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Environmental Monitoring and Health Risk Assessment of African Catfish *Clarias gariepinus* (Burchell, 1822) Cultured in Rural Ponds, India

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Abstract Water quality monitoring of *Clarias gariepinus* culture ponds (n = 27) revealed poor physico-chemical conditions and metal contaminants in fish tissues (n = 324). Human health risk assessment for some heavy metal contamination delineated low risk in general except for Aluminium (Al), Iron (Fe) and Lead (Pb) which accumulated significantly ($p < 0.05$) high in tissues. Health risks values were 6.3×10^{-3}–9.6×10^{-3} for Al; 3×10^{-3}–9.7×10^{-3} for Fe and 1.15×10^{-5}–9.3×10^{-6} for Pb respectively suggesting that contamination of Pb particularly in ponds fed with chicken waste (CW) was posing high risks.

Keywords African catfish · Metal contaminants · Animal waste feeding · Health risks

Culture of introduced African catfish *Clarias gariepinus* in rural ponds, tanks, cement cisterns and even derelict waters using animal wastes as feed is very common in India. The fish tolerates harsh environmental conditions, and grows fast even through feeding of animal wastes (Singh and Lakra 2011). Heavy metals are most frequently occurring toxic contaminants (Fu-quan et al. 2010) and several elemental accumulations have been reported in fish due to waste recycling and/or poor aquatic environment (Malik et al. 2010). Metals that accumulate in the tissue of aquatic organisms pose a threat to the survivorship of individual species as well as the ecosystem and energy flow of the food web (Vander Oost et al. 2003). Therefore, biological effect monitoring (BEM) is needed to evaluate environmental changes in water quality and food safety (Vander Oost et al. 2003), for regular use of living aquatic organisms, particularly fish as food. Fishes are notorious for their ability to concentrate heavy metals and since they play important role in human nutrition, they need to be carefully screened to ensure that toxic metals are not being transferred to man through fish consumption (Adeniyi et al. 2008; Lakshmanan et al. 2009; Malik et al. 2010). Since, farmers have been cultivating *C. gariepinus* in ponds and tanks in a very unhygienic conditions and by feeding chicken and slaughter house wastes under poor husbandry. This study was undertaken to monitor the water quality of culture ponds and to examine possible presence of metals in culture pond as well as fish tissues. The presence of some heavy metals and their accumulation may be injurious to human health, if such fish is consumed (Kumar et al. 2011). In this study, we have assessed the health risks of such heavy metals that may pose health risks. This study also aimed to develop a model to quantify health risk in relation to some heavy metal contaminant in fish for biosafety purpose.

Materials and Methods

This study was conducted at Amrahi village of Lucknow district in Utter Pradesh (N 26°51′ and E 80°51.12′) in a cluster of over 100 ponds spread in approximately 30 km^2 area having approximately 5 km^2 culture water area. We analyzed metal contaminants in randomly selected ponds' water (n = 27) and fish tissues (n = 324) from the culture ponds using commercial pellet feed, slaughter house waste or chicken waste as feed. Water samples collected from

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ponds with different feed types were subjected to analysis of water temperature, pH, free CO_2, dissolved oxygen (DO), hardness, ammonia, chlorides and alkalinity either in situ or in the laboratory following the standard methods of APHA (2005). Water quality rating for physico-chemical parameters was done by calculating the water quality index (WQI) which was divided into four stages viz. permissible, slight, moderate and sever and was rated on a scale of 0–100. Selected parameters were calculated with the help of a software (BIOPATRA) water quality calculator (www.textbookx.com).

For estimation of metals, water samples were collected in sterilised polyethylene bottles, brought to the laboratory and kept in refrigerator until analysis (CPCB 2008). Similarly, tissues (muscles, gills, gonad and liver) of *C. gariepinus* were collected and washed with double-distilled water and put in Petri dishes to dry at 120°C until reaching a constant weight. One gram of dried tissue (in three replicates) was then digested with di-acid (HNO3 and $HClO_4$ in 2:1 ratio on a hot plate set at 80°C (gradually increased) until all materials were dissolved. Stored water samples and digested tissue samples were subjected to heavy metals estimations using a UNICAM-flame atomic absorption spectrophotometer (AAS, Agilent) using the methods of APHA (2005). Analysis of sample was done according to standard, reagent blank and sample replicate randomly inserted in the analysis process to assess contamination and precision. The transportation error contamination was 0.0207–0.0857. Recovery studies of metals determination were conducted to demonstrate the efficiency of the method. The recovery rates ranged from 83.3 % to 92.2 %.

Human health risk assessment (USEPA) was carried out in three stages: (a) hazard identification, (b) exposure assessment, and (c) risk characterization (Li et al. 2011). The hazard identification was done by monitoring of heavy metals in ponds water as well as fish organs/muscle as described above. For quantification of exposure, a multiple pathway exposure model (SEDISOIL) was used (Harma et al. 1999) and calculated with the following equation:

$$\text{Ingestion of fish (mg/kg/day)} = \frac{\text{CF} \times \text{IRF} \times \text{FI} \times \text{AF}}{\text{BW}}$$

where CF = concentration of the metal contaminant in fish [mg/kg flesh weight (fw)], IRf = ingestion rate of fish (fish weight (kg)/day), and FI = fraction contaminated (unitless), AF = absorption factor (unitless), and BW = body weight (Kg).

The hazard quotient (HQ), was calculated following the method of USEPA (2001) using the formula.

$$\text{HQ} = \text{CDI}/\text{RfD}$$

where HQ is hazard quotient (unitless); CDI is the cumulative daily intake and RfD is reference dose (mg/(kg/day).

The health risk was determined by using USEPA (2001).

$$\text{Risk} = \text{CDI} \times \text{slope factor}\,[\text{mg}/(\text{kg}/\text{day})].$$

The above was calculated with the help of risk calculator (www.ajdesigner.com).

All values from chemical analyses were presented as mean ± SD. Data obtained from the experiment were subjected to one way analysis of variance (ANOVA) test using the Statistical Package for the Social Sciences (SPSS). The correlation coefficients between the quality parameter pairs of the water samples were calculated by the application of Pearson correlation analysis. Parameters were analysed statistically (at 5 %) and significance was calculated by student's 't' test with the use of computer programmed statistical tool SPSS, version 8.1.

Results and Discussion

The water analysis of African catfish culture ponds fed with different feed types showed varied values for pH, dissolved oxygen, ammonia, total dissolved solid, total hardness, free CO_2, alkalinity, chlorides and conductivity. Which were significantly different in ponds using different feed types and the water quality was miserably poor much below the optimum or desirable levels (Table 1) (Alaa and Kloas 2010). The poor environmental conditions in all the culture ponds confirmed that the *C. gariepinus* is a hardy fish and tolerated harsh environments (Singh and Lakra 2011). Among metals detected in ponds water were lead (Pb), cadmium (Cd), aluminium(Al), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), zinc (Zn), molybdenum (Mo), nickel (Ni) and cobalt (Co). However, the levels of most of these detected heavy metals were much below the permissible levels (WHO 2011) except for Al and Fe (Table 1). The health importance of the present metals in African catfish culture ponds were divided into three major groups: (1) Cu, Zn, Co, Cr, Mn and Fe were which classified as essential metals (2) Al, was non-essential metal and (3) cadmium (Cd) and lead (Pb) were classified highly toxic metals (Malik et al. 2010). Further examination of metals in the tissues (gonad, liver, gills and muscles) of *C. gariepinus* cultured with different feed types revealed the presence of Al, Cd, Co, Cu, Cr, Fe, Mn, Mo, Ni, Pb and Zn (Fig. 1). However, Al level was found high in most of the tissues but highest (5.230 ± 0.547 mg/kg) in gonads of the fish followed by muscle, particularly in chicken waste fed fishes (CW) where Al level was 1.034 ± 0.058 mg/kg. Co was detected significantly ($p < 0.05$) high in liver of *C. gariepinus* collected from ponds fed with slaughter house waste (SH). Cr was found significantly ($p < 0.001$) high in gonads of the fish collected from all the three feed types. Cu was detected high

Table 1 Physico-chemical parameters of the African catfish culture ponds fed with different feed types (values are mean ± SD)

Parameters	CoF	SH	CW	WHO Limits^{b}
pH	8.98 ± 0.043	8.42 ± 0.12	8.71 ± 0.15	7–8.5
Temperature (°C)	29.5 ± 0.64	29.7 ± 0.32	28.5 ± 0.43	0–35°C
Conductivity (μs/cm)	473.3 ± 60.41	789.3 ± 61.66	684 ± 60.71	–
TDS (mg/L)	157.6 ± 7.21	94.6 ± 12.0**	138.3 ± 14.83*	500
Total hardness (mg/L)	345.3 ± 14.49	336.7 ± 6.32	346.7 ± 13.03	200
DO (mg/L)	5.0 ± 0.12	5.1 ± 0.39	4.9 ± 0.14	3.00
Free CO_2 (mg/L)	7.33 ± 0.048	7.11 ± 0.18	6.9 ± 0.1	5-10
Ammonia (mg/L)	0.18 ± 0.049	0.56 ± 0.17*	2.67 ± 0.074**	0.50
Alkalinity (mg/L)	62 ± 2.00	58.3 ± 7.91	61.6 ± 2.23	200
Chlorides (mg/L)	73.74 ± 1.19	71.40 ± 2.10	73.09 ± 2.31	251
Lead^{a} (mg/L)	0.007 ± 0.002	0.005 ± 0.003	0.014 ± 0.012	0.05
Cadmium^{a} (mg/L)	0.004 ± 0.004	0.003 ± 0.001	0.003 ± 0.001	0.01
Aluminium^{a} (mg/L)	0.951 ± 0.103	0.972 ± 0.391*	0.964 ± 0.748**	0.03
Copper^{a} (mg/L)	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.05
Chromium^{a} (mg/L)	0.011 ± 0.007	0.011 ± 0.004	0.013 ± 0.011	0.05
Iron^{a} (mg/L)	0.815 ± 0.464	0.966 ± 0.417*	0.887 ± 0.827	0.01
Manganese^{a} (mg/L)	0.027 ± 0.012	0.045 ± 0.002	0.042 ± 0.018	0.05
Zinc^{a} (mg/L)	0.063 ± 0.002	0.069 ± 0.043	0.083 ± 0.012	5.00
Molybdenum^{a} (mg/L)	0.002 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.025
Nickel^{a} (mg/L)	0.002 ± 0.001	0.001 ± 0.001	0.004 ± 0.001	0.01
Cobalt^{a} (mg/L)	0.004 ± 0.004	0.003 ± 0.001	0.003 ± 0.001	0.002
WQI	67.394	55.033	46.970	–

Feed types: *CoF* commercial feed, *SH* slaughter house waste, *CW* chicken waste

Significance levels: * $p<0.05$; ** $p<0.01$ (when compared with culture ponds fed with CoF)

[a] Limit of detection: Pb-30; Cd-1; Al-1; Cu-4, Cr-4; Fe-10; Mn-1; Zn-5; Mo-5; Ni-5, Co-1

[b] WHO (2011)

(0.126 ± 0.011 mg/kg) in muscle of *C. gariepinus* collected from pond fed with CW diet. Fe was detected high (9.238 ± 0.413, 11.70 ± 0.548 and 18.777 ± 0.598 mg/kg) in fish muscles collected from all the three feed types as compared to other tissues. Mg was detected significantly ($p < 0.001$) high (1.681 ± 0.49 mg/kg) in liver and muscle (1.069 ± 0.714 mg/kg) collected from SH and CW feeds as compared to other tissues in CoF fed pond. Mo was detected significantly ($p < 0.05$) high (0.139 ± 0.065 mg/kg) in muscle of *C. gariepinus* collected from ponds fed with CW and SH. Ni was high (0.327 ± 0.006 mg/kg; 0.468 ± 0.016 mg/kg) in muscles in *C. gariepinus* collected from pond fed with CW and SH. Pb was detected significantly ($p < 0.05$) high in the muscle tissues of *C. gariepinus* raised particularly with chicken waste (CW) amongst all the three feed types where it was 0.192 ± 0.072 mg/kg. Zn was observed significantly ($p < 0.001$) high (4.076 ± 1.23 mg/kg) in liver in comparison to other tissues in *C. gariepinus* fed with all the three feed types.

Our results revealed that the metals contaminant in fish tissues was mainly from animal waste and not from ponds water since the levels of metals in the water were very low. The cumulative average value of accumulated metals in fish tissues was calculated for different feed types and the same is presented (Fig. 1). It showed that the level of most of the metals contaminants were very low individually but was found considerably high when calculated as cumulative value (Fig. 1; Table 2). Heavy metals are of ecological significance due to their toxicity and their ability to accumulate in living beings (Luk and Au-Yeung 2006). The monitoring of the metals contamination in various tissues although revealed elevated levels but was not found alarming in general. However, few metals contaminants present in high concentrations in tissues were found alarming (Table 2). The risk of different metals contaminants was calculated taking their values in fish tissues. We found that the ingestion value for Cd was 1.3×10^{-3} for conventional feed type (CoF); 1.4×10^{-3} for slaughter house waste (SH) and 2.2×10^{-3} for chicken waste feed (CW) group. The hazard quotient was 0.013 for CoF; 0.014 for SH and 0.022 for CW respectively. At the same time, risk values were found very low when taken into consideration of the reference dose (Table 2). For CoF, the ingestion value was 1.3×10^{-3}; for SH it was 1.4×10^{-3} and for CW it was 2.2×10^{-3} respectively. The risk for Cu was 0.019 (CoF); 0.001 (SH) and the same was for CW. The ingestion of fish for Cu was nil in case of CoF but it was 5.5×10^{-4} for SH and 4.7×10^{-4} for CW. It showed that the presence of Cu in the muscular tissues of fish was although very low or nil, yet its health risk values for ingestion were close to the risk level in case of waste

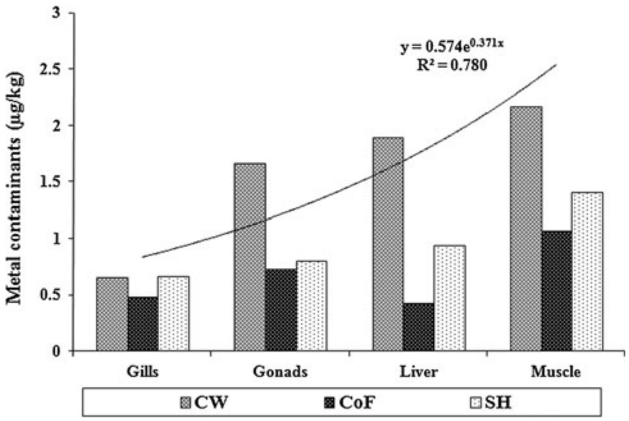

Fig. 1 Cumulative values of metal contaminants in different tissues of *C. gariepinus* raised in rural ponds with different feed types

feedings (SH & CW). The level of Cr was found 0.04 in CoF; 0.025 in SH and 0.008 in CW. The risk for Cr was 5.9×10^{-4} in CoF; the 7.7×10^{-4} in SH and 8.5×10^{-4} in CW (Table 2). Oral RfDs for Cr is reported 1.5 for water and 0.003 mg Cr/kg/day for food respectively (corresponding to 105 and 0.21 mg/day based on EPA assuming at 70 kg body weight). A tolerable daily intake (TDI) of 5 μg/kg/day for oral exposure to chromium has also been reported (Lijzen et al. 2001). The maximum intake of chromium has been reported to be 0.17 mg/day for food, up to 0.002 mg/day for drinking water and up to 0.6 mg/day for supplements with maximum to the extent of 0.77 (Lijzen et al. 2001). Intestinal absorption of Cr is low (up to 2 %) in human (SARA Group 2008). The risk for Mn was assessed to be 0.001 in CoF; 0.002 in SH and 0.003 in the CW. The ingestion of fish was 4.3×10^{-2} for CoF, 2.2×10^{-2} for SH and 4.2×10^{-2} for CW (Table 2). The risk of ingestion of this metal was slightly high in this study. The USEPA (2001) has reported that 0.14 mg/kg/day is an appropriate reference dose for manganese. Assessment for Molybdenum (Mo) was 0.009 in CoF, and the same (0.009) was found in SH while it was 0.108 in case of CW.

The health risk for adults for Mo was found to have a value of 4.3×10^{-3} in CoF; 4.5×10^{-3} in SH and 5.7×10^{-3} in CW. According to WHO (2011) the daily requirement for Mo is 0.015–0.15 mg/day for adults, i.e. about 1–5 μg/kg/day (USEPA 2001). Hazard quotient of Ni was found to be 0.005 in CoF; 0.006 in SH and 0.009 in CW respectively. The ingestion of fish was 1.1×10^{-4} for CoF; 1.2×10^{-4} for SH and 1.8×10^{-4} for CW. The risk of ingestion of this metal was low. There is no reported evidence suggesting that Ni compounds are carcinogenic by the oral route (Lijzen et al. 2001). As per WHO (2011) report, Tolerable Daily intake (TDI) of 5 μg/kg/day for Ni through use of an uncertainty factor of 1,000 (to compensate for the absence of reliable chronic toxicity/carcinogenicity/reproductive toxicity data) does not cause any risk. As per USEPA (2001) RfD of 20 μg Ni/kg/day is reported. The hazard quotient for Zn was 0.001 for all the three feed types. The calculated risk for ingestion of Zn was 2.4×10^{-3} in CoF; 2.5×10^{-3} for SH and 3.1×10^{-3} for CW. Recommendations on limits for the tolerable intake of Zn can be confusing, sometimes conflicting to some extent with recommended nutrient intakes. WHO proposed a TDI of 0.3–1.0 mg/kg Zn, corresponding to 18–60 mg/day for a 60 kg adult. In consideration of the data on dietary intakes (up to 0.2 mg Zn/kg/day), risk of Al was also found similar

Table 2 Risk values of each metal contaminants in tissues of *C.gariepinus* raised using differen feed types

Risk assessment components	Ingestion of fish(CF × IRf × FI × AF/BW)			Hazard quotient (CDI/RfD)			Risk values (CDI × Slope factor)		
Feed types	CW	SH	CoF	CW	SH	CoF	CW	SH	CoF
Various metal contaminants									
Cd	2.2×10^{-3}	1.4×10^{-3}	1.3×10^{-3}	0.022	0.014	0.013	2.20E−05	1.40E−05	1.30E−05
Co	2.2×10^{-3}	1.4×10^{-3}	1.3×10^{-3}	0.001	0.001	0.007	2.2E−05	1.40E−05	1.30E−05
Cu	4.7×10^{-4}	5.5×10^{-4}	0	0.001	0.001	0.019	4.70E−05	5.50E−05	NA
Cr	8.5×10^{-4}	7.7×10^{-4}	5.9×10^{-4}	0.008	0.025	0.04	8.50E−05	7.70E−05	5.90E−05
Mn	4.2×10^{-2}	2.8×10^{-2}	4.3×10^{-2}	0.003	0.002	0.001	NA	NA	4.30E−05
Mo	5.7×10^{-3}	4.5×10^{-3}	4.3×10^{-3}	0.108	0.009	0.009	NA	4.50E−05	4.30E−05
Ni	1.8×10^{-4}	1.2×10^{-4}	1.1×10^{-4}	0.009	0.006	0.005	NA	NA	NA
Zn	3.1×10^{-3}	2.5×10^{-3}	2.4×10^{-3}	0.001	0.001	0.001	NA	NA	NA
Al	4.0×10^{-2}	2.6×10^{-2}	2.7×10^{-2}	0.019	0.005	0.002	9.60E−03	6.30E−03	6.30E−03
Fe	19.4×10^{-2}	4.5×10^{-2}	3.6×10^{-2}	0.001	0.001	0.001	9.70E−04	4.50E−05	3.60E−03
Pb	4.1×10^{-2}	2.1×10^{-2}	1.0×10^{-2}	0.001	0.002	0.002	9.35E−06	2.25E−05	1.15E−05

NA not applicable

to Zn hazard and it was similar in CoF, SH and CW feed types. The ingestion of fish for Al was 1.7×10^{-2} for CoF, 2.1×10^{-2} for SH and 4.0×10^{-2} for CW fed fishes. Hazard analysis for Fe was found 0.001 which was similar in all the three types of diets given to the fish. The ingestion of fish was 3.6 for CoF, 4.5 for SH and 19.4 for CW fed fishes. The risk of ingestion for these metals was highest. Human toxicity is well documented, particularly in respect of fatalities in children associated with ingestion of adult Fe supplements. A single dose of 20 mg Fe/kg is sufficient to produce gastrointestinal symptoms (USEPA 2001). In the absence of any regulatory assessment, the oral PDE for Fe was set arbitrarily at 13 mg/day (260 µg/kg/day in a 50 kg patient), based on the value given by USRDA and the UK guidance for supplemental intake. Ingestion of fish for Pb was 1.7×10^{-2} in case of CoF, 2.1×10^{-2} in SH and 4.1×10^{-2} in CW fed fishes. Hazard quotient for Pb was found 0.001 in all the three diets. The risk for adults for Pb was assessed very high in this study (USEPA 2001). The levels of non carcinogenic toxic oral risk ranged from 1.3×10^{-5} to 8.5×10^{-5} for all the examined metals. The toxic risk value for Fe ranged from 3.6×10^{-3} to 9.6×10^{-3} and was found highest of all the values of total risks of metals. Since the risk value of Fe has not been reported earlier by any previous workers therefore, Pb was only considered to pose the greatest risk to human health in this study. It is to mention that risks in the range of 1×10^{-6}–1×10^{-4} typically have been adjudged to be acceptable (USEPA 2001). The potential ingestion risk exposure to Al, Fe and Pb was 6.3×10^{-3}–9.6×10^{-3}, 3×10^{-3}–9.7×10^{-3} and 1.15×10^{-5}–9.3×10^{-6} respectively (Table 2; Obiri et al. 2006).

In this study, risk assessment of metal contaminant was analyzed only on the basis of the ingestion of fish because metals contaminants were observed in fish through waste feeding mainly and not through pond water as observed in this study. The risks would have been much more higher than the presently calculated value when the ingestion of surface water, suspended material, dermal contacts of the sediments were simultaneously considered as described in the risk assessment model (SEDISOIL). We have assessed risk based on the tolerable daily intake (TDI) which refers to the reference dose of substance that can be taken daily without identifiable risk at lifetime exposure.

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