Analysis and Evaluation of Pollutant Residues in Freshwater Fish at Jiamusi Section of Songhua River

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Abstract. Carp from Jiamusi section of Songhua River were collected to analyze and evaluate the residues of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons and volatile organic compounds in muscle tissue. The results showed that 100% of heavy metals, organochlorine pesticides and polycyclic aromatic hydrocarbons were up to standard, and a small amount of volatile organic compounds were detected. The structure and morphology of fish tissue samples were not abnormal, and the structure of nucleus and cytoplasm was normal. No pathological changes or morphological abnormalities were found. Fish were healthy.

1. Introduction

Songhua River is a large freshwater fish farm in northeast China, rich in fish resources, "three flowers and five luo", big white fish, mandarin fish and other precious varieties have long been famous in the world, the whole basin fish species up to 77, is an important source of freshwater fish in north China, rich carp, grass carp, catfish and so on. Aquatic organisms are an important part of the water environment. Biological monitoring can reflect the comprehensive impact of various pollutants on organisms under natural conditions. Fish of higher nutritional level in aquatic organisms can enrich various pollutants from water and sediments, and as an important part of people's diet, the analysis of them is not only one of the important contents of the evaluation of water pollution status, but also closely related to people's health.

2. Sample collection and processing

The samples were collected from Jiamusi section of Songhua River, and all the samples were wild freshwater fish. Fishing methods vary depending on local fishing conditions, and nets are usually chosen to keep fish samples alive. Collect and transport process to ensure low temperature preservation, and make samples as soon as possible.

Fish samples should be made to keep them fresh. Dissecting the fish, collecting the back muscles above the side line of the fish, removing the skin of the fish, removing the large spines to make samples, and cryopreservation.
2.1. Sample sizes

In this paper, three stages of monitoring of pollutants in freshwater fish (carp) were completed in three consecutive years. Carp weighing more than 1.5kg were made into a single sample of carp; Carps weighing 0.5 ~ 1kg were prepared as mixed carps.

In the past three years, 90 fish individuals were collected and 30 samples were prepared. Analytical items include: heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, volatile organic compounds, chlorophenols. At the same time, the liver and reproductive system of fish were collected for tissue section experiment. The annual sample situation is shown in Table 1.

### Table 1. A list of fish samples for analysis.

| Annual phase | Collection of individual fish (strips) | Analysis item | Sample number |
|--------------|---------------------------------------|---------------|---------------|
| phase one    | 30                                    | Heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, volatile organic compounds, chlorophenols | 10             |
| phase II     | 30                                    |               | 10             |
| phase III    | 30                                    |               | 10             |
| total        | 90                                    |               | 30             |

3. Results and evaluation of pollutant analysis

3.1. Heavy metal

Five kinds of heavy metal pollutants such as arsenic, lead, chromium, cadmium and mercury were analyzed. The statistics of monitoring results are shown in table 2 and 3. The comparison and analysis of the data showed that the mean and maximum detected concentrations of cadmium, chromium and mercury showed a gradually decreasing trend in the past three years, while the inter-annual changes of arsenic and lead were relatively small.

### Table 2. Statistical table of heavy metal residue monitoring results unit: mg/kg

| Index | Phase I                           | Phase II                          | Phase III                          |
|-------|-----------------------------------|-----------------------------------|------------------------------------|
| As    | Concentration range 0.0171~0.0888 | 0.0218~0.111                      | 0.0240~0.107                       |
|       | Average value 0.0430              | 0.0575                            | 0.0531                             |
| Pb    | Concentration range 0.0416~0.154  | 0.0171~0.135                      | 0.0370~0.163                       |
|       | Average value 0.0901              | 0.0680                            | 0.0737                             |
| Cd    | Concentration range 0.00190~0.00631 | 0.00126~0.00416 | 0.00055~0.00102 |
|       | Average value 0.00386              | 0.00240                           | 0.00087                            |
| Cr    | Concentration range 0.244~0.391   | 0.182~0.376                       | 0.0771~0.264                       |
|       | Average value 0.334                | 0.238                             | 0.169                              |
| Hg    | Concentration range 0.0602~0.112  | 0.0591~0.121                     | 0.0577~0.111                      |
|       | Average value 0.0851               | 0.0817                            | 0.0786                             |

Since there is no complete evaluation method for freshwater aquatic organisms in China, and the reference standards of mercury and arsenic in GB2672-2005 "limits of pollutants in food" are methyl mercury and inorganic arsenic, which are not total mercury and arsenic. The reference evaluation standards for mercury and arsenic in this paper are NY5073-2006 "limits of toxic and harmful substances in pollution-free food and aquatic products", the reference evaluation standards for mercury 0.5mg/kg, arsenic 0.5mg/kg, lead, cadmium and chromium are GB2672-2005 "limits of pollutants in food", lead 0.5mg/kg, cadmium 0.1mg/kg and chromium 2.0mg/kg.
The biological quality index was used to evaluate the biological quality, and the single factor evaluation method was adopted, that is,

$$P_i = \frac{C_i}{S_i}$$

(1)

$P_i$ -- Biological quality index of pollutants in item I;
$C_i$ -- Measured mass concentration of pollutants in item I;
$S_i$ -- Mass concentration standard value of pollutant in item I.

The biological quality index and evaluation range are shown in Table 3.

**Table 3. Biological quality index and evaluation range.**

| Biological quality index $P_i$ | Pollution level          |
|--------------------------------|--------------------------|
| $P_i < 0.2$                   | Normal background value  |
| $0.2 \leq P_i \leq 0.6$      | Mild contamination       |
| $0.6 < P_i \leq 1.0$         | Middle level pollution   |
| $P_i > 1.0$                  | Serious contamination    |

When using the single factor evaluation method to evaluate the enrichment degree of heavy metals, it was found that the average biological mass index of lead, cadmium, chromium, arsenic and mercury in the carps samples with a section of Jiamusi was less than 0.2, within the range of normal background value. Lead, cadmium, chromium, arsenic and mercury were all detected in 30 fish samples, and all were lower than the limit of the reference evaluation standard.

3.2. Polyaromatic hydrocarbon

The main analysis items include: naphthalene, acenaphthene, fluorene, phenanthrene, benzo (a) anthracene and other 11 substances. The analysis results are shown in Table 4. Through comparison, it was found that the maximum concentration and mean value of anthracene and fluoranthene in the mixed samples of carp were significantly decreased, and the data of benzanthenrene and flexor were relatively stable.
Table 4. Statistical table of pahs residue monitoring results  unit: μg/kg

| Index            | Phase I                  | Phase II                  | Phase III                  |
|------------------|--------------------------|---------------------------|----------------------------|
| Naphthalene      | Concentration range      | No detected               | No detected               | No detected               |
|                  | Average value            | —                         | —                          | —                          |
| Acenaphthylene   | Concentration range      | No detected               | No detected               | No detected               |
|                  | Average value            | —                         | —                          | —                          |
| Acenaphthene     | Concentration range      | No detected               | No detected               | No detected               |
|                  | Average value            | —                         | —                          | —                          |
| Fluorene         | Concentration range      | Qualitative detection     | No detected               | Qualitative detection     |
|                  | Average value            | —                         | —                          | —                          |
| Phenanthrene     | Concentration range      | Qualitative detection     | No detected               | Qualitative detection     |
|                  | Average value            | —                         | —                          | —                          |
| Anthracene       | Concentration range      | 0.035~0.071               | 0.031~0.070               | Qualitative detection     |
|                  | Average value            | 0.051                     | 0.055                      | —                          |
| Fluoranthene     | Concentration range      | 0.059~0.082               | 0.048~0.79                 | Qualitative detection     |
|                  | Average value            | 0.068                     | 0.061                      | —                          |
| Pyrene           | Concentration range      | Qualitative detection     | Qualitative detection     | Qualitative detection     |
|                  | Average value            | —                         | —                          | —                          |
| [1,2]benzanthracene | Concentration range   | 0.013~0.017               | 0.012~0.016               | Qualitative detection     |
|                  | Average value            | 0.015                     | 0.014                      | —                          |
| Chrysene         | Concentration range      | 0.018~0.022               | 0.017~0.022               | Qualitative detection     |
|                  | Average value            | 0.020                     | 0.020                      | —                          |
| Benzopyrene      | Concentration range      | No detected               | not detected              | No detected               |
|                  | Average value            | —                         | —                          | —                          |

Note: L is the limit of quantitative detection;
The evaluation standard adopted in this paper is based on the European Union (EC)1881/2006 directive "maximum limits of specific pollutants in food". The standard value of benzo (a) pyrene in fish flesh specified in the standard is 2.0 g/kg, and benzo (a) pyrene is used as a marker to evaluate pahs.

Monitoring results show that the annual benzo (a) pyrene were not detected, and measured the fish in the ∑ PAHS wet weight levels related literature at home and abroad have reported a similar order of magnitude, and belongs to the low level, due to both at home and abroad was not about ∑ PAHS total relevant standards, so according to above standard and research status at home and abroad, a preliminary thought polycyclic aromatic hydrocarbons in a relatively safe level.

3.3. Organo-chlorine pesticide

The main analysis items include:benzene hexachloride, DDT, hexachlorobenzene and other 11 substances. The analysis results are shown in table 5. The results show that the concentration of pollutants fluctuates slightly and the change range is small[1].
Table 5. Statistical table of monitoring results of organochlorine pesticide residues. unit: μg/kg

| Index                  | Phase I          | Phase II         | Phase III         |
|------------------------|------------------|------------------|-------------------|
| Benzene hexachloride   | Concentration    | 0.091L~0.199     | 0.091L~0.15       | 0.091L~0.199     |
|                        | Average value    | 0.129            | 0.105             | 0.129            |
| DDT                    | Concentration    | 0.076~0.163      | 0.021L~0.27       | 0.076~0.163      |
|                        | Average value    | 0.125            | 0.062             | 0.125            |
| Hexachlorobenzene      | Concentration    | 0.011L~0.252     | 0.011L~0.17       | 0.011L~0.252     |
|                        | Average value    | 0.109            | 0.102             | 0.109            |
| Heptachlor             | Concentration    | No detected      | No detected       | No detected      |
|                        | Average value    | —                | —                 | —                |
| Penta nitrochlorobenzene| Concentration  | No detected      | No detected       | No detected      |
|                        | Average value    | —                | —                 | —                |
| Endosulfan - I        | Concentration    | No detected      | No detected       | No detected      |
|                        | Average value    | —                | —                 | —                |
| Endosulfan - II       | Concentration    | No detected      | No detected       | No detected      |
|                        | Average value    | —                | —                 | —                |
| Cis-chlordane         | Concentration    | 0.0021L~0.040    | No detected       | No detected      |
|                        | Average value    | 0.0088           | —                 | —                |
| Endrin                | Concentration    | 0.0024L~0.309    | 0.0024~0.100      | 0.0024L~0.2160   |
|                        | Average value    | 0.0794           | 0.0341            | 0.0487           |
| Endrin aldehyde       | Concentration    | 0.0028L~0.048    | No detected       | No detected      |
|                        | Average value    | 0.020            | —                 | —                |
| Endosulfan sulfate    | Concentration    | 0.0057L~0.011    | No detected       | No detected      |
|                        | Average value    | 0.0061           | —                 | —                |

Note: L is the limit of quantitative detection;

According to the national standard of the People's Republic of China GB2763-2005 "maximum residue limits of pesticides in food", the maximum residue limits of benzene hexachloride and DDT in aquatic products are 0.5mg/kg and 0.1mg/kg, respectively[2].

In terms of its content, the annual mean value of detected substances within 3 years was below 0.2μg/kg, and the total amount of organochlorine pesticides in Jiamusi cross-section fish samples was less than 1.0μg/kg, far less than the maximum DDT limit of 0.1mg/kg specified in GB2763-2005, which was at a safe level.

According to the analysis of organochlorine content, the maximum value of samples detected within 3 years was less than 1.0μg/kg, and the mean value was less than 0.2μg/kg. The residues of benzene hexachloride and DDT were far less than the requirements of 0.5mg/kg and 0.1mg/kg specified in GB 2763-2005 maximum residue limits of pesticides in food. As other organochlorine components have not been used or used less in China, their residues are small and their pollution is small, specific information of relevant limits has not been found yet. Preliminary conclusion: the content of organochlorine pesticides in carps collected from Jiamusi section in this analysis is very low, and the limit of organochlorine pesticides in fish muscle tissue is at a relatively safe level.

3.4. Volatile organic compound

The samples were analyzed for 13 volatile organic compounds, including dichloromethane, trichloromethane, tetrachloride, trichloroethylene, tetrachloroethylene, benzene, toluene, ethylbenzene, m-p-xylene, o-xylene, isopropyl benzene, mercaptan and sulfide. The analysis results are shown in table 6.
### Table 6. Statistical table of monitoring results of VOCs residue unit: μg/kg

| Index          | Concentration range | Phase I     | Phase II     | Phase III    |
|----------------|---------------------|-------------|-------------|-------------|
| Dichloromethane| No detected         | No detected | No detected |
| Average value  | —                   | —           | —           |
| Trichloromethane| 10L ~ 30.5          | 10L ~ 27.5  | 10L ~ 25.8  |
| Average value  | 13.2                | 11.4        | 12.0        |
| Benzene        | No detected         | No detected | —           |
| Average value  | —                   | —           | —           |
| Methylbenzene  | 11.9 ~ 27.6         | No detected | No detected |
| Average value  | 19.7                | —           | —           |

Note: L is the limit of quantitative detection; Other items trichloroethylene, tetrachloroethylene, carbon tetrachloride, ethylbenzene, m-p-xylene, o-xylene, isopropyl benzene, mercaptan and sulfide were not detected. Dichloromethane, trichloromethane, toluene and benzene were detected in some samples with low detection rate. Trichloroethylene, tetrachloroethylene, carbon tetrachloride, ethylbenzene, m-p-xylene, o-xylene, isopropyl benzene, mercaptan and sulfide were not detected. Therefore, it is concluded that the residual volatile organic compounds in carp of Jiamusi section in the Songhua River basin are at a safe level.

#### 3.5. Tissue slice

The main object of detection is the reproductive system, liver and other organs of the collected carp. Through the histological analysis of each tissue section, whether there is abnormal development of the fish is detected from the perspective of morphology.

This test mainly used hematoxylin-eosin staining (HE staining for short) to observe tissue sections. The main principle of this method is that the alkaline hematoxylin-eosin staining solution can make the chromatin in the nucleus and the ribosome in the cytoplasm purple blue. However, the acidic eosin dye mainly reddens the cytoplasm and extracellular matrix. This characteristic can be used as an effective and extensive HE staining method for structural analysis in histology, embryology, pathology teaching and scientific research.

![Figure 2. Male and female fish.](image)

HE staining analysis of the internal organs, testicles, ovaries and other tissue sections of the collected carp showed that there were no abnormalities in the structure and morphology of the fish tissue samples collected in each year. The nuclear and cytoplasmic structures of the tissues were normal, and no tissue lesions or morphological abnormalities were observed.

#### 4. Conclusion

Within 3 years, all the heavy metals (As, Cd, Cr, Pb, Hg) in the muscle tissue of wild carp in Jiamusi section of Songhua River were detected, the detection rate was 100%, but the content did not exceed the standard limit, the standard rate was 100%, the heavy metal residue was relatively safe. Polycyclic aromatic hydrocarbons, organochlorine and volatile organic compounds were partially
detected, but their contents did not exceed the standard limit, reaching the standard rate of 100%. The persistent organic pollutants of wild fish in the watershed were less enriched.

Tissue sections and genetic toxicity tests showed no abnormalities in the structure and morphology of fish tissue samples, normal nuclear and cytoplasmic structure of tissues, no tissue lesions or morphological abnormalities, and healthy fish. On the whole, fish in the Songhua River basin are in good living condition and relatively safe for eating.

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