



Determination of four perfluorinated compounds (PFAS) in muscle and liver tissues of common pheasants (*Phasianus colchicus*) from two locations in northern Serbia

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ARTICLE INFO

Keywords:

PFAS

Biomonitoring

Phasianus colchicus

LC-MS/MS

Game animals

Serbia

ABSTRACT

This study examined and established the presence of perfluoroalkyl substances (PFAS) (perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS)) in the muscle and liver tissues of common pheasants (*Phasianus colchicus*) collected from two locations in northern Serbia. Breast muscle and liver samples from twenty-five pheasants were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Samples were collected during the 2024–2025 hunting season. Higher concentrations of PFAS were detected in liver (40.0% of all examined samples) compared to breast muscle tissue (8.0% of all examined samples). Total PFAS concentrations in pheasant liver ranged from less than the limit of quantification (<LOQ) to 5.774 µg/kg, while only concentrations detected in muscle tissue were 0.123 µg/kg and 0.171 µg/kg. The most commonly found of the four examined compounds was PFOS, which was followed by PFNA. The aim of this research was to determine the levels of four PFAS in game pheasant tissue collected from two locations in Northern Serbia and compare the results with those similar or different game birds from other countries or regions. According to available literature, this is the first study to examine the presence of PFAS in pheasant tissues in Serbia.

1. Introduction

Perfluoroalkyl substances (PFAS) have been widely used in many industries over the past eighty years. Primarily these compounds are utilised in the paper and electronics industries, cosmetics, household products, fire extinguishers, and general consumer goods. Due to the strong C-F bond, PFAS can be resistant to photochemical, oxidative, and microbial degradation. PFAS are divided into those with a shorter chain (less than 8 carbon atoms) and those with a long chain (more than 8 carbon atoms). The length of the chain affects their toxicity, since PFAS with a small-

er number of carbon atoms in the chain remain in the human body for several days or months, while those with a longer chain can remain in the human body for several years. (Felder *et al.*, 2023). PFAS do not accumulate in adipose tissue, but primary in serum, liver, and kidney (Death *et al.*, 2021). These compounds are connected with hepatotoxicity, neurotoxicity, fertility problems, impacts on the development of foetuses and children, and weakened immune response to vaccines (Mikolajczyk *et al.*, 2024).

Four PFAS compounds, i.e., perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), and per-

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Paper received April 4th 2026. Paper accepted 30th 2026.

Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

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fluorooctanesulfonic acid (PFOS), have been classified as toxic by the European Food Safety Authority (EFSA, 2020). The maximum allowable concentrations of PFAS in meat and offal of game animals are set by the European Commission Regulation 915/2023 (European Commission, 2023) and are: for meat, PFOS — 5.0 µg/kg, PFOA — 3.5 µg/kg, PFNA — 1.5 µg/kg, PFHxS — 0.6 µg/kg and the sum of all four PFAS — 9.0 µg/kg; for offal, PFOS — 50.0 µg/kg, PFOA — 25.0 µg/kg, PFNA — 45.0 µg/kg, PFHxS — 3.0 µg/kg and the sum of all four PFAS — 50.0 µg/kg. These values are also set in the legal infrastructure of Serbia (Official Gazette of the Republic of Serbia, 2024).

Over the last two decades, many studies have been conducted on the presence of PFAS compounds in game animals, mainly waterfowl birds (Sinclair et al., 2006; Sharp et al., 2021; Szabo et al., 2022; Dayan et al., 2025) and large mammals (Kowalczyk et al., 2018; Stahl et al., 2012; Felder et al., 2023; Schröder et al., 2024; Arioli et al., 2019). The common pheasant (*Phasianus colchicus*) is one of the most important and widespread game species in Serbia, which makes it a good choice for bio-monitoring of PFAS compounds. The greatest distribution of the common pheasant is in the northern, lowland, and agricultural regions of Vojvodina, in the north of Serbia, from where the samples in the current study were taken. Pheasant habitats are near rivers, agricultural fields, and on the edges of forests. The animal's diet includes seeds, cereals, fruits, insects, and small invertebrates (Popović et al., 2019).

In Serbia, the presence of PFAS compounds was investigated in samples of surface water, sediment, and soil in the basin of large rivers in northern Serbia (Beškoski et al., 2013; Buljovčić et al., 2022). A study that examined the concentration of PFAS compounds in wild boar muscle tissue was conducted on animals from central regions of Serbia (Krnjaja et al., 2025).

The aim of this research was to determine the levels of four different types of PFAS (PFOA, PFNA, PFOS, and PFHxS) in game pheasant tissue collected from two different agricultural regions of Vojvodina (Northern Serbia) and compare the results with those similar or different game birds from other countries or regions. According to available literature, this is the first study in Serbia to determine the presence of PFAS compounds in tissues of common pheasant using liquid mass chromatography LC-MS/MS.

2. Materials and Methods

2.1 Sample collection

Breast muscles and livers were collected from 25 pheasants during one hunting season (autumn-winter 2024–25) from two locations in Vojvodina, in northern Serbia. Twelve birds were collected from the hunting ground DUNAV-SREM, in the municipality of Stara Pazova, Srem district (44.9850° N 20.1608° E), and thirteen were collected from the hunting ground BRZAVA, in the municipality of Plandište, South Banat district (45.2272° N 21.1217° E). Pheasants were collected within the framework of the national residue monitoring program in Serbia, and no animals were killed specifically for this research. Sex, weight, and age were measured by authorised hunters. All animals

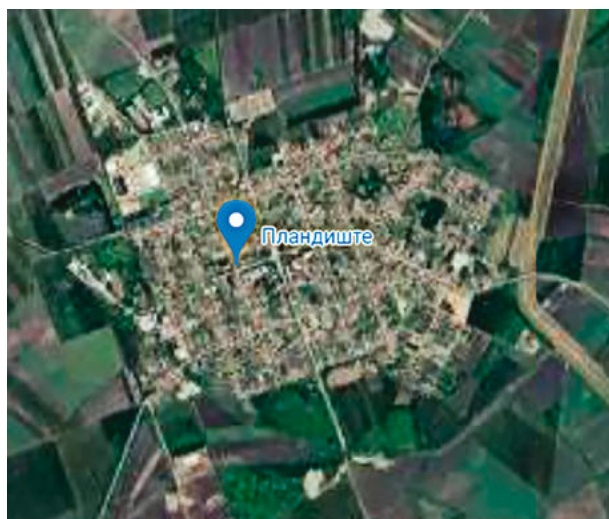


Figure 1. Plandište sampling location

Source: Google Maps, accessed 22 April 2026.



Figure 2. Stara Pazova sampling location

Source: Google Maps, accessed 22 April 2026.

examined in this study were males aged 2 to 3 years, weighing 1.2 to 1.5 kg. After sampling, the tissue samples were placed in polypropylene tubes and stored at -18°C , and were thawed at 4°C , and homogenised in a blender one day before analysis by LC-MS/MS.

2.2 Reagents and Standards

The solvents and chemicals used during this study were HPLC grade. Acetonitrile (Honeywell, Germany), methanol (Honeywell, Germany), water (Honeywell, Germany), ammonium acetate (Sigma Aldrich, Germany) and a QuEChERS kit containing 4 g MgSO_4 , 1 g NaCl, 1 g sodium citrate dibasic sesquihydrate (SCDS), and 0.5 g sodium citrate tribasic dihydrate (SCTD) (Phenomenex, USA) were used. The PFAS standards, containing the four PFAS compounds examined in this study, as well as internal standards containing their ^{13}C -labeled analogues, were manufactured by Wellington Laboratories Inc., Canada. Working standards were prepared by diluting the stock standards in methanol.

2.3 Sample Preparation and Instrumental Analysis

After adding internal standards, each sample was additionally homogenised with acetonitrile on an UltraTurrax machine. The homogenised material was then centrifuged and the supernatant separated. QuEChERS salts were added to the supernatant, after which the mixture was centrifuged again. The resulting supernatant was evaporated to dryness under a stream of nitrogen, and the dry residue was dissolved again in acetonitrile. After vortexing, the dissolved residue was transferred to a polypropylene cuvette and centrifuged again to obtain the supernatant that was subjected to HPLC analysis. Pheasant

tissue samples, in which no PFAS were previously found, were used as blank samples, and after spiking with standard, as calibration materials and the spiked control (QC spike). Calibration range was from 0.1 to 5 $\mu\text{g}/\text{kg}$. The limit of quantification (LOQ) was determined during the validation process and was 0.1 $\mu\text{g}/\text{kg}$ for each compound.

For analysis and detection, we used a HPLC with mass detector LCMS 8050 (Shimadzu, Japan). The HPLC system was equipped with a RP Kinetex C18 column 100 mm $\times$ 2.1 mm, particle size 2.6 μm , and PFAS Delay Column Restek 50 $\times$ 2.1 mm, particle size 5 μm . HPLC analysis included a gradient with 2mM ammonium acetate in water/acetonitrile, 95:5, v/v, and 10% of acetonitrile/methanol, 60:40, v/v, as mobile phases. Multiple reaction monitoring (MRM) mode was used for quantification. Ion transitions for MS/MS are presented in Table 1.

2.4 Quality Control

Quality control materials (QC) Fish/T0687QC and Dried Egg/T06142QC were purchased from the Food Analysis Performance Assessment Scheme (FAPAS, UK). During the instrumental analysis, the results obtained for the QC materials were within the range specified in the manufacturer's certificate. Another control was conducted with blank samples spiked with calibration standards (QC spike). Recovery values were in the acceptable range of 80–120%.

3. Results and Discussion

The concentrations of PFAS, as well as the number of pheasant tissues in which PFAS were detected in birds from the two sampling locations, are shown in Table 2.

Table 1. Optimised multiple reaction monitoring (MRM) transitions for target PFAS compounds and corresponding isotopically labelled internal standards.

Analyte	Internal Standard	Precursor Ion (m/z)	Quantifier Product Ion (m/z)	Qualifier Product Ion (m/z)
PFOS	$^{13}\text{C}_8$]PFOS	499.0	80.0	99.0/169.0
PFOA	$^{13}\text{C}_8$]PFOA	413.0	369.0	169.0
PFNA	$^{13}\text{C}_9$]PFNA	463.0	419.0	219.0
PFHxS	$^{13}\text{C}_3$]PFHxS	399.0	80.0	99.0

Table 2. Concentrations of PFAS (µg/kg) registered in tissue samples of pheasants from the two sampling locations

Sampling location	Tissue	N	N (%)	Range
Plandište	Liver	13	4 (30.8%)	0.431–1.268
	Muscle	13	nr	-
Stara Pazova	Liver	12	6 (50.0%)	0.115–5.774
	Muscle	12	2 (16.7%)	0.123–0.171
Total	Liver	25	10 (40.0%)	/
	Muscle	25	2 (8.0%)	/

n — number of examined samples; N (%) — number and percentage of samples in which PFAS registered; nr — not registered, so below the LOQ (0.1 µg/kg)

Table 3. Concentrations of PFAS compounds (µg/kg) in pheasant liver samples from the two sampling locations

Sampling location	Sample ID	PFOS	PFOA	PFNA	PFHxS	Range (total)
Plandište	1	0.847	nr	0.421	nr	0.431–1.268
	2	0.779	nr	nr	nr	
	3	nr	nr	nr	nr	
	4	nr	nr	nr	nr	
	5	nr	nr	nr	nr	
	6	nr	nr	nr	nr	
	7	nr	nr	nr	nr	
	8	nr	nr	nr	nr	
	9	nr	nr	nr	nr	
	10	nr	nr	nr	nr	
	11	0.431	nr	nr	nr	
	12	0.448	nr	0.314	nr	
	13	nr	nr	nr	nr	
Stara Pazova	1	nr	nr	nr	nr	0.115–5.774
	2	nr	nr	nr	nr	
	3	nr	nr	nr	nr	
	4	nr	nr	nr	nr	
	5	nr	nr	nr	nr	
	6	nr	nr	nr	nr	
	7	0.253	nr	nr	nr	
	8	nr	nr	0.115	nr	
	9	0.915	nr	0.906	nr	
	10	nr	nr	0.167	nr	
	11	0.999	nr	0.320	nr	
	12	2.580	nr	3.194	nr	

nr — not registered, so below the LOQ (0.1 µg/kg)

PFAS were detected more often in the pheasants liver than in breast muscle. The PFAS detection frequency was higher at the Stara Pazova location (50%) than at the Plandište location (30.8%). In samples in which PFAS were detected, total PFAS concentrations in liver ranged from 0.431 $\mu\text{g/kg}$ to 1.268 $\mu\text{g/kg}$ in birds from Plandište, and from 0.115 $\mu\text{g/kg}$ to 5.774 $\mu\text{g/kg}$ in those from Stara Pazova. In breast muscle samples, PFAS were detected in only two samples from the Stara Pazova location with concentrations of 0.123 $\mu\text{g/kg}$ and 0.171 $\mu\text{g/kg}$. Higher concentrations and more frequent occurrence of PFAS in liver, compared with those in muscle, could be explained by the affinity of PFAS for organs that are metabolically active and rich in proteins.

Because of the greater distribution of PFAS in pheasant liver, these results are given separately in Table 3.

Table 3 shows that PFOA and PFHxS were not detected in any of the collected liver samples. PFNA was detected in the livers of two birds from the Plandište location at concentrations of 0.421 $\mu\text{g/kg}$ and 0.314 $\mu\text{g/kg}$. At Stara Pazova, the frequency was higher, and PFNA was detected in the livers of five pheasants in concentrations from 0.115 $\mu\text{g/kg}$ to 3.194 $\mu\text{g/kg}$. PFOS was detected in four birds from the Plandište location in concentrations from 0.431 $\mu\text{g/kg}$ to 0.847 $\mu\text{g/kg}$, and in four birds from the Stara Pazova location with concentrations from 0.253 $\mu\text{g/kg}$ to 2.580 $\mu\text{g/kg}$. Graphical presentations of the obtained results for PFNA and PFOS from the singular sampling locations are given in Figure 3 and 4.

Due to the large number of samples in which the concentrations of PFAS compounds were below the LOQ, it was not possible to make any significant statistical comparison between the two locations or between breast muscle and liver samples.

Compared to similar studies that examined the concentration of PFAS compounds in the tissues of wild birds, this study shows lower values. In a study that examined waterfowl in the Northeast Atlantic flyway, concentrations of PFOS in breast muscle ranged from LOQ to 46.6 $\mu\text{g/kg}$, and of PFNA were up to a maximum of 4.06 $\mu\text{g/kg}$ (Dayan *et al.*, 2025). PFOS and PFNA were also the most common PFAS found in livers of ducks from isolated areas in Australia (Szabo *et al.*, 2022). PFOS was found in 30% of duck livers with a maximum concentration upto 5.3 $\mu\text{g/kg}$, while PFNA was found in 22% of the livers with a maximum concentration of 0.16 $\mu\text{g/kg}$ (Szabo *et al.*, 2022). Also, in Victoria,

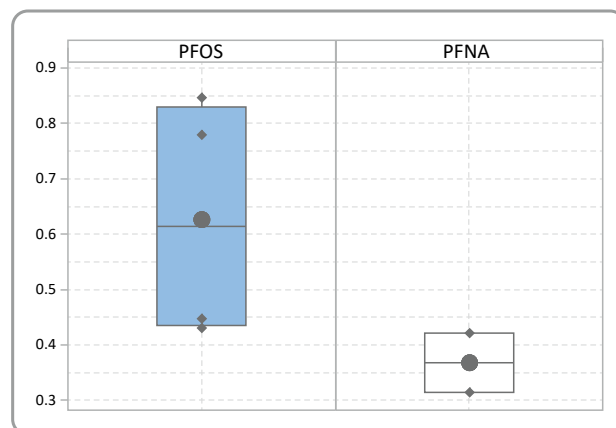


Figure 3. Box plot of registered per-and polyfluoroalkyl substances in the liver of pheasants from the Plandište sampling location

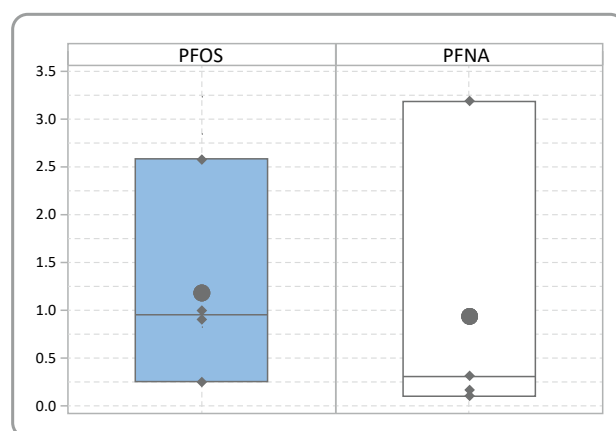


Figure 4. Box plot of registered per-and polyfluoroalkyl substances in liver of pheasants from the Stara Pazova sampling location

Australia, the maximum concentration of PFOS was 33 $\mu\text{g/kg}$ in wild duck breast muscle, and 340 $\mu\text{g/kg}$ in wild duck liver (Sharp *et al.*, 2021). In animals from the Niagara River (United States), the concentration of PFOS in mallard livers ranged from 28 $\mu\text{g/kg}$ to 425 $\mu\text{g/kg}$ (Sinclair *et al.*, 2006).

4. Conclusion

This study shows results of examination of four PFAS compounds (PFOS, PFOA, PFNA, PFHxS) in the liver and breast muscle of common pheasant from two locations in northern Serbia. Higher concentrations in pheasant liver compared to breast muscle were likely due to the well-known bioaccumulation of PFAS in protein rich and metabolically active organs. The higher prevalence of PFAS in the pheasants from Stara Pazova suggests possible local contamination. Although in a large number of sam-

ples the values were below the detection limits, this study shows that PFAS compounds are present in tissues of common pheasants. Because pheasant is an important game species in Serbia, and these wild

birds are consumed by humans, these results indicate the need for further testing and continued bio-monitoring of PFAS, and a larger number of samples from different locations in Serbia.

Određivanje četiri perfluorovana jedinjenja (PFAS) u mišićnom tkivu i jetri fazana (*Phasianus colchicus*) sa dve lokacije u severnoj Srbiji

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INFORMACIJE O RADU

Ključne reči:

PFAS
Biomonitoring
Phasianus colchicus
LC-MS/MS
Lovna divljač
Srbija

APSTRAKT

Ova studija je ispitala i utvrdila prisustvo perfluoroalkilnih supstanci (PFAS) (perfluorooktansulfonska kiselina (PFOS), perfluorooktanoinska kiselina (PFOA), perfluorononanoinska kiselina (PFNA) i perfluoroheksansulfonska kiselina (PFHxS)) u mišićnom tkivu i jetri fazana (*Phasianus colchicus*) prikupljenih sa dve lokacije u severnoj Srbiji. Uzorci grudnog mišića i jetre dvadeset pet fazana analizirani su tečnom hromatografijom i tandemskom masenom spektrometrijom (LC-MS/MS). Uzorci su prikupljeni tokom lovne sezone 2024–25. Veće koncentracije PFASa su otkrivene u uzorcima jetre (40,0% svih ispitivanih uzoraka) u poređenju sa mišićnim tkivom (8,0% svih ispitivanih uzoraka). Ukupne koncentracije PFAS u uzorcima jetre kretale su se od <LOQ do 5,774 µg/kg, dok su jedinje koncentracije detektovane u mišićnom tkivu bile 0,123 µg/kg i 0,171 µg/kg. Najčešće detektovano jedinjenje bio je PFOS, a posle njega PFNA. Cilj ove studije je da se pokaže potencijal fazana kao bioindikatora kontaminacije PFAS. Prema dostupnoj literaturi, ovo je prva studija koja je ispitala prisustvo PFAS u tkivima u Srbiji.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: The research results presented in this paper were funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, based on the Agreement on the Implementation and Financing of Scientific Research Work NIO in 2026, No. 451-03-33/2026-03/200050 dated 05.02.2026.

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