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First multi-contaminant assessment of the non-native American mink (*Neogale vison*) in Iberian freshwater ecosystems

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HIGHLIGHTS

- PBDEs, MeO-BDEs, EEs, PTEs, and TCEs across multiple tissues of the American mink.
- PBDE and MeO-BDE levels were low, indicating limited local pollution.
- Liver concentrated PBDEs, while fur accumulated most elements.
- Contaminant levels were not influenced by sex or body size.
- American mink can be used as a sentinel species.

GRAPHICAL ABSTRACT

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ABSTRACT

Non-native predators can act simultaneously as stressors and sentinels of environmental quality. This study aimed to quantify multi-contaminant burdens, characterize tissue-specific accumulation patterns, and evaluate the influence of biological factors (sex and body size) and trophic ecology ($\delta^{13}C$, $\delta^{15}N$) on contaminant exposure in 49 American mink (*Neogale vison*) collected during control campaigns (2023–2024) from two freshwater ecosystems in northern Portugal Angueira and Maçãs. We quantified legacy contaminants, including polybrominated diphenyl ethers (PBDEs) and methoxylated PBDEs (MeO-BDEs) in liver, together with essential (EEs),

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potentially toxic (PTEs), and emerging technology-critical (TCEs) elements in kidney, muscle, and fur. Thirteen PBDE and MeO-BDE congeners and 63 elements were determined to characterize multi-contaminant burdens and tissue-specific accumulation patterns, and stable isotopes ($\delta^{13}C$, $\delta^{15}N$) to assess trophic position and contaminant–diet relationships. Liver $\sum$PBDE (0.0712 ng g^{-1} w.w.) and $\sum$MeO-BDE (0.0645 ng g^{-1} w.w.) concentrations were low compared with other regions or controlled exposure studies, suggesting limited regional inputs of these contaminants in the study area. No sex-related differences were detected. Fur accumulated most EEs and several PTEs (e.g., As, Sr), supporting its value as a non-invasive matrix. Stable isotopes indicated similar trophic positions between sexes ($\delta^{13}C \approx -25‰$; $\delta^{15}N \approx 12‰$) with moderate trophic niche overlap (38%). Weak-to-moderate positive correlations between $\delta^{15}N$ and some PBDE congeners suggest a potential trophic dietary contribution to exposure. Overall, American mink accumulated multiple contaminant classes at relatively low levels, with exposure mainly influenced by local environmental conditions and diet. Given that American mink individuals are routinely culled during management programs, our findings support their use as a multi-contaminant sentinel and highlight fur and liver as practical matrices for freshwater monitoring.

1. Introduction

Predators play a fundamental role in maintaining ecosystem structure and function by regulating prey populations and influencing trophic dynamics [1]. When the predator is non-native, however, its ecological role becomes dual: besides exerting potential ecological impacts on native communities, it can also serve as a sentinel of environmental change [2,3], as differences in foraging behavior, prey selection, and habitat use relative to native predators may alter local fluxes of energy, matter, and contaminants. As a result, non-native predators may integrate signals from multiple trophic pathways, providing insights into ecological dynamics and contaminant transfer within invaded ecosystems [3].

The American mink (*Neogale vison*) exemplifies this dual role. Introduced to Europe in the 20th century for fur farming, it has successfully established feral populations across a wide range of freshwater and coastal habitats, due to its generalist diet, high mobility, and behavioral plasticity [4–6]. These same traits that make the American mink a successful invader also make it a useful sentinel species, as it forages across aquatic and terrestrial resources and reflects exposure to contaminants originating from diverse prey and habitats (Basu et al., 2007).

The American mink is an opportunistic and generalist predator that feeds on fish, amphibians, small mammals, birds, and aquatic and terrestrial invertebrates, with diet varying according to habitat, season, and prey availability [7]. In freshwater ecosystems, fish and crayfish often constitute important dietary components, while terrestrial prey such as mammals and birds may increase in importance in riparian and agricultural landscapes [8–10]. This broad trophic niche allows the American mink to integrate aquatic and terrestrial contaminant pathways, strongly influencing patterns of bioaccumulation and trophic transfer [2,4,11].

European freshwater ecosystems, where American mink are now widespread, are particularly vulnerable not only to biological invasions but also to multiple other anthropogenic stressors such as chemical pollution, hydromorphological alteration, and emerging pressures related to climate change and land-use intensification [12,13]. In this context, predators such as the American mink may simultaneously accumulate multiple classes of contaminants with distinct origins and environmental behaviors. These include inorganic elements: such as essential elements (EEs), which are required for physiological functions but may become toxic at elevated concentrations [14,15]; potentially toxic elements (PTEs), which have no known biological function and can exert adverse effects even at low exposure levels [14,15]; and technology-critical elements (TCEs), a group of metals increasingly used in modern technologies (e.g., rare earth elements, high-tech and platinum-group metals) whose environmental pathways, bioaccumulation patterns and ecological risks are still poorly understood [16-18].

In addition, persistent organic pollutants (POPs) such as polybrominated diphenyl ethers (PBDEs) are synthetic flame retardants that remain widespread in aquatic ecosystems despite regulatory restrictions, while methoxylated PBDEs (MeO-BDEs) may originate from natural biosynthesis and from metabolic or environmental transformation of PBDEs have also raised increasing concern due to their persistence, bioaccumulation potential, and capacity for trophic transfer [19,20]. The interactions among these contaminant classes and their combined accumulation within food webs remain poorly understood, particularly in systems where non-native predators such as the American mink exploit both native and non-native prey, such as the non-native crayfish (*Pacifastacus leniusculus* and *Procambarus clarkii*) species, potentially reshaping exposure pathways and contaminant fluxes across trophic levels [10,16,21].

Most ecotoxicological research on European freshwater predators has focused on the native Eurasian otter (*Lutra lutra*), which is widely used in biomonitoring programs that rely primarily on non-invasive samples such as spraints, as ethical and legal constraints limit direct access to the animals [22,23]. However, these non-invasive samples often provide limited information on tissue-specific accumulation, metabolic pathways, and the potential interactions among multiple contaminants [24]. In contrast, the non-native American mink, controlled across several European countries, provides a unique opportunity to obtain internal organs and perform multi-matrix assessments of contaminant bioaccumulation (Bonesi & Palazón, 2007b). Because these animals are already culled during control campaigns, their use overcomes the ethical concerns of invasive sampling in native predators and helps fill a key gap in ecotoxicology, the scarcity of tissue-based contaminant profiles for free-ranging predators. As a semi-aquatic predator integrating aquatic and terrestrial prey, the American mink serves as an ideal model to evaluate cross-ecosystem pollutant exposure and complement biomonitoring programs based on native species [2,6].

Understanding contaminant burdens in *N. vison* provides a twofold advantage: (i) it enhances our knowledge of multi-contaminant bioaccumulation patterns in freshwater predators, and (ii) it clarifies how non-native species may contribute to contaminant redistribution and ecological risk in invaded freshwater ecosystems. In this context, the present study aimed to: (i) quantify legacy (PBDEs and MeO-PBDEs) and emerging contaminants (TCEs), together with trace elements (EEs and PTEs), in American mink from northern Portugal, and test whether this non-native predator can serve as a reliable multi-contaminant sentinel in invaded Iberian freshwater ecosystems; (ii) characterize tissue-specific accumulation patterns; (iii) evaluate the influence of biological factors (sex and body size) on contaminant burdens; and (iv) integrate contaminant profiles with stable isotope data ($\delta^{13}C$ and $\delta^{15}N$), to assess trophic ecology, test for sex-related differences in trophic niche and explore trophic transfer and biomagnification trends.

Based on this framework, we hypothesized that: (i) non-native American mink from northern Portuguese rivers would show detectable but comparatively low burdens of legacy and emerging contaminants, reflecting limited regional environmental disturbance and contamination; (ii) organic contaminants would be detectable in liver

samples, while trace elements would show tissue-specific accumulation patterns, with higher concentrations expected in fur and kidney compared to muscle, reflecting differences in uptake, storage, and excretion pathways; (iii) contaminant loads would also vary according to biological factors, with potential sex-related differences and higher concentrations in heavier (and likely older) individuals due to progressive bioaccumulation over time; (iv) stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) would indicate similar trophic niches between males and females, consistent with niche overlap in invaded systems; and (v) positive relationships between δ^{1} ^{5}N and selected contaminants would indicate trophic transfer and incipient biomagnification along local food webs.

2. Materials and methods

2.1. Study area and sampling

American mink were captured in the Angueira and Maçãs Rivers (Fig. 1). The Angueira River is a small transboundary tributary that rises in Spain, near Alcañices (Zamora), and enters Portugal at São Martinho de Angueira, flowing southwards through a predominantly rural landscape before discharging into the left bank of the Maças River [25]. This area of northeastern Portugal, located between the Montesinho Natural Park and the Douro International Natural Park protected areas, still preserves relatively well-structured riparian corridors and traditional water-use systems. However, local water abstraction, small weirs, and reduced summer flows introduce hydromorphological pressure at the reach scale [26].

The Maçãs River has a similar hydrographic and socioecological setting: it rises in the Sierra de la Culebra (Zamora), forms short stretches at the Portuguese–Spanish border, and then crosses sparsely populated agricultural areas in northeastern Portugal before joining the Sabor River as a left-bank tributary [27]. Although it drains a relatively undisturbed area, the Maçãs River has been exposed to fragmentation due to the presence of multiple weirs, and typical interior-plateau pressures such as small-scale irrigation, sediment inputs from rural areas, and progressive alteration of flow regimes downstream related to regulation in the Sabor–Douro system [28].

Within the Douro River Basin Management Plan, the Sabor basin, where both Maças and Angueira Rivers belong, is classified as a water body of high ecological value but vulnerable to low-flow periods and to stressors linked to land use, making this basin suitable for monitoring contaminant inputs and for evaluating the effects of non-native species management [27]. Because both rivers lie in a region where biological invasions (e.g., signal crayfish, red swamp crayfish *Procambarus clarkii*, American mink) and multiple-stressor scenarios have been documented for northern Iberian freshwaters [16], they constitute an appropriate setting to test whether a controlled, non-native predator can integrate contaminants from aquatic, riparian, and agriculture-related sources.

In the Angueira River, 24 sites were surveyed along an approximately 25 km section, resulting in the capture of 44 individuals between September and November 2024. In the Maçãs River, 5 individuals were captured along a 20 km stretch between September and October 2023. All capture sites are shown in Fig. 1.

American minks were captured using tomahawk traps under LICENSE No. 231 / 2024 / CAPT (ICNF). All individuals were captured and removed from the environment in accordance with the invasive species control guidelines, then transported to the Veterinary Teaching Hospital of the University of Trás-os-Montes and Alto Douro (UTAD), in Vila Real, Portugal. At this facility, the animals were humanely euthanized by licensed veterinarians in accordance with the European Directive 2010/63/EU and the Portuguese Decree-Law No. 113/2013 on the protection of animals used for scientific purposes, as well as national and European Union guidelines for invasive species management (Regulation EU No. 1143/2014).

Before necropsy, biometric parameters, including total length, body weight, and sex, were recorded. Liver, kidney, muscle, and fur samples were then collected, placed in pre-cleaned glass vials, and frozen at −20 °C prior to chemical analyses. These tissues were selected because they integrate contaminants with different metabolic and temporal signatures [2,19,29].

Before analysis, tissues were thawed at room temperature, weighed individually, and subsequently frozen at −80 °C for 12 h before freeze-drying for 48 h at −20 °C and 0.35 bar. Dried tissues were ground in a mortar to a homogeneous powder. Fur samples were decontaminated by three consecutive washes with Milli-Q water, followed by drying in an Infors HT Multitron Pro oven at 40 °C for 48 h. All processed samples were stored in glass vials until analysis.

2.2. Determination of organic contaminants

Due to limited tissue mass after necropsy, it was not possible to analyze all contaminant groups in every matrix. Therefore, PBDE and MeO-BDE analyses were restricted to liver samples, which provided sufficient material and represent the most informative tissue for hydrophobic persistent compounds in carnivores.

For each homogenized liver powder sample from the American mink,

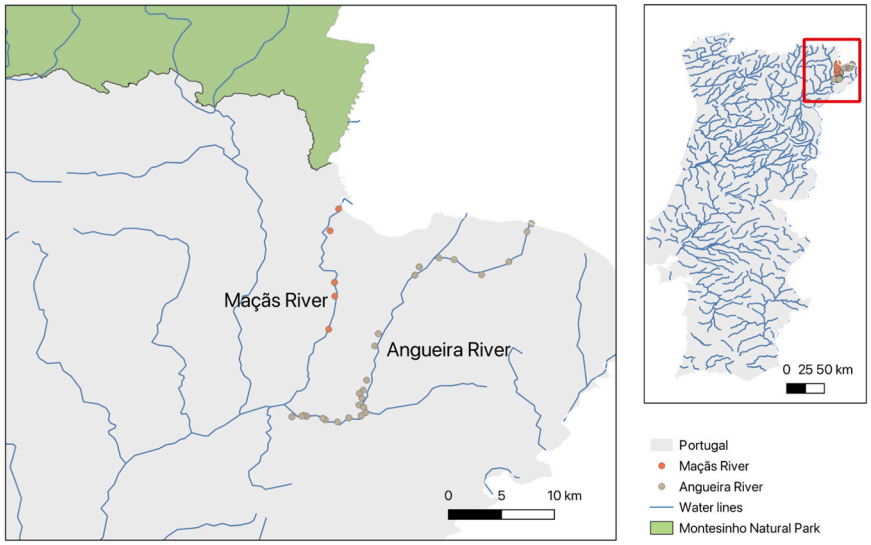

Fig. 1. Sampling sites of American Mink in the Maças and Angueira Rivers (Sabor basin, NW of Portugal).

500 mg of dry weight was weighed and transferred to 15 mL amber glass vials. To each vial, 2 mL of ultrapure water was added, followed by 2.5 mL of acetonitrile: toluene (4:1, v/v). The samples were extracted for 24 h in an orbital shaker (D-72379 Hechingen, Edmund Bühler GmbH, Germany) at 250 rpm, under controlled temperature conditions of 25 °C.

After this period, 1 g of magnesium sulfate ($MgSO_4$) and 0.5 g of sodium chloride (NaCl) were added, followed by vigorous vortexing for 1 min and centrifugation at 2000 rpm for 5 min at room temperature to promote phase separation. The resulting supernatant was transferred to 4 mL vials containing EMR-lipid (Agilent Technologies) for sample cleanup, following the protocol described by Fernández-Cruz et al. [30]. Briefly, EMR-lipid is a sorbent specifically designed to selectively retain lipids through size exclusion and hydrophobic interactions, minimizing matrix effects while preserving target analytes. After another centrifugation under the same conditions, the supernatant was collected and evaporated to dryness under a nitrogen stream in a sample concentrator (Stuart SBHCONC/1 Sample Concentrator) at 40 °C (Stuart SBH200D/3 Block Heater).

The dry fraction was then resuspended in 0.5 mL of n-hexane, to which 0.250 mL of sulfuric acid (H_2SO_4) was added to promote degradation of residual organic matter. The solution was vortexed and centrifuged for 5 min at 2000 rpm. The resulting supernatant was collected, 0.5 mL of ultrapure water was added, and the mixture was vortexed and centrifuged again under the same conditions.

The final supernatant was passed through a column containing 200 mg of neutral alumina previously activated with n-hexane to retain remaining impurities and eluted with 0.5 mL of the same solvent. The samples were then evaporated again under the same conditions and resuspended in 70 µL of trichloroethylene. Subsequently, 15 µL of an internal standard mixture containing BDE-37, BDE-77, FBDE-126, and $^{1\ 3}$C-6-MeO-BDE-47 was added to each sample before extraction (following [31]. Finally, the extracts were transferred to 2 mL vials containing 100 µL inserts for subsequent analysis.

Then, the concentrations of PBDEs were measured using a gas chromatograph (GC) Agilent 7890B (Agilent Technologies, USA), coupled to a triple quadrupole mass spectrometer Agilent 7000 C operating in electron impact ionization (EI) mode. The GC–MS/MS system was equipped with an electronic pressure control (EPC) and an automatic sampler 7683 (Agilent Technologies, USA). Quantitative analysis was performed using MassHunter software (version B.02.03).

All standards (BDE-28, BDE-47, BDE-99, BDE-153, BDE-154, BDE-183, 2-MeO-BDE-68, 4-MeO-BDE-49, 4-MeO-BDE-101, 4-MeO-BDE-103, 5-MeO-BDE-47, 5-MeO-BDE-99, 5-MeO-BDE-100) were purchased from Wellington Laboratories, Inc. (Guelph, Ontario, Canada) with purity above 99%. The PBDE and MeO-PBDE standard mixtures for calibration were prepared in n-hexane (Merck, Darmstadt, Germany), while the standard mixtures used for the American mink samples were prepared in n-hexane after receiving the standards in nonane and stored in amber glass vials at 4 °C. Stock solutions containing the internal standards BDE37, BDE77, FBDE-126, and 2′-MeO-BDE-68 were prepared in n-hexane.

For the analysis of the compounds present in the samples, the injector was maintained at 280 °C, and 1 µL of extract was injected in splitless pulsed mode (pulse pressure of 32 psi for 1 min and purge flow of 50 mL min^{-1}). Compound separation was achieved using a Zebron 5MS capillary column (30 m × 0.25 mm × 0.25 µm, Phenomenex, Torrance, CA, USA). The oven was programmed to start at 150 °C (held for 1.5 min), ramped at 40 °C min^{-1} to 250 °C, then at 7 °C min^{-1} to 320 °C, held for 3 min, for a total run time of 18.5 min. The helium flow rate was 1.3 mL min^{-1}. The transfer line, ion source, first and second quadrupole temperatures were 280, 230, 150, and 150 °C, respectively. Collision cell gases were nitrogen (1.5 mL min^{-1}) and helium (2.25 mL min^{-1}). The triple quadrupole mass spectrometer was operated in multiple reaction monitoring (MRM) mode (Table S1, Supplementary Material), detecting two transitions per analyte [31].

Method validation was conducted according to previously published protocols (e.g., [31]. Procedural blanks were measured with each batch of samples and were prepared simultaneously using the same chemical reagents and volumes as for the samples. The limit of detection (LOD) was defined as the lowest concentration in a spiked blank sample that gave a signal-to-noise ratio of 3, and the limit of quantification (LOQ) was set as the lowest concentration in the sample that could be quantified with precision (RDS <20%). The linearity of the method was evaluated by preparing calibration curves using matrix-matching, with seven calibration levels spanning the linear range. Calibration curves were generated by least squares linear regression, plotting the peak area ratios of target compounds and their respective internal standard against the concentration of each target compound. For all targeted compounds, a good linearity was obtained throughout the studied range (liver: 0.5 ng g^{-1} to 10 ng g^{-1} dry weight). All analytes provided a coefficient of determination (R2), higher than 0.90. Detailed method performance parameters, including spiking levels, recoveries, repeatability, linear range, and coefficients of determination (r^2) for all PBDE and MeO-BDE congeners, are provided in Table S2.

2.3. Determination of inorganic contaminants

Approximately 40 mg of fur and 200 mg of muscle and kidney of American mink individuals were digested with 3 mL of 65% HNO_3 using a microwave digestion system (Mars 6, CEM Corporation, USA). After digestion, extracts were adjusted to a final volume of 10 mL with ultrapure Milli-Q water (Direct-Q System, Millipore, Germany). Before the analysis, a 500 µl aliquot was further diluted 1:20 with 1% (v/v) HNO_3.

Elemental quantification was performed using Inductively Coupled Plasma Mass Spectrometry (PlasmaQuant® MS Q, Analytik Jena, Germany). Instrumental conditions included a nebulizer gas flow of 1.02 L min^{-1}, auxiliary gas flow of 1.5 L min^{-1}, plasma gas flow of 9.0 L min^{-1}, and radio frequency power of 1.20 kW, sampling depth 5 mm. Detection was carried out sequentially after ionization of the elements, in standard mode (no gas), and using the integrated Collision Reaction Cell (iCRC) with hydrogen (reaction gas) or helium (collision gas) to minimize polyatomic interferences. Each sample was analyzed using 20 scans and 5 replicates, with a dwell time of 20 ms. Hydrogen and helium flows were 80 mL min^{-1}. The exceptions were the determinations of Si and S, where the hydrogen flow was 180 mL min^{-1}, and the determinations of Na, Mg, P, K, Ca, and Fe, where the helium flow was 150 mL min^{-1}. The high gas flows resulted from higher concentrations of these elements in the samples, and combined with the tunable sensitivity of the ReflexION, extended the analytical range to higher concentrations.

Quality assurance procedures were applied throughout sample preparation and ICP MS analysis. Procedural blanks (n = 4) were digested and analyzed in parallel with samples using identical reagents, vessels, and digestion conditions to assess potential contamination. Blank concentrations were subtracted from sample values. Calibration was performed using multi-element standard solutions, and linearity was verified across the working range for each element ($R^2 > 0.995$). Instrumental performance was monitored by repeated analysis of a 10 ng g^{-1} multi-element standard solution.

An internal standard solution (5 ng g^{-1} in 1% HNO_3) containing Bi, Ir, Li, Rh, Sc, and Y was continuously introduced during analysis to correct for instrumental drift and matrix effects. Internal standard correction was applied automatically through ISTD-to-analyte pairing in the PlasmaQuant® MS software. Internal standard recoveries remained within 70–130%, confirming stable instrumental performance.

Instrument detection limits (IDLs) were calculated as $t \times SD$ of six instrumental blanks (n = 6; t = 4.032, 99% confidence level). IDLs ranged from 0.002 ng g^{-1} (Re) to 3404 ng g^{-1} (S). Method detection limits (MDLs) were determined from four procedural blanks using $t \times SD$ (n = 4; t = 5.841, 99% confidence level) and converted to tissue concentrations considering average sample mass and dilution factors.

MDLs ranged from 0.012 ng g^{-1} (Pt) to 5811 ng g^{-1} (Ca; dry weight) in kidney/liver and from 0.059 to 29055 ng g^{-1} in fur. High MDLs values were obtained by lowering the sensitivity to increase the analysis range. Accuracy was evaluated using certified reference materials (DORM-5 fish protein and BCR-668 mussel tissue), analyzed approximately once every 10 samples. For non-certified elements, accuracy was assessed using a 10 ng g^{-1} multi-element standard solution. Recoveries for certified elements ranged between 80–120%, meeting internationally accepted QA/QC criteria (Table S3), and elements not fulfilling this criterion were excluded. Precision, expressed as relative standard deviation (RSD%), was below 20% for all quantified elements.

A total of 63 elements were detected and quantified. However, sixteen elements (B, Sn, Sm, Eu, Gd, Tb, Dy, Er, Tm, Hg, Mo, Sb, Ba, Pb, Th, and Si) that showed CRM recoveries outside the accepted range were excluded from further analysis. The remaining 48 elements were grouped into three distinct categories according to their biological and functional characteristics: (1) Essential Elements (EEs): Mn, Co, Ni, Cu, Zn, Mg, Ca, Fe, Se, V, S, Na, K, P; (2) Potentially Toxic Elements (PTEs): Cd, U, As, Sr, Cr, Zr, Cs, Tl, Ag, Be, Al; and (3) Technology-Critical Elements (TCEs): Ti, Rb, La, Ce, Pr, Ho, Yb, Ga, Ge, Hf, Ta, In, Re, Te, Pt, Nd, Lu, Nb, Ru, Pd, W, Os and Au).

2.4. Stable isotope analysis

Before the stable isotope analysis, lipid extraction was performed on kidney and muscle samples of American mink. Aliquots of the tissues were transferred to glass vials, followed by the addition of a chloroform: methanol solution (2:1, v/v) and vigorous shaking. After each cycle, the supernatant was discarded, and the process was repeated three times per sample to ensure efficient removal of lipids. Each vial was then dried under controlled temperature conditions in an oven (Infors HT, Multitron Pro) for 48 h at 50 °C.

Subsequently, the samples were sent to CIIMAR (Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal), where $\delta^{15}N$ and $\delta^{13}C$ isotope compositions were determined using an Elemental Analyzer (EA), model Flash 2000, Organic Elemental Analyzer (Thermo Scientific), coupled to an Isotope Ratio Mass Spectrometer (IRMS), Delta V Advantage, via a Conflo IV interface. The EA experimental conditions were: oxidation reactor at 1020 °C, reduction reactor at 650 °C, GC column at 45 °C, with a run time of 500 s, and gas flows of 90 mL/min for helium (He), 180 mL/min for oxygen (O_2), and 200 mL/min for carbon dioxide (CO_2) and nitrogen (N_2).

To ensure measurement accuracy, $\delta^{15}N$ and $\delta^{13}C$ values were normalized using certified reference materials: IAEA-N-1, IAEA-N-2, and IAEA-NO_3 for nitrogen; USGS-24 and USGS-40 for carbon, with an analytical error of approximately 0.1‰.

In addition to isotopic composition, total carbon and nitrogen contents were quantified using a conversion factor (K factor) based on *Chlorella* algae. Analytical control was maintained by analyzing an internal seabass tissue standard every 12 samples to monitor system stability. Corrections for signal intensity effects were performed using a calibration curve constructed with nitrogen and carbon delta values as a function of the amplitude of different caffeine standard masses. All samples were analyzed in duplicate, and only results with a coefficient of variation (CV) < 10% were accepted to ensure reproducibility.

2.5. Statistical analysis

All data processing and statistical analyses were performed using RStudio software [32] (version 4.5.0). The packages readxl, dplyr, tidyr, reshape2, multcomp, multcompView, ggplot2, ggpubr, rstatix, viridis, corrplot, and PerformanceAnalytics were used for data import, organization, visualization, and statistical analysis.

The analyses included variable transformation, calculation of total concentrations for congeners and elements, and the application of normality (Shapiro–Wilk) and homogeneity of variance (Levene) tests. Concentrations of PBDEs and MeO-BDEs were $\log_{10}$-transformed to improve normality and reduce heteroscedasticity. Concentrations of PBDEs and MeO-BDEs were originally quantified on a dry weight (d.w.) basis to ensure analytical consistency among tissues and samples. Wet weight (w.w.) values were subsequently calculated using measured moisture content to facilitate comparison with studies reporting fresh tissue concentrations. When assumptions were met, ANOVA and Tukey post-hoc tests were performed. Pearson's correlation was applied to evaluate the potential relationships between stable isotope values and pollutant concentrations, as well as to explore dietary influences on contaminant exposure, using a significance level of $\alpha = 0.05$. Only contaminants that exhibited significant or biologically relevant correlations were displayed in the figures, while non-significant relationships were omitted for clarity. Generalized Linear Models (GLMs) were also applied to evaluate potential relationships between the different variables studied, including sex, size, weight, and tissue type, for both PBDEs and trace elements, identifying significant effects.

3. Results

3.1. PBDEs

Liver ∑PBDE and ∑MeO-BDE concentrations in American mink were low (3.78 and 4.51 ng g^{-1} d. w., respectively; Table S4). Generalized linear models showed no significant effects of sex, body size, or body mass on PBDE concentrations (all $p > 0.05$). At the congener level, no significant effects were detected for BDE-153 (sex: $p = 0.58$; body size: $p = 0.41$; body mass: $p = 0.40$). Similar non-significant patterns were observed for BDE-154 (all $p > 0.31$), BDE-47 (all $p > 0.11$), and BDE-99 (all $p > 0.46$). BDE-183 was excluded due to insufficient detection, and BDE-28 due to low sample size ($n < 5$) (Fig. 2).

Among detected PBDE congeners in American mink, 4-MeO-BDE-103, BDE-99, and BDE-28 contributed most to the total burden, whereas BDE-183 showed the lowest contribution (Figure S1).

3.2. Inorganic contaminants

Mean concentrations of EEs, PTEs, and TCEs detected in the different matrices of the American mink are presented in Table S5.

Tissue type was a significant predictor for most EEs ($p < 0.05$; Table S6). For EEs, Fur samples had significantly higher concentrations of Cu, Mn, Ni, S, V, and Zn (Fig. 3). Potassium also differed significantly among tissues, increasing from fur < kidney < muscle, while sodium was similar in kidney and muscle, both significantly higher than in fur. Iron concentrations were higher in the kidney, fur showed intermediate values, and did not differ significantly from those in either kidney or muscle. Fig. 4

For PTEs, only As and Sr varied significantly among tissues, both having higher concentrations in fur, with no differences between the kidney and muscle

Regarding TCEs, tissue-related differences were limited. Pr and Ru also varied significantly among tissues, whereas Rb differed only between muscle and kidney. Rubidium concentrations were higher in muscle, Ru in fur. In contrast, Pr showed no significant differences among tissues (Fig. 5). Overall, fur consistently had higher concentrations for most trace elements, whereas muscle and kidney showed lower and more comparable levels.

3.3. Stable isotope analysis

Stable isotope analysis was conducted on individuals from the Angueira River ($n < 5$ for Maçãs). Mean $\delta^{13}C$ values were −24.9‰ for males and −26.0‰ for females, while $\delta^{15}N$ values were 12.1‰ and 12.4‰, respectively. No significant differences were detected for either $\delta^{13}C$ ($p = 0.10$) or $\delta^{15}N$ ($p = 0.35$).

Isotopic niche analysis indicated substantial overlap between sexes

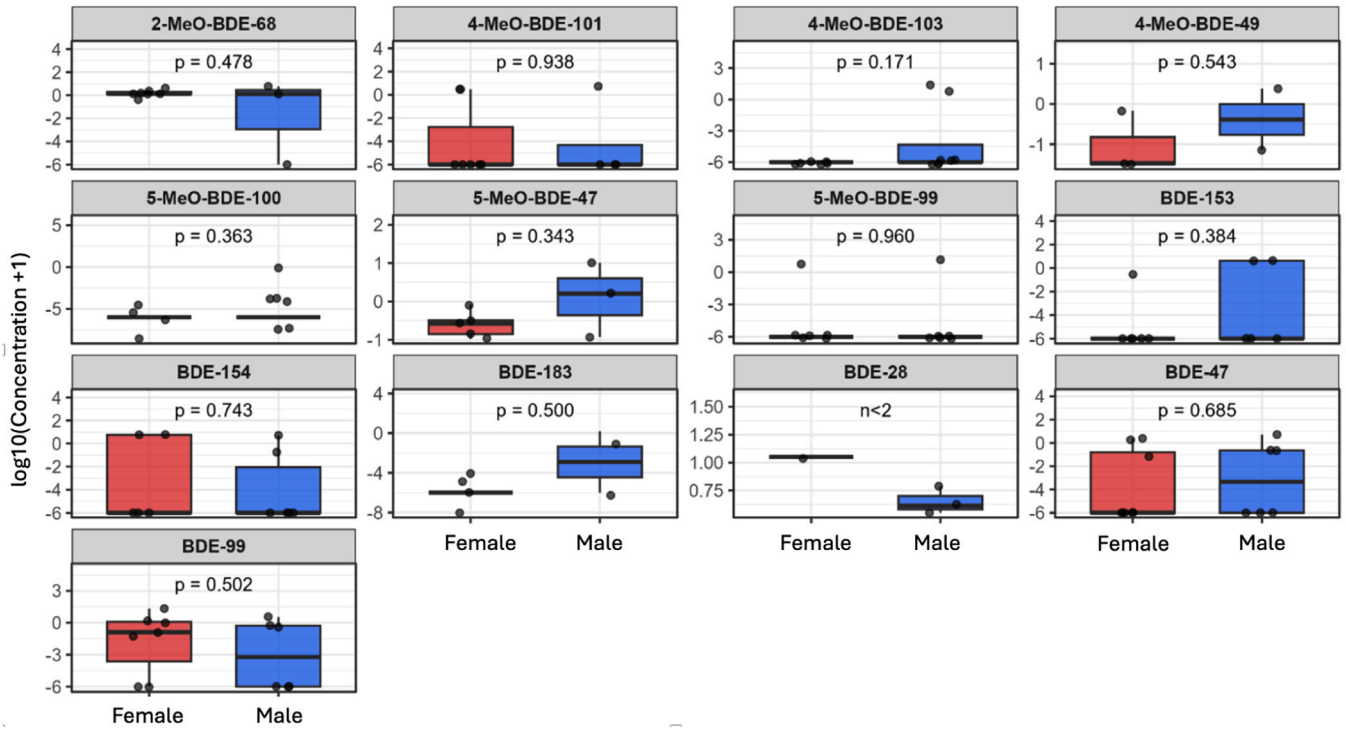

Fig. 2. Boxplots of the base-10 logarithm of PBDE congener concentrations in American mink (*Neogale vison*) livers according to sex. Boxes represent the interquartile range (25th–75th percentiles), horizontal lines indicate median values, and whiskers represent minimum and maximum values. P-values were obtained from generalized linear models (GLMs) testing the effects of sex on individual PBDE congeners. No statistically significant differences were detected ($p > 0.05$).

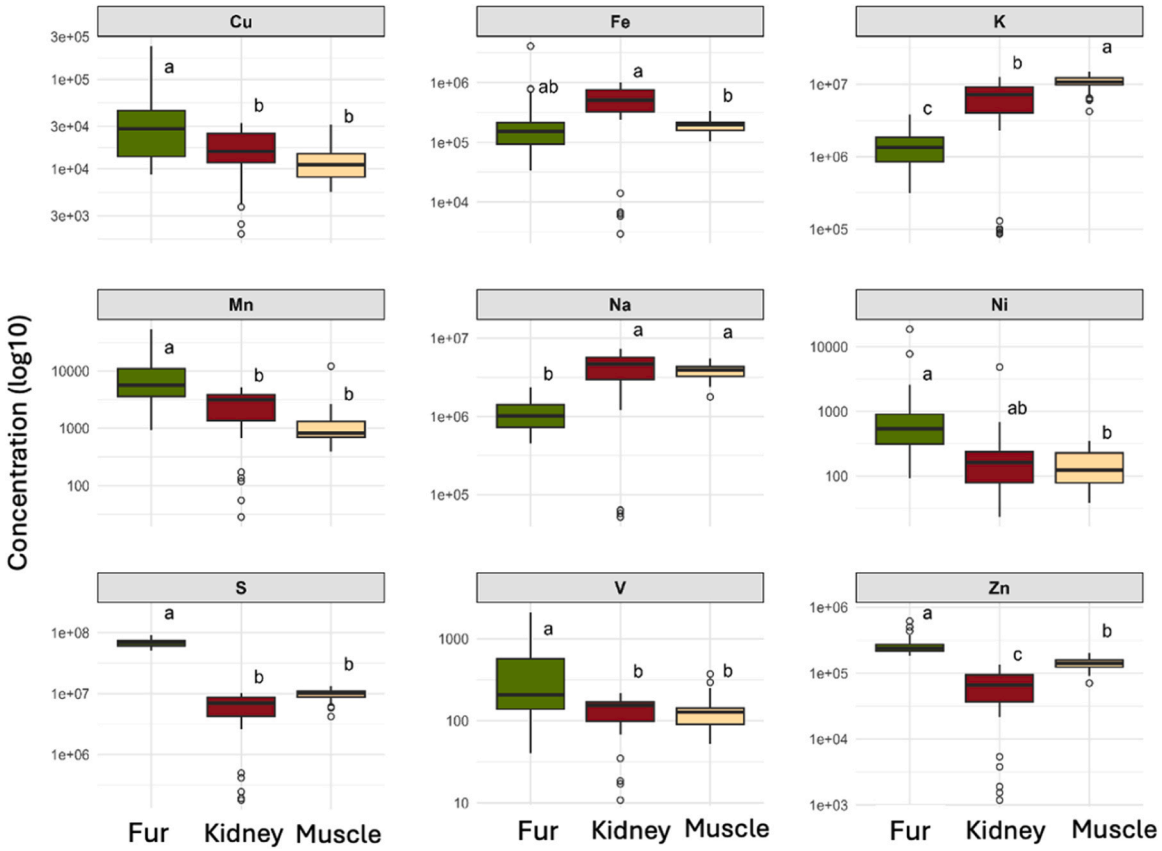

Fig. 3. Boxplots of essential element (EEs) concentrations in different tissues of American mink (*Neogale vison*), represented on a $\log_{10}$ scale. Boxes represent the interquartile range (25th–75th percentiles), horizontal lines indicate median values, and whiskers represent minimum and maximum values. Different letters indicate statistically significant differences among tissues according to one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

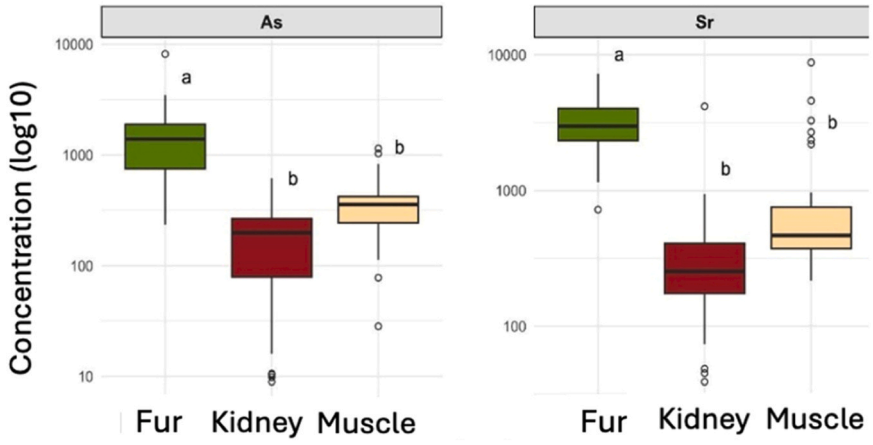

Fig. 4. Boxplots of potentially toxic elements (PTEs) concentrations in different tissues of American mink (*Neogale vison*), represented on a $\log_{10}$ scale. Boxes represent the interquartile range (25th–75th percentiles), horizontal lines indicate median values, and whiskers represent minimum and maximum values. Different letters indicate statistically significant differences among tissues according to one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

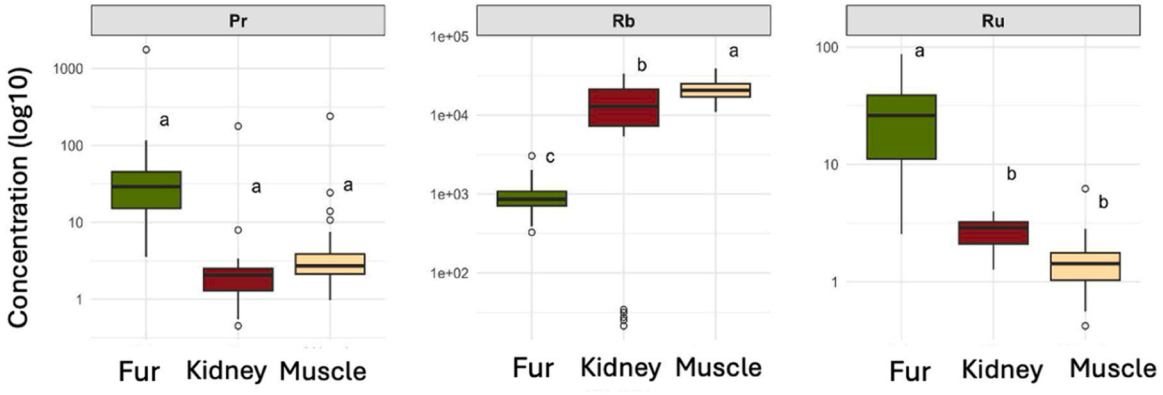

Fig. 5. Boxplots of technology-critical elements (TCEs) concentrations in different tissues of American mink (*Neogale vison*), represented on a $\log_{10}$ scale. Boxes represent the interquartile range (25th–75th percentiles), horizontal lines indicate median values, and whiskers represent minimum and maximum values. Different letters indicate statistically significant differences among tissues according to one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

(38% of total ellipse area; Fig. 6a). Standard ellipse areas (SEA) were 6.3‰^{2} for males and 4.7‰^{2} for females (Fig. 6b).

Pearson correlation analysis (Fig. 7a) between stable isotopes and contaminants was generally weak. δ^{15}N showed moderate positive correlations with Cu ($r = 0.39$) and Ni ($r = 0.34$), while δ^{13}C showed mostly weak relationships.

Correlations between PBDE congeners and isotopes were also low to moderate, with the highest values observed for 5-MeO-BDE-47 (δ^{13}C: $r = 0.56$ and δ^{15}N: $r = 0.34$) and PBDE-153 (δ^{15}N: $r = 0.69$) (Fig. 7).

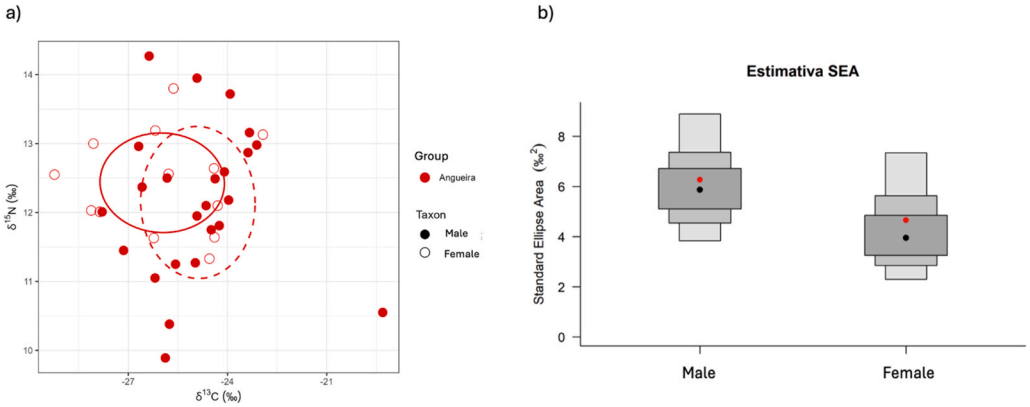

Fig. 6. a) Isotopic niche of American mink (*Neogale vison*) individuals from the Angueira River, showing males, filled circles, solid ellipses, and females, open circles, dashed ellipses. b) Estimated standard ellipse area, SEA, for male and female American mink, with 95%, 75%, and 50% confidence intervals; black and red dots indicate the mode and maximum likelihood estimate, respectively.

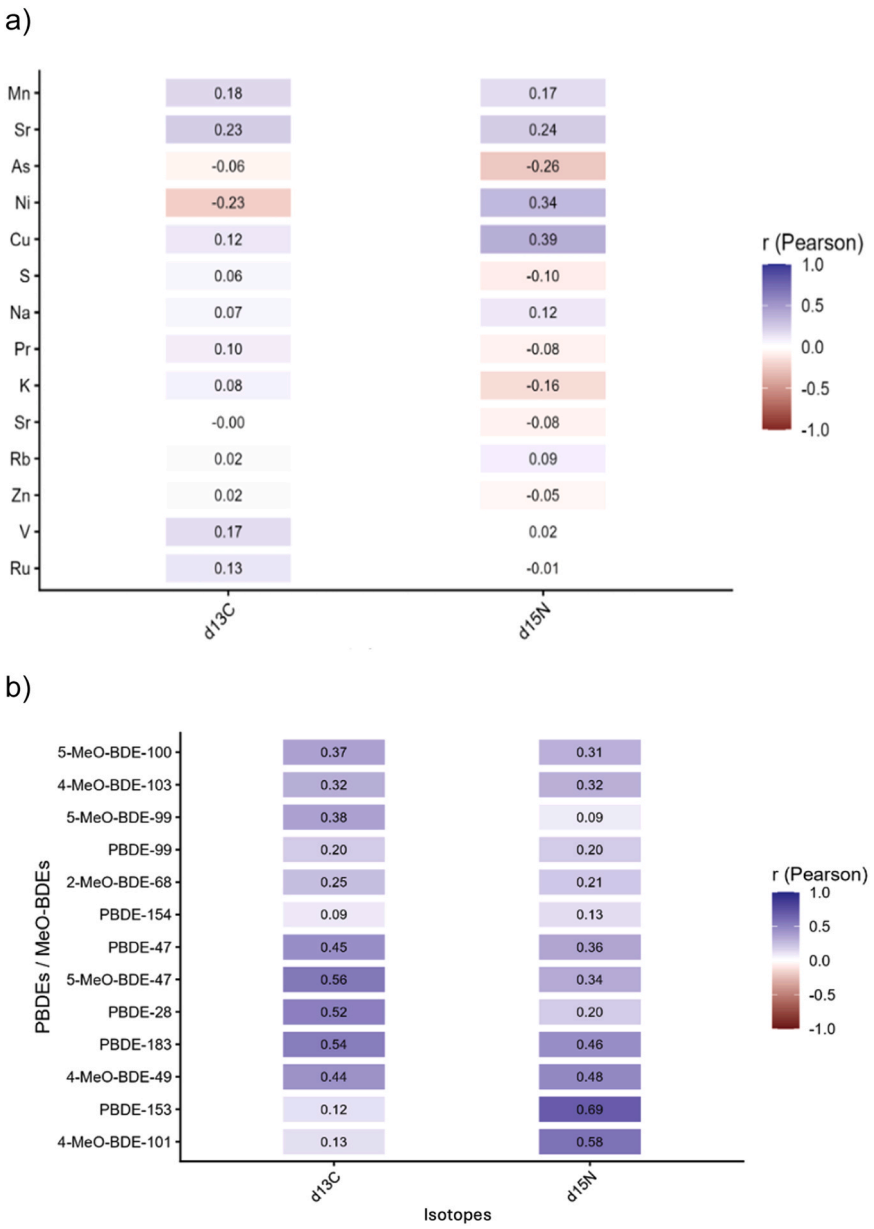

Fig. 7. Pearson correlation between (a) trace/technology-critical elements and stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and (b) 13 PBDE congeners in American mink (*Neogale vison*). Blue cells indicate positive and red cells negative correlations; values 0–0.3 = weak, 0.3–0.7 = moderate, and 0.7–1 = strong correlation. Asterisks (BH): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. General contamination patterns

American mink from the Angueira and Maçãs Rivers showed detectable but generally low levels of PBDEs and trace elements, consistent with relatively low anthropogenic pressure in these systems. The observed concentrations fall within the lower range reported for mustelids and other semi-aquatic predators [33,34], supporting the interpretation of limited regional contamination. Higher PBDE burdens have been documented in American mink and Eurasian otter populations from more industrialized regions of Europe and North America [20,35], while elevated trace element levels are commonly reported in predators inhabiting contaminated and urbanized river basins [33,34]. In fact, the concentrations measured in this study are comparable to or lower than those reported for populations from less impacted environments. Regional baselines further support this interpretation. For example, European otters from northwestern Spain showed mean concentrations (mg kg^{-1} dw) of Zn around 134 in fur and 155 in liver, Cd around 0.02 in fur and 1.15 in liver, and Pb around 1.31 in fur and 0.28 in liver [23]. Similarly, in northeastern Portuguese rivers characterized by low human disturbance, signal crayfish showed mean concentrations (mg kg^{-1} dw) of Cu ranging from 63.5 to 81.7, Zn from 76.7 to 110, Mn from 7.2 to 35.2, Cd from 0.15 to 0.21, and Al from 42.4 to 129 [16], indicating low to moderate metal burdens in less disturbed freshwater systems. Nevertheless, the detection of PTEs and TCEs, such as As, Sr, Ru, Rb, and Pr, even in low-disturbance areas, highlights their widespread occurrence in freshwater ecosystems. Their consistent presence across tissues reinforces the role of top predators as integrators of environmental contamination, while their higher concentrations in fur support its use as a non-invasive matrix for ecotoxicological monitoring.

Isotopic results further indicated similar trophic positions and diets between sexes. Consistently, PBDE and trace element concentrations were not influenced by sex or body size, varying only according to the

type of analyzed tissue. These results suggest that exposure and bioaccumulation are primarily driven by environmental and dietary factors.

4.2. Trophic dynamics and contamination by PBDEs and MeO-BDEs

In the present study, PBDE concentrations were not influenced by sex or body size, aligning with previous findings in *L. canadensis* and *L. lutra* [36,37]. Conversely, tissue type played a major role in PBDE detection, as internal organs, particularly those with higher lipid content such as the liver, tended to exhibit higher concentrations [19]. In our study, PBDEs and MeO-BDEs were quantified exclusively in the liver due to limited tissue availability and the recognized suitability of this matrix for hydrophobic contaminants. Therefore, alternative tissues could not be evaluated for PBDE analysis within this dataset. Previous studies have shown that non-invasive matrices, such as feces and fur, are reliable indicators of PBDE exposure, as their concentrations correlate well with blood and tissue burdens [38–40]. These findings are presented here as external evidence from the literature. In contrast, our results provide direct empirical support for the use of fur as a non-invasive matrix for multi-element monitoring, highlighting the need for future research to assess its applicability for PBDE monitoring under Iberian freshwater conditions.

Although information on PBDE contamination in *N. vison* remains scarce, studies in other mustelids (e.g., *L. lutra*, North American river otter *Lontra canadensis*) demonstrate efficient accumulation through trophic transfer [36]. *In vivo* studies have demonstrated a clear positive relationship between fish-based diets and PBDE accumulation in hepatic tissues [41], indicating an efficient dietary uptake and bioaccumulation within aquatic food webs. Consistent with other predators, BDE-47 typically dominates the congener profile, followed by BDE-99 and BDE-153 [42,43], reflecting similar patterns as reported for the Eurasian otter and other piscivorous mammals.

Mustelids are generally recognized as highly sensitive to lipophilic contaminants, with several studies showing stronger physiological and reproductive impacts [37,44]. For example, experimental exposure studies with *N. vison* have shown that dietary intake of PBDE mixtures (e.g., DE-71) results in marked physiological and immune alterations, including increased liver enzyme activity and organ hypertrophy [45]. This heightened susceptibility underscores the importance of *N. vison* as a sentinel species for assessing contaminant exposure in freshwater food webs.

In the present study, most PBDE concentrations were below ranges previously associated with overt toxicological effects in controlled exposure experiments with American mink. Experimental studies have shown that dietary exposure to PBDE mixtures can induce physiological and reproductive effects, those adverse effects generally occur at substantially higher dietary and tissue concentrations than those observed here, particularly in American mink exposed to technical PBDE mixtures such as DE-71 [41,42,45]. Nevertheless, chronic exposure to low-level mixtures may still contribute to sub-lethal effects, including endocrine disruption, immunological stress, and altered vitamin A and thyroid hormone metabolism, particularly under long-term environmental exposure scenarios [36,44,45].

Information on MeO-BDEs in aquatic mammals and other taxonomic groups remains particularly limited. Most available data derive from marine systems, where natural biosynthesis of MeO-BDEs has been attributed to microbial or algal pathways [46,47]. In crustaceans from Portuguese estuaries, MeO-BDE concentrations ranging from 1.72 to 5.66 ng g^{-1} d.w. were reported in Menezes-Sousa et al. [48]. In another study, in nearby freshwater ecosystems (Rabaçal and Tuela Rivers), markedly lower concentrations (mean = 0.174 ng g^{-1} d.w.) were reported for the signal crayfish [49], which are an order of magnitude lower than those typically found in marine and estuarine organisms. This likely reflects the limited anthropogenic pressure and the absence of major point sources in these basins. Similarly, *N. vison* from Sweden exhibited MeO-BDE levels ranging from 1.60 to 2.10 ng g^{-1} lipid weight [50], often lower than in their fish prey, suggesting either rapid metabolism of these contaminants or a diet mainly relying on terrestrial prey. Such variability emphasizes the combined influence of local contamination sources, trophic habits, and metabolic capacity in shaping contaminant burdens in mustelids. In comparison, the MeO-BDE concentrations observed in the present study are higher than those reported for low-impact areas, suggesting additional sources or enhanced bioaccumulation potential in the studied populations.

Comparisons with the Eurasian otter provide a relevant native benchmark for interpreting contaminant exposure, as both species occupy high trophic positions but may differ in ecological strategies and foraging behavior. Otters are more specialized piscivores with a strong reliance on fish, whereas American mink exploit a broader spectrum of aquatic and terrestrial prey [51,52]. These ecological differences may result in distinct contaminant profiles within the same system, particularly for lipophilic compounds such as PBDEs, which are strongly associated with fish-based food webs [36,41]. In addition, interspecific differences in metabolic capacity, detoxification pathways, and elimination rates may further contribute to divergent congener patterns between native and non-native predators [37,45]. As a result, American mink and otters, although occupying similar trophic levels, may integrate contaminant signals through partially distinct ecological and physiological pathways, reinforcing their complementary value as sentinels of freshwater contamination.

4.3. Trace elements distribution and bioaccumulation in the American mink

Trace element distribution was strongly tissue-dependent, with fur consistently showing higher concentrations for most elements. Among EEs, Cu showed the highest concentrations, consistent with previous studies reporting its preferential accumulation in soft tissues, approximately 30% in the liver, 30% in fur, 20% in the kidney, and 10% in muscle and bone of mammals, including the American mink [53]. This heterogeneous distribution reflects its biological functions, as Cu is primarily stored in the liver as a cofactor for enzymatic processes, excreted and regulated through the kidneys, and bound to sulfur-rich proteins such as keratin in fur, which explains its relatively high proportion in this tissue [53]. In contrast, Fe showed similar concentrations across tissues, consistent with its fundamental role in oxygen transport and enzymatic processes [54]. The values recorded in the kidney (504 μg g^{-1}) were within the range reported for *L. lutra* in southern Italy (65.4–507 μg g^{-1}) [22]. Manganese, Na, and K followed their expected physiological patterns. Manganese, an essential cofactor in enzymatic biochemical reactions but toxic at elevated concentrations [55], showed lower values in fur (mean = 8.28 μg g^{-1}) compared to those reported in fur of wild mustelids from Northern Italy (27 μg g^{-1} dry weight; [34], analyzed using comparable ICP-based methods. This difference may reflect lower anthropogenic inputs in the study area, since Mn primarily derives from human activities [56]. Sodium and K are both key elements for osmotic regulation and cellular function, showing the highest concentrations in muscle (mean K = 10628 μg g^{-1}; mean Na = 3860 μg g^{-1}), consistent with the high metabolic activity of this tissue that functions as reservoirs [57,58].

Nickel (mean = 1.24 μg g^{-1}), S (mean = 68000 μg g^{-1}), and V (mean = 0.43 μg g^{-1}) also accumulated in higher concentrations in fur compared to the other tissues. These values align with the literature, as S is present in amino acids and therefore in keratin, while Ni and V have a strong affinity for this protein, which is abundant in fur [34,59]. Similar results were obtained for Zn, an element important for enzymes, proteins, and transcription factors, with higher concentrations in fur (mean = 265 μg g^{-1}), comparable to those described for mustelids in European populations (131 μg g^{-1}; 134 μg g^{-1} d.w.) [22,23,34].

Among PTEs, such as As (mean = 1.61 μg g^{-1}) and Sr (mean = 3.20 μg g^{-1}) showed higher concentrations in fur, exceeding values

reported for mustelids and other mammals (As = 0.43 μg g^{-1}) [23,34,60]. This pattern may reflect both internal redistribution and binding to keratin. Garcia-Muñoz et al. (2025) reported that the kidney accumulates As values similar to the liver, as the former is the main organ responsible for excretion, while the latter is a storage site. For Sr, studies suggest competition with Ca, leading to preferential accumulation in mineralized tissues such as bones [61], although its protein-binding nature also allows its presence in fur, reflecting internal concentrations [62].

Finally, TCEs showed more variable patterns, with Praseodymium, a relatively understudied element found in the environment and fertilizers [63], showing relatively low values. Exposure to Pr can alter Fe homeostasis, mitochondrial respiration, and gene expression, and can adversely affect heart morphology and fish swimming behavior [63,64]. Rubidium was more abundant in muscle (mean = 21.4 ng g^{-1}), consistent with its physiological similarity to K [65]. Some authors suggest it may play an essential biological role but warn about its narrow margin between necessity and toxicity (Garcia-Muñoz et al., 2025; [65]. Ruthenium, although present in low concentrations, was preferentially detected in fur, supporting the role of keratin in binding circulating elements from the bloodstream [66,67]. Moreover, studies indicate that Ru tends to accumulate in the liver, kidneys, and bones after systemic absorption, suggesting that studying these matrices could be informative [66].

Authors have argued that the concentrations of PTEs vary with seasonality, river flow, anthropogenic contamination, and factors such as geological processes or the reproductive cycle of the monitored species [68]. Intraspecific variation, though already reported in other studies [69], was not observed here, as no differences were found between males and females. Accumulation may depend on trophic transfers, meaning predators such as the American mink may be more susceptible to the effects of these contaminants due to their bioaccumulative potential.

High pollutant concentrations have been associated with poor physical condition, potentially affecting individual survival. Additionally, some authors have suggested that exposure of mustelids to contaminants such as PCBs may enhance metal accumulation in their tissues [70]. In the present study, although PBDE levels in American mink were moderate compared with values reported in previous studies, the concentrations of trace elements and metals may also be influenced by this interaction. This hypothesis could be further explored through a combined analysis of datasets on organic and inorganic contaminants.

Regarding tissue-specific patterns, only Rb and K showed higher concentrations in muscle compared with other matrices. In contrast, the kidney generally had higher concentrations than muscle for most elements except Fe, emphasizing the physiological importance of this organ in element accumulation. This pattern reflects the role of the kidney in detoxification, where primary bioaccumulation occurs, leading to generally higher concentrations of several trace elements (Garcia-Muñoz et al., 2025; [57].

The data from the present study indicate that most trace elements showing significant differences occurred at higher concentrations in the fur of the American mink. This pattern reflects the high metal-binding capacity of keratin, the main structural protein in fur, which is rich in sulfur-containing amino acids such as cysteine. The thiol (-SH) functional group of cysteine exhibits a strong affinity for several inorganic elements, promoting their incorporation into the growing fur during the anagen (active growth phase), when elements circulating in the bloodstream are incorporated into the follicular matrix and become structurally embedded within the keratin shaft [34,67]. As a result, fur provides a time-integrated record of internal exposure rather than a short-term reflection of recent uptake. Once incorporated into the keratin structure, elements are largely retained and no longer subject to metabolic redistribution, contributing to the accumulation of elements in fur compared with soft tissues [67,71]. Element speciation may further influence this pattern, as elements capable of forming stable complexes with sulfur or protein ligands are more efficiently sequestered in keratinized tissues, helping to explain the higher concentrations of As, Sr, and Ru observed in fur relative to liver or kidney. Consequently, fur serves as a reliable, non-invasive matrix for assessing trace element accumulation. Similar trends have been reported in controlled studies involving several mustelid species, in which higher concentrations of trace elements were consistently observed in fur, followed by liver and kidney [29,33].

Additionally, element accumulation in our study was not influenced by sex, weight, or body size, although previous studies report variable patterns depending on species and environmental context. The literature presents a wide range of interpretations, as some authors studying mustelids report no significant influence of age or sex on accumulation [29,53], while others report positive trends on accumulation associated with age [72,73] and higher concentrations in males (Garcia-Muñoz et al., 2025).

4.4. Trophic ecology of the American mink

Stable isotope analysis provides information about the origin and assimilation of food resources, allowing the inference of trophic niches and ecological relationships among organisms [74]. Variation in $\delta^{13}C$ values did not indicate differences in the origin of food sources. Although the mean value in females (−26.0‰²) was slightly lower than in males (−24.9‰²), no significant intraspecific differences were observed, suggesting that both male and female American mink individuals from the Angueira River share a similar carbon source, predominantly rich in C_3 carbon [75], which may indicate a diet based on terrestrial prey [8,9]. Likewise, the $\delta^{15}N$ values showed no difference in trophic level between males and females since the mean values were very close (12.1‰² for males and 12.4‰² for females). Moreover, the high $\delta^{15}N$ values detected for both groups suggest that these individuals occupy a similarly high trophic position in the local food web, consistent with their status as predators. Studies conducted on captive and wild American mink have reported similar values for both isotopes [11,76]. The observed 38% niche overlap between males and females from the Angueira River indicates substantial sharing of food resources, although with some differentiation. Studies have reported sexual dimorphism in mustelids such as the American mink, with males preying on larger prey [77]. Comparable studies have also found no sex-related differences in the diet of this species [11,78].

The higher SEA value in males suggests a broader trophic niche, potentially reflecting greater dietary variability, whereas females, with a smaller SEA, may have a more specialized or restricted feeding strategy. This pattern aligns with the ecological consequences of sexual dimorphism in mustelids, where the physiological characteristics of individuals influence the prey consumed [77,78].

Relationships between muscle isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) and hepatic accumulation of PBDEs were explored. However, none of the correlations remained statistically significant after correction for multiple comparisons. Therefore, these data do not support strong inference regarding trophic transfer patterns or biomagnification in this species. More conclusive evidence would require food web–based approaches that incorporate prey species and trophic magnification factors (TMFs) [19,79].

5. Conclusions

This study demonstrates that the non-native American mink can be effectively used as a sentinel of multiple contaminant classes in invaded freshwater ecosystems. By simultaneously quantifying PBDEs, MeO-BDEs, and 63 elements – including essential, potentially toxic, and technology-critical elements – across several tissues, we show that this species integrates a broad contaminant signal from its environment and diet. Overall, ∑PBDE and ∑MeO-BDE concentrations were low compared with values reported for other mustelids and more

contaminated regions, indicating that the Angueira and Maçãs Rivers were under relatively low pressure from these persistent organic pollutants during the sampling period (2023–2024).

Tissue type, rather than sex, size, or body mass, was the main factor structuring contaminant patterns. Taken together, findings of this study support three key findings: (i) even in less disturbed basins, the American mink may accumulate a mixed burden of organic and inorganic contaminants that deserves monitoring; (ii) fur can be adopted in surveillance programs, including for other mammals, as it reflects essential and some toxic elements and reduces the need for lethal sampling; and (iii) the presence of a non-native predator can be leveraged as a scientific advantage, serving as a sentinel of environmental contamination in areas where population control is already ongoing.

Future work should (i) include the American mink's main prey and sympatric native predators such as the Eurasian otter to directly assess trophic transfer and biomagnification pathways, (ii) incorporate additional emerging contaminants (e.g., PFAS, pharmaceuticals), and (iii) evaluate spatial and seasonal variation, allowing ongoing control campaigns to be integrated into long-term environmental monitoring programs. In this context, assessing trophic niche overlap between species occupying similar ecological roles will be particularly important. The American mink and Eurasian otter are highly mobile and have been reported to coexist in various ecosystems, including the present study area [80,81]. Integrating contaminant, isotopic, and dietary information across native and non-native predators will be essential to better understand the dynamics of local trophic niches, pollutant redistribution, and the ecological role of non-native species. This integrated approach will strengthen both non-native species management and the surveillance of freshwater ecosystem health.

Environmental implication

Non-native American mink (*Neogale vison*) are routinely culled in European control programs, yet their carcasses remain an underused source of environmental information. By integrating legacy organic pollutants, essential and potentially toxic elements, and emerging technology-critical elements across multiple tissues, this study shows that mink can serve as effective sentinels of contaminant exposure in transboundary freshwater ecosystems. The relatively low but diverse contaminant burdens detected highlight the persistence of hazardous substances even in sparsely populated basins. Incorporating mink tissue analyses into ongoing management actions would provide a cost-effective tool for long-term biomonitoring and assessing ecosystem health.

CRediT authorship contribution statement

Bárbara Lages: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Ronaldo Sousa:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sara C. Cunha:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis. **Juliana Souza-Kasprzyk:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. **José O. Fernandes:** Writing – review & editing, Validation, Software, Resources, Methodology, Funding acquisition. **Alejandro Nieto Gámez:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation. **Miguel Nóvoa:** Writing – review & editing, Visualization, Resources, Project administration, Investigation. **Americo Guedes:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation, Funding acquisition. **Pedro Alves:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation, Data curation. **Przemyslaw Niedzielski:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Data curation. **Paulo Cortez:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Methodology, Data curation. **Roberto Sargo:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Methodology, Data curation. **Andreia Garcês:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Data curation. **Filipa Loureiro:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Data curation. **Amilcar Teixeira:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Janeide Padilha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2026.141895.

Data availability

Data will be made available on request.

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