

DETERMINATION OF SELECTED HEAVY METALS CONCENTRATION IN FARMED AND FRESH WATER FISH CONSUMED IN PESHAWAR

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ABSTRACT

Objective: To assess and compare the concentration of heavy metals in farm and fresh water fish commonly consumed in district Peshawar.

Material and Methods: A Cross-sectional, analytical study was conducted at community medicine department of Khyber Medical college, Peshawar-Pakistan. Eighty Samples were selected using Non probability convenient sampling technique. Atomic absorption spectroscopy was used for analysis of heavy metals. Data was analyzed using SPSS version 23.

Results: Out of all fishes 80(100%), there were 32(40%) fresh water fishes and 48(60%) were farm fishes. Mean lead level in fishes was $-0.0771\text{ug/g} \pm 0.05\text{SD}$. Mean Cadmium level was $0.0475\text{ug/g} \pm 0.04\text{SD}$. Mean mercury level was $8.1101\text{ug/g} \pm 1.25\text{SD}$. Mean Arsenic level was $0.166\text{ug/g} \pm 0.112\text{SD}$. Out of all fish 80(100%), Fresh water contain more lead $-0.1046 \pm 0.05\text{SD}$ as compare to farm fish $-0.0587 \pm 0.593\text{SD}$ ($P=0.000$) Mean cadmium level in farm fish was $0.0509 \pm 0.06\text{SD}$. while mean cadmium level in fresh water fish was $0.0425 \pm 0.097\text{SD}$ ($P=0.441$). Out of all fishes 40(100%), Fresh water fish contain high mercury $8.46712 \pm 0.739\text{SD}$ as compare to WHO standard and approximately similar values are reported in farm fishes $7.87220 \pm 1.472\text{SD}$ ($p=0.000$). Farm fish contain high level of arsenic $0.21275 \pm 0.110\text{SD}$ as compare to fresh fish $0.09668 \pm 0.07\text{SD}$ ($p=0.001$)

Conclusion: High level of mercury and arsenic are found in this study. However, concentration of mercury is above WHO defined limits as compare to other metals in all samples.

Keywords: Heavy metals, farm, fresh water, fish, pollutants.

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INTRODUCTION

Heavy metal term refers to metalloid elements or any metal that is toxic and poisonous at low concentration¹. Heavy metals have density as high as ranging from 3.5-7 g cm. Heavy metals include Zinc, mercury, chromium, cadmium, arsenic, nickel, lead, thallium and copper². Heavy metals are widespread pollutants of soil and water bodies. Heavy metals are found in earth crust and most commonly are biodegradable in nature³.

These metal got entry in human body through water, air and food. Small concentration of these elements is essential for human metabolism. However, higher

concentration of heavy metals in human and animals leads to toxicity and several health hazards. Bio-accumulative nature of heavy metals in biotic system induces more hazardous effect in metals. These metals enter into environments through several human activities including mining activities, household application and industrial discharge in water bodies⁴.

Chemical properties of heavy metals differ from each other. These metals are extensively used in artifacts, machines and electronics and in high tech applications. These heavy metals enter in human and animal food chains through anthropogenic sources and natural geochemical weathering. Contamination of food chains is mainly due to mining waste, urban runoff, industrial waste water and landfill leaches (Particularly from electroplating, metal finishing industries and electronic industries)⁵.

Waste disposal is an important paramount today due to increasing generation of metals worldwide.

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Several aquatic environments are facing severe metal concentration exceeding protection limits designed for living beings. Metals had significant tendency to bio-accumulate food chains. These metals can be transported with sediments or remained persistent within environment. Major environmental pollution cases in world are due to mercury, lead, copper and other heavy toxic metals⁶.

Most common water pollutants are heavy metals. Severe toxicity is reported by heavy metals in low concentration and pose health threats for human and animals. Nervous system, reproductive system and kidneys are usually affected by leads in water. Irreversible brain damage and encephalopathic symptoms are associated with lead exposure⁷. Cadmium is very commonly used in solders, television, insecticides, electroplating industries, ceramics, metal finishing, metallurgical activities and photography. Cadmium is affecting environment through cadmium containing pigments, cadmium containing phosphate fertilizers, metal ore refining, alloys and electronic compounds, detergents and petroleum refining products. Major source of cadmium is rechargeable batteries containing nickel cadmium compounds. Cadmium exposure is associated with bone degeneration, blood cell destruction, liver damage and renal dysfunction. Literature reported that there is enough evidence for carcinogenicity of cadmium^{8,9,10}. Copper is an essential trace element of human biological system. It is essential for activation of enzymes in human body for photosynthesis. However at higher concentration it poses harmful effects in body. Exposure of humans to copper dust in high concentration leads to mouth, eye and nose irritation and associated with diarrhea and nausea. Continuous exposure is also associated with kidney damage and even mortality in some cases. Copper is harmful for several aquatic living beings even in low concentration. Industrial applications, metallurgy and mining are major source of environmental pollution with copper¹¹.

Zinc is essential element in human diet. Zinc showed harmful effects in high concentration. Zinc toxicity in children is associated with vomiting and nausea. High concentration of zinc in human beings results in cholesterol issues and anemia. Major sources of zinc in water are mining, metallurgical processing of zinc ores and industrial applications. Burning of coal is also an important source of zinc^{12,13}. Nickel is naturally found in volcanic rocks and within soil. Nickel and its elements are excessively used in automobile industries, electroplating, batteries, stainless steel, spark plugs, coins, cosmetics and nickel cadmium batteries construction on industrial scale. Nickel usually introduced into human body through soil, leaching minerals and

weathering rocks. Major nickel contamination in aquatic system is due to water soluble salts of nickel. Enameling and paint formation industries left their industrial discharge containing nickel elements in water bodies. Nickel is also known as nickel carbonyl, commonly used in cigarettes^{14,15}. Arsenic is found in earth's crust, worldwide. Arsenic means yellow orpiment, derived from Zarnik in Persian literature. The element was initially isolated in 1250 AD by Albert Magnus. Arsenic is reported in crystalline or amorphous forms in ores. Arsenic is found in high concentration in some areas as compare to others leading human and other living beings towards several health hazards. Arsenic enters in to environment through anthropogenic activities, coal combustion, smelting process, mining and pesticides use. High toxicity of ground water bodies and surface water is associated with arsenic. Arsenate (V) and arsenite (III) are found mostly in ground water. Process of adsorption and PH control process results in controlling arsenic in aqueous environment. Adsorption of arsenic in aquatic bodies is maintained with metal oxides of iron, aluminum and magnesium. Groundwater of India, Chile, Bangladesh, Taiwan and Brazil are rich in high concentration of arsenic leading towards toxic health effects on humans and other living beings^{16,17}.

Mercury toxicity is very important to understand worldwide. Its major example is Minamata Bay of Japan. It was reported that physically deformed babies and mentally disturbed children are borne due to exposure of mothers to mercury contaminated fish. Mercury is naturally found in volcanic eruptions, soil, weathering rocks while anthropogenic mercury found as a result of use of mercury in industrial application (mining, mercury vapor lamps, and application in batteries). Most toxic specie of mercury is methyl mercury^{18,19}. Excessive chromium utilization in industrial application leads huge amount of toxic chromium to enter in water bodies. Natural inputs and anthropogenic sources are responsible for environmental contamination with chromium. Natural sources of chromium are volcanic eruption, soil, sediments and geological weathering of rocks. However, fossil fuels, plastic manufacturing, leather industry, electroplating metals, production of chromates and tannery industries are major source of anthropogenic chromium. Trivalent chromium is less toxic than hexavalent chromium^{20,21}.

With ever-increasing pollution in natural water bodies, the risk of fish with heavy metal contamination is increasing day by day. The aim of our study is to assess the concentration of some heavy metals like cadmium, lead and chromium in the tissues of the commonly consumed fish in Peshawar city. The results then will be compared to permissible limits from the World Health Organization (WHO) to detect whether the heavy metal

contamination levels in these fish exceed the safe consumption permissible limit. The measurement of heavy metals in fish can be useful to assessing potential health risks to humans associated with the consumption of fish. As, no local data is available that evaluated the concentration of heavy metal in the commonly consumed fish species sold in this region of Pakistan, this study will establish that whether the use of these fish is safe and presence of heavy metal is within normal limits. The study results will help the consumers to select the better species for daily use that will improve the overall health status of the local community.

MATERIAL AND METHODS

A Cross sectional, analytical study was conducted at community medicine department of Khyber Medical College, Peshawar from February 2019 to July 2019. Samples were selected from different areas of Peshawar. Using WHO sample size calculator was used to calculate the sample size using 95% Confidence level 2.5% absolute precision and 0.26 as population mean and 0.072 standard deviation from previous study¹³ Convenient Non Probability Sampling Technique was used for samples collection.

Data was collected after synopsis is approved by Graduate Committee, Ethical Board and Advance Study Review Board (ASRB). Forty sample of fish were collected. Ten sample each from four identified Fish farms in Peshawar. Similarly ten sample were collected from four identified fresh water river sites. The samples were coded for privacy. After recording the necessary biological parameters, i.e. wet body weight and total length, the samples were washed, preserved in ice boxes, and transported to Quality Control Labs, for heavy metal analysis and kept in freezer. The fish samples were thoroughly washed with distilled water to remove any adhering contaminants, and they were put on dissection tray and thawed at room temperature. They were dissected using a knife and forceps, and the intestine, guts and bones removed. Then the only muscle portion was taken in a clean Petri dish and dried in an oven at $120 \pm 20^\circ\text{C}$ for 48 hours at which time the weight was constant weight. Before acid digestion, the dried muscle were ground to a fine powder using a mortar and pestle, and stored in a deep freezer (-40°C) prior to analysis. Then 1 gram of each of the powder sample were mixed with 20 ml distilled water and digested using 2:1 mixture of conc. HNO_3 (55%) and conc. HClO_4 (70%) in 100 ml Erlenmeyer flask on a heating digester (200 to 250°C) until a clear solution will be obtained and the volume reduced to approximately 15 ml. Then, this solution was filtered using Whatman no. 1 filter paper into the volumetric flask and diluted to 50 ml. The samples

thus prepared were analyzed using Atomic Absorption Spectrophotometer.

All the gathered data was analyzed by using Statistical Package for Social Sciences (SPSS) Version 17.0. Mean \pm SD was calculated for all the quantitative data like, fish weight, fish length and concentration of Arsenic, Cadmium, Lead and Mercury in each species of fish. All the fish samples were compared with the WHO permissible limit for Ar, Cd, Hg and Pb concentration and were presented as frequency and percentage for each species. The P value <0.05 was considered significant. As the test is done between two related variables so paired T-test was done. All the results were presented in the form of tables and graphs.

RESULTS

Total 80 fishes were included in study. Out of 80(100%), 12(15%) fishes were taken from khazana farm, 12(15%) were taken from Warask Road farm, 12(15%) Pustun Gari farm, 12(15%) swat khila river, 12(15%) sardayab river and 8(10%) were taken from Nagumen river. Length of fish was $\leq 5.5\text{cm}$ in 42(52.5%) and $>5.5\text{cm}$ in 38(47.5%) fishes. Weight of fish was $\leq 400\text{ gm}$ in 40(50%) and $>400\text{ gm}$ in 40(50%) fishes as shown in Table 1.

Out of all fishes 80(100%), there were 32(40%) fresh water fishes and 48(60%) were farm fishes. Out of all fish 80(100%), Fresh was contain more lead $-0.1046 \pm 0.05\text{SD}$ as compare to farm fish $-0.0587 \pm 0.593\text{SD}$ ($P=0.000$) as shown in Table 1. Out of all 80(100%), Mean cadmium level in farm fish was $0.0509 \pm 0.06\text{SD}$. while mean cadmium level in fresh water fish was $0.0425 \pm 0.097\text{SD}$ ($P=0.447$) as shown in Table 2

Out of all fishes 80(100%), Fresh water fish contain high mercury $8.46712 \pm 0.739\text{SD}$ as compare to WHO standard and approximately similar values are reported in farm fishes $7.87220 \pm 1.472\text{SD}$ ($p=0.03$) as shown in Table 3

Out of all fishes 80(100%), Farm fish contain high level of arsenic $0.21275 \pm 0.110\text{SD}$ as compare to fresh fish $0.09668 \pm 0.07\text{SD}$ ($p=0.001$) as shown in Table 4.

Arsenic level was according WHO standard in 36(45%) fishes while above standard in 44(55%) fishes. Mercury level was high from WHO standard in all fishes 80(100%). Cadmium level was within WHO standard limits in all fishes 80(100%). Lead level was also within WHO limits in all fishes as shown in figure. WHO standard limit for lead was $0.123 \mu\text{g/g}$ while mean lead level in current samples was $0.077 \mu\text{g/g}$. Cadmium level according to WHO standard was $0.195 \mu\text{g/g}$ while in current samples $0.047 \mu\text{g/g}$ reported. Mercury level according to WHO

standard was 0.145 µg/g while in current samples mean 8.11 µg/g was found. Arsenic level in WHO limits was 0.12 µg/g while in current samples 0.166 µg/g was reported as shown in figure



Fig 1: Heavy metals level according to WHO standard

Table 1: Comparison of lead level in farm and fresh water fish.

Type of fish (N=80)	Mean ± Standard error	T value	P value
Farm fish (N=48)	-0.058750±0.593211	2.543	0.000
Fresh fish(N=32)	-0.104650±0.04945		

Table 2: Comparison of cadmium level in farm and fresh water fish.

Type of fish (N=80)	Mean ± Standard error	T value	P value
Farm fish (N=48)	0.050917±0.0619305	0.533	0.447
Fresh fish (N=32)	0.042563±0.097020		

Table 3: Comparison of mercury level in farm and fresh water fish.

Type of fish (N=80)	Mean ± Standard error	T value	P value
Farm fish (N=48)	7.872208±1.4728	-1.491	0.03
Fresh fish (N=32)	8.467125±0.739072		

Table 4: Comparison of arsenic level in farm and fresh water fish.

Type of fish (N=80)	Mean ± Standard error	T value	P value
Farm fish (N=48)	0.212750±0.1091	3.673	0.001
Fresh fish (N=32)	0.096688±0.0737		

DISCUSSION

In present study, out of all fish samples 80(100%) Fresh fish contain more lead -0.1046±0.05SD as compare to farm fish -0.0587±0.593SD (P=0.000). However, Yilmaz et al reported that farm fish samples had significantly high concentration of lead as compare to fresh water fish (p=0.05)²². Similarly Yilmaz et al found high concentration of lead in fresh river water (4.556ug/g) as compare to farm fish (0.012ug/g) due to of industrial waste dumping in natural water reservoirs²³.

In present study, out of all 80(100%) mean cadmium level in farm fish was 0.0509±0.06SD. while mean cadmium level in fresh water fish was 0.0425±0.097SD (P=0.44). chi et al reported that fresh water fish is significantly associated with cadmium accumulation leading towards liver damage(p=0.00)²⁴. Evidence exist that fresh water fish is more prone to be effected by cadmium metal (OR;1.2, 95% C.I, p=0.01) due to agriculture waste, nickel cadmium batteries waste and industrial waste dumping in fresh water^{25,26,27}.

In present study, Out of all fishes 80(100%) Fresh water fish contain high mercury 8.46712±0.739SD as compare to WHO standard and approximately similar values are reported in farm fishes 7.87220±1.472SD (p=0.03). chen et al reported that mercury level are found to be consistent within farm and fresh fish (p=0.34)²⁸. However, Qin et al reported that fresh fish sample contain high concentration of mercury(5.667ug/g) as compare to farm fish leading towards human health hazards(p=0.05). They also reported respiratory, liver dys-functioning, vomiting and nausea in living beings due to mercury affected fish utilization²⁹.

In present study, Out of all fish samples 80(100%) Farm fish contain high level of arsenic 0.21275±0.110SD

as compare to fresh fish $0.09668 \pm 0.07SD$ ($p=0.001$). Li et al reported arsenic is very common in *Cyprinus carpio* Linnaeus and *Pelteobagrus fluvidraco* leading towards liver and kidney disorders³⁰. A similar study reported that arsenic is more likely to accumulate in fresh water as compare to farm fish (OR; 1.3, 95% C.I, $p=0.02$) due to water pollution caused by industrial waste and mining waste³¹.

Present study found out that WHO standard limit for lead was $0.123 \mu\text{g/g}$ while mean lead level in current samples was $0.077 \mu\text{g/g}$. Cadmium level according to WHO standard was $0.195 \mu\text{g/g}$ while in current samples $0.047 \mu\text{g/g}$ reported. Mercury level according to WHO standard was $0.145 \mu\text{g/g}$ while in current samples mean $8.11 \mu\text{g/g}$ was found. Arsenic level in WHO limits was $0.12 \mu\text{g/g}$ while in current samples $0.166 \mu\text{g/g}$ was reported. Varol et al reported that lead content is highest in fish samples liver while cadmium content was similar in all organs of fish according to WHO standards of heavy metals concentration. They also reported copper highest content in gills and chromium highest concentration in kidney and liver while our study didn't consider these metals^{32,33}. Uysel et al analyzed seven metals in fresh and farm fish samples. These metals include As, Cd, Fe, Cu, Hg, Pb, Hg. All metals sediments exceed limits and quality assessment standards. However, potential ecological risk index showed moderate to high risk for living beings^{34,35}. Sankar et al reported that contaminated environment leads to food chain pollution resulting in health hazards. In this study analysis of heavy metals Cu, Mn, Zn, Cr, As, Hg, Fe and Pd was done in 5 fish samples and 5 crustacean species including shrimp, 3 crabs and one lobster. Atomic absorption spectrometer was utilized for understanding metal concentration in samples. Heavy metals concentration was reported in hierarchy $\text{Fe} > \text{Cd} > \text{Pb} > \text{Cr} > \text{Mn} > \text{Zn} > \text{Hg}$. Concentration of lead was significantly higher than Australian guidelines. A high concentration of heavy metals was found in crabs as compare to shrimp and lobster ($p=0.01$). Three fish species contain acceptable range of carcinogenic metals as compare to other samples ($p=0.05$)^{36,37}. Sivaperumal et al reported that 3 fish species were collected from xiang river in china. A high concentration of As, Cd, Cu, Mn, Pb and Fe was reported in muscles, gills

and liver. These metal concentration was measured through inductive coupled plasma mass spectrometer. They reported that Cd and Cu is most common in livers. High concentration of Mn and Pb was found in gills. High toxic levels of As, Cd and Pb was found in *P. Fulvidraco* while a high concentration of nutrients elements was reported in omnivorous species. They reported significant association between metal concentration and fish sizes³⁸.

Another similar study measures concentration of heavy metals Hg, Cr, Zn, Pb and Cd in muscle tissue of fish samples from Morava river. Atomic absorption spectrometer was utilized for metals analysis. They reported a significant positive correlation between metals (Hg, Zn, Cd and Cr) and fish age ($p=0.00$). Concentration of metals was $0.045\text{-}0.405$, $0.005\text{-}0.034$, $0.006\text{-}0.023$, $0.017\text{-}0.041$ and $5.57\text{-}61.32(\text{mg.kg}^{-1})$ in Hg, Pb, Cd, Cr and Zn respectively. This value did not exceed Czech Republic standard values. The analyzed content were found to be lower at monitoring sites as compare to other sites and the measured value were admissible in Czech Republic. Although total accumulation was highest in *P. fluvidraco* as compare to *C. carpio*^{39,40,41}.

CONCLUSION

Heavy metals accumulation in fresh and farm fish is an emerging global health issue. High level of mercury and arsenic are found in this study. However, concentration of mercury is above WHO defined limits as compare to other metals in all samples.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

- Bilal S:** Developed study, manuscript writing.
Junaid N: Methodology and laboratory analysis.
Khalil KUR: Statistical analysis.
Ayub R: Referencing and critical analysis.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.