



Evaluation of some Essential and Toxic Metals in farmed *Ctenopharyngodon idella* in relation to Body Size and Condition Factor

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Abstract: Basic aim of this research was to determine levels of some metals (Ca, Cd, Cr, Co, Cu, Fe, K, Mn, Ni, Na, Pb Zn) and their relationships to body size and condition factor in the whole body of *Ctenopharyngodon idella* (Grass carp). Concentrations of these elements were assessed in 55 fish samples of different size, randomly collected in January, 2015, from a private fish farm by ICP-OES after wet digestion method. Results of the study showed that analyzed elements were found within normal ranges. Ca, Cu, Fe, K, Mn, Na and Zn had highly significant relationships with fish size. Cd, Pb, Cu, K, Mn, Ni, Na, increased isometrically while Cr, Co, Zn, had negative allometric relationship and Fe and Ca showed positive allometric relationship with rise in weight of *C. idella*. Moreover, Ni, Mn, Pb and Cd represented isometric increase whereas Na, K, Zn, Fe and Ca had positive allometry; and Co, Cu, Cr had negative allometry with increase in total length of the fish. Most of inter-elemental relationships showed. Multiple regressions analyses showed positive correlation for Fe, Cu, Zn, Mn, Pb, Na, K, Ca and Ni had highly significant relations with total length and body weight.

Keywords: *Ctenopharyngodon idella*, Metal concentration, Regression analysis, Fish size

1. **INTRODUCTION**

Metals are considered as a major source of natural contaminations creating mutagenic, cytotoxic and cancer-causing impacts in animals (More *et al.*, 2003). Numerous metals are vital but all metals are lethal at higher range value, on the grounds that they pose oxidative stress by producing of free radicals. Metals might be harmful in that they can supplant crucial metals in pigments and enzyme, disturbing their roles. Consequently, metals demolish the biodiversity (Ghosh, 2005). Cu, Zn, Fe, Co, Cr are essential elements as they play pivotal functions in metabolism and as co-factor in biosynthesis reactions in living organism. Cd, As, Pb and Hg are non-essential elements and play no advantageous job. Assessment impact of metals is essential from three purposes of view toxicological, biological, human wellbeing (Svobodova *et al.*, 1996). Fishes are considered as a superior example for the examination of contamination burdens than water test in view of the critical level of metal they bioaccumulate (Atuma and Egborge, 1986). These metals become health hazard for fish, its predators, along with consumers like humans. Obviously, fishes are the connection for the exchange of dangerous devastating metals from water to people (Ashraf, *et al.*, 2010).

Metal pollution is an extraordinary concern because of steadiness for long time, bioaccumulation as well as biomagnifications in food chain (Sharma *et al.*, 2007; Rahman *et al.*, 2013), eventually postures lethality both in humans and aquatic fauna (Ahmed *et al.*, 2015).

There are five potential progressions for a heavy metal to enter in the fish body: (i) by means of nourishment, (ii) non-sustenance particles, (iii) gills, (iv) oral utilization of water, and (v) the skin (Nussey, 2000). Since it is realized that fish presents protein nourishment all around the globe, dangerous impacts of heavy metals in their body can bring about risky consequences for human wellbeing. The heavy metals are considered as basic poisonous contaminants of aquatic ecosystem, because of their high potential to enter and aggregate in food chain (Olojo *et al.*, 2005). It has been observed that metals contamination in aquatic environment is indicated through fish (Ahmad and Shuhaimi-Othman, 2010).

Fishes are helpful animals to study metal pollution due to their explore nature at the diverse trophic levels of aquatic environment (Dubey *et al.*, 2012). Concentration of various elements in the fish depend upon the size i.e. length and weight of fish. Comparatively smaller and juvenile fishes have greater concentrations of metals than big and older ones.

Keeping in view of general welfare, metals concentration should be checked over quality of fish and its products in order to secure human. Thus, the purpose of present work was to assess the concentration levels of Ca, Cd, Cr, Co, Cu, Fe, K, Mn, Ni, Na, Pb and Zn to find potential human risk of consumption of *Ctenopharyngodon idella* and their relationship with body size (length, weight) and condition factor.

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2. MATERIALS AND METHODS

Fifty-five specimens of *Ctenopharyngodon idella* were collected by using cast net from the ponds of a private fish farm Muzaffar Garh (30.1787° N, 71. 2800° E), Punjab, Pakistan, in January, 2019. Total length (TL) and body weight of each fish specimen was recorded.

Each sample was put into the pre-weighted aluminum foil plates, dried to a consistent weight in oven and powdered. 1g of powder sample was ashed in muffle furnace at 500°C for 12 hr. The obtained ash was processed further by wet digestion method, by heating at 82-100°C on hotplate to dryness; and diluted to make 25ml solution (Naeem et al., 2011). These solutions were examined to determine the concentrations of Chromium (Cr), Cadmium (Cd), Cobalt (Co), Sodium (Na), Calcium (Ca), Lead (Pb), Copper (Co), Potassium

(K), Manganese (Mn), Nickel (Ni), Zinc (Zn) and Iron (Fe) in the whole body of *C. idella* by ICAP-OES 6500 at Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad Pakistan.

Statistical Analysis

Regression analysis performed to find the relation of metal concentration with fish size and condition factor. The value of correlation coefficient was significant at $P < 0.001$, $P < 0.01$, and $P < 0.05$.

3. RESULTS

Fifty-five samples of *C. idella* (Grass carp) were used for the analysis of Cr, Cd, Co, Na, Ca, Pb, Co, K, Mn, Ni, Zn and Fe. Their mean (\pm SE) values of different metal concentrations found in *C. idella* are shown in (Table 1).

Table 1: Elemental composition of Grass carp (n = 55)

| Elements | Concentration | | Permissible Limit | Reference |
|----------|------------------------------|------------------------------|-------------------|--------------------|
| | $\mu\text{g/g}$ (dry weight) | $\mu\text{g/g}$ (wet weight) | | |
| | Mean \pm S.E. | Mean \pm S.E. | | |
| Ca | 48094.17 \pm 1101.00 | 9808.90 \pm 317.7 | 190– 8810 | WHO,1985 |
| Cd | 0.02 \pm 0.01 | 0.004 \pm 0.003 | 2 | WHO,1985 |
| Cr | 4.35 \pm 0.30 | 0.85 \pm 0.06 | 2,20 | WHO,1984,1985 |
| Co | 0.09 \pm 0.02 | 0.02 \pm 0.01 | 0.10 | WYSE et al. (2003) |
| Cu | 8.91 \pm 1.17 | 1.81 \pm 0.23 | 30 | WHO,1989 |
| Fe | 232.45 \pm 19.43 | 49.16 \pm 4.81 | 100 | FAO,WHO,1989 |
| K | 9465.82 \pm 215.63 | 1965.18 \pm 75.35 | Not reported yet | Not reported yet |
| Mn | 9.98 \pm 0.80 | 2.15 \pm 0.22 | 20 | Dural et al (2007) |
| Ni | 2.80 \pm 0.16 | 0.56 \pm 0.04 | 7.67 | FAO. 1984 |
| Na | 4427.08 \pm 119.61 | 904.04 \pm 904.04 | 300– 1340 | WHO,1985 |
| Pb | 1.18 \pm 0.13 | 0.25 \pm 0.03 | 2 | WHO,1985 |
| Zn | 108.25 \pm 3.01 | 22.29 \pm 0.94 | 100 | WHO,1989 |

S.E = Standard Error

Table.2: Regression analysis of elemental composition of Grass carp (n = 55) log wet body weight in gram against log body burden element

| Body weight in gram (wet) | Name of Element | Correlation coefficient | Intercept | Slope | Standard error | t value (b = 1) |
|---------------------------|-----------------|-------------------------|-----------|--------|----------------|------------------------|
| 87.00 to 722.00 | Ca | 0.919*** | 3.799 | 1.078 | 0.064 | -14.547*** |
| | Cd | 0.006 ns | 0.047 | 0.007 | 0.172 | -5.80695*** |
| | Cr | 0.298* | 1.266 | 0.386 | 0.171 | -5.46195*** |
| | Co | 0.284* | 1.784 | -0.649 | 0.304 | -3.93847*** |
| | Cu | 0.670*** | 0.196 | 0.989 | 0.152 | -5.58995*** |
| | Fe | 0.770*** | 0.840 | 1.334 | 0.153 | -5.20195*** |
| | K | 0.876*** | 2.993 | 1.122 | 0.086 | -10.5059*** |
| | Mn | 0.653*** | -0.021 | 1.112 | 0.179 | -4.47459*** |
| | Ni | 0.346* | -0.330 | 0.970 | 0.365 | -1.76973 ^{ns} |
| | Na | 0.896*** | 2.809 | 1.058 | 0.073 | -12.6406*** |
| | Pb | 0.200 ns | -0.312 | 0.728 | 0.494 | -1.29629 ^{ns} |
| Zn | 0.845*** | 1.545 | 0.907 | 0.079 | -11.7512*** | |

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; n.s. = > 0.05

Regression analyses of metals concentration with wet weight for *C. idella* are shown in (Table 2). Concentration of Fe, Mn, Zn, Na, Ca, Cu and K were observed to be highly significant ($P < 0.001$) with wet weight, while Cr, Co, Ni showed significant relationship ($P < 0.01$) with wet weight of body. Na, Ni, Mn, K, Cu and Ca showed isometric increase while Cd, Cr, Co, Pb and Zn showed negative allometry, while Fe showed positive allometry with body weight of *C. idella*. Pb and Cd showed insignificant correlation ($P > 0.05$) with wet body weight.

Regression between metals (Zn, Mn, Fe, Ca, Cu, K and Na) and TL were strongly significant ($P < 0.001$). Cr and Ni represented significant ($P < 0.05$) relationship while Pb, Cd and Co showed non-significant ($P > 0.05$) relation with TL. Cu represented

isometry ($b = 3$); Mn, K, Fe, Ca and Na had positive allometric ($b > 3$) while Zn, Ni, Cr, Pb, Cd and Co showed negative allometric relationship with total length (Table 3).

Non-significant relationship was observed between elements (Cd, Cr, Co, Cu, Fe, Pb, Na, Ni, Mn and K) and condition factor while Co, Ca and Zn showed significant relation ($P < 0.05$) with condition factor (Table 4).

In multiple regression results showed that Fe, Cu, Zn, Mn, Pb, Na, K, Ca and Ni highly significant relationship ($P < 0.001$) however Co had significant relation ($p < 0.01$). Although Cr and Cd showed non-significant relationship ($P > 0.05$) (Table 5-8).

Table 3: Regression analysis of elemental composition of Grass carp (n=55) of Log of total length (cm) against log of body burden elements (µg/g)

| Total length in centimeter | Name of Element | Correlation coefficient | Intercept | Slope | Standard error | t value (b = 3) |
|----------------------------|-----------------|-------------------------|-----------|--------|----------------|------------------------|
| 19.60 To 39.70 | Ca | 0.927*** | 1.679 | 3.268 | 0.184 | -13.0363*** |
| | Cd | 0.011 ns | 0.004 | 0.042 | 0.516 | -5.77195*** |
| | Cr | 0.267* | 0.693 | 1.038 | 0.520 | -4.73123*** |
| | Co | 0.240 ns | 2.608 | -1.648 | 0.926 | -4.88774*** |
| | Cu | 0.676*** | -1.748 | 2.997 | 0.454 | -3.61093*** |
| | Fe | 0.762*** | -1.674 | 3.966 | 0.468 | -2.44426* |
| | K | 0.880*** | 0.806 | 3.387 | 0.254 | -8.42402*** |
| | Mn | 0.649*** | -2.143 | 3.325 | 0.540 | -2.23056* |
| | Ni | 0.294* | -1.582 | 2.474 | 1.116 | -0.21417 ^{ns} |
| | Na | 0.895*** | 0.771 | 3.176 | 0.220 | -10.4604*** |
| | Pb | 0.208 ns | -1.843 | 2.277 | 1.483 | 0.254073 ^{ns} |
| Zn | 0.860*** | -0.271 | 2.771 | 0.228 | -10.3869*** | |

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; n.s. = > 0.05

Table 4: Regression analysis of elemental composition of Grass carp (n = 55) of condition factor against concentrations of elements

| Cond. Factor (K) | Name of Element | Correlation coefficient | Intercept | Slope | Standard error | t value (b = 0) |
|--------------------|-----------------|-------------------------|-----------|-----------|----------------|------------------------|
| 1.05 to 1.41 | Ca | 0.279* | 18694.751 | -7420.963 | 3541.041 | -2.0957* |
| | Cd | 0.013 ns | 0.007 | -0.003 | 0.029 | -0.10345 ^{ns} |
| | Cr | 0.034 ns | 0.646 | 0.169 | 0.684 | 0.247076 ^{ns} |
| | Co | 0.310* | 0.202 | -0.150 | 0.064 | -2.34375* |
| | Cu | 0.086 ns | 3.817 | -1.679 | 2.693 | -0.62347 ^{ns} |
| | Fe | 0.022 ns | 38.637 | 8.790 | 55.835 | 0.157428 ^{ns} |
| | K | 0.166 ns | 3217.228 | -1045.636 | 862.281 | -1.21264 ^{ns} |
| | Mn | 0.074 ns | 3.743 | -1.329 | 2.493 | -0.53309 ^{ns} |
| | Ni | 0.241 ns | -0.315 | 0.733 | 0.409 | 1.792176 ^{ns} |
| | Na | 0.113 ns | 1237.022 | -278.087 | 339.045 | -0.82021 ^{ns} |
| | Pb | 0.101 ns | 0.553 | -0.250 | 0.342 | -0.73099 ^{ns} |
| Zn | 0.294* | 49.944 | -23.092 | 10.398 | -2.22081* | |

*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; n.s. = > 0.05

Table 5: Interelemental regression analysis of Ca µg/g against various metals in µg/g in body weight

| Name of Element | Correlation coefficient | Intercept | Slope | Standard error |
|-----------------|-------------------------|-----------|-------|----------------|
| Cd | 0.155 ns | 0.016 | 0.000 | 0.000 |
| Cr | 0.371** | 1.524 | 0.000 | 0.000 |
| Co | 0.493*** | -0.065 | 8.959 | 2.194 |
| Cu | 0.077 ns | 1.255 | 0.000 | 0.000 |
| Fe | 0.525*** | -28.786 | 0.008 | 0.002 |
| K | 0.808*** | 85.301 | 0.192 | 0.019 |
| Mn | 0.722*** | -2.647 | 0.000 | 0.000 |
| Ni | 0.253 ns | 0.846 | 0.000 | 0.000 |
| Na | 0.711*** | 258.719 | 0.066 | 0.009 |
| Pb | 0.516*** | -0.219 | 0.000 | 0.000 |
| Zn | 0.764*** | 0.182 | 0.002 | 0.000 |

*** = P<0.001; ** = P < 0.01; * = P < 0.05; n.s. = > 0.05

Table.6: Interelemental regression analysis of Grass carp (n = 55) of Zn in µg/g against dissimilar metals in µg/g in wet body weight .

| Name of Element | Correlation coefficient | Intercept | Slope | Standard error |
|-----------------|-------------------------|-----------|---------|----------------|
| Ca | 0.764 ns | 4036.077 | 258.940 | 30.324 |
| Cd | 0.019 ns | 0.005 | -0.000 | 0.000 |
| Cr | 0.038 ns | 0.903 | -0.002 | 0.009 |
| Co | 0.776*** | -0.084 | 0.005 | 0.000 |
| Cu | 0.158 ns | 0.933 | 0.039 | 0.034 |
| Fe | 0.483*** | -6.126 | 2.480 | 0.623 |
| K | 0.766*** | 592.877 | 61.555 | 7.165 |
| Mn | 0.767*** | -1.779 | 0.176 | 0.020 |
| Ni | 0.024 ns | 0.583 | -0.001 | 0.005 |
| Na | 0.604*** | 481.981 | 18.931 | 3.467 |
| Pb | 0.532*** | -0.122 | 0.017 | 0.004 |

*** = P<0.001; ** = P < 0.01; * = P < 0.05; n.s. = > 0.05

Table.7: Multiple Regression Relationships in Grass carp for body weight, condition factor and body Burden Element.

| Multiple regression Relationships | Multiple correlation coefficient | Intercept | b ₁ ± S. E | b ₂ ± S. E | r ² | V I F |
|--|----------------------------------|-----------|-----------------------|-----------------------|----------------|-------|
| Fe = a + b ₁ W + b ₂ K | 0.816*** | -15241 | 100.06±9.93 | 4306±17426 | 0.666 | 0.334 |
| Cu = a + b ₁ W + b ₂ K | 0.580*** | 615.6 | 1.2953±0.25 | -434.5±452.5 | 0.336 | 0.664 |
| Zn = a + b ₁ W + b ₂ K | 0.937*** | 5388 | 19.00±0.99 | -4015±1747 | 0.878 | 0.122 |
| Cr= a + b ₁ W + b ₂ K | 0.184 ns | 35.5 | 0.082±0.07 | 95.0±131.2 | 0.034 | 0.966 |
| Co = a + b ₁ W + b ₂ K | 0.363** | 33.24 | -0.010±0.006 | -22.70±10.60 | 0.132 | 0.868 |
| Cd = a + b ₁ W + b ₂ K | 0.045 ns | -1.782 | 0.0001± 0.004 | 2.227±7.362 | 0.002 | 0.998 |
| Mn = a + b ₁ W + b ₂ K | 0.784*** | -88.4 | 2.778±0.30 | -37.9±541.2 | 0.614 | 0.386 |
| Pb= a + b ₁ W + b ₂ K | 0.672*** | 18.4 | 0.33±0.05 | -29.08 ± 89.34 | 0.452 | 0.548 |
| Na = a + b ₁ W + b ₂ K | 0.960*** | 86480 | 985.54±40.12 | -85339±70361 | 0.922 | 0.078 |
| K = a + b ₁ W + b ₂ K | 0.949*** | 228073 | 2321.8±108.1 | -246871±189612 | 0.901 | 0.099 |
| Ca = a + b ₁ W + b ₂ K | 0.969*** | 1887887 | 11891.2±428.2 | -1924628±751043 | 0.938 | 0.062 |
| Ni = a + b ₁ W + b ₂ K | 0.797*** | -233.6 | 0.62236±0.06719 | 185.0±117.8 | 0.636 | 0.364 |

b₁ ± S.E and b₂ ± S.E = regression coefficient; r² = proportion of variance due to regression; VIF = Variance inflation factor, *** P < 0.001; N.S. > 0.05

Table 8: Multiple regression analysis of Grass carp for body weight (wet), total length & body burden element.

| Relationships | Multiple correlation coefficient | Intercept | $b_1 \pm S.E$ | $b_2 \pm S.E$ | R^2 | VIF |
|---------------------------|----------------------------------|-----------|----------------|---------------|-------|-------|
| $Fe = a + b_1 W + b_2 TL$ | 0.846*** | 74900 | 236.81±46.35 | -4489±1492 | 0.716 | 0.284 |
| $Cu = a + b_1 W + b_2 TL$ | 0.580*** | -834.6 | -0.210±1.299 | 49.20±41.83 | 0.336 | 0.664 |
| $Zn = a + b_1 W + b_2 TL$ | 0.937*** | -1176 | 16.078±5.278 | 93.6±169.9 | 0.878 | 0.122 |
| $Cr = a + b_1 W + b_2 TL$ | 0.184 ^{ns} | -494.0 | -0.9500±0.3506 | 33.95±11.29 | 0.034 | 0.966 |
| $Co = a + b_1 W + b_2 TL$ | 0.363** | -17.00 | -0.047±0.03 | 1.223±1.01 | 0.132 | 0.868 |
| $Cd = a + b_1 W + b_2 TL$ | 0.045 ^{ns} | -9.90 | -0.017±0.02 | 0.56±0.67 | 0.002 | 0.998 |
| $Mn = a + b_1 W + b_2 TL$ | 0.784*** | 2047.3 | 6.28±1.47 | -115.16±47.60 | 0.614 | 0.386 |
| $Pb = a + b_1 W + b_2 TL$ | 0.672*** | 246.1 | 0.75±0.25 | -13.85±8.07 | 0.452 | 0.548 |
| $Na = a + b_1 W + b_2 TL$ | 0.960*** | -80930 | 878.6±205.3 | 3463±6609 | 0.922 | 0.078 |
| $K = a + b_1 W + b_2 TL$ | 0.949*** | -258477 | 2008.8±554.1 | 10137±17840 | 0.901 | 0.099 |
| $Ca = a + b_1 W + b_2 TL$ | 0.969*** | 161287 | 12774±2298 | -30096±73972 | 0.938 | 0.062 |
| $Ni = a + b_1 W + b_2 TL$ | 0.797*** | 463.4 | 1.3914±0.33 | -25.15±10.63 | 0.636 | 0.364 |

$b_1 \pm S.E$ and $b_2 \pm S.E$ = regression coefficient; r^2 = proportion of variance due to regression; VIF = Variance inflation factor, *** $P < 0.001$; N.S. > 0.05

4. DISCUSSION

Accumulation order of the studied elements in the body of farmed *Ctenopharyngodonidella* was as $Ca > K > Na > Fe > Zn > Mn > Cu > Cr > Ni > Pb > Co > Cd$ (Table 1). The same trend was reported by Naeem *et al.* (2010) in farmed *Oncorhynchus mykiss* except Mn. The lowest heavy metal concentration (wet weight) value was observed for Cd ($0.004 \pm 0.003 \mu\text{g g}^{-1}$) while Ca showed highest value of concentration ($9808.90 \pm 317.7 \mu\text{g g}^{-1}$) in the fish. On the other hand, maximum concentration of Ca has also been reported in *Aristichthys nobilis* (Naeem *et al.*, 2011) and *Oncorhynchus mykiss* (Naeem *et al.*, 2010). The present data on Ca are also compatible with already reported data by Salam *et al.* (1993, 1996, 1998, and 2002) and Ansari *et al.* (2000). Zhang *et al.* (2017) have also reported Cd, Cr, Cu, Pb and Zn concentration, 0.0032, 0.5369, 0.4502, 0.0111 and 17.29 mg/kg, respectively in the muscles of *C. idella*.

The permissible limit for Cd ($\mu\text{g/g}$) as recommended by FAO (2011), FAO/WHO (1989) and Burger (2010) is 0.05–2 $\mu\text{g/g}$. Thus the values encountered in the present study were within the normal range. This implies that this heavy metal (Cd) may not be taken up above the higher limit in aquatic environments and thus may be non-toxic for consumers to utilize from environment. Also, the obtained concentration of Cd is in according to findings of previous work of Ashraf *et al.* (2010), Tole and Shitsama (2003) and Chatta *et al.* (2016).

The concentration of Cobalt was found to be 0.09 ± 0.02 and $0.02 \pm 0.01 \mu\text{g/g}$ in dry and wet weight,

respectively, which concentrations are within the permissible limit ($0.10 \mu\text{g/g}$) (WYSE *et al.* 2003). Co was found in very small quantities (0.02 ± 0.01 , wet weight) in the present study and found in agreement with those reported previously in *Cyprinus carpio* (Andreji *et al.*, 2006). Moreover, cobalt concentration ($\mu\text{g/g}$) in different fish species have been reported 3.52 ± 0.024 and $0.67 \pm 0.04 \mu\text{g/g}$ in *Aristichthys nobilis* (Naeem *et al.*, 2011), 1.048 ± 0.026 and $0.241 \pm 0.015 \mu\text{g/g}$ in *Wallago attu*, in dry and wet weight of fish species (Yousaf *et al.*, 2012). These reported concentrations of cobalt were much higher than the results of the present study and this might have been due to the presence of pollutants in the investigated aquatic environments as well as due to variation in fish species and their habitats.

The mean accumulations of Pb observed in grass carp were $1.88 \pm 0.13 \mu\text{g/g}$ (dry weight) and $0.25 \pm 0.33 \mu\text{g/g}$ (wet weight). These concentration values are below the maximum permissible limit. Yousaf *et al.* (2012) and Naeem *et al.* (2010, 2011) had reported higher levels of Pb in *Wallago attu*, *Onchorynchus mykiss* and *Aristichthys nobilis*. However, Chatta *et al.* (2016) had reported similar concentration of Pb in *Labeo rohita*, *Cirrhinus mrigala*, and *C. idella* (wet body weight) as recorded in the present study.

The mean concentration of Ni analyzed in grass carp was $2.80 \pm 0.16 \mu\text{g/g}$ (dry weight) and this result is similar to the report of Emeka (2014). However, the observed concentration of Ni in grass carp wet weight 0.56 ± 0.04 in the present study was higher than the

reported values of 2.96 µg/g in *Eutropius niloticus* and 1.35 µg/g in *Citharinus citharus* by Ere *et al.* (2014).

The mean accumulation values of Cr in grass carp were 4.35±0.30 µg/g (dry weight) and 0.85±0.06 µg/g (wet weight); these values are within the permissible range according to the USFDA (1993). The mean wet weight value of Cr (0.85±0.06 µg/g) in the present study is closely matched with that reported by Shearer (1984). However, Naeem *et al.* (2010), Shabanda and Itodo (2012) and Emeka (2014) had reported higher levels of Cr than observed in the present study and this might have been due to difference in fish species, habitats and the prevailing pollution levels in the studied aquatic habitats.

The mean concentration of Cu in grass carp was 8.91±1.17µg/g (dry weight) and 1.81±0.23 (wet weight). These observed values are also under the maximum permissible limit by FAO/WHO (1984, 1989), and are similar to those already reported by Shearer (1984), Yousaf *et al.* (2012) and Naeem *et al.* (2012).

The mean concentrations of Mn in grass carp was found 9.98±0.80 (dry weight) and 2.15±0.22 µg/g (wet weight). These Mn concentrations recorded in the present study are within the permissible range as recommended by FAO/WHO (1989) and Dural *et al.* (2007). The present study results concerning Mn are compatible to the findings of Naeem *et al.* (2011) and Ansari *et al.* (2006) in various fish species. However, Naeem *et al.* (2010) and Naeem *et al.* (2012) had reported higher concentration of Mn in different fish species, compared to the concentrations recorded in the present study. These high values might be due to difference in the investigated fish species as well as their feeding habits and behaviour (Olatunde, 1978).

The concentration of Zn in the present study was found to be within the normal range as described by FAO/WHO (1989). The analyzed mean concentrations (µg/g) of Zn in grass carp were 108.25±3.01 (wet weight) and 22.29±0.94 (dry weight). The observed values are in agreement with those of Naeem *et al.* (2011) in *Oreochromis niloticus*, Naeem *et al.* (2011) in *Aristichthys nobilis*, Naeem *et al.* (2012) in *Mystus bleekeri*, Salam *et al.* (1998) in *Catla catla*, Salam *et al.* (1993) in *Tor putitora*, Shearer, (1984) in *Oreochromis mykiss*, and Yousaf *et al.* (2012) in *Wallago attu*. While, Ansari *et al.* (2006) reported rather high level of Zn in *Puntius cola* which may have been due to pollution of its habitat.

The mean concentrations of Fe were 232.45 and 49.16±4.18 µg/g in the dry and wet body weight of *C. idella*. Already reported concentrations of Fe in dry and wet weight in *Wallago attu* and *Aristichthys nobilis* were

94.017±42.688 and 21.672±7.133; 311.23±30.04 and 61.64±6.21 by Yousaf *et al.* (2012) and Naeem *et al.* (2010). Concentration of Fe, 538±238 and 133.6±75.2 in dry and wet weight, respectively, in *Puntius cola* was reported by Ansari (2006). Maximum permissible limit for Fe according to WHO/FAO (1989) is 100 µg/g which indicates that the Fe values in the present study were higher than the permissible value by WHO/FAO.

The mean value of Na in grass carp was 4427.08±119.61 and 904.04 ± 904.04 µg/g in dry and wet weight, respectively. The permissible range of Na by FAO/WHO (2001) is 300-1340 µg/g showing that concentration of Na in the present study was within the normal range. Previously, the mean concentration of Na reported in *Puntius sophore* and *Tara baim* were found 3335 and 3746 µg/g (Rashid *et al.*, 2012).

The mean concentration µg/g of K was 9465.82± 215.63 and 1965.18±75.3 µg/g in the present study. Naeem *et al.* (2010) analyzed K and reported its concentration to be 6877.20±282.145 µg/g (dry weight) and 147.07 ±38.893 µg/g (wet weight) in *Onchorynchus mykiss*. In *Aristichthys nobilis* the reported mean concentration (µg/g) of K was 8503.91±319.88 µg/g (dry weight) (Naeem *et al.*, 2011); whereas Mashaii *et al.* (2011) reported 12272.75 µg/g to 13715.25 µg/g K (wet weight) in *Oncorhynchus mykiss*. In *Catla catla*, concentration of K dry and wet weight was reported 9636±913.5 µg/g and 2046±470 µg/g in dry and wet weight respectively (Salam *et al.*, 1998).

Results of the regression analyses shows that only Cd and Pb showed non-significant correlation with wet body weight increase representing no effect of wet weight of the fish on these metal concentrations. Na, Ni, Mn, K, Cu, Ca showed isometric (b=1 or not significantly different from 1.0) increase indicating an increase of the metal concentrations in direct proportion to increase in body weight of the fish. While Cd, Cr, Co, Pb and Zn indicated negative allometric (b < 1) trend with an increase in wet weight of *C. idella*, which suggests that these metals probably accumulated at a lesser rate compared to its rate of excretion as the fish increases in weight. However, Fe showed represented proportional increase in metal concentration with growing fish weight. A previous study conducted by Naeem *et al.* (2011) on *Aristichthys nobilis* had described isometric pattern in many of their reported metals while Mn, Fe and Co as positive allometric pattern with the increase in body weight of the fish. Similarly, according to Salam *et al.* (2002) Cu, Fe, Mn, Na, K and Zn increases isometrically with an increase in body weight of *Cirrhinus mrigala*. While, Naeem *et al.* (2012) had studied metal concentrations in a wild catfish (*Mystus bleekeri*) and reported positive allometry

in Mn and Zn with an increase in the fish size. The variation may be due to difference in foraging technique, size of fish and metabolism rate.

In the present study, Cu showed isometric ($b=3$) pattern with an increase in total length of *C. idella*, which is in general agreement with the results of Naeem *et al.* (2010), who has also reported that Cu increases in the same proportion as the length of the *O. mykiss*. Mn, K, Fe, Ca and Na showed positive allometric pattern ($b > 3$) with an increase in total length of *C. idella*. On the contrary, Naeem *et al.* (2010) reported isometry in Mn and Fe, while negative allometry in K, Ca and Na in *O. mykiss*. Ansari *et al.* (2006) documented positive allometry in manganese for *Puntius chola* and Yousof *et al.* (2012) had also documented positive allometry relation in Fe concentration of wild *Wallago attu* with an increase in total length, as found in the present study in *C. idella*. Whereas, Zn, Ni, Cr, Pb, Cd and Co showed negative allometric relation ($b < 3$) with an increase in total length of *C. idella* in the present work, indicating a significant proportional decrease in the concentration of Zn, Ni, Cr, Pb, Cd and Co with an increase in total length of total length. It also suggests that these metals may accumulate at lesser rate as compared to its rate of excretion as the fish grows. Moreover, non-significant correlation of Cd with length was also previously reported by Naeem *et al.* (2012), as observed in the present study which indicates that the concentration of Cd do not change considerably in large and small sized fish.

In the present study, condition factor remained constant with the most of the studied metal concentrations. Similar results in different fish species are also documented by Farkas *et al.* (2002) and Naeem *et al.* (2011, 2012). These results shows that condition factor does not influence the metal concentrations in fish body.

Results for multiple regression analyses shows that Fe, Cu, Zn, Mn, Pb, Na, K, Ca, Ni found significantly ($P < 0.001$) correlated with wet body weight along with total length; body weight along with condition factor as also reported by Naeem *et al.* (2010, 2011). All VIF values were less than 10 as reported .

5.

CONCLUSIONS

It is concluded that different metal concentrations in farmed *C. idella* are in permissible limit as described by different organizations. Hence the fish is safe for human consumption in term of heavy metals, and might be a good source of essential metals. Fish size has a definite influence on the metal accumulation in the fish, however condition factor does not effects the metal concentration in fish body.

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