.2130

r = Correlation coefficient; Values in parentheses are the standard errors; Means with similar letters in a single column are statistically similar at p<0.05

The contributions of pH towards fish yield increments in T_3, T_5 and T_7 were significant. Fehmida (1990) observed that in major carp culture ponds the pH of water fluctuated within alkaline range, which was close to optimal fish requirements. However, this alkalinity range exerted a positive influence on fish growth. Hussain and Kenar (1990), while working on growth and production of fish in ponds, reported that the ponds with higher pH range (6.5-9.5) produced higher fish yields than the control pond with low pH. However, negative correlation between fish yield and water pH was reported by Tave and Anderson (1993). Planktonic productivity, determined in terms of dry weight of all the ponds except T_2 and T_4 showed positive but non-significant correlation with fish yield increments (Table IV). The negative regression of fish yield on planktonic productivity of these ponds demonstrated that the fish have utilized the planktonic productivity comparatively better than the other ponds as evident from the higher net fish yields obtained from these ponds at the final harvest (Hassan & Javed, 1999).

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