

# Heavy metal residues in imported frozen fish and *Pangasius hypophthalmus* (Basa) fish fillets

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## ABSTRACT

To estimate the levels of mercury, cadmium and lead in imported frozen *Pangasius hypophthalmus* (*P. hypophthalmus*) fillets and Sardine; 70 random samples (35 of each) were collected randomly from different fish markets in Qalyubia and Gharbia governorates. The obtained results revealed that the mean levels of mercury, cadmium and lead were  $0.57 \pm 0.06$ ,  $0.11 \pm 0.01$  mg/kg and  $0.15 \pm 0.01$  in the examined samples of *P. hypophthalmus* and  $0.51 \pm 0.04$ ,  $0.14 \pm 0.02$  and  $0.09 \pm 0.01$  mg/kg in sardine, respectively. The levels of mercury and lead were significantly ( $p < 0.05$ ) higher in *P. hypophthalmus* as compared with sardine. Moreover, the majority of the examined fish samples, particularly, *P. hypophthalmus* exceeded the safe permissible limits. In this respect, 25.71%, 14.29% and 20% of the examined samples of *P. hypophthalmus* and 17.14%, 11.43% and 8.57% of the examined samples of sardine were over the higher permissible limits recommended by EOS [10] for mercury, cadmium and lead levels, respectively. In conclusion, the examined samples of *P. hypophthalmus* were significantly more polluted with high levels of toxic metals especially mercury and lead which seriously distress human health. Therefore, measures should be put in place to reduce the exposure to a minimum, and the daily intake should be set in accordance with the present findings.

**Keywords:** Basa, Cadmium, Lead, Mercury, Sardine

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## INTRODUCTION

Fish consumption is recommended because it is a good source of high quality protein, minerals, vitamins and omega-3 polyunsaturated fatty acids. The later composite protects consumers against coronary heart disease, reducing arrhythmias and thrombosis and risk of fatal heart attack and sudden death, lowers plasma triglyceride levels. Moreover, the intake of fish is beneficial to children's growth and development and against some diseases such as rheumatoid arthritis, psychiatric disorders and lung disease [26]. However, the presence of toxic heavy metals in fish makes it difficult to establish clearly the role of fish consumption on a healthy diet [5].

*Pangasius hypophthalmus* (*P. hypophthalmus*) (striped, river and sutchi catfish); commonly referred to as Basa fish; is present in the main rivers (e.g the Mekong and Chao Phraya) in Vietnam. The product is almost totally exported to over 100 countries including Egypt as frozen fillets, and conceivably its acceptability and popularity is growing. The absence of fishy odour, spines, small bones and skin, white or pale pink flesh, delicate flavour and firm texture when cooked, allow a wide range of preparations of *Pangasius* fillets. Taken together with their availability on the market in standard size, make Basa fillets suitable to the demands of the food service industry and restaurants. Also, *Pangasius* fillets are characterized by high moisture levels, low protein, lipid, cholesterol and polyunsaturated fatty acids, high percentage of saturated fatty acids and sodium content probably partially due to the sodium tripolyphosphate used to retain moisture [24].

The pollution of aquatic environment with heavy metals is considered a serious problem during the recent years. Owing to their toxicity persistence and tendency to accumulate in water and sediment, thus, become permanent additives to the aquatic environment. Heavy metals, when occurring in higher concentrations, become severe poisons for all living organisms, leading to devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [12]. Untreated public and industrial wastes together with inputs from the atmosphere are the major sources of heavy metals in the river and sea and subsequently inside the fish tissues. There are two major pathways for entrance of the heavy metals to fish; absorption across the gill surface and through the intestinal mucosa. Fish can bioaccumulate mercury and cadmium dissolved in water, but they cannot bioaccumulate lead, due to the low binding ability of lead with sulphhydryl group in fish muscles as well as low solubility of lead salts [26]. The consumption of fish contaminated with heavy metals is the most likely route for human exposure leading to potential health risks especially for children [29]. The main threats to human health from heavy metals are associated with exposure to mercury, cadmium and lead, due to their known toxicity to human being, even at low concentrations when ingested over a long period [21]. These toxicants accumulate in the body, especially in liver leads to defects in cellular uptake mechanism in the mammalian liver and kidney and inhibit hepatic and renal sulfate/bicarbonate transporter through various mechanisms, leading to sulfaturia following heavy metal intoxication. Furthermore, freezing or heat treatment of fish for long period of time cannot destroy the heavy metals [8]. Thus, there is great risk associated with consumption of the frozen fish.

Starting from these considerations, the objective of this work is to investigate the presence of some heavy metals (mercury, cadmium and lead) in frozen *P. hypophthalmus* fillets and sardine, collected from different Egyptian fish markets and comparing the obtained results with the EOS [10] permissible limits.

## **MATERIAL AND METHODS**

### **Fish samples:**

70 random samples of imported frozen Basa (*Pangasius hypophthalmus*) fillets and Sardine (35 of each) were collected from different fish markets at Qalyubia and Gharbia governorates. Each sample was kept in a separate sterile plastic bag and transferred to the laboratory in an insulated ice box as quickly as possible.

### **Determination of heavy metals [wet digestion technique]:**

The samples were prepared and digested according to the technique described by Shibamoto and Bjeldanes [28]. Washing of equipment is an important process to avoid contamination especially when trace elements or heavy metals are to be analyzed. The tubes, plastic film and glassware were soaked in water and soap for 2 hours and then rinsed several times with tap water, they were rinsed once with distilled water, once with mixture (520 ml deionized water, 200 ml conc. HCl and 80 ml H<sub>2</sub>O<sub>2</sub>) and once with washing acid (consisted of 900ml deionized water and 100 ml conc. HCl) then followed by washing with deionized water and air –dried in an incubator away from contamination or dust. Briefly; after washing, one gram from each sample of dorsal muscle was digested by 10 ml of digestion mixture (60 ml Nitric acid 65% and 40 ml Perchloric acid 70-72 %) in screw capped tube after maceration by sharp scalpel. The tubes were tightly closed and the contents were vigorously shaken, stand overnight at room temperature and heated for 4 hours in

water bath at 70°C to ensure complete digestion of samples. Cooled samples were diluted with 10 ml deionized water, thoroughly mixed, filtered with Whatman filter paper and kept at room temperature until analyzed for heavy metal contents. Blank and standard solutions were prepared in the same manner. Digested samples, blanks and standard solutions were analyzed for mercury, lead and cadmium contents by Atomic Absorption Spectrophotometer (AAS) (UNICAM969AA Spectrophotometer) at the adjusted conditions (table 1)

Table 1 Analysis conditions of mercury, lead and cadmium by Atomic Absorption Spectrophotometer

Heavy metal condition	Mercury	Lead	Cadmium
Lamp wave length (nm)	253.7	217.0	228.8
Lamp current (m. amp)	10	15	8
Fuel flow rate	1.2	14	1.2
Measurement time (seconds)	4.0	4.0	4.0
Detection limit (mg/kg)	0.01	0.01	0.01

### Calculation and statistical Analysis:

Mercury absorbency was recorded directly from the digital scale of AAS and its concentration (mg/kg) was calculated according to the following equation:

$$C_1 = \frac{A_1}{A_2} \times C \times \frac{D}{W}$$

C<sub>1</sub>= Concentration of mercury (mg/kg) wet weight. A<sub>1</sub>= Absorbency reading of sample solution. A<sub>2</sub>= Absorbency reading of standard solution. C= Concentration of mercury on the standard solution. D= Dilution factor of sample. W= Weight of each sample.

The concentration of lead and cadmium (mg/kg) was estimated according to the following equation:

$$C = R \times \frac{D}{W}$$

C=concentration of lead (mg/kg) wet weight. R=reading of digital scale of AAS. D= Dilution of prepared sample. W= Weight of the sample.

The obtained results were statistically evaluated by using student *t*-test according to Feldman *et al.* [13].

## RESULTS and DISCUSSION

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 [30].

The results presented in table (2) showed that the concentrations of mercury ranged from 0.27 to 1.04 and 0.19 to 0.85 in imported frozen *P. hypophthalmus* and sardine samples, respectively. Statistical analysis revealed a significant (P<0.05) higher mercury content in *P. hypophthalmus* (0.57 ± 0.06 mg/kg) than in sardine (0.51 ± 0.04 mg/kg). Moreover, 25.71% and 17.14% of the examined *P. hypophthalmus* and sardine samples, respectively, were found to exceed the permissible limits (0.50 mg/kg) recommended by EOS [10]. Such variations in mercury concentrations among the examined fish samples could be attributed to variation in feeding habits, species and age of the fish under investigation [19]. Nevertheless, higher findings were reported by El-Sayed [11] who recorded that the mean mercury concentration in sardine was 0.74 mg/kg. Lower results were obtained by Hassan and Salem (0.32 ± 0.02 mg/kg) [14].

Mercury is ubiquitous in the environment and therefore every human being, irrespective of age and location, is exposed to one form of mercury or another. The major sources of environmental mercury are urban discharges, agriculture materials, mining, combustion and industrial discharges [31].

Methyl mercury is very stable and bioaccumulates in the food chain. Therefore, fish, especially predatory fish and marine mammals, are major sources of methyl mercury. Accurately, mercury is recognized as a highly toxic metal and a cumulative poison. The toxicity of mercury may be due to its competition with essential metals for zinc and copper binding sites, and due to its great affinity for thiol or sulphhydryl groups of proteins [20].

Methyl mercury is more toxic to man than inorganic form, because it cannot be excreted from the body and can cross the blood brain barrier to the nervous system, so it is a well – known neurotoxicant, resulting in progressive and irreversible brain damage [2].

Consumption of mercury contaminated fish may result in Mina Mata disease in human which is characterized by loss of sensation at extremities of the fingers and toes and areas around the mouth, muscular weakness, loss of vision, loss of hearing, slurred speech, cerebral palsy, paralysis and coma. In addition, mercury poisoning may cause gonadotoxic, mutagenic and nephrotoxic effects, mental retardation in children, myocardial infarction, coronary heart disease (CHD), congestive impairment, dyspnoea, chills, bilateral infiltration in the lung [22].

Cadmium is an industrial and environmental pollutant that affects adversely human health, and progressively accumulates inside the body particularly kidneys. Results given in table (3) indicated that the concentrations of cadmium ranged from 0.03 to 0.24 and 0.04 to 0.42 mg/kg in imported frozen *P. hypophthalmus* and sardine samples, respectively. There was no significant difference in the cadmium content of *P. hypophthalmus* ( $0.11 \pm 0.01$  mg/kg) and sardine ( $0.14 \pm 0.02$  mg/kg) samples. Furthermore, 14.29% of *P. hypophthalmus* and 11.43% of sardine samples under investigation exceeded the permissible limits of the cadmium (0.10 mg/kg) recommended by EOS [10]. Nearly similar results were recorded by El-Sayed ( $0.14 \pm 0.01$  mg/kg) [11]. However, lower results were previously obtained by Hassan ( $0.05 \pm 0.01$  mg/kg) [15].

Cadmium occurs in nature as a natural component of rock and sediment, soil and dust, air and water, plant and animal tissues. Cadmium exposure occurs mainly through two sources, the first is the oral route through water and food contaminated with cadmium, since food contributes 80-90% of the cadmium dose received by most people and the second source is through inhalation of cadmium particles, which arise from tobacco smoke and cigarette smoking. Mining, metal processing, house dust, electroplating, jewelry industry, zinc and lead refining, smelting of cadmium and lead and the burning of coal and other fossil fuels. Cadmium is also present in several unlicensed medicinal herbal remedies used widely in parts of India and Nigeria [3].

Cadmium is a cumulative toxic agent with a biological half – life of 10-30 years. Cadmium burden of the body increases with age and found to be greater in smokers than in non smokers. Accurately, cadmium acts on sulphhydryl groups of essential enzymes and also binds to albumin, phospholipids and nucleic acids, interferes with oxidative phosphorylation and replaces zinc in enzymes so changing their activities [4].

Cadmium is a severe pulmonary and gastrointestinal irritant, which can be fatal if inhaled or ingested. Furthermore, cadmium plays a role in hypertension, prediabetes

and diabetes mellitus in human, through injury of adrenal gland, adipose, hepatic, and pancreatic tissue, especially cells within islets of Langerhans, reducing insulin levels, altering glucose metabolism and / or glucose uptake that ultimately results in increased blood glucose. Elevated blood glucose levels coupled with the direct effects of cadmium on renal tissue eventually leads to diabetic nephropathy [9].

Cadmium is an industrial and environmental pollutant that affects adversely a number of organs in human, and progressively accumulates inside the body particularly kidneys, which is the target organ, whatever the source and portal of entry of cadmium containing more than half of the body burden of cadmium after long exposure. Therefore, it is nephrotoxic pollutant, causing kidney damage, end stage renal disease (ESRD), irreversible proximal tubular reabsorptive dysfunction, glomerular damage with decreased glomerular filtration rate, irreversible renal failure, renal Fanconi syndrome, nephrolithiasis, nephritis and nephrosis, kidney stones and overall mortality [18].

Moreover, cadmium poisoning may result in a case called Itai-Itai or Ouch-Ouch disease, which is a combination of osteomalacia and osteoporosis [17]. Cadmium is classified as a probable human carcinogen (group I) [16]. Interestingly cadmium is not directly genotoxic, but only weakly mutagenic in mammalian cells [7].

Lead is one of the most ubiquitous metals known to humans. It reaches the aquatic system because of superficial soil erosion, atmospheric deposition. The results presented in table (4) showed that lead level ranged from 0.05 to 0.31 and 0.02 to 0.18 in imported frozen *P. hypophthalmus* and sardine samples, respectively. Statistical analysis revealed a significant ( $P < 0.05$ ) higher lead content in *P. hypophthalmus* ( $0.15 \pm 0.01$  mg/kg) than in sardine ( $0.09 \pm 0.01$  mg/kg). Moreover, 20% and 8.57% of the examined *P. hypophthalmus* and sardine samples, respectively, were found to exceed the permissible limits (0.10 mg/kg) recommended by EOS [10]. Lower results were obtained by Hassan and Salem ( $0.03 \pm 0.01$  mg/kg) [14]. In contrast, higher findings ( $0.18 \pm 0.02$  mg/kg) were reported by El-Sayed [11].

Environmental lead toxicity is an old but persistent public health problem throughout the world. The absorption and biological fate of lead are affected by a variety of factors, including an individual's nutritional status, health and age. Children, pregnant women, and the malnourished can absorb 40-70% of ingested lead. Dietary deficiencies of iron, calcium, zinc and ascorbic acid can result in increased gastrointestinal absorption of lead [27].

Lead is one of the most ubiquitous metals known to humans. It reaches the aquatic system because of superficial soil erosion, atmospheric deposition. The main route of exposure for general population is food and air. The main sources of lead are diet, old lead – based paint, lead in soil and dust from contaminated leaded paint and gasoline, mining and industrial activity, indoor floor dust, hand – to – mouth behavior, concurrent malnourishment and pica activity in children and lead smelting. Crops grown in soils contaminated with lead residues from pesticides or atmospheric lead and packaged in cans made with lead solder, lead emissions from petrol, improperly prepared infant formula, metal welding, car accumulators and lead – containing scraps and sewage effluents [1].

The most common routes of lead absorption in adults and children are inhalation, ingestion and skin, respectively. The most sensitive targets for lead toxicity are the developing nervous system, the haematological, gastrointestinal, renal, reproductive and cardiovascular systems. Excretion of lead is primarily via the kidneys and can also be excreted with bile through the gastrointestinal tract, and the half life of lead in

blood is about 30 days 20-30 years in bone which contains up to 94% of the body burden of lead [23].

Lead exposure has also been associated with reduced bone growth in fetuses and children, resulting in reduced head circumference and stature. Lead interferes with bone formation, maturation and resorption and may also be a potential risk factor for osteoporosis. Lead may exert both indirect and direct actions on bone turnover. Signs and symptoms of acute lead poisoning in adults may include abdominal pain, anorexia, nausea, severe vomiting, intestinal cramps, epigastric, colic, constipation, headache, joint and muscle pain, convulsions, hemolytic anemia [6].

## CONCLUSION

In conclusion, the current study proved that there are great variations in the levels of mercury and lead in the examined samples of fish. In addition, the examined samples of *P. hypophthalmus* were significantly polluted with high levels of toxic metals which seriously affect the human health. In other words, the continuous consumption of these contaminated fish may result in public health hazard through progressive irreversible accumulation of such toxic pollutants in the human body. The potential harm from these metals suggested that people should not eat smaller quantities of fish known to accumulate heavy metals only, but also they should eat a diversity of fish in order to avoid consuming unhealthy quantities of heavy metals.

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Table 2 Concentration of mercury (mg/kg) in the examined imported frozen fish samples and their acceptability according to EOS [10]

Samples	Permissible limits	Positive samples		Rejected samples		Min.	Max.	Mean $\pm$ S.E. (n=35)
		N	%	n	%			
<i>Pangasius hypophthalmus</i>	0.5	18	51.43	9	25.71	0.27	1.04	0.57 $\pm$ 0.06*
Sardine	0.5	15	42.86	6	17.14	0.19	0.85	0.51 $\pm$ 0.04

n= number of samples. \* indicated significant differences (P < 0.05) between the examined *Pangasius hypophthalmus* and sardine samples estimated with student *t*-test.

Table 3 Concentration of cadmium (mg/kg) in the examined imported frozen fish samples and their acceptability according to EOS [10]

Samples	Permissible limits	Positive samples		Rejected samples		Min.	Max.	Mean $\pm$ S.E. (n=35)
		N	%	n	%			
<i>Pangasius hypophthalmus</i>	0.1	11	31.43	5	14.29	0.03	0.24	0.11 $\pm$ 0.01 <sup>NS</sup>
Sardine	0.1	10	28.57	4	11.43	0.04	0.42	0.14 $\pm$ 0.02

n= number of samples. <sup>NS</sup> indicated a non-significant difference between the examined *Pangasius hypophthalmus* and sardine samples.

Table 4 Concentration of lead (mg/kg) in the examined imported frozen fish samples and their acceptability according to EOS [10]

Samples	Permissible limits	Positive samples		Rejected samples		Min.	Max.	Mean $\pm$ S.E. (n=35)
		N	%	n	%			
<i>Pangasius hypophthalmus</i>	0.1	12	34.29	7	20	0.05	0.31	0.15 $\pm$ 0.01*
Sardine	0.1	8	22.86	3	8.57	0.02	0.18	0.09 $\pm$ 0.01

n= number of samples. \* indicated significant differences (P < 0.05) between the examined *Pangasius hypophthalmus* and sardine samples estimated with student *t*-test.

### الملخص العربي متبقيات المعادن الثقيلة في الأسماك المجمدة و شرائح سمك الباسا ريهام عبد العزيز أمين قسم مراقبة الأغذية - كلية الطب البيطري بمشنتهر - جامعة بنها

أجريت هذه الدراسة لمعرفة مدى تلوث بعض الأسماك و منتجاتها ببعض المعادن الثقيلة مثل الزئبق، الكاديوم و الرصاص لما لها من آثار سمية تراكمية تمثل خطورة بالغة على صحة المستهلك. استخدم في هذه الدراسة سبعون عينة من الأسماك المجمدة و تشمل البانجيس هيبوفثالامس "سمكة الباسا" و السردين، بواقع خمسة و ثلاثون عينة من كل نوع تم تجميعا من محلات مختلفة بمحافظة القليوبية والغربية. دلت نتائج الدراسة على أن متوسطات تركيزات الزئبق، الكاديوم و الرصاص (مجم/كجم) كانت 0.57 ، 0.15 و 0.11 في عينات الباسا بينما كانت 0.51 ، 0.09 و 0.14 في عينات السردين على التوالي. كما أوضحت النتائج أن عينات الباسا كانت الأكثر تلوثا بالمعادن الثقيلة عند مقارنتها بالسردين خصوصا الزئبق والرصاص التي تؤثر جديا على صحة المستهلك. وفي هذا الصدد فإن 25.70%، 14.29%، و 20.00% من عينات الباسا، وكذلك 17.14%، 11.43% و 8.57% من عينات السردين قد تجاوزت الحدود المسموح بها لعناصر الزئبق، الكاديوم و الرصاص على الترتيب. من هذه الدراسة نخلص الى أنه استهلاك هذه الأسماك الملوثة ستؤدي الى مخاطر صحية نتيجة لتراكم مثل هذه الملوثات السامة في جسم الانسان. لذلك لابد من وضع بعض الاحتياطات لتقليل معدل التلوث لأقل حد ممكن و أيضا لابد من توافق معدل تناول السمك اليومي مع النتائج التي تم الحصول عليها.