Occurrence of trace elements and organochlorine compounds in farmed rainbow trout *(Oncorhynchus mykiss)*

Djinovic-Stojanovic J., Vranic D., Trbovic D., Jankovic S., Petrovic Z., Matekalo-Sverak V. and Spiric A.

Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

Abstract— **Consumption of fish has increased worldwide due to its high nutritional value and health benefit. However, the benefits from fish consumption may be offset by the presence of polychlorinated biphenyls (PCBs), chlorinated pesticides (OClPs), toxic elements, etc.**

The purpose of this study was to evaluate the presence of trace elements (Hg, As, Cd, Pb, Cu, Fe, Zn, Mn and Se), PCBs (sum of seven individual congeners) and OClPs in thirteen muscle tissues of marketable size rainbow trout.

The content of trace elements was determined by atomic absorption spectroscopy (AAS) by applying different techniques: Cu, Zn, Fe and Mn - flame; Cd and Pb – graphite furnace; As and Se – hydride generation; Hg – cold vapor. Of the tested elements, Zn (9.76 mg kg^{-1}) and Fe (4.76 mg kg^{-1}) had the highest average concentration in fish tissue, followed by Se (0.035 mg kg^{-1}) and Hg (0.014 mg kg^{-1}). Cd (0.006-0.018 mg kg^{-1}) and Mn (0.94-2.14 mg kg^{-1}) were detected in three, Cu (0.22-1.09 mg kg^{-1}) in six samples, while in the remaining samples concentrations of these elements were above the LD. As and Pb were detected in traces. Statistical analyses of the concentration of elements showed that only between concentration of Fe and Zn (R=0.900) in analyzed fish samples there was significant correlation (at 95% confidence).

Quantities of OClPs and PCBs were determined by GC/ECD. Quantities of PCBs and OClPs (except for p,p'-DDE 0.002-0.003 mg kg^{-1}) in all tested samples were above LD of the applied method.

Keywords— **Trace elements, OClPs, PCBs.**

I. INTRODUCTION

Consumption of fish and fish products has increased worldwide. This high-quality food item is demonstrated to prevent coronary heart disease, hypertension and cancer, due to its high-quality proteins, n-3 polyunsaturated fatty acids, liposoluble vitamins and essential elements, as well as and low cholesterol and saturated fatty acids content [1-4]. The importance of fish and fish products in diet is well documented in different studies [3, 5].

However, several organic and inorganic compounds might be present in fish and seafood as contaminants. These compounds can be divided into three major groups:

- Inorganic chemicals: arsenic, cadmium, lead, mercury, selenium, copper, zinc and iron.
- Organic compounds: polychlorinated biphenyls (PCBs), dioxins and insecticides (chlorinated pesticides). This is a very diverse group with a wide range of industrial uses and a chemical stability that allows them to accumulate and persist in the environment.
- Processing-related compounds: sulphites (used in shrimp processing), polyphosphates, nitrosamines and residues of drugs used in aquaculture (e.g. antibiotics or hormones).

Heavy metals are natural trace components of the aquatic environment. In the last several decades their levels have increased due to industrial wastes, geochemical structure, agricultural and mining activities [6, 7]. This is in contrast with organic compounds, most of which are of anthropogenic origin brought to the aquatic environment by humans [8].

Heavy metals like copper, iron and zinc are essential for fish metabolism, while some others such as mercury, cadmium, arsenic and lead have no distinguished role in biological systems [9, 10] and are toxic even at low concentrations when ingested over a long period. Bioaccumulated contaminants (resulting from pollution) can cause some lethal and sub lethal impacts on fish, which can be expressed as physiologic effects on individuals or fish population at various life stages [11]. Fish is known to contribute significantly to human exposure to toxic elements (mainly mercury). Therefore, trace metals have been quantified in muscle tissue of a variety of fish species [12, 13]. Many of the studies have been undertaken with the aim to assess food safety in relation to toxic elements' presence in fish population and public health interests. Elements like Ca, Mg, K, Mn, Fe, Cu, Zn and Se are required for most biological functions. The shortage or excess intake of these elements

promotes organ malfunctions, chronic diseases and ultimately death, and therefore a balanced diet is fundamental [14, 15]. On the other hand, toxic elements including As, Cd, Hg and Pb accumulate in tissues of aquatic organisms at levels that can cause physiological impairment at higher trophic levels, including humans. In the study of Minganti et al. [16] the phenomenon of bioaccumulation and biomagnifications, especially of mercury, was investigated by comparing contamination of farmed and wild fish specimens of the same species. Farmed fish species showed mercury and arsenic concentrations significantly lower than wild species, and no relationship was found between mercury concentration and fish body size. The level of methyl mercury in fish has important implications on consumer health.

The goal of the present study was to determine concentration of environmental contaminants (trace elements, polychlorinated biphenyls and chlorinated pesticides) in marketable size rainbow trout, samples collected from two fish pond with intensive production situated in Bosnia and Herzegovina- Republic of Srpska. In other to establish a possible relationship between the examined elements in fish samples, correlation analysis in relation to their content was applied (with significance of correlation $p \leq 0.05$).

II. MATERIALS AND METHODS

A. Materials

Investigation was carried out on 13 samples of marketable size rainbow trout of similar lenght and weight, collected during August and September 2010 from the fish pond with intensive production situated in Bosnia and Herzegovina - Republic of Srpska. Fish was fed formulated feed consisting of fish meal, soybean protein products and corn and wheat. Along the year, water temperature in fish pond was 8^0C. Analysis was carried out on homogenized samples of fish muscle after evisceration and deprivation of skin, tail, head, fins and bones.

B. Methods

For trace elements determination, samples were prepared by microwave digestion (MILESTONE ETHOS TC), 0.5g sample + 8mL nitric acid (HNO_3 J.T. BAKER) + 2mL hydrogen peroxide (H_2O_2 J.T. BAKER). Analysis was carried out on Atomic Absorption Spectrometer, Varian "SpectrAA 220", by different techniques: Cu, Zn, Fe and Mn – by flame technique; Cd and Pb – by graphite furnace; As and Se – by hydride generation and Hg – by cold vapor technique.

For chlorinated pesticides and PCBs determination extraction of fat from fish samples has been performed by petroleum ether (fraction 40-60^{0}C). After drying trough anhydrous Na_2SO_4, the extracts were evaporated under stream of N_2 to dryness. Dry residues were dissolved in n-hexane and cleaned up on column filled with Al_2O_3, previously heated at 800^0 C during 5h.

After extraction of samples, gas chromatograph (GC) Varian, model 3800, equipped with a 63-Ni electron capture detector (ECD) and Varian VF 5-ms capillary column was applied for determination of sixteen organochlorine pesticides (OClPs), as well as of sum of seven polychlorinated biphenyls' individual congeners (28, 53, 101, 118, 138, 153, 180). The following OClPs were determined: Lindane, Hexachlorocyclohexane, HCH (α, β and δ isomeres), Aldrine, Dieldrine, Heptahlor, Heptahlorepokside (cis- and trans-), p,p'-DDE, p,p'-DDD, p,p'-DDT, Endrine, Hexachlorobenzene and Chlordane (α- i γ-).

III. RESULTS

The content of trace elements, as well as the content of OClPs and PCBs in filets of rainbow trout is presented in Tables 1-2.

Table 1 Content of trace elements in fish tissue (n=13)

Trace elements	Content (MEAN ± SD) [mg kg^{-1}]
Hg	0.014 ± 0.002
As	< 0.10
Cd	0.006 – 0.018[1]
Pb	< 0.05
Cu	0.220 – 1.090[1]
Fe	4.756 ± 4.971
Zn	9.762 ± 8.038
Mn	0.940 – 2.140[1]
Se	0.035 ± 0.012

[1]*Residues of Cd and Mn were detected in tree samples; in case of Cu residues were detected in six samples.*

Table 2 Content of OClPs and PCBs in fish tissue (n=13)

Organochlorinated pesticides (OClPs)	Content [mg kg^{-1}]
Lindane	< 0.001
HCH (α, β and δ)	< 0.001
Aldrine & Dieldrine	< 0.001
Heptahlor & Heptahlorepokside (cis-, trans-)	< 0.001
p,p'-DDE + p,p'-DDD + p,p'-DDT	0.002 – 0.003
Endrine	< 0.001
Hexachlorobenzene	< 0.001
Chlordane (α- i γ-)	< 0.001
Polychlorinated biphenyls (PCBs)	< 0.001
Sum PCBs (28, 53, 101, 118, 138, 153, 180)	< 0.001

IV. DISCUSSION

Results for content of trace elements in the analyzed fish tissue samples are summarized in Table 1 as mean value ± standard deviation. From the presented results it could be seen that, in case of Fe and Zn, the standard deviation is very similar to their mean value. Fe and Zn were detected in all analyzed samples. It could be mentioned that at the beginning of the sampling period their content was much higher than later on, what, as a consequence, gave rise to high values of standard deviations. Besides that, these two elements had the highest average concentration in fish tissues (Zn: 9.76 mg kg^{-1}; Fe: 4.76 mg kg^{-1}). Se and Hg were detected in all fish samples too, but their concentrations were rather uniform during the sampling period. Namely, standard deviations for Se and Hg were much lower than their mean values (Table 1). Cd and Mn were detected in three, Cu in six samples, while in the remaining samples concentrations of these elements were above the LD (Cd < 0.005 mg kg^{-1}, Mn < 0.2 mg kg^{-1}, Cu < 0.2 mg kg^{-1}). As and Pb were detected in traces (LD: As < 0.10 mg kg^{-1}, Pb < 0.05 mg kg^{-1}).

Correlation analysis for the concentrations of elements detected in fish samples can reveal some facts about possible associations of different elements. Namely, from the obtained data it might be seen that there is a significant positive correlation (with the significance of correlation $p \leq 0.05$) only between concentrations of iron and zinc (Figure 1). This data might indicate to a possible association of this two elements in the fish tissue, as well as to their common origin from some environmental pollution source.

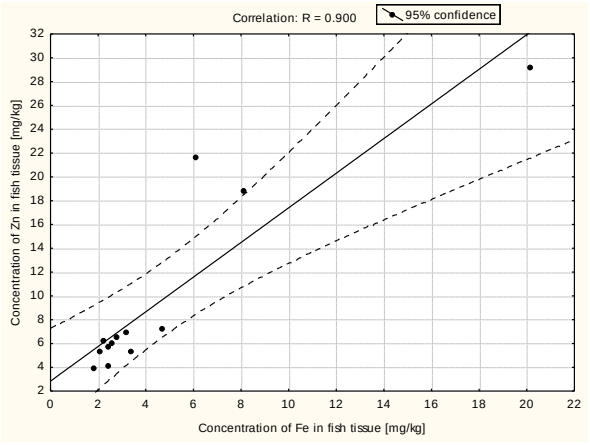

Fig. 1 Significant correlation between concentration of Fe and Zn in fish tissue

Results obtained for chlorinated pesticides and PCB congeners in muscle tissue of rainbow trout are summarized in Table 2. The obtained data indicate that the concentrations of fifteen analyzed pesticides were above the limit detection of the applied method. Only in case of p,p'-DDE the detected quantities were very similar in all the threeteen analysed samles and ranged from 0.002 to 0.003 mg kg^{-1}.

V. CONCLUSIONS

On the basis of the obtained results it might be concluded that marketable size rainbow trout from aquaculture do not contain residues of environmental contaminants and do not pose any risk to consumers.

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