

**Comprehensive Profiling of Chemical Contaminants in Farmed Beluga
Caviar: Multi-Residue Analysis of Pesticides, Synthetic Dyes, Heavy Metals,
and Antibiotics**

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Abstract:

Introduction: Caviar, a luxury food product derived from sturgeon, may contain pesticides, dyes, antibiotics, and heavy metal residues, making a comprehensive risk assessment essential for consumer safety.

Objective: This study specifically investigated the concentrations of diverse contaminant residues in aquaculture-produced caviar. Multiple analytical approaches can be employed to identify and measure contaminant residues in caviar.

Material and Methods: In this study, liquid chromatography-tandem mass spectrometry, gas chromatography-tandem mass spectrometry, and inductively coupled plasma mass spectrometry were employed to determine 55 target residues, including three heavy metals, nineteen organochlorine pesticides, seven organophosphate pesticides, twenty-two antibiotics, and four dye residues in Beluga sturgeon (*Huso huso*) caviar samples. The samples were collected from major Iranian aquaculture facilities (16 sturgeon farms) located in the provinces of Gilan, Mazandaran, Golestan, Qom, and Fars during the 2024-2025 production cycle.

Results: The study revealed that heavy metal levels did not exceed 10 µg/kg, dye residues were beneath 0.2 µg/kg, and the pesticide residues were under 5 µg/kg. Additionally, the antibiotic residues present in the samples were below the detection threshold and in compliance with European Commission food regulations.

Conclusion: The results of this survey can be attributed to the effectiveness of modern, controlled farming practices, such as monitoring water quality, optimized feeding schedules, and notice to veterinary drug withdrawal periods. Furthermore, it can be concluded that the levels of pesticide, synthetic dye, heavy metals, and antibiotic residues in Iranian cultivated caviar were beneath the method's limit of detection (LOD) and do not pose health concerns for consumers.

Keywords: Anti-Bacterial Agents; Coloring Agents; Metals, Heavy; Pesticides

1. Introduction

As people's lifestyles evolve and societies become more industrialized, public concern about potential and actual chemical and microbial hazards in various food products has increased. In this context, the rise in the use of agricultural chemicals and pesticides, climate change, and the adoption of new technologies have led to excessive levels of these contaminants entering consumers' diets, exceeding safe limits, and ultimately increasing public concern, prompting regulatory actions by veterinary and food authorities in various countries [1].

Caviar, a raw animal product, is derived from the sturgeon fish. The value of sturgeon fish is not only for their meat but mainly for the caviar obtained from them, which has exceptionally high economic value. The production volume of farmed caviar in Iran is 21 tons [2-4].

Twenty-seven sturgeon species are found throughout the Northern Hemisphere, with six of the most important global species inhabiting the Caspian Sea, which alone contains 93% of the world's sturgeon populations. These include *Acipenser stellatus*, *Acipenser ruthenus*, *Acipenser persicus*, *Acipenser gueldenstaedtii*, *Huso huso*, and *Acipenser nudiiventris*. These species are now listed as critically endangered [5-7].

Monitoring and analyzing residual contaminants, including hormones, antibiotics, anthelmintics, dyes, pesticides, and heavy metals, in farmed caviar has become critically important due to the potential use of pharmaceuticals and chemicals in aquaculture practices, as well as possible environmental contaminant exposure. With the recent increase in farmed caviar production, ensuring the safety and quality of these products through comprehensive evaluation and control of residues has become a top priority for both regulatory compliance and consumer safety [8-10].

According to EU guidelines, monitoring heavy metals and various analytes (such as antibiotics, anthelmintics, organochlorine/organophosphate/pyrethroid pesticides, hormones, dyes, and heavy metals) in farmed fish becomes especially important.

A study conducted from March to April 2011 determined the accumulation of Zinc (Zn), lead (Pb) and cadmium (Cd), in Persian sturgeon caviar sourced from the southern Caspian Sea coast, the mean levels of Zn, Pb, and Cd in the caviar were 44.73, 2.11, and 0.01 µg/g, respectively [11].

In a study conducted on Persian sturgeon caviar collected from a sampling location in the Southern Caspian Sea, the average concentrations (mg kg⁻¹) of As were 0.01, Cd 0.05, Cu 1.42, Co 0.01, Pb 0.01, and Sn 0.28 various heavy metals and metalloids were determined as follows: [12].

In a comprehensive environmental contaminant assessment, concentrations of both organic contaminants (such as polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and hexachlorocyclohexanes (HCHs)) and inorganic pollutants (a suite of 23 metals) were determined in caviar obtained from *Acipenser stellatus*, *Acipenser gueldenstaedtii*, and *Huso huso* sourced from Iran, Bulgaria, Azerbaijan, and Russia. Analytical results showed average contaminant concentrations of 15.47 ± 25.8 ng/g ww for PCBs, 797 ± 139 ng/g ww for DDT, and 9607 ± 486 ng/g ww for arsenic, with PBDEs being detected in all samples [8].

According to those mentioned above, this study was performed to assess 55 target residues (pesticides, antibiotics, heavy metals, and dyes) in sturgeon caviar samples collected from major Iranian aquaculture facilities during the 2024-2025 production cycle.

2. Materials and Methods:

The research method employed a quantitative approach, based on data collected from samples of farmed caviar produced in Iran and the results of laboratory tests performed on these samples. Beluga caviar samples were obtained from *Huso huso* fish (1.6–2 m in length, 35–70 kg in weight, and 10–14 years of age) that were cultured in 16 sturgeon farm in the Gilan, Mazandaran, Golestan, Qom, and Fars provinces of Iran (Table 1).

Table 1. Geographical distribution of the sampled farms.

Province	Number of caviar producing farms	Number of sampled farms
Gilan	15	9
Mazandaran	5	3
Golestan	2	1
Qom	3	1
Fars	2	1

2.1. Sample preparation and heavy metal analysis

Heavy metal levels in the samples were determined following standard protocols BS EN 13806:2002 and BS EN 1550:2007 for sample preparation and measurement. Approximately 50 g of the sample underwent homogenization to ensure uniformity, after which a 0.3 g aliquot was transferred into the microwave digestion vessels (Anton Paar GmbH, Germany). Subsequently, 2 mL of 30% H_2O_2 and 3 mL of 65% HNO_3 were added, followed by a 20-minute pre-digestion period to allow for reaction stabilization. The vials were then placed in a microwave and subjected to an appropriate digestion cycle. Following digestion, the vials were cooled at ambient temperature for 20 minutes, after which the resulting digest was quantitatively transferred into 30 mL volumetric flasks and made up to volume with double-distilled water. For analysis, the solutions were transferred to special vials for atomic absorption spectroscopy (AAS). Lead and cadmium concentrations were measured using a graphite furnace atomic absorption spectrometer (Agilent, USA). Mercury (Hg) was analyzed with a mercury analyzer employing the cold vapor technique. The sample was converted into mercury vapor and directed into a gas cell, where absorption was measured at the specific wavelength of a mercury lamp [13].

2.2. Sample preparation and analysis of organochlorine pesticides

To quantify organochlorine pesticides, including β -HCH, γ -HCH, hexachlorobenzene (HCB), α -hexachlorocyclohexane (HCH), aldrin, chlordane (cis- and trans-), endrin, heptachlor, methoxychlor, heptachlor epoxide, DDT isomers, pendimethalin, dieldrin, endosulfan (α -, β -, and sulfate), dicofol, and chlorothalonil, approximately 1 g of caviar was homogenized with anhydrous sodium sulfate. The resulting mixture was then transferred to a pre-washed extraction thimble that had been rinsed with hexane. Prior to the extraction step, the following internal standards were added: PCBs 46 and 143 (10 ng each), ϵ -HCH (7.5 ng), brominated biphenyl 103 (2 ng), and brominated biphenyl 15 (0.5 ng). After that, the sample was extracted for two h at hot temperature with 75 mL of a hexane/acetone blend in a 3:1 volume ratio. After concentrating the extract to roughly 5 mL, an aliquot of about one-sixth of the volume was taken for fat content analysis, performed by solvent evaporating at 105°C for 1 h. The remaining extract was subsequently loaded onto a cartridge packed with 8 g of silica gel that had been pre-treated with 44% concentrated sulfuric acid. The target compounds, including polybrominated diphenyl ethers, polychlorinated

biphenyls, and organochlorine pesticides, were completely eluted with 15 mL of *n*-hexane followed by 10 mL of dichloromethane. The collected eluate was then concentrated using rotary evaporation and further reduced to near dryness (Agilent, USA) under a gentle nitrogen stream. The residue was reconstituted in 80% iso-octane, after which the solution was subjected to analysis for persistent organohalogenated pollutants [14, 15].

2.3. Sample preparation and measurement of organophosphate pesticides

The analytical method for six organophosphate pesticides (trichlorfon, malathion, chlorpyrifos, phoxim, profenofos, diazinon, and fenitrothion) began with homogenization; To a 50 mL polypropylene centrifuge tube, 1 g of the homogenized sample was added, followed by the addition of internal standards and target analytes for recovery evaluation. After equilibrating for 15 min, the sample was supplemented with 10 mL of acetonitrile and shaken vigorously for 30 s. The extract was then transferred to another 50 mL centrifuge tube pre-loaded with 4 g of anhydrous MgSO₄ and 1 g of NaCl. This was followed by vigorous shaking for 1 minute and centrifugation at 3,250 rpm for 2 min. Subsequently, a 1 mL aliquot of the supernatant was added to a 2 mL centrifuge tube pre-loaded with anhydrous MgSO₄ (150 mg), C18 (50 mg), PSA (50 mg), and ZrO₂ (50 mg). The mixture was vigorously shaken (30 s) and centrifuged (3,250 rpm for 2 min), after which 0.5 mL of the purified extract was dispensed into an autosampler vial for GC-MS/MS analysis [15, 16].

2.4. Sample Preparation and Analysis of Dye Residues

Detection of dye residues (Leucomalachite green, Crystal violet, Leuco crystal violet and Malachite green) was performed as follows: Two grams of homogenized sample were weighed, followed by the integration of 40 µL of internal standard mix (100 ng/mL). Subsequently, 500 µL of hydroxylamine solution (9.5 g/L) was introduced, and the mixture was vortexed for 2 minutes before being incubated in the dark for 10 min. Following that, 8 mL of acetonitrile and 0.1 g of anhydrous MgSO₄ were introduced. Next, vortexing of the mixture was carried out for 2 min, and then it was shaken at 100 rpm for 10 minutes. Following centrifugation for 5 minutes, the supernatant (4 mL) was gently pipetted into a clean tube and dried completely at 50°C using a gentle flow of nitrogen.

The residue after drying was dissolved in 1000 μ L of a solution containing acetonitrile and 1 g/L ascorbic acid (1:100, v/v), then vortexed for 2 min, and finally filtered via a 0.22 μ m PVDF syringe filter. Quality control measures included spiking selected samples at levels of 0.1, 0.25, 0.5, and 1 ng/g. The spiked samples were equilibrated for 1 h prior to extraction, which was performed using the method detailed above. Ultimately, a calibration curve was created using the spiked samples, enabling the determination of analyte concentrations by comparing peak areas with the calibration equation [17].

2.5. Sample preparation and analysis of antibiotic residues

First, 3 g of the sample was introduced into a 50 mL screw cap tube. The procedure continued with the addition of 15 mL of methanol (70%) and 200 μ L of EDTA (0.1 M). The homogenization of the mixture was performed for 1 min using a high-efficiency disperser, and subsequently centrifugation at $3800 \times g$ for 5 min. Once centrifugation was complete, the supernatant (500 μ L) was carefully transferred to polypropylene tubes, admixed with purified water (2 mL), and passed through 0.45 μ m filters before analysis. The prepared samples were analyzed using an LC-MS/MS, which was fitted with a Turbo V ion source and an electrospray ionization probe. For chromatographic separation, a C18 Synergi column was used, while data collection and process were conducted with Analyst 1.6.2 software. The mobile phase comprised two solvents: phase A, containing 0.2% formic acid and 0.1 mM oxalic acid in water, and phase B, consisting of 0.2% formic acid and 0.1 mM oxalic acid in acetonitrile.

The optimized gradient program started with an increase in mobile phase B from 0% to 75% within 1 min, followed by a hold at 75% for 1.6 min. Subsequently, the composition was returned to 0% B over 4.6 min, giving a total run time of 7.2 min per injection. For mass spectrometric detection, the instrument operated in negative ionization mode for AMFs and in positive ionization mode for antibiotic residues, following the method described by Yipel et al. (2017) [18].

3. Results

According to the results, the tested caviar poses no significant health risk from heavy metal contamination, since the levels of lead, mercury, and cadmium were all beneath the quantifiable limit of 10 μ g/kg. This finding underscores the high quality and safety of this luxury product (Table 2).

Table 2- Concentrations of heavy metals in farmed beluga caviar determined by ICP-MS

Heavy metal	Detection limit (µg/Kg)	Result (µg/Kg)
Lead	10	<10
Mercury	10	<10
Cadmium	10	<10

Analytical results indicated that all tested samples contained organochlorine and organophosphate pesticide residues at levels below the 5 µg/kg threshold (Tables 3 and 4).

Table 3- Concentration of organochlorine pesticide residues in farmed beluga caviar determined by GC-MS/MS

Organochlorine pesticide	Detection limit (µg/Kg)	MRL(µg/Kg)	Result (µg/Kg)
HCB	5	10	<10
HCH alpha	5	10	<10
HCH beta	5	10	<10
HCH gamma(Lindane)	5	10	<10
Heptachlor	5	10	<10
Heptachlore epoxyde	5	10	<10
Aldrine	5	10	<10
Chlordane cis	5	10	<10
Chlordane trans	5	10	<10
Endrin	5	10	<10
Methoxychlore	5	10	<10
DDT isomers	5	10	<10
Pendimethaline	5	10	<10
Dieldrin	5	10	<10
Endosulfan alpha	5	10	<10

Endosulfan beta	5	10	<10
Endosulfan sulphate	5	10	<10
Dicofol	5	10	<10
Chlorothalonil	5	10	<10

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197 Table 4- Concentration of organophosphate pesticide residues in farmed beluga caviar

Organophosphate pesticide	Method	Detection limit (µg/Kg)	MRL(µg/Kg)	Result (µg/Kg)
Trichlorfon	LC/MS/MS	5	10	<10
Malathion	GC/MS/MS	5	10	<10
Chlorpyrifos	LC/MS/MS	5	10	<10
Phoxim	LC/MS/MS	5	10	<10
Profenofos	LC/MS/MS	5	10	<10
Diazinon	LC/MS/MS	5	10	<10
Fenitrothion	GC/MS/MS	5	10	<10

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199 The findings of this revealed that dye residues were consistently lower than the stringent threshold
200 of 0.2 µg/Kg, indicating that the product is free from significant artificial coloring and poses no
201 safety concerns regarding dye contamination (Table 5).

202 Table 5- Concentration of dye residues in farmed beluga caviar by LC-MS/MS

Dye	Detection limit (µg/kg)	MRL (µg/kg)	Result (µg/Kg)
Malachite green	0.2	0.5 (Sum of	<0.2
leucomalachite	0.2	MG&LMG	<0.2
green		)*	
Crystal violet	0.2	0.2	<0.2
Leuco crystal	0.2	0.2	<0.2
violet			

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According to the data in Table 6, LC-MS/MS analysis confirmed that any antibiotic residues present in the samples were below the detection threshold and compliant with Commission Regulation (EU) No 37/2010.

Table 6- Concentration of antibiotic residues in farmed beluga caviar by LC-MS/MS

Antibiotic	Detection limit (µg/kg)	Result (µg/Kg)	MRPL (ppb)
Oxytetracycline	25	<25	25
Tetracycline	25	<25	25
Chlortetracycline	25	<25	25
Doxycycline	25	<25	25
Ciprofloxacin	15	<15	15
Enrofloxacin	15	<15	15
Danofloxacin	25	<25	25
Difloxacin	75	<75	75
Marbofloxacin	40	<40	40
Sarafloxacin	10	<10	10
Erythromycin	50	<50	50
Sulfadiazine	25	<25	25
Sulfadimethoxine	25	<25	25
Sulfachloropyridazine	25	<25	25
Sulfamethoxypyridazine	25	<25	25
Sulfaquinoxalin	25	<25	25
Sulfadimidine	25	<25	25
Trimethoprim	15	<15	15
Florfenicol	15	<15	15
Norfloxacin	5	<5	5
Nalidixic Acid	4	<4	4
Cefuroxime	5	<5	5

226 4.Discussion

227 High concentrations of heavy metal residues in caviar above the maximum residue limits should
228 be considered as a serious risk to consumers. The results of this study indicated that the levels of
229 heavy metals in the analyzed matrix were below the limit of detection. Similar results were
230 reported by Fath El-Bab and Seadawy (2006), who recorded average heavy metal levels in caviar
231 samples as follows: Pb (0.064 ppm), Hg (0.162 ppm), and Cd (0.052 ppm) [19]. Adel et al. (2026)
232 measured toxic elements (cadmium, lead, chromium, arsenic, mercury) in caviar from farmed
233 Siberian sturgeon (*Acipenser baerii*) in Tehran Province, Iran. The results indicated that toxic
234 element levels were minimal and complied with international regulatory standards [20]. Hosseini
235 et al. (2013) evaluated the concentrations of Ba, Se, Hg, and Sn in the caviar of wild *Acipenser*
236 *persicus*. Their findings indicated that, except for mercury, the mean levels of the other metals
237 were considerably under the permissible limits, and thus not harmful for human consumption [21].
238 Ni et al. (2021), detected four heavy metals in the samples, including cadmium, lead, copper, and
239 mercury. Furthermore, they reported the presence of copper and mercury in all analyzed samples;
240 notably, lead exhibited the highest prevalence (77.4%), followed by cadmium (34.0%) [22].

241 According to the results of this study, the dye residues were below 0.2 µg/Kg. Similar findings
242 were noted by Kirschbaum et al. (2006), who reported that genuine *Sevruga Malosol* caviar from
243 *Acipenser sturio* L. contained no detectable colorant [23]. However, they detected and E 151 E
244 110 in German caviar made from lumpfish. Consistent with our findings, Rejczak and Tuzimski
245 (2017) reported that the concentrations of dyes in fish roe specimens (ranging from 67.3 to 237.8
246 µg/g) were below the maximum permitted level of 300 µg/g set by Commission Regulation (EC)
247 N o 9 4 / 3 6 / E C (1 9 9 4) f o r t h i s f o o d c a t e g o r y [2 4] .

248 Pesticides are broken down slowly in the environment, showing resistance to both hydrolysis and
249 biodegradation. In the present study, the pesticides levels were lower than 5 µg/kg. Multiple studies
250 have consistently reported higher levels of organochlorine compounds in Black Sea caviar in
251 comparison with specimens from the Caspian Sea [25, 26]. Wang et al. (2008) noted that
252 Romanian *Huso huso* caviar had the highest ΣPCB concentration (388 ng/g lipid weight) among
253 samples from seven countries, including Iran, Azerbaijan, France, Kazakhstan, Russia, Romania
254 and Moldova [8].

Despite the widespread use of antibiotics, analysis of the samples in this study revealed that antibiotic residue levels were below the detection limit. In contrast, Ni et al. (2021) identified ten antibiotics, including oxytetracycline, ampicillin, ciprofloxacin, chloramphenicol, pefloxacin, sulfapyridine, sulfisomidine, enrofloxacin, sulfadiazine, and ofloxacin, in 106 seafood samples from Shanghai, China. Chloramphenicol showed the highest prevalence (47.2%), followed by ampicillin (31.1%) and ciprofloxacin (15.1%). Conversely, the occurrence of pefloxacin (0.9%), sulfapyridine (1.9%), and sulfisomidine (1.9%) was markedly lower [22].

5. Conclusion

Ensuring that luxury food products such as caviar are both safe and of high quality is important not only owing to their economic significance but also because of their potential direct effects on consumer health. Advanced analytical techniques, including ICP-MS, LC-MS/MS, and GC-MS/MS, confirmed that the levels of Pb, Hg, and Cd were below 10 µg/kg, antibiotics in line with Commission Regulation (EU) No 37/2010, dye residues were lower than 0.2 µg/kg, and the pesticide residues were below 5 µg/kg. The findings of this study, based on caviar samples collected from major Iranian aquaculture facilities during the 2024–2025 production cycle, demonstrate that the levels of all tested contaminants were below the analytical limits of detection (LOD), therefore posing no health risks to consumers.

Ethical Considerations

Not Applicable.

Data availability

All data analyzed during this study are included in this article.

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Authors' contributions

Conceptualization, study design and experiments: Hadi Tabarraee; Abbasali Motallebi; Data acquisition: Hadi Tabarraee; Project administration, technical, and material support, data analysis, interpretation Amir Ali Anvar; Afshin Akhondzadeh Basti; Writing the original draft: Hadi Tabarraee; Review and editing: Abbasali Motallebi; Amir Ali Anvar; Maryam Tala

Conflict of interest

The authors declared no conflict of interest.

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364