

Determination of Trace Elements Level of Pikeperch Collected from the Caspian Sea

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Abstract Toxic and essential elements levels (chromium, copper, iron, manganese, nickel, lead, cadmium, and zinc) have been determined in the brain, heart, liver, gill, gonads, spleen, bile and muscle of *S. lucioperca*, collected from the Caspian Sea by employing Flame- Atomic absorption spectrometry. Results indicated that nearly all of the toxic metals concentrations (nickel, lead and cadmium) in tissues were higher than limits for fish suggested by Food and Agricultural Organization, World Health Organization and European Union. Lead was higher in spleen than other tissues. Levels of essential metals (iron, copper, zinc and manganese) were below the limits suggested by European Union and Food and Agricultural Organization/World Health Organization and Turkish Food Codex for fish. Iron distribution pattern in tissues was in the following order: heart ($88.67 \pm 2.74 \mu\text{g g}^{-1}$ wet wt) > spleen ($70.96 \pm 2.05 \mu\text{g g}^{-1}$ wet wt) > bile ($29.35 \pm 0.94 \mu\text{g g}^{-1}$ wet wt) > brain ($14.29 \pm 0.51 \mu\text{g g}^{-1}$ wet wt) > liver ($8.57 \pm 0.29 \mu\text{g g}^{-1}$ wet wt) > gill ($3.20 \pm 0.14 \mu\text{g g}^{-1}$ wet wt) > red ($2.79 \pm 0.11 \mu\text{g g}^{-1}$ wet wt) and white muscles ($2.79 \pm 0.09 \mu\text{g g}^{-1}$ wet wt) > gonads ($2.57 \pm 0.07 \mu\text{g g}^{-1}$ wet wt).

Keywords Toxic and essential elements · *Sander lucioperca* · Flame-Atomic absorption

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Recently interest in consumption of aquatic foods, especially fish, is widely increases in different areas of the world, because of their high protein content and low saturated fats. They also contain omega fatty acids and also they can support good health and protect body against cardiovascular diseases (Tuzen 2009). Metals pollution especially in sea-originated foods such as fish represents serious food safety concern in both of developed and undeveloped countries (Mendil et al. 2005). Mainly pollution sources leads to fish contamination with metals originated from industrial and domestic wastewaters (Tuzen 2009). Metals can be classified as potentially toxic (Cd, Pb, Hg), semi-essential Ni, Co and essential Cu, Zn, and Se (Yildirim et al. 2009). The essential metals can be toxic when present in excess level; elevated intake is of concerns. Toxic metals can damage to both of marine species diversity and ecosystems, due to their toxicity and accumulative behaviors and it can be subsequently transferred to human through the food chain (Turkmen et al. 2009). Therefore, it is important to determine the chemical quality of the marine organisms, particularly the levels of metals, in order to evaluate the possible risk to human health (Cid et al. 2001). *Sander lucioperca* (Percidae family) is known as freshwater and briny water fish, and a semi-anadromous, found in watershed and in the basins of Caspian Sea (Abdolmalaki and Psuty 2007). It seems to prefer salinities below 12%. Pikeperch has considerable economic and ecologic importance at a regional level of the Caspian ichthyofauna (Abdolmalaki and Psuty 2007). Also it occurrence in the Caspian Sea is restricted to estuaries and coastal zones, so it confronts the pollutions (Abdolmalaki and Psuty 2007; Kazancheyev 1981). The fishes were collected from the Caspian Sea, the world's largest lake with $386,400 \text{ km}^2$ (Dumont 1998) and bordered by five countries i.e. Azerbaijan, Iran, the Russian

Federation, Kazakhstan and Turkmenistan. Many factors may influence metal levels in fish such as fish species, living area and type of nutrition, development levels and sexuality as well as the physical and chemical characteristics of the waters.

Mainly, the fish absorb metals via two uptake routes: digestive tract (diet exposure) and gill surface (water exposure) (Turkmen et al. 2009). Then, metals are transferred via blood to other target organs, such as liver. Previous studies have been shown that trace metals accumulate mainly in liver, where metals were stored for detoxification through metallothioneins. The Muscle is not important tissue in metals agglomeration, except mercury, the study of potential metals accumulations in this tissue of fish is justified because it has been consumed by humans (De Carvalho and Hartz 2009). The aim of present study was to determine the metal levels (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in brain, heart, liver, gill, gonads, spleen, bile and muscle (red and white) of *S. lucioperca* collected from the Caspian Sea. The results of this study will be important to understand the level of metal pollution metals in common fish species of the Caspian Sea.

Materials and Methods

Twenty-four specimens of *S. lucioperca* were collected from three sampling locations (I-Babolsar; II-Chapakrood; III- Farah Abad at the Caspian Sea in November 2010 (Fig. 1). Fishes were transferred to the laboratory to record sex, age, total body length, and total wet weight. The results are given in Table 1. The age of *S. lucioperca* was determined from computation of annual growth rings on fish (between 1 and 2 years). All experiments were performed according to the norms of the ethical committee of University of Mazandaran, Babolsar which is in accordance with the national guidelines for animal care and use.

Trace elements levels were measured using flame-atomic absorption spectroscopy as described in the 1984 Perkin-Elmer manual. Briefly, 0.1 g of samples were weighed and placed in metal-free glass tubes (washed with hydrochloric acid). The samples were dried in oven at 115°C for 5 h; 0.3 ml of concentrated sulphuric acid was added, and the samples were digested for 2 days. Subsequently, 0.1 ml of concentrated nitric acid and 50 µL of hydrogen peroxide were added, and the freed metal salts were determined using atomic absorption spectroscopy (Perkin-Elmer AAS 100 Wellesley, MA, USA). The results were expressed as microgram of element per gram of tissue-wet weight (Eslami et al. 2011). The digestion and analytical procedures were checked by the analysis of standard reference material (DORM-2 and DOLT-3 National Research Council Canada, Ottawa, ON, Canada). The results are given in Table 2.

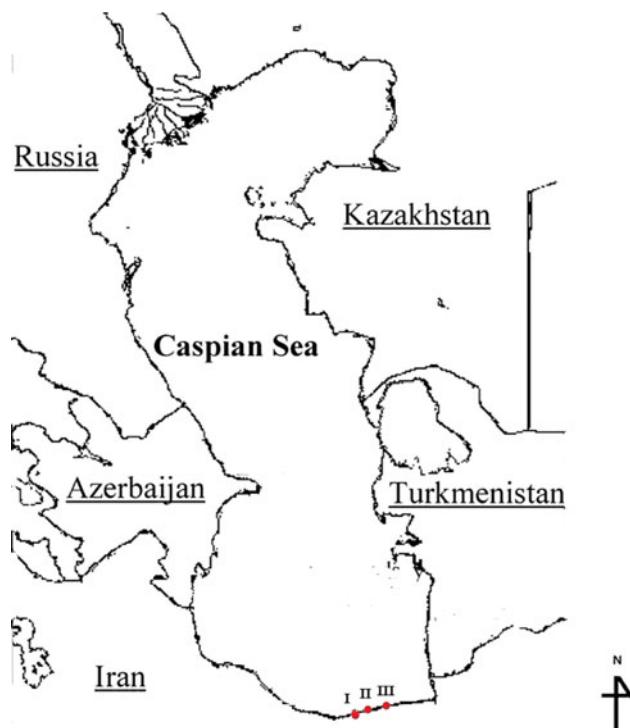


Fig. 1 Sampling locations (I—Babolsar; II—Chapakrood; III—Farah abad) in Caspian Sea

Table 1 Some morphometric and biological characteristics (Mean \pm SD) of *Stizostedion lucioperca*

| Sex | n | Age (year) | Fork length (mm) | Length (mm) | Body weight range (g) |
|-----|----|------------|--------------------------|-----------------------------------|-------------------------------|
| ♂♂ | 24 | 1–2 | 80 \pm 5.65 (76–84) | 346.5 \pm 16.7 (332.2–361.5) | 542.5 \pm 68.1 (485–640) |

Values in parentheses indicate minimum—maximum values

Table 2 Certified metal concentration in reference material

| Reference material | Element | Certified ($\mu\text{g g}^{-1}$) | Found ($\mu\text{g g}^{-1}$) | RSD (%) |
|--------------------|---------|------------------------------------|--------------------------------|---------|
| DOLT-3 | Cu | 31.2 \pm 1.0 | 33.7 \pm 0.4 | +8 |
| | Zn | 86.6 \pm 2.4 | 94.3 \pm 0.2 | +9 |
| | Ni | 2.72 \pm 0.35 | 2.52 \pm 0.18 | -7 |
| | Cd | 19.4 \pm 0.6 | 17.8 \pm 0.8 | -8 |
| DORM-2 | Fe | 142 \pm 10 | 138 \pm 8 | -3 |
| | Cu | 2.34 \pm 0.16 | 2.68 \pm 0.2 | +7 |
| | Mn | 3.66 \pm 0.34 | 3.96 \pm 0.24 | +8 |

Data are mean (\pm SD) of five determinations

Sixteen blank control solutions were used to estimate the detection limits of the investigated elements following the same analytical procedures. Three times of the standard deviation was used as detection limit (Table 3).

Table 3 Operating conditions of the atomic absorption spectrometer (AAS 100)

| Detection limits ($\mu\text{g g}^{-1}$) | Melting point (°C) | Drying temp. (°C) | Detection wave length (nm) | Elements |
|---|--------------------|-------------------|----------------------------|----------|
| 0.033 | 1085 | 120 | 324.8 | Cu |
| 0.047 | 1535 | 120 | 248.3 | Fe |
| 0.035 | 450 | 120 | 213.9 | Zn |
| 0.009 | 1246 | 120 | 279.5 | Mn |
| 0.056 | 1455 | 120 | 232.0 | Ni |
| 0.018 | 1900 | 120 | 357.9 | Cr |
| 0.020 | 327.46 | 120 | 283.4 | Pb |
| 0.007 | 321.07 | 120 | 228.8 | Cd |

For all elements, the slit width was 0.2 nm. The air and acetylene flow rates were 4.0 and 0.5 L min^{-1} , respectively

Descriptive statistical analysis was used. Student Newman–Keuls test was employed for the comparison of means. The significance was set at $p < 0.05$.

Results and Discussion

The concentrations of trace elements (microgram per gram wet-tissue) in brain, heart, liver, gill, gonads, spleen, bile and red and white muscles of *S. lucioperca* were summarized in Table 4. Fe, Zn, Cu, Pb, Mn, Cd and Ni were detected in nearly all of samples. Fe levels were higher than other metals in bile, heart, liver and spleen. Distribution patterns of metal concentrations in the brain, heart, liver, gill, gonads, spleen, bile, red and white muscle of *S. lucioperca* follow the sequence: Zn > Fe > Pb > Mn > Ni > Cu > Cd; Fe > Zn > Ni > Pb > Mn > Cu > Cd; Fe > Zn > Pb > Ni > Mn > Cu > Cd; Zn > Fe > Pb > Ni > Mn > Cu > Cd; Pb > Ni > Fe > Mn > Cu > Cd; Fe > Zn > Pb > Ni > Mn > Cu > Cd; Fe > Zn > Pb > Ni > Mn > Cu > Cd; and Zn > Fe > Pb > Ni > Mn > Cu > Cd, respectively. The distribution

patterns of Fe in tissues of *S. lucioperca* follow the order: heart > spleen > bile > brain > liver > gill > white muscle > red muscle > gonads; of Zn following the order: Spleen > brain > heart > bile > gill > red muscle > white muscle > liver. The distribution patterns of Cu follow the order: Spleen > heart > bile > brain > gonads > gill > white muscle > liver > red muscle. The distribution patterns of Mn follow the order: Bile > spleen > brain > heart > gonads > liver > gill > white muscle > red muscle. The distribution patterns of Ni follow the sequence: Heart > bile > spleen > gonads > brain > liver > white muscle > gill > red muscle; the distribution patterns of Pb follow the order: Spleen > brain > heart > bile > gonads > liver > white muscle > gill > red muscle; the distribution patterns of Cd follow the order: Heart > spleen > bile > brain > gonads > liver > gill > red muscle > white muscle. Metal contents (Cd, Ni) were higher in heart compared to other metals. Metal levels varied significantly in different tissues of the same species.

The metals may accumulate in the tissues of fish, which are often at the top of the aquatic food chain and they might have toxic effect on human health (Turan et al. 2009). Therefore, recently great attentions have been paid to determination of the trace metals in fish (Turan et al. 2009; Oymak et al. 2009; Dural et al. 2007). The present study focused on the accumulation of metals in different tissues of *S. lucioperca*. Mainly, metabolically active tissues such as the liver are accumulation place of metals. In the liver, metals are bound to metallothioneins, low molecular weight proteins with high cysteine content (Oymak et al. 2009). It is the major organ involved in xenobiotic metabolisms, while the gills are the primary site of metal uptake from the water, especially if metals are bound to particulate matters (Klavins et al. 2009). Zinc is an essential element involved in many metabolic activities, and its deficiency can lead to loss of appetite and growth, skin damages and immunological abnormalities. Samples had lower levels of Zn when compared with the corresponding Turkish Food Codex (TFC) limits of 50 mg kg^{-1}

Table 4 Metal concentrations ($\mu\text{g g}^{-1}$ wet wt) in tissues of *Sander lucioperca* from Caspian Sea

| Tissues | Liver | Heart | Spleen | Brain | Red-Muscle | Gill | White-Muscle | Gonad | Bile |
|---------|-------------|--------------|--------------|--------------|-------------|-------------|--------------|-------------|--------------|
| Fe | 8.57 ± 0.29 | 88.67 ± 2.74 | 70.96 ± 2.05 | 14.29 ± 0.51 | 2.79 ± 0.11 | 3.20 ± 0.14 | 2.79 ± 0.09 | 2.57 ± 0.07 | 29.35 ± 0.94 |
| Cu | 0.43 ± 0.01 | 2.97 ± 0.10 | 3.62 ± 0.13 | 1.82 ± 0.06 | 0.36 ± 0.01 | 0.55 ± 0.02 | 0.50 ± 0.01 | 0.62 ± 0.02 | 2.24 ± 0.12 |
| Ni | 1.95 ± 0.05 | 17.64 ± 0.64 | 7.83 ± 0.28 | 3.30 ± 0.14 | 1.46 ± 0.04 | 1.63 ± 0.05 | 1.77 ± 0.04 | 4.74 ± 0.18 | 9.82 ± 0.34 |
| Zn | 3.99 ± 0.19 | 31.80 ± 0.96 | 40.44 ± 1.34 | 39.63 ± 1.01 | 4.79 ± 0.24 | 6.08 ± 0.31 | 4.72 ± 0.21 | ND | 28.78 ± 0.87 |
| Pb | 3.89 ± 0.17 | 12.82 ± 0.47 | 15.60 ± 0.58 | 13.13 ± 0.53 | 1.94 ± 0.06 | 2.00 ± 0.08 | 2.18 ± 0.09 | 5.59 ± 0.27 | 12.06 ± 0.44 |
| Cd | 0.33 ± 0.02 | 1.61 ± 0.06 | 1.54 ± 0.07 | 0.58 ± 0.02 | 0.23 ± 0.00 | 0.26 ± 0.01 | 0.17 ± 0.00 | 0.48 ± 0.01 | 0.97 ± 0.03 |
| Mn | 1.43 ± 0.07 | 5.23 ± 0.31 | 5.97 ± 0.36 | 5.70 ± 0.34 | 0.79 ± 0.02 | 1.23 ± 0.04 | 1.11 ± 0.05 | 1.93 ± 0.07 | 6.18 ± 0.46 |

ND not detected. Values are mean ± SD

(Yildirim et al. 2009; Mendil et al. 2010). In addition, Cu is an essential element for good health, but the elevated intake can cause adverse health problems (Uriu-Adams and Keen 2005). The Cu level in all of samples was below the TFC limits of 20 mg kg^{-1} (Yildirim et al. 2009; Mendil et al. 2010). All samples were less than the permissible limits for human consumptions in comparisons to the Canadian food standards (Cu, 100 mg kg^{-1} ; Zn, 100 mg kg^{-1}) (De Carvalho and Hartz 2009). It shows that the levels of these metals in the edible tissues of the fish are lower than the above-mentioned guidelines. Some trace elements are essential to all cells and deficiencies of essential metals cause different diseases. In diabetes, Cr, Cu, and Zn have indirect roles in the insulin secretion process from beta cells of the langerhans islets (Chausmer 1998). Cr (III) is another essential element that influences the carbohydrates, lipids and proteins metabolisms. However, Cr (VI) is highly carcinogenic (Yildirim et al. 2009). There is little evidence of Cr accumulations in fish (De Carvalho and Hartz 2009). In the literature, Cr levels in fish have been reported in the range of $0.06\text{--}2.63 \text{ mg kg}^{-1}$ for muscles and $0.20\text{--}3.97 \text{ mg kg}^{-1}$ for livers of fish from Turkish seas (Turkmen et al. 2009). No limits were established for Cr concentrations in fish and fishery products in TFC. No Cr was found in fish in our study. Excessive Cd in human body and may induce renal failure and sterility (Yildirim et al. 2009). In fish, Cd can cause metal blocks thiol group (SH) in enzymes and leads to enzymatic dysfunction (De Carvalho and Hartz 2009). The limit value for cadmium in the edible part of fish, proposed by the European Commission is 0.05 mg kg^{-1} wet weights and according to TFC is 0.1 mg kg^{-1} wet weights (Mendil 2010; Commission of the European Communities 2001). In this study, all analyzed samples presented concentrations more than the proposed limits and Cd exists in high-risk level especially in heart and spleen. Pb poisoning is generally ranked as the most common environmental health hazards. Impairments of hearing abilities, anemia, renal failures, weakened immune systems, low birth weights, still births and miscarriages, premature births, elevated blood, and urine Pb levels are the most common symptoms of Pb poisoning (Yildirim et al. 2009; Mel et al. 2007). The European Community established threshold values of non-essential metal concentration of fish muscles is $0.3 \text{ } \mu\text{g g}^{-1}$ per wet weight for lead (Regulation (EC) 2008). The maximum Pb levels permitted for fish are 0.4 mg kg^{-1} according to TFC (Mendil et al. 2010). Concentrations of Pb measured in fish were so much higher than the levels issued by FAO and TFC limits (De Carvalho and Hartz 2009). Ni is a problematic element for human life (Yildirim et al. 2009; Kong et al. 2007). The maximum daily intake for an adult man (a 70 kg person) is $25 \text{ } \mu\text{g}$ (Leterme et al. 2006). The concentrations of Ni measured in many of

tissues especially spleen, bile and heart of fish were higher than the permissible limit for human. There is no information about maximum Ni levels in fish samples in Turkish standards (Mendil et al. 2010). Mn plays a vital role in biochemical processes and improvement of impaired glucose tolerance and has an indirect role in the management of diabetes mellitus (Choudhury et al. 2007). Also, it can protect against skeletal deformities and gonadal dysfunctions (Eslami et al. 2011). The daily mineral requirement of an adult man (a 70 kg person) is 2.8 mg (Kong et al. 2007). The reason for presence of Pb in *S. lucioperca* tissues is probably due to the increase of industrial activities of petroleum companies and sailing as well as pollution in the Caspian Sea. In addition, it might originate from agricultural waste shed to the Caspian Sea water basins. The range of international standards for Pb in fish is $0.5\text{--}10 \text{ mg kg}^{-1}$ wet weight depending on the types of fish (Commission of the European Communities 2001; TFC 2002). In this study, Zn level of the spleen and brain were higher than in other tissues. In addition, the level of Zn was higher in liver because of its role in the enzymatic processes. Metal levels in the gill could be because of metal complexion with the mucus, which is impossible to be completely eliminated from the lamellae prior to tissues are prepared for analysis. The metals adsorption into the gill surface can affect the metal levels of the gill (Oymak et al. 2009). Furthermore the liver tissues act as metals storing. Thus, the liver and the gill in fishes have been known as environmental indicator organs of water pollutions (Oymak et al. 2009). In this study, the gill metal concentrations were not so high except Cd that was higher than standards. Gills are critical organs in fish for respiratory and excretory functions. A high rate of metal absorption through the gills also makes fish susceptible to its exposure (Oymak et al. 2009). There were no reported data about trace elements determination in *S. lucioperca*. Of the nine tissue types analyzed, humans only consume muscle. Furthermore, the sampling and preparation of muscle tissue is easy. Metal concentrations found in the tissues of *S. lucioperca* varied considerably, and levels of toxic metals (Ni, Cd, Pb) were higher in heart and spleen than liver and other tissues (Table 4). The concentrations of these elements were higher than minimum limit of detection for the males. Numerous studies have shown that concentrations of metals are usually increased in liver with comparison of muscle tissue (Canli and Atli 2003; Moiseenko and Kudryavtseva 2001). The high concentrations of Zn and Fe metal in the red and white muscles examined in this work could reflect the high levels of these binding proteins in the muscle tissue (Allen-Gil and Martynov 1995). On the other hand, the increase of Cu level in liver was likely to be due to the fact that this metal could be bio-accumulated by some fish species without any

toxic effects (De Carvalho and Hartz 2009). Some of toxic metal levels detected in all of the tissues were higher than limits for fish proposed by Food and Agriculture Organization/World Health Organization, (FAO/WHO), European Union (EU), and TFC. *Sander lucioperca* was associated with enhanced toxic metal (Cd) contents in the red and white muscle and was not within the safe limits for human consumptions. Levels of essential metals (Fe, Cu, Zn, and Mn) were below the limits proposed by EU and FAO/WHO and TFC for fish. Because of high levels of metals, potential danger may emerge in the future depending on the domestic wastewaters and industrial activities in this region. Accordingly, it was recommended that monitoring studies be periodically performed to assess the human exposure to these toxic elements through fish and fishery product consumptions. These results can be used to find the chemical quality of fish in order to evaluate the possible risk associated with their consumptions by humans. The present study shows that precautions need to be taken in order to prevent metal's pollutions. Otherwise, these pollutions can be dangerous for fish and human health.

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