ISSN: 2347-5129
IJFAS 2014; 1(5): 176-181

www.fisheriesjournal.com
Received: 08-04-2014
Accepted: 25-05-2014

Sharad C. Srivastava
Exotic Fish Germplasm Section of Fish Health Management, National Bureau of fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow-226002 (Uttar Pradesh), India.

Pankaj Verma
Exotic Fish Germplasm Section of Fish Health Management, National Bureau of fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow-226002 (Uttar Pradesh), India

Ambrish K Verma
Uttar PradeshPollution Control Board, Lucknow-226016, India

Atul K. Singh
Exotic Fish Germplasm Section of Fish Health Management, National Bureau of fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow-226002 (Uttar Pradesh), India.

Correspondence:
Sharad C. Srivastava
Exotic Fish Germplasm Section of Fish Health Management, National Bureau of fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow-226002 (Uttar Pradesh), India.

Assessment for possible metal contamination and human health risk of *Pangasianodon hypophthalmus* (Sauvage, 1878) farming, India

Sharad C. Srivastava, Pankaj Verma, Ambrish K Verma, Atul K. Singh

ABSTRACT
Pangasianodon hypophthalmus commonly known as pangas or pyaasi in India, the total aquaculture production, catfish particularly *Pangasius* is receiving popularity. Cultured fishes may absorb dissolved trace metals from its feeding diets and/or habitat leading to the accumulation in various tissues. Irrespective of the source, the potential bioaccumulation of heavy metals in fish to a degree that may constitute a potential threat to human health when ingested is of great concern. Therefore, unregulated culture of *P. hypophthalmus* may have ecological implications if its culture continues without any precautionary and contaminant measures. We have determined the concentrations of heavy metals in *P. hypophthalmus* in order to evaluate the possible risk of consumption. Copper, lead, nickel, cadmium, chromium, and zinc were estimated in the muscles of fish. Health index (HI) for all observed metals for the fishes from market was 1.2531, while it was 0.1687 in the fish cultured locally in Lucknow. The value of HI was more than one in fishes examined from the market suggesting that it may cause health risks.

Keywords: Aquaculture, *Pangasianodon hypophthalmus*, Metals, Health risk, Health index.

1. Introduction

Pangasianodon hypophthalmus is popularly known as pangas or pyaasi in India, belonging to the family pangasiidae, under the order Siluriformes. The origin of *P. hypophthalmus* is reported from the Mekong River of Vietnam to the Chao Phraya River to Thailand [1]. Later the fish was introduced to several countries such as Malaysia, Indonesia, Philippines, Bangladesh and China, where it spread and was adopted under aquaculture. In India, the fish was introduced from Bangladesh [2]. *Pangasius sp.* is highly tolerant to salinity, pH, dissolved oxygen, temperature or even pollution [3]. *P. hypophthalmus* is omnivorous, feeding on algae, higher plants, zooplankton, and insects, while larger specimens also take fruit, crustaceans and fish [2]. The aquaculture production of pangasius, catfish is an important industry in Vietnam and it received popularity even in India in terms of commercial culture. Pangas culture has proved itself as a profitable enterprise due to year round production, quick growth and high productivity [4, 15].

In India, the *Pangasius hypophthalmus* is produced in Andhra Pradesh and West Bengal on a commercial scale. However, its production moved into the states like Uttar Pradesh, Punjab, Rajasthan, Bihar, Chhattisgarh and Haryana. There is a good potential for *P. hypophthalmus* culture as this species can be reared in any condition of fresh water be a small or large area. *P. hypophthalmus* in India is generally cultivated in highly eutrophic waters. Metal contaminant is reported from several types of water body in India. [16, 17]. Since the fish raised under eutrophic condition raises the possibility of heavy metal contamination [16, 17 & 18]. We have attempted to investigate the presence of any heavy metals such as Cu, Pb, Ni, Cd, Cr and Zn levels in muscle tissues of *P. hypophthalmus* and determined the possible health risk posed by fish ingestion through consumption of the fish. It was conceptualised that the fish coming in the whole sale market was a commercial produce where the fish was raised in high density and consequently there was eutrophic condition in the pond since there is hardly any practice of exchange of water in the Pangasius culture [2]. However, we also considered some of the recently initiated culture produce of Pangasius under farm condition which was under semi-intensive culture. Under this perspectives, we examined the possible accumulation of heavy.

metal in pangasius obtained from local fish farm at Lucknow raised under semi-intensive culture as well as the fish available in the market being transported from the Andhra Pradesh where the fish is raised under intensive farming. The risk assessment of the present heavy metals was done by hazard identification, characterization, exposure assessment, and risk characterization, using data from toxicity studies on the specific heavy metals.

P. hypophthalmus is well accepted by a wide range of people and therefore, it has been a good source of protein and calorie for poor, medium and better-off people in rural as well as urban areas. In 2008, about 81% (115 million tons) of estimated world fish production was used as human food with an average per capita of 17 kg [5] & [19]. As fish constitute an important part of human diet, it is not surprising that the quality and safety aspects of fish are of particular interest. Over the past several decades, the concentrations of heavy metals in fish have been extensively studied in various places around the world. Since the diet is the main route of human exposure to heavy metals [20].

Cultured fishes may absorb dissolved elements and trace metals from its feeding diets and surrounding water leading to their accumulation in various tissues in significant amounts [2]. Metals can enter into the food web through direct consumption of water or organisms or through uptake processes and be potentially accumulated in edible fish and other wildlife [6]. As fishes are constantly exposed to pollutants in contaminated water they could be used as excellent biological markers of heavy metals because non-essential metals are also taken up by fish and have accumulated in their tissues [7]. Consumption of the contaminated fishes have been reported to show the risk potential for human [8, 9, 10, 11 & 2]. Irrespective of their source, the potential accumulation of heavy metals in fish available in the Utter Pradesh market to such a degree that may constitute a potential threat to human health when ingested is of great concern. Heavy metals have been considered as dangerous substances causing serious health hazards to human being and other living organisms through progressive irreversible accumulation in their bodies [21]. In view of above mentioned literature and reports, we have attempted to determine the concentrations of heavy metals in *P. hypophthalmus* in order to evaluate the possible bio-accumulation risk of fish consumption. Accordingly, the objective of the current study was to determine the levels of heavy metals like copper (Cu), lead (Pb), nickel (Ni), cadmium (Cd), chromium (Cr), and zinc (Zn) in the muscles of *P. hypophthalmus* available in Utter Pradesh fish market. These concentrations were then compared against the possible accumulation of heavy metals in *P. hypophthalmus* raised in local farms of Lucknow, Uttar Pradesh. The concept of this study was the 'safe levels of heavy metal exposure' for humans, which could be identified as the risk of known toxicological profiles keeping in view of the reports on the contamination of different fish species to determine their heavy metal contamination and human health risk [12, 13]. The traditional approach to risk assessment was separated into hazard identification, hazard characterization, exposure assessment, and risk characterization, and using data from toxicity studies on the specific heavy metals for hazard identification and characterization.

2. Materials and Methods

Six to eight fish species of *P. hypophthalmus* was collected from the local culture pond and also fish market of Lucknow, Utter Pradesh. The fishes were brought to the laboratory and dissected with clean instruments. Tissues were washed with double-distilled water and put in petri dishes to dry at 120 ^{0}C until reaching a constant weight. One gram of each dried tissue (in three replications) was digested with di-acid (HNO3 and $HClO_4$ in 2:1 ratio; [7] on a hot plate set at 80 ^{0}C (gradually increased) until all materials were dissolved. Digested samples were diluted with double-distilled water appropriately in the range of the standards which were prepared from the stock standard solution of the metals (Sd-Fine). Metal concentrations in the samples were measured using a UNICAM- flame atomic absorption spectrophotometer (AAS). The absorption wavelengths were 217.0 nm for Pb, 267.7 nm for Cr, 231.6 nm for Ni and 324.7 nm for Cu, 228.8 nm for Cd, 213.9 nm for Zn. The QC sample was run at a frequency of once in every 10 samples; to check for the calibration accuracy and the instrument drift. The results within 5% of the known QC values were deemed acceptable. Blank samples were run in duplicate in each analysis batch in a randomized order and were used to calculate the method detection limit (MDL) [22]. The results were expressed as micrograms per gram dry weight for fish.

$$\text{Metal concentration in fish tissue} = [C \times V] / W$$

Where,
C = concentration in µg/L (read from AAS); V= volume of sample (ml); W= weight of sample (g)

Potential exposures to heavy metal such as lead, copper, nickel, and chromium via ingestion of fish (muscle) was calculated using edible tissue samples collected from the local culture pond and the fish market. Deterministic and probabilistic exposure assessment techniques SEDISOIL model was used [14] to characterize risks. The evaluation considered published fish total daily intake (TDI) from market/culture pond surveys limited access areas as well as accessible recreational areas. The deterministic risk assessment used a TDI from a market/culture survey conducted in a culture area with relatively few access points for market. For the purpose of comparison, the probabilistic assessment was used as a fish consumption rate distribution that included values from other culture areas in India, as well as the TDI from the culture pond. Human health risk assessment (USEPA) was carried out in three stages: (1) hazard identification, (2) exposure assessment, and (3) risk characterization [2, 23]. The hazard identification was done by monitoring of heavy metals in pond water as well as fish muscle as described above. For quantification of exposure, a pathway exposure model (SEDISOIL) was used [14] and calculated with the following equation:

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

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 using formula [24].

$$HQ = CDI / RfD$$

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

To assess the overall potential for carcinogenic effects posed by more than one metals the HQs calculated for each metals was summed and expressed as a HI [25],

$$HI = HQ1 + HQ2 + . . . + HQ_n$$

In cases where the carcinogenic HI did not exceed unity, it was assumed that no chronic risks were likely to occur at the site. If the HI is greater than unity as a consequence of summing several HQs, it would be appropriate to segregate to compounds by effect and by mechanism of action and to derive HI for human health.

The health risk was determined by using [24],

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

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

The level of total cancer risk that is of concern is a matter of personal, community, and regulatory judgment, risks above 1E-04 to be sufficiently large that some sort of remediation is desirable. Excess cancer risks that range between 1E-06 and 1E-04 are generally considered to be acceptable, although this is evaluated on a case-by-case basis and EPA may determine that risks lower than 1E-04 are not sufficiently protective and warrant remedial action [26, 27].

2.1 Data analysis

Mean concentrations ± SD. (the standard deviation of the mean) in µg/kg wet weight were calculated. One-way analysis of variance (ANOVA) was used after the logarithmic transformation was done on the data to improve normality followed by the Duncan multiple range test as a multiple comparison procedure to assess whether the means of metal concentrations varied significantly among fish species. Possibilities less than 0.05 were considered statistically significant ($p<0.05$). All statistical calculations were performed with SPSS 13.0 for Windows.

2.2 Results and Discussion: The study revealed that the concentration of heavy metals in cultured fishes ranged between 0.012±0.001 for Cu, 0.096±0.033 for Pb,0.014±0.001 for Cd,0.524±0.053 for Cr,1.112±0.251for Zn. Among all these metals Ni was not detected in cultured fishes. Studied heavy metal concentration in cultured pond fishes did not exceed the permissible limit set by [28]. As compared to the concentration of heavy metals in cultured fishes, market fishes have reported a higher range of heavy metals i.e. 0.053±.001 for Cu, 0.942±0.321 for Pb,0.11±0.01 for Ni, 0.48±0.011 for Cd, 2.231±0.91 for Cr, 1.121±0.662 for Zn (fig. 1).In market fishes, Pb and Cr were significantly ($p>0.001$) very high. Observed Cu was also significantly ($p>0.005$) high. Concentration of all studied metals in market fishes was more than the permissible limit decided by [28].

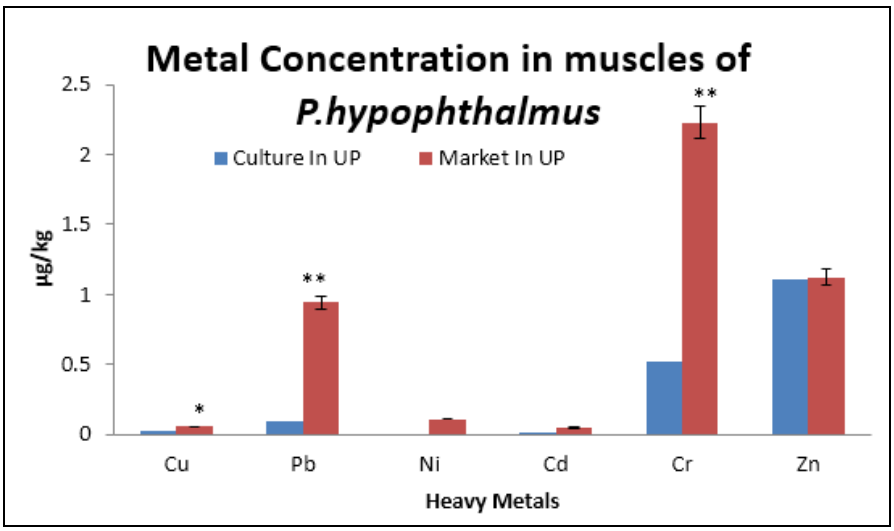

Fig 1: Metal concentration of the *P. hypophthalmus* in cultured pond and market fish available

Significance levels: *p > 0.05; ** p > 0.01 (when compared with cultured fish and fish available from market) Limit of detection: Cu-4, Pb-30; Ni-5, Cd-1; Cr-4; Zn-5.

The maximum amount of heavy metals reached in human body through the ingestion of market fishes as compared to the cultured one. In studied metals maximum amount of Zn i.e. $8.7\times10^{-3}$was reached in human body through the marketfishes. Heavy metals like Cu, Pb, Cr, Cd and Ni was also ingested in human through the consumption of market fishes (Table 1). HQ value was also calculated for studied metals (Table 1). HQ value for market fishes was higher than cultured fishes the and maximum HQ value was observed for Pbi.e 0.9252 in market fishes. Combined HQ means calculated health index (HI) was 0.1687 for cultured fishes and 1.2531 for market fishes. Outcomes from HI value stated that cultured fishes were safe for human consumption than market fishes because the observed HI value for market fishes was more than 1 that can

create the carcinogenic risk in human. The carcinogenic risk value in cultured fishes ranged between 1E-06 to 1E-04 (table 1). These values generally considered to be acceptable [29]. But carcinogenic risk values for market fishes ranged between 3.70E-03 for Pb, 4.40E- 03 for Cr, 2.60E-03 for Zn. These risk value in market fishes was more than (1E-06 to 1E-04) that creates carcinogenicity in human beings during 70 year life time ingestion of market fishes.

The distribution and potential bioaccumulation of heavy metals in muscle of fish (*P. hypophthalmus*), a major aquaculture species, were studied in relation to two different fish farming systems. It has long been recognized that heavy metals in the environment have a particular significance in the eco-toxicology, since they are highly persistent and can be toxic in traces [30]. During the last few decades, great attention has been paid to the possible dangers of heavy metal in human due to the consumption of contaminated fish. Fish absorbed heavy metals from water through the gills, skin and digestive tract. The heavy metals of the most widespread concern to human health are lead, copper, mercury and cadmium [31, 32 & 33]. Heavy metals are recognized as toxic substances due to their low rate of elimination from the consumer body; either man or animals [33, 34 & 35]. The results of this study demonstrate that inside the fish, the fate of the toxic elements is different in terms of distributions into different organs as well as possible biotransformation and, eventually, elimination. The results also show significant differences in heavy metal accumulation among the different types of fish culture. The observed level of heavy metals in cultured fishes was lower than the permissible limit decided by WHO means cultured fishes are safe for human consumption. The concentration of heavy metals like Pb, Cd, Cr, Zn was maximum in market fishes compared to cultured fishes and it was more than the acceptable limit set by WHO. That can create carcinogenic risk. Although Ni was not detected in cultured fishes and it was present in market fishes, but the concentration of Ni did not exceed than the safe limit (Figure. 1). The maximum amount of all studied metals accumulated in the human body through the ingestion of market fishes. For threshold contaminants, the risk to a human receptor from being exposed to a chemical via a single pathway can be expressed as an exposure ratio, commonly called a Hazard Quotient (HQ). HQ is advantageous in that whether or not there is risk can be simply determined only by checking whether HQ is larger or smaller than one. The observed HQ value was less than one in cultured as well as market fishes it was slightly more in market fishes (Table 2). Detected Value of HQ for Pb (0.9252) in market fishes it was slightly less than one, means market fishes was marginally safe for carcinogenic risk for Pb.

Table 1: Risk values of each metal contaminant in muscle of *P. hypophthalmus* was analyzed in cultured fish as well as in fish from market

Metals	Ingestion of fish (CF × IRf × FI×AF/BW)		Hazard quotient (HQ =CDI/RfD)		Risk values (CDI × Slope factor)		Health Index ($HI=HQ_1+HQ_2+\ldots HQ_n$)	
	Cultured	Market	Cultured	Market	Cultured	Market	Cultured	Market
Cu	4.7×10^{-5}	2.0×10^{-4}	0.0011	0.0051	1.89 E-06	8.33 E-06	0.1687	1.2531
Pb	3.7×10^{-4}	3.7×10^{-3}	0.0943	0.9252	3.77 E-05	3.70 E-03		
Ni	ND	4.3×10^{-4}	ND	0.0216	ND	8.64 E-06		
Cd	5.5×10^{-5}	1.8×10^{-4}	0.0018	0.0029	5.51 E-0	1.89 E-06		
Cr	4.3×10^{-3}	4.4×10^{-3}	0.0029	0.0062	4.36 E-05	4.40 E-03		
Zn	2.0×10^{-3}	8.7×10^{-3}	0.0686	0.2921	6.17 E-04	2.60 E-03		

Total HQ means health index (HI) was less than 1 (0.1687) for cultured fish (*P. hypophthalmus*) it demonstrated that ingestion of fish from cultured ponds does not result in over exposure of studied metals. Thus no adverse effect poses to the health of consumers [36]. We need to perform a detailed analysis to determine whether the potential for noncancerous health effects is accurately estimated by the total HI. This is because the toxicological effects associated with exposure to multiple chemicals, often through different exposure pathways, may not be additive. The total HI might therefore overestimate the potential for noncancerous health effects. HI was more than 1(1.2531) for market fishes. It implies that the estimated exposure exceeds the USEPA reference dose for the contaminant of interest and it may have the probability of contracting cancer. Risk description provides information important for interpreting the risk results and identifies a level for harmful effects on the plants and animals of concern in an ecological risk assessment.

Calculated carcinogenic risk value in cultured fishes ranged between 1E-06 to 1E-04 for all studied metals except Zn for which observed risk value was 6.17 E-04 and it was near to the standard value. In market fishes observed risk value ranged between 3.70 E-03 for Pb, 4.40 E-03 for Cr and 2.60 E-03 for Zn. These values were more than 1E-06 to 1E-04. It indicated that the consumption of market fishes leads to the daily intake of heavy metals in local residents, which pose a potential health threat from long-life time exposure of 70 years are more in the future. Hazard index of metals suggested that contamination in most of the fish had potential for human health risk due to consumption of muscle. Thus, regular monitoring of heavy metals contamination in the fish species grown at contaminated water is necessary for avoiding the possible human health risk owing to the consumption of contaminated fish.

3. Conclusion

Consumption of fish with elevated levels of heavy metals may lead to high level carcinogenic risk to human health. It is thus advocated that regular monitoring of heavy metal contamination in the fish species thriving at contaminated water must be carried out to ascertain the food safety. This initiative will be an important step to undertake measures to remove heavy metals from the cultured methods as per need in

view of the environmental and food safety.

4. Acknowledgment
Author was thankful to Dr. J. K. Jena, Director of National Bureau of Fish Genetic Resources (NBFGR) Lucknow, India for providing the lab facilities.

5. References

1. Roberts TR, Vidhayanon C. Systematic revision of the Asian catfish family Pangasiidae, with biological observation and descriptions of three new species. Proceedings of the Academy of Natural Sciences of Philadelphia 1991; 143:97-144.
2. Singh AK, Lakara WS. Culture of Pungasious hypophthalmus in India: impact and present scenario. Pakistan Journal of Biological Sciences 2012; 15(1):19-26.
3. David A. Brief taxonomic account of the Gangetic *Pangasiuspangasius* (Ham.) with description of a new sub-species from the Godavari Proceedings of the Indian Academy of science 1962; 34(3):136-156.
4. Ali MZ, Hossain MA, Mazid MA. Effect of mixed feeding schedules with varying dietary protein levels on the growth of sutchi Cat fish, Pangasiushypophthalmus (Sauvage) with silver carp, Hypophthalmichthys molitrix (Valenciennes) in ponds. Aquaculture Research 2005; 36:627-634.
5. Dural M, Goksu MZI, Ozak AA. Investigation of heavy metal levels in economically important fish species captured from the Tuzla Lagoon. Food Chemistry 2007; 102:415-421.
6. Paquin RR, Farley K, Santore RC, Kavvadas CD, Mooney KG, Winfield RP *et al.* Metals in aquatic systems a review of exposure, bioaccumulation, and toxicity models. Society of Environmental Toxicology and Chemistry SETAC Pensacola 2003; 61–90.
7. Canli M, Atli G. The relationships between heavy metal (Cd,Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fishspecies. *Environmental Pollution*, 121:129–136. Netherlands. Environment Health Prospects 2003; 107:27–35
8. USEPA. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis. Ed 3, Office of Science and Technology of Water, Washington, DC EPA 823-B-00-007: 2000; 1-200.
9. Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food and Chemical Toxicology 2008; 46:2782–2788.
10. Michael AM, Matthew RM, Michael FEW. Elevated levels of metals and organic pollutants in fish and clams in the Cape Fear River watershed. Archives of Environmental Contamination And Toxicology 2011; 61:461–471
11. Imar MR, Carlos JRS. Metal Levels in Fish Captured in Puerto Rico and Estimation of Risk from Fish Consumption. Archives of Environmental Contamination and Toxicology 2011; 60:132–144.
12. Laar C, Fianko JR, Akiti TT, Osae S, Brimah AK. Determination of heavy metals in the blackchin tilapia from the Sakumo Lagoon, Ghana. Research Journal of Environmental and Earth Sciences 2011; 3(1):8-13.
13. Anim AK, Ahialey EK, Duodu GO, Ackah M, Bentil NO. Accumulation profile of heavy metals in fish samples from Nsawam, along Densu River, Ghana. Research Journal of Environmental and Earth Sciences 2011; 3(1):56-60.
14. Harma JA, Jean-Paul R, Edwin JCM, Jurian AH, Jos CSK. Human health risk assessment in relation to environmental pollution of two artificial fresh water lakes in the Netherlands. Environmental Health Perspectives 1999; 107:27–35.
15. Corneillie S. The role of prebiotics in pangasius production International Aqua Feed 2014; 10-12.
16. Singh KP, Mallik A, Mohan D, Sinha S. Multivariate statistical techniques for the evaluation of spatial and temporal variations in water quality of Gomti River (India): A case study. Water Research 2004; 38(18):3980-3992
17. Kosygin LVW. Fish fauna of the Ukhrul district, Manipur with a note onconservation strategies. The Journal of Advanced Zoology 2006; 27(2):61-66.
18. Singh AK, Srivastava SC, Ansari A, Kumar D, Singh R. Environmental Monitoring and Health Risk Assessment of African Catfish *Clarias gariepinus* (Burchell, 1822) Cultured in Rural Ponds, India. Bulletin of Environmental Contamination and Toxicology 2012; 89:1142–1147.
19. FAO. World Review of Fisheries and Aquaculture Part 1 2010. http://www.fao.org/docrep/013/i1820e/i1820e01.pdf. 3 june 2014.
20. Castro-Gonzeza MI, Méndez-Armentab M. Heavy metals: implications associated to fish consumption, Environmental Toxicologyand Pharmacology 2008; 26:263–271.
21. Wang X, Sato T, Xing B, Tao S. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Science of the Total Environment 2005; 350:28-37.
22. CPCB, Guidelines for water quality monitoring, Control pollution Control Board.2008; http://www.cpcb.nic.in/upload/NewItems/NewItem_116_Guidelinesof20waterqualitymonitoring_31.07.08.pdf
23. Li Y, Jingling L, Zhiguo C, Chao L, Zhifeng Y. Spatial distribution and health risk of arsenicand polycyclic aromatic hydrocarbons (PAHs) in the water of the Luanhe River Basin, China. Environmental Monitoring and Assessment 2010; 163:1–13.
24. US EPA. Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Office of Research and Development, Washington, DC. 2001; http://www.epa.gov/iris/ Office of Solid Waste and Emergency Response.
25. USEPA. Risk assessment guidance for superfund, Vol. I, Human health evaluation manual. Part A (interim final), EPA/540/1–89/002. Washington, DC: Office of Emergency and Remedial Response, US Environmental Protection Agency. 1989.
26. US EPA. National oil and hazardous substances pollution contingency plan, 40 CRF part 300. Washington, DC: US Environmental Protection Agency Publication 9285.7–01B. Washington, DC: Office of Emergency and Remedial Response, 1990.

27. US EPA. Risk assessment guidance for super- fund, Vol. I, Human health evaluation manual. Part B. Development of risk-based preliminary remedia- tion goals (interim), PB92–963333. US Environmental Protection Agency.1991.
28. WHO/FAO. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission 13th Session. Report of the Thirty Eight Session of the Codex Committee on Food Hygiene, Houston, United States of America, ALINORM 07/ 30/13. 2007.
29. USEPA. USEPA Regional Screening Level (RSL) Summary Table: November 2011. 2011; http://www.epa.gov/regshwmd/risk/human/Index.htm. 3. June 2014.
30. Langston WJ. Toxic effects of metals and the incidence of metal pollution in marine ecosystem. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, FL, 1990; 101–122.
31. Chen YQ, Edwards JJ, Kridel SJ, Thornburg T, Berquin IM. Dietary fat gene interactions in cancer. Cancer metastasis Reviews2007; 26: 535-551
32. Bhouri AM, Bouhlel IL, Chouba M, Hammami CEl, Chaouch A. Total lipid content, fatty acid and mineral compositions of muscles and liver in wild and farmed sea bass (Dicentrarchuslabrax). African Journal of Food Science 2010; 4(8):522-530.
33. Elnimr T. Evaluation of some heavy metals in *Pangasius hypothalamus* and Tilapia nilotica and the role of acetic acid in lowering their levels. International Journal of Fisheries and Aquaculture 2011; 3(8):151-157.
34. WHO, Environmental health criteria. No.134. Cadmium. WHO. Geneva. 1992; http// www.freepatentsonline.com/5958248.htm.
35. Wafaa E, Saleh G, Dief M. Some studies on heavy metal pollution in water and fishes. Egyptian Journal of Basicand Applied Physiology 2003; 2(2):185-200.
36. Kar S, Maity JP, Jean JS, Liu CC, Liu CW, Bundschuh J, Lu HY. Health risks for human intake of aquacultural fish: Arsenic bioaccumulation and contamination. Journal of Environmental Science and Health, Part a 2011; 46:1266–1273.