Health Risk Assessment of Potentially Toxic Elements, Persistence of NDL-PCB, PAHs, and Microplastics in the Translocated Edible Freshwater Sinotaia quadrata (Gasteropoda, Viviparidae): A Case Study from the Arno River Basin (Central Italy)

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Abstract

With this study we investigated the accumulation of potentially toxic elements (As, Cd, Cr, Cu, Hg, Pb, Zn), six indicators (28, 52, 101, 138, 153, 180) of non-dioxin-like polychlorinated biphenyls (Σ_6 NDL-PCBs), polycyclic aromatic hydrocarbons (PAHs), and microplastics in *S. quadrata* (edible part) collected from two sampling sites (1 and 2) from the Arno River Basin (Central Italy). A risk assessment of the implications for human health was also performed. Levels of potentially toxic elements in gastropods from site 2 were slightly higher and the Σ_6 NDL-PCB concentration was significantly higher (7.32 ng g^{-1} *vs*. 3.07 ng g^{-1}) compared to site 1 due to higher anthropogenic pressures. The concentration of chrysene, benzo[b]fluoranthene, and benzo(a)pyrene was below the limit of quantification (0.5 μg kg^{-1}). Benzo[a]anthracene was detected in gastropods from both sites (0.5 ± 0.02 μg kg^{-1} and 0.7 ± 0.02 μg kg^{-1} from site 1 and 2, respectively). The microplastics frequency (mainly polyethylene terephthalate) differed significantly between the sites (site 1, 0.8 ± 1.30; site 2, 1 ± 0.37 items/specimen). All contaminant levels were compliant with international regulatory limits and guidelines. Incremental lifetime cancer risk (ILCR) values for As, Cd, Cr, and Pb were far below the safety values of 1×10^{-4}. Similarly, the ILCR values from the Monte Carlo simulation model were all within the safety region of 1×10^{-4} and $1 \times 1\ 0^{-6}$. Findings from the health risk assessment indicated no adverse effects for human health from any of the contaminants analysed here, except for microplastics for which no limits or legislation are currently in force.

Keywords River Arno, Benzo[a]anthracene, Environmental monitoring, Polyethylene terephthalate, Risk assessment

Introduction

Evaluation of polluting compounds and knowledge of the ecological condition of an ecosystem are requisite for management and prevention of health risks to humans and for environmental protection (Cunningham et al. 2009). Biomonitoring involves the use of organisms to evaluate changes in water or air quality and measure the bioavailable fraction of the compound and its fate along the food web (McIntyre and Beauchamp 2007; Needham et al. 2007).

Among freshwater macroinvertebrates, gastropods are benthic organisms that colonize a wide variety of aquatic environments (Strong et al. 2007). Found in typically lotic and lentic environments, many species have been translocated and become an invasive species in non-native environments (Cianfanelli et al. 2017). The geographical area comprising Florence, Prato, and Pistoia (Tuscany, central Italy) is home to a large Asian community of more than 50,000 inhabitants (over 8% of which are Chinese), ranking second to the Milan metropolitan area (north Italy) population size (ISTAT 2019). In keeping with their traditional cuisine, they have introduced animal and plant species to urban non-native environments outside China. The freshwater gastropod *Sinotaia quadrata* (Caenogastropoda, Viviparidae) is widely used in Chinese cuisine. Its recent discovery in the Arno river basin is the first recorded occurrence in Europe (Cianfanelli et al. 2017). The potential creation of an extensive mollusk farm using the Arno as a natural habitat for *S. quadrata* for human consumption raises concern about the threat to biodiversity and to human health from consumption of this alien species (Cianfanelli et al. 2017).

Cianfanelli et al. (2017) reported evidence for a wellstructured population of *S. quadrata* in sites on the Arno river (downstream of Florence), where the water is rich in organic substances and nutrients that facilitate the action of tolerant suspension feeders like *S. quadrata*. The main anthropogenic inputs to the Arno occur downstream of Florence and derive from direct civil and industrial wastewater discharges. The River Arno is the longest in Tuscany (about 245 km length, catchment area about 8228 km^2) and is ranked fifth among the largest basins in Italy. It rises in the northern Apennines and empties into the Tyrrhenian Sea near Pisa. It is polluted by industrial and untreated civil wastewaters from Florence and other cities along the river (Arezzo, upper stretch of the river; Pistoia and Prato north of Florence). Adding to the potential threat to the environment are the wastewaters from industrial zones and nurseries around Pistoia and Prato carried by its tributaries, the Bisenzio and the Ombrone.

The bioaccumulation of contaminants by *S. quadrata* may pose a potential health risk to humans: potentially toxic elements, persistent and accumulative substances such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), plus emerging contaminants such as microplastics can accumulate in aquatic organisms, including gastropods.

Metal contamination is a major environmental concern in aquatic ecosystems because the elements tend to accumulate in the body of organisms at concentrations higher than those found in abiotic compartments (Jia et al. 2017; Pastorino et al. 2019). Some have nutritional functions and are essential for life (copper and zinc) and for maintenance of animal (Esposito et al. 2018), even if become toxic at higher concentrations (Yuan et al. 2017; Pastorino et al. 2020a). Others still, like lead, cadmium,

mercury, and arsenic, have no biological function and are extremely toxic even at low concentration (Halder et al. 2020).

PCBs comprise a series of 209 aromatic compounds made up of variously chlorinated biphenyl molecules synthesized at the beginning of the last century and produced commercially since 1930 (Howell et al. 2008); they are currently largely banned due to their toxicity and tendency to bioaccumulate (Oziolor et al. 2018). Only 12 of the 209 PCBs congeners have chemical-physical and toxicological characteristics comparable to dioxins and furans: these are defined as dioxin-like PCBs (DL-PCBs). The sum of the six congeners termed indicators (28, 52, 101, 138, 153, 180) includes about half of all non-dioxin-like PCBs (NDLPCBs) present in feed and food products (Malisch and Kotz 2014). The European Food Safety Authority (EFSA) stated that the sum of these PCBs constitutes an adequate indicator of the occurrence and human exposure to NDL-PCBs since they are analytically predominant in environmental matrices (EFSA 2005; Squadrone et al. 2013).

Polycyclic aromatic hydrocarbons are organic compounds formed by two or more aromatic rings that derive mainly from the incomplete combustion of organic matter (e.g., coal, petroleum derivatives, oil or biomass) (Marzooghi and Di Toro 2017; Wang and Wang 2018; Molsen et al. 2019). Because of their hydrophobicity and long-range transportation, PAHs are widely distributed and can accumulate in diverse environmental compartments, including living organisms (Honda and Suzuki 2020). Generally, PAHs are substances of toxicological interest as some are considered probable or possible carcinogens. The most extensively studied component is benzo(a)pyrene (B[a]P) widespread in the environment at significant concentrations and extremely toxic (Zhang et al. 2019); the PAH4 index (the sum of benzo[a]anthracene-B[a]A, chrysene-Chr, benzo[b]fluoranthene-B[b]FL, and B[a]P) was recognized as an indicator of cancer risk due to dietary exposure to PAHs (EFSA 2008).

Another major environmental issue is plastic pollution because ubiquitous in marine and freshwater ecosystems worldwide (Borrelle et al. 2020). An emergent threat to wildlife and human health is the formation of microplastics from the breakdown of large plastic pieces into tiny particles (< 5 mm) (Mitrano and Wohlleben 2020). Human health and well-being are intimately linked to environmental quality (USEPA, 2021). Supra-optimal levels of pollutants can induce or increase the risk of developing diseases of the respiratory, reproductive, and immune system and cancer in humans (Shahid et al. 2020).

To our best knowledge, there are no investigations into the occurrence of contaminants in *S. quadrata* from the Arno River basin. Furthermore, a risk assessment of implications for human health by their consumption is warranted. The aims of the present study were to: (a) measure the accumulation of potentially toxic elements (As, Cd, Cr, Cu, Hg, Pb, Zn), PAHs (B[a]A, Chr, B[b]FL, and B[a]P), NDL-PCBs (28, 52, 101, 138, 153, 180), and microplastics in tissue samples of *S. quadrata* collected from two sites on the Arno; (b) determine whether contaminant levels are compliant with regulatory limits and guidelines; (c) assess the possible human health risks associated with gastropod consumption.

Material and Methods

Study Area

Two sampling sites were selected according to their accessibility and the presence/abundance of *Sinotaia quadrata* (Cianfanelli et al. 2017). Site 1 (Samminiatello; 1663666X 4845701Y) and site 2 (Fibbiana; 1660695X 4844688Y) are both located in the Municipality of Montelupo Fiorentino

(Florence Province) (Fig. 1) where the riverbed is largely disconnected from the perifluvial belt, as it is intensely urbanized. The sites are located near the River Arno Valdarno Inferiore Capraia e Limite (code MAS-108) stations, which is monitored by the Regional Environmental Protection Agency of Tuscany (ARPAT) for assessment of the ecological status sensu Water Framework Directive. At the station site, the River Arno receives the Ombrone Pistoiese and Bisenzio tributaries on the right. The river water quality begins to decrease due to inflow of wastewater from the Baciacavallo treatment plant (Prato Province), which serves the towns of this textile manufacturing area. This stretch of the Arno is noted for its low quality: the ecological status of MAS-108 is "poor" according to the last report (ARPAT 2021).

Sampling and Processing of *Sinotaia quadrata*

Fifty specimens homogeneous in size (33.9 ± 0.12 mm in height and 19.4 ± 0.21 mm in diameter) were collected by hand at each site directly from stones, pebbles, and riverbed mud (30–100 cm in depth). Samples were placed in precleaned glass beakers to prevent contamination, covered with aluminum foil, and transported refrigerated (+ 4 °C) to the laboratory within a few hours after collection and immediately frozen (−20 °C) whole (in their shell) until chemical analyses.

Detection of Potentially Toxic Elements

Determination of As, Cd, Cr, Cu, Hg, Pb, and Zn was performed on the whole soft body of fifteen *S. quadrata* per site by inductively coupled plasma-optic emission spectrometry (ICP-OES) on a Perkin Elmer Optima 2100 DV instrument (PerkinElmer, Inc., Shelton, CT, USA), coupled with a CETAC U5000AT + ultrasound nebulizer (Cetac Technologies, Inc., Omaha, NE, USA) for mercury, as previously reported by Pastorino et al. (2020b). Briefly, samples were homogenized and microwave-digested in a Milestone ETHOS ONE oven using 4 mL H NO_3 and 1 mL H $_2O_2$. All reagents were from Merck (Darmstadt, Germany); acids were of Suprapur grade. Results are presented as μg g^{-1}wet weight (w.w.). Analytical performance was determined by processing certified reference materials (HISS-1) in six replicates for corresponding elements, along with blank reagents in each analytical run. All reference material values were within certified limits. Table S1 presents the limit of detection (LOD), the reference material values, and the percentages of recovery.

PAH and PCBs Analyses

Fifteen specimens of *S. quadrata* per site underwent PAHs analysis. Determination of benzo[a]anthracene-B[a]A, chrysene-Chr, benzo[b]fluoranthene-B[b]FL, and B[a]P) was performed using HPLC-FLD. Fifteen specimens of *S. quadrata* per site underwent NDL-PCBs (28, 52, 101, 138, 153, 180) analysis by GC–MS.

The HPLC system consisted of a 1100 Series quaternary pump (Agilent Technologies, Santa Clara, CA, USA). Data acquisition and analysis were performed using ChemStation software. The $\lambda_{ex}/\lambda_{em}$ for the fluorescence was 294/404 nm; the gain was 12x; the injection volume 1 mL; the stop time at 36 min; and the column temperature 25 °C. Chromatographic separation was performed using a reversed-phase column Envirosep pp (125 m × 4.6 mm, i.d. 5 μm). The eluents were: water (A) and acetonitrile (B). The flow rate was 1 mL min^{-1} and the program was 80% B for 15 min, then 100% B for 10.0 min, and then held for 10 min.

GC–MS analyses were performed on a gas-chromatograph coupled with single quadrupole GC DSQ 70 FOCUS (Thermo Fisher Scientific, Waltham, MA, USA), equipped with an AS 3000 autosampler. Xalibur software was for mass spectrometer control, data acquisition and analysis. The GC column was a DB-5MS (30 m × 0.25 mm, 0.25 µm), the working conditions were: drying gas He (purity > 99.9%) at 1.2 mL min^{-1}; EI voltage 70 eV; injector temperature 250 °C; splitless mode; split flow 50 mL min^{-1}; gas saver flow 10 mL min^{-1} (5 min); injection volume 1 µL. The oven temperature program was started at 100 °C for 1 min, then increased by 20 °C min^{-1} to 190 °C, and held for 2 min, increased by 3 °C min^{-1} to 250 °C and by 50 °C min^{-1} to 300 °C, and held for 20 min. The mass spectrometer operated in EI ionization in positive mode, the MS transfer line was 270 °C. Table S2 presents the transitions used for detection.

PAHs analysis: 2 g of sample were saponified with 10 mL of a solution of KOH 2 N in EtOH for 2 h and extracted three times with 20 mL of cyclohexane. The organic phase was dried in a rotavapor at 60 °C and added with 4 mL of acetonitrile. The samples were purified with SPE columns and eluted with 12 mL of acetonitrile. The organic phase was dried at 60 ± 5 °C, reconstituted in 1 mL of acetonitrile, and injected into the HPLC system. Analytical blanks and two spiked samples at a concentration of 2 µg kg^{-1} for all analytes were run in the same way as the samples; concentrations were determined using a calibration curve prepared in acetonitrile using PAH-Mix 9 standard (Dr. Ehrenstorfer GmbH, Germany) at 1 ng mL^{-1}, 2 ng mL^{-1}, 5 ng mL^{-1}, 10 ng mL^{-1}, 20 ng mL^{-1}, 50 ng mL^{-1}, 100 ng mL^{-1}, 200 ng mL^{-1}. The recovery sample results ranged between 81% and 90%. The LOQ for all analytes was 0.5 µg Kg^{-1}.

NDL-PCBs analysis: 5 g of each sample was added with 20 µL of internal standard (PCB No.198 and PCB No.155, Dr. Ehrenstorfer GmbH). The PCBs were extracted from fat tissue using a hexane/acetone mixture (50/50 v/v) and shaken overnight. For the clean-up, an Extrelut-SPE cartage system was built by putting Extrelut between a glass flask and a SPE cartage; 3 mL of sulfuric acid was maintained in contact with the fat for 10 min in the Extrelut cartage. The sample was eluted with 13 mL of n-hexane. The organic phase was dried at 60 ± 5 °C, added with 0.1 mL of iso-octane, and injected into the GC system. Quantification was based on peak areas and relative response factors derived from calibration curves constructed using PCB-Mix, PCB No.198 and PCB No.155 (Dr. Ehrenstorfer GmbH) at 5 ng mL^{-1}, 10 ng mL^{-1}, 50 ng mL^{-1}, 100 ng mL^{-1}, 200 ng mL^{-1} in isooctane. For quality assurance, an analytical blank and two spiked samples at a concentration of 12 µg kg^{-1} for all determinations were used, together with each batch of samples, to check for the absence of matrix interferent and the recovery, which was found to range between 94% and 101%. World Health Organization toxic equivalents (WHO-TEQs) are expressed as lower bound concentrations, assuming that all values of the specific NDL-PCB congeners below the LOQ are equal to zero. The LOQ for all analytes was 1.2 ng g^{-1}.

Microplastics Analysis

Frozen specimens of *S. quadrata* (n = 5 per site) were pooled and analyzed, as reported by Pastorino et al. (2021a). Briefly, analysis was performed under air-controlled conditions to prevent airborne microplastic pollution in a dedicated clean chamber equipped with double HEPA filtration to minimize microplastic pollution. Accurately rinsed glassware was used at each stage and blanks and spiked samples were analyzed to evaluate the performance of the process. Blanks were performed using

extraction solutions as samples (n = 5), because of results obtained on blanks (mean 0.004 ± 0.009 items/L, recovered items was a white filament of polyethylene terephthalate-PET), data reported in this study were not corrected by microplastics recorded in blanks because it was negligible. Spiked samples were extracted to evaluate recovery. Pooled gastropods tissues were added with 0.14 items g^{-1} of marked microplastics (PP 23–60 µm blue and PET 35–100 µm white; n = 5) to evaluate recovery (PP 97.1 ± 7.8%; PET 94.3 ± 6.4%). Pooled samples were extracted by ultrasonic cavitation (35 °C, 50 min, frequency 40 kHz) using an extraction solution of H_2O_2 (15% w/v). The extract was collected by vacuum filtration on an Anodisc™ membrane (Merck) for chemical analysis. The samples were analyzed by microscopy associated with Fourier transform infrared spectroscopy (µFT-IR Nicolet iN 10MX, Thermo Fischer Scientific) using a liquid nitrogencooled MCT-A detector that operates in the spectral range of 7800–650 cm^{-1} by transmission technique. Spectral matches acquired on unknown particles were compared with the reference library (OMNIC™ Picta™ software libraries integrated with libraries collected by the Bioscience Research Center on reference materials); only spectral matches >90% were considered; matches between 70% and 90% of correspondence were evaluated by skilled researchers before being excluded. Limit of detection of the chemical analysis was a particle size of 10 µm. Detailed quality assurance/ quality control activities to ensure the general quality of the method were the same as those recently published elsewhere (Pastorino et al. 2021a).

Statistical Analysis and Risk Assessment

Statistical Analysis

The Shapiro–Wilk test was performed to determine whether our dataset was well-modeled by normal distribution. Differences in contaminant (trace elements, NDL-PCBs, PAHs, microplastics) concentration between the two sites were analyzed using the non-parametric Mann–Whitney U test since the null hypothesis for normal distribution could not be rejected. Differences in microplastics shape, type, and color between the samples from the two sites were tested using the chi-square test. Statistical significance was set at p-value < 0.05. Statistical analyses were performed using R software (RStudio, Inc., version 1.1.463). A probabilistic approach using Monte Carlo simulation (MCS) was employed for a more realistic assessment of cancer risk related to toxic metals (Orosun et al. 2020, 2021). MCS was performed using Oracle Crystal Ball software version 11.1.2.4.900.

Risk Assessment: Trace Elements

Exposure parameters for human health risk are presented in Table S3. To evaluate potential hazardous exposure to potentially toxic elements from gastropod consumption by the local Chinese population, the estimated daily intake (EDI; µg kg^{-1} day^{-1}) and the target hazard quotient (THQ) were calculated for each element using the following formulas, respectively (USEPA 2000a):

$$\mathrm{EDI} = \frac{Cm * CR}{ABW}$$

where Cm is the average element concentration between the two sites (µg g^{-1} w.w.); CR is the consumption rate obtained from Cheng and Yap (2015) that estimated the potential human health risk

from metals via mangrove snail consumption (17.86 g day^{-1} and 35.70 g day^{-1} for average and high-level mollusk consumers, respectively); ABW is the average human body weight (70 kg for adults);

$$\text{THQ} = \frac{EF * ED * CR * Cm}{RfD * ABW * AET} * 10 - 3$$

where EF is exposure frequency (365 days year^{-1}); ED is the exposure duration (70 years), equivalent to an average lifetime; CR is the consumption rate (17.86 g day^{-1} and 35.7 g days^{-1} [edible part] for average and high-level mollusk consumers, respectively); RfD is the oral reference dose for Cr, Cu, Pb, Zn, Cd, Hg, and As (1500 μg kg^{-1} day^{-1}, 40 μg kg^{-1} day^{-1}, 3.5 μg kg $^{-1}$ day^{-1}, 300 μg kg^{-1} day^{-1}, 1.0 μg kg^{-1} day^{-1}, 0.7 μg kg^{-1} day^{-1}, 50 μg kg $^{-1}$ day^{-1}, respectively) as reported by literature (Hang et al. 2009; Cheng and Yap 2015); ABW is the average body weight (70 kg for adults); AET is the average exposure time for non-carcinogens (365 days year^{-1} * ED); and 10^{-3} is the unit conversion factor. The total target hazard quotient (TTHQ) of trace elements for *S. quadrata* is the sum of each THQ obtained from each element. Values of THQ or TTHQ > 1 mean that the exposed population is likely to experience obvious deleterious effects after the consumption of contaminated gastropods.

The carcinogenic risk assessment was calculated to estimate the possibility of developing cancer after exposure to toxic metals (Orosun et al., 2020, 2021). To do this, we calculated the average daily intake (ADI) for cancer risk (CR). The ADI was calculated (Zeng et al. 2015):

$$\text{ADI} = \frac{Cm * CR * ED * EF}{ABW * AET}$$

where Cm is the average element concentration between the two sites (mg kg^{-1} w.w.), CR is the consumption rate (0.01786 g days^{-1} and 0.0357 g days^{-1} for average and high-level mollusk consumers, respectively), ED is the exposure duration (70 years), EF is the exposure frequency (365 days year^{-1}), ABW is the average body weight (70 kg for adults), and AET is the average exposure time for noncarcinogens (365 days year^{-1} * ED).

The incremental lifetime cancer risk (ILCR) was calculated for each metal by multiplying the ADI (mg/kg/day) by the carcinogenic slope factor (SF; As: 1.5, Cd: 0.38, Cr:
0.5, Pb: 8.5 × 1 0^{-3} [mg/kg/day]$^{-1}$) (Orosun et al. 2020):

$$\text{ILCR} = \text{ADI} * \text{SF}$$

For example, an ILCR of 10^{-4} indicates a probability of 1 in 10,000 individuals developing cancer. The risk was then categorized in seven risk levels (level I: extremely low risk, < 1 0^{-6}; level VII: extremely high risk, >10^{-3}) according to Li et al. (2017) and Orosun et al. (2020). The acceptable range lies between 1 × 1 0^{-4} and 1 × 10^{-6}.

Finally, we used a probabilistic approach with Monte Carlo simulation (MCS) for a more realistic risk assessment related to potentially toxic metals (As, Cd, Cr, Pb). While overestimation of risk can lead to wastage of limited means on needless mitigatory actions, underestimation can cause preventable health consequences to the dwellers (Orosun et al. 2020, 2021). The MCS method allows for creating probability distributions of risk that describe the uncertainty surrounding specific input variables (i.e., body weight, daily intake, concentration of contaminant, and cancer potency factor). Rather than one-point values, the MCS method uses several values to repeatedly calculate and obtain results with

different assurance levels that range from 1 to 99%. The MCS model was run for a total of 10,000 trials. The mean, 5th and 95th percentiles of ILCR distribution were determined.

Risk Assessment: NDL-PCB

Dietary daily exposure to NDL-PCBs was calculated as follows (Lee et al. 2021):

$$\text{ADD} = \frac{C * CR}{ABW}$$

where ADD (ng k g^{-1} b.w. d ay^{-1}) is the average daily dose exposure to NDL-PCBs; C (ng g^{-1} w.w.) is the average concentration for the two sites of each NDL-PCBs, considering that the values of the congeners below the LOQ were considered equal to the LOQ (1.2 ng g^{-1}); CR is the consumption rate (17.86 g $days^{-1}$ and 35.7 g $days^{-1}$ for average and high-level mollusk consumers, respectively); and ABW is the average body weight (70 kg).

The non-cancer hazard quotient (HQ) for each of the six congeners was calculated as follows (Lee et al. 2021):

$$\text{HQ} = \frac{ADD}{TDI}$$

where tolerable daily intake (TDI) is set at 10 ng kg^{-1} b.w. day^{-1} as proposed by the World Health Organization (AFFSA 2007), assuming that the six indicators of PCB account for about 50% of total dietary NDL-PCB intake (Johnson 2007).

Finally, the hazard index (HI) was calculated according to the formula (Lee et al. 2021):

$$\text{HI} = \Sigma HQ$$

where ΣHQ is the sum of the individual HQs of each of the six congeners. HQ or HI < 1 indicates a daily exposure dose that might not cause adverse health effects.

Risk Assessment: PAHs

Dietary daily exposure to PAHs was calculated for each of the four congeners as follows (Tongo et al. 2017):

$$\text{DDI} = \frac{C * CR}{ABW}$$

where DDI is the dietary daily intake (µg kg^{-1}b.w. day^{-1}), C (µg kg^{-1} w.w.) is the average concentration for the two sites of each PAH; CR is the consumption rate (0.0175 kg $days^{-1}$ and 0.0357 kg $days^{-1}$ for average and high-level mollusk consumers, respectively); and ABW is the average body weight (70 kg).

Carcinogenic risk assessment of PAHs was assessed using the PAH4 index, individual PAH carcinogenic potencies, and carcinogenic toxic equivalents (TEQs). The PAH4 index was calculated as follows (Tongo et al. 2017):

PAH4 Index (PAH4) = ∑(B[a]A + Chr + B[b]FL + B[a]P) where ∑(B[a]A + Chr + B[b]FL + B[a]P) is the sum of the concentration (μg kg^{-1} w.w.) of benzo[a]anthracene (B[a] A), chrysene (Chr), benzo[b]fluoranthene (B[b]FL), and benzo[a]pyrene (B[a]P).

The carcinogenic potency of each PAH congener was calculated by multiplying the PAH concentration by the individual toxicity equivalency factor (TEF) (Tongo et al. 2017):

Carcinogenic potencies of individual PAHs (B(A)Pteq) = Ci ∗ TEFi where Ci (μg kg^{-1} w.w.) is the measured concentration of the PAH congener (i) in *S. quadrata* (mean of site 1 and site 2) and TEF is the individual toxicity equivalency factor of the PAH congener (i). TEF are (Nisbet and LaGoy 1992): B[a] A = 0.1; Chr = 0.01; B[b]FL = 1; B[a]P = 0.1.

Carcinogenic toxic equivalents (TEQs) were calculated for the PAH4 index as follows (Ding et al. 2012):

TEQ = ∑B(A)Pteq

The TEQ was compared with a screening value (SV) to determine the health risks of PAH4 to humans from gastropod consumption. The SV indicates the threshold of chemicals in edible tissue which is of potential public health concern (Cheung et al. 2007):

$$SV = \frac{\left(\frac{RL}{SF}\right) * ABW}{CR}$$

where RL is the maximum acceptable risk level dimensionless = 10^{-5} (USEPA 2000b), SF is the oral slope factor (SF): 1 mg kg^{-1} day^{-1} (USEPA 1993), CR is the consumption rate (0.0175 kg $days^{-1}$ and 0.0357 kg $days^{-1}$ for average and high-level mollusk consumers, respectively), and ABW is the average body weight (70 kg).

Results

Potentially Toxic Element Accumulation

Table 1 presents the mean toxic element concentration in *S. quadrata* from the two sampling sites. The mean concentration of elements (μg g^{-1}) at site 1 was: Zn (3.55) > Cu (1.89) > Pb (0.113) > As (0.112) > Hg (0.045) > Cr (0.009) > Cd (0.001); the mean concentration (μg g^{-1}) at site 2 was: Zn (3.68) > Cu (2.39) > As (0.685) > Pb (0.141) > Hg (0.060) > Cr (0.021) > Cd (0.002). The concentrations of all elements were slightly higher at site 2; however, the difference in element concentration between the two sites was not statistically significant (Mann–Whitney U test, $p > 0.05$).

NDL-PCB Accumulation

The concentration of Σ_6 PCB was significantly higher in the samples from site 2 (7.32 ng g^{-1} vs. 3.07 ng g^{-1}) (Mann–Whitney U test, $p = 0.001$) (Table 2). Among the single congeners, the concentration of PCB-138 and PCB-153 was analytically predominant: PCB-138 accounted for 53.4% and 29.6% of the Σ_6 PCB for site 1 and site 2, respectively; PCB-153 accounted for 46.9% and 24.2% of the Σ_6 PCB for

site 1 and site 2, respectively; PCB-180 was detected only in samples from site 2 and accounted for 22% of the Σ_6 PCB.

PAHs Accumulation

Among the single congeners, the concentration of Chr, B[b]FL and B[a]P was below the limit of quantification (0.5 μg kg^{-1}). B[a]A was detected in the samples from both sites (mean values: 0.5 and 0.7 μg kg^{-1} for site 1 and site 2, respectively) (Table 3).

Microplastics

The number of microplastics frequency was 0.8 ± 1.30 item/ specimen for site 1, and 1 ± 0.37 item/specimen for site 2 (Table 4). There was a significant difference in the items/ specimens between the two sites (Mann–Whitney U test, $p = 0.008$). Fibers ($n = 6$) were found in samples from site 1 ($n = 2$) and site 2 ($n = 4$); fragments ($n = 3$) were detected in samples from site 1 ($n = 1$) and site 2 ($n = 2$). There were no differences in shape frequency between the two sites (χ^2 test, $p > 0.05$). The predominant colors were green (33.33%) and tan (33.33%), followed by blue (22.22%) and black (11.12%). There were no differences in color frequency between the two sites (χ^2 test, $p > 0.05$).

The chemical composition of microplastic was predominantly PET polyethylene terephthalate (66.67%), followed by nylon (22.22%) and PP polypropylene (11.11%). There were no differences in chemical composition frequencies between the two sites (χ^2 test, $p > 0.05$).

Risk Assessment

Potentially Toxic Elements

Table 5 reports the estimated weekly intake (EDI) and the target hazard quotient (THQ) for potentially toxic elements by the consumption of *S. quadrata* in an average and a high-level mollusk consumer. In an average mollusk consumer, the EDI ranged from 0.0003 μg kg^{-1} day^{-1} for Cd to 0.92 μg kg^{-1} day^{-1} for Zn. A similar scenario was observed for a high-level mollusk consumer: EDI ranged from 0.0007 μg kg^{-1} day^{-1} for Cd to 1.84 μg kg^{-1} day^{-1} for Zn. The target hazard quotient (THQ) for all trace elements was < 1, as was the total target hazard quotient (TTHQ) for an average and a high-level mollusk consumer: 0.0026 and 0.0051, respectively. Average daily intake (ADI) (Table 6), calculated to obtain the ILCR, ranged from 0.00000013 for As to 0.0000039 for Cr and from 0.00000025 for As to 0.0000078 for Cr, for an average and a high consumption level, respectively. The ILCR was also estimated; the mean (for average and high consumption, respectively) was: As (1.90E−7; 3.81E−7), Cd (1.26E−7 ; 2.51E−7), Cr (1.94E−6; 3.88E−6), and Pb (2.76E−7; 5.51E−7), with Cr contributing the most to cancer risk, followed by Pb, As, and Cd (Table 6).

The MCS results are presented in Fig. 2 and Tables S4 and S5. According to the 95% cumulative probability, Cr (1.24E − 5) posed the highest risk, followed by As (1.85E − 6), Pb (1.49E − 6), and Cd (7.01E − 7). The sensitivity chart from the MCS model (Table S5) revealed that carcinogenic slope factor ranks highest, followed by the daily intake of gastropods, concentration of toxic element in *S. quadrata*, and then average adult body weight, which was negative for all potentially toxic elements.

NDL-PCB

Table 7 presents the average daily dose exposure (ADD) to NDL-PCBs for an average and a high-level mollusk consumer. The ADD ranged from 0.31 ng kg^{-1} day^{-1} for PCB28 and PCB-101 to 0.49 ng kg^{-1} day^{-1} for PCB-138 for an average mollusk consumer and from 0.61 ng kg^{-1} day^{-1} for PCB-28 and PCB-101 to 0.97 ng kg^{-1} day^{-1} for PCB138 for a high-level mollusk consumer. The ADD values for the Σ_6 PCB ranged from 2.24 ng kg^{-1} day^{-1} to 4.49 ng kg^{-1} day^{-1} for an average and a high-level mollusk consumer, respectively. All the hazard quotient (HQ) values were < 1. The same results were obtained by calculating the Hazard Index (HI) (sum of the individual HQs), which was < 1 for an average (0.22) and a high-level (0.45) mollusk consumer alike.3.5.3 PAHs.

Table 8 presents the dietary daily intake (DDI) (μg kg^{-1}b.w. day^{-1}) for an average and a high-level mollusk consumer. The DDI ranged from 0.0001 μg kg^{-1} day^{-1} for B[a]P, Chr and B[b]FL to 0.0002 μg kg^{-1} day^{-1} for B[a]A for an average mollusk consumer and from 0.0003 μg kg^{-1} day^{-1} for B[a]P, Chr and B[b]FL to
0.0004 μg kg^{-1} day^{-1} for B[a]A for a high-level mollusk consumer.

The carcinogenic potencies of PAHs (B(A)Pteq) were: B[a]P: 0.05 μg kg^{-1}; B[a]A: 0.06 μg kg^{-1}; Chr: 0.005 μg kg^{-1}; B[b]FL: 0.5 μg kg^{-1}. The TEQ obtained from PAH4 was 0.615 μg kg^{-1}(0.000615 mg kg^{-1}). The SV was 0.04 and 0.02 mg kg^{-1} for an average and a high-level consumer, respectively.

Discussion

Contaminants Accumulation

The literature documents the use of mollusks as bioindicators to assess water quality in compliance with ecological and human safety legislation (Tietze and De Francesco 2010; Kumar et al. 2017). Generally, freshwater gastropods are considered good indicators of water quality owing to their relatively long-life cycle and low mobility (de Freitas Tallarico 2015). They are highly sensitive to chemical pollution; high levels can cause their disappearance or inhibit their reproduction (Krupnova et al. 2018; Caixeta et al. 2020). They are especially sensitive to pollution by heavy metals (i.e., cadmium, mercury, lead, zinc, copper) (Krupnova et al. 2018). Since contaminants can have a negative impact on freshwater mollusks at environmental concentrations lower than for other invertebrates or vertebrates, mollusks are studied as part of an ecological early warning system (de Freitas Tallarico 2015). In the present study the two sampling sites are located downstream of wastewater treatment plants and civil and industrial discharges, but the levels of contaminants were not of particular concern for human safety.

Although the difference was not statistically significant, the trace element concentration was higher in samples from site 2. This is easily explained by the fact that site 2 is located downstream of site 1 and receives the waters from the Pesa River near Montelupo Fiorentino (Florence Province). The Pesa River is under anthropogenic pressures that deteriorate the water quality at site 2. Despite this, the concentration was low. Cortecci et al. (2009) measured the main toxic trace elements (As, Cu, Zn, Ni, Pb, Cd) in water samples from the River Arno and found that concentrations were below the acceptable limits of current European regulations for the quality of waters for potable use and fish life. In a later study, Mecatti et al. (2017) measured the concentration of Hg, Cd, As, Pb, and Cr in the muscle of the European catfish (*Silurus glanis*) from the Arno and found very low levels of these toxic elements.

Unfortunately, there are no studies on trace element accumulation in *S. quadrata* or other mollusk species for comparison with our study. However, drawing on data for other members of the same gastropod family (Viviparide), the concentrations we measured were much lower than those reported by Gundacker (2000) who detected Cd, Cu, Pb, and Zn in *Viviparus* sp. soft bodies at five sampling sites in Vienna (Austria). Of note is that *S. quadrata* is a filter-feeder organism, which is why the trace elements accumulating in its soft body are more closely related to ingestion of suspended matter and filtrate from river water. Metals concentration in water recorded by ARPAT at the MAS-108 station (located near the two sampling sites) and during the same month (July 2020) as our sampling revealed low values for all elements (As 1 μg L^{-1}; Cd < 0.2 μg L^{-1}; Cr < 1 μg L^{-1}; Pb < 1 μg L^{-1}; Cu 5 μg L^{-1}; Zn 35 μg L^{-1}; Hg 0.035 μg L^{-1}) (ARPAT 2021).

Among the NDL-PCBs, the six congeners (28, 52, 101, 138, 153, 180) were selected for this study because they are easily quantified compared to other PCBs (Squadrone et al. 2013). Moreover, they account for all relevant levels of chlorination. The EFSA stated that the sum of these six congeners is an adequate indicator of the occurrence of NDLPCBs and human exposure to them (EFSA 2005). As for the trace elements, the Σ_6 NDL-PCB was also higher at site 2 compared to site 1. Congeners PCB-153 and PCB-138 were analytically predominant compared to the other congeners. PCB-52 and PCB-180 were also detected in samples from site 2. The non-planar PCB-138, PCB-153, and PCB-180 are the most predominant and persistent congeners reported in humans (Humphrey et al. 2000; Leijs et al. 2019). Squadrone et al. (2013) found that congeners PCB-153 and PCB-138 were analytically predominant (40% and 30%, respectively) in the muscle of *Silurus glanis* from north Italian rivers. The concentrations we measured are in line with those detected by Nhan et al. (2001) in the soft tissue of the freshwater mollusk (*Angulyagra* sp.; Viviparidae) from water canals in the region of Hanoi City (Vietnam).

The PAHs concentration was below the LOQ
(0.5 μg kg^{-1}) for the majority of the PAHs congeners (B[a]
P, Chr and B[b]FL), except for B[a]A which was near the LOQ for both sites. Primary exposure to benz[a]anthracene occurs mainly from smoking or second-hand smoke, air polluted with combustion products or food and water polluted with combustion products (Librando et al. 2014). Generally, B[a]A do not accumulate in fish (due to biotransformation) (Bleeker and Verbruggen 2009). Its accumulation in *Sinotaia quadrata* may be related to the absence of an enzyme for metabolic activation. Moreover, aquatic mollusks in general are limited in their ability to excrete pollutants directly through their excretory organs and tissues compared to other invertebrates such as arthropods or vertebrates, which is why gastropods can be used in ecological early warning systems (Oehlmann and Schulte-Oehlmann 2003).

The number of microplastic items/specimen was similar to those reported by Akindele et al. (2019): 1.70 ± 0.42 items/specimen in the freshwater gastropod *Melanoides tuberculate* (Thiaridae) from the Osun River system (Nigeria). PET (polyethylene terephthalate) was the main chemical type we found, as reported in other freshwater ecosystems (Li et al. 2020; Pastorino et al. 2021a, 2021b). PET is a synthetic material in the polyester family made from oil, natural gas or vegetable raw materials. Thanks to its properties, PET is used in diverse areas: from the production of containers (plastic bottles) to textiles (Piccardo et al. 2020). The dominant microplastics shape in the samples from both sites was fibers. This finding is shared by previous studies that reported fibers as the most dominant shape in freshwater environments (McCormick et al. 2014; Baldwin et al. 2016; Pastorino et al. 2021a). The

presence of fibers can be attributed to their diverse origins: washing, domestic wastewater, and other anthropogenic activities. Their occurrence in our samples could be related to the wastewater from textile factories in Prato that reaches the River Arno through its Bisenzio tributary.

Compliance with National and International Regulatory Bodies and Health Risk Assessment

Generally, analysis of the results for accumulation highlights low concentrations for all contaminates analyzed. Since no arsenic limit has been established by any regulatory body, its concentration in *S. quadrata* could not be assessed for potential risk by direct safety guideline comparison. Cd concentration was within the limit of 1.0 μg g^{-1} imposed by Regulation (EC) 1881/2006 (EC 2006). Cr concentration in samples from both sampling sites were well below the maximum allowable limit of 13 μg g^{-1} for shellfish as established by the US Food and Drug Administration (USFDA 2011). The Cu levels were below the limits recommended by the US Environmental Protection Agency (USEPA 1983) (120 μg g^{-1}), the UK Ministry of Agriculture, Fisheries and Food (MAFF 2000) (20 μg g^{-1}), and the WHO (1996) (30 μg g^{-1}). The Hg levels were below the limit of 0.5 μg g^{-1} imposed by Regulation (EC) 1881/2006 (EC 2006). Pb levels were below the limit of 4.0 μg g^{-1} recommended by the USEPA (USEPA 1983), 2.0 μg g^{-1} by the UK Ministry of Agriculture, Fisheries and Food (MAFF 2000), 2.0 μg g^{-1} by the WHO (WHO 1996) 0.5 μg g^{-1} by the UN Food and Agriculture Organization (FAO 1983) and 1.5 μg g^{-1} imposed by Regulation (EC) 1881/2006 (EC 2006). Zn levels were also within the limits (480 μg g^{-1}) established by the USEPA (1983), the UK Ministry of Agriculture, Fisheries and Food (MAFF 2000) (50 μg g^{-1}), and those (30 μg g^{-1}) determined by FAO (1983).

The sum of the six indicators NDL-PCBs 28, 52, 101, 138, 153, 180 (Σ_6 NDL-PCBs) was well below the maximum tolerable levels (MLs) of 75 ng g^{-1} established by Regulation 1259/2011 (EC 2011b).

Knowledge of the dietary intake of *S. quadrata* is an important factor in assessing risk to human health from potentially harmful chemicals in food. For both an average and a high-level consumer the trace elements EDI was lower than the oral reference dose (Cheng and Yap 2015). Generally, the EDI we calculated was lower than that reported in a study by Cheng and Yap (2015) that estimated the health risks from toxic metals via mangrove snail consumption from the Malaysian Peninsula.

Target hazard quotients (THQ) were also calculated since they are widely used in the risk assessment of heavy metals in contaminated foods (Storelli 2008). Our findings revealed that all THQs were < 1 for all elements, indicating that the health risks associated with As, Cd, Cr, Cu, Hg, Pb, Zn exposure for average and high-level mollusk consumers are insignificant. The TTHQ was also < 1, suggesting that the consumption of *S. quadrata* does not carry health risks.

The ILCR was far below the safety values of 1×10^{-4} as recommended by the USEPA (USEPA 2021) for the toxic elements studied here. The ILCR calculated by the MCS model was within the safety region of $1 \times 1\ 0^{-4}$ and 1×10^{-6}, as recommended by the USEPA (USEPA 2021). Based on the Delphi method (Li et al. 2017), the risk ranged from level I to level II, from completely acceptable to not willing to care about the risk.

The average daily dose (ADD) of the Σ_6 NDL-PCB was 2.24 and 4.49 ng kg^{-1} day^{-1} b.w. for an average and a highlevel mollusk consumer, respectively. The two ADD were much lower than the reference dose of the Σ_6 NDL-PCB (10 ng kg^{-1} day^{-1} b.w.) (Lee et al. 2021). The average daily intake of total NDL-PCBs for adults in Europe is estimated to be between 10 ng kg^{-1} day^{-1} and 45 ng kg^{-1} day^{-1} b.w. (EFSA 2005) and lower than the ADD we calculated.

The non-cancer risk (HQ) and the hazard index (HI) associated with NDL-PCBs exposure from an average and a high consumption of gastropods were all < 1, indicating no adverse effects for human health. Unfortunately, the lack of similar studies on freshwater gastropods precludes comparison with the literature.

The dietary daily intake (DDI) for the PAH4 index was much lower than the range (0.0195–0.0345 μg kg day^{-1} b.w.) estimated by the EFSA (EFSA 2008) for each congener. Also, B[a]P daily intake was below the acceptable daily intake of 0.01 μg kg^{-1} day^{-1} suggested by the Joint FAO/ WHO Expert Committee on Food Additives (JFCFA) and for the range 0.039–0.065 μg kg^{-1} day^{-1} reported by the EFSA (EFSA 2008). Concentrations of B[a]P and PAH4 index were lower than the legal limit (fishery products) of 2 μg kg^{-1} and 12 μg kg^{-1}, respectively, imposed by Regulation (EC) 835/2011 (EC 2011a).

The SV for average and high consumption was compared to the TEQ to determine the health risk to humans via gastropod consumption. The TEQ (0.000615 mg kg^{-1}) was far below the SV (0.04 and 0.02 mg kg^{-1}, respectively), indicating no risk to human health from the consumption of *S. quadrata.*

We were unable to assess the health risk from exposure to microplastics, since too little data are available to fully understand their implications for human health, as declared by the EFSA (German Federal Institute for Risk Assessment et al. 2020). Currently, there is no legislation concerning microplastics as contaminants in food. Nonetheless, negative effects may be expected due to their physical (size, and shape) and chemical proprieties, concentration, occurrence of contaminants adsorbed or microbial biofilm growth (Campanale et al. 2020).

Conclusions

Contaminant levels in specimens of *S. quadrata* collected from two sites on the Arno were compliant with regulatory limits and guidelines. The findings from health indices indicated no adverse effects for human health by exposure to trace elements, NDL-PCB, and PAHs from gastropod consumption. However, the ingestion of microplastics could expose humans to chemicals known to be harmful. Substantial data gaps with respect to exposure as well as toxicity of such particles impede the risk assessment. Finally, regular monitoring of the River Arno for organic and inorganic pollutants is warranted to detect eventual changes in contaminant levels.

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Data Availability The data supporting the findings of this study are available on request from the corresponding author.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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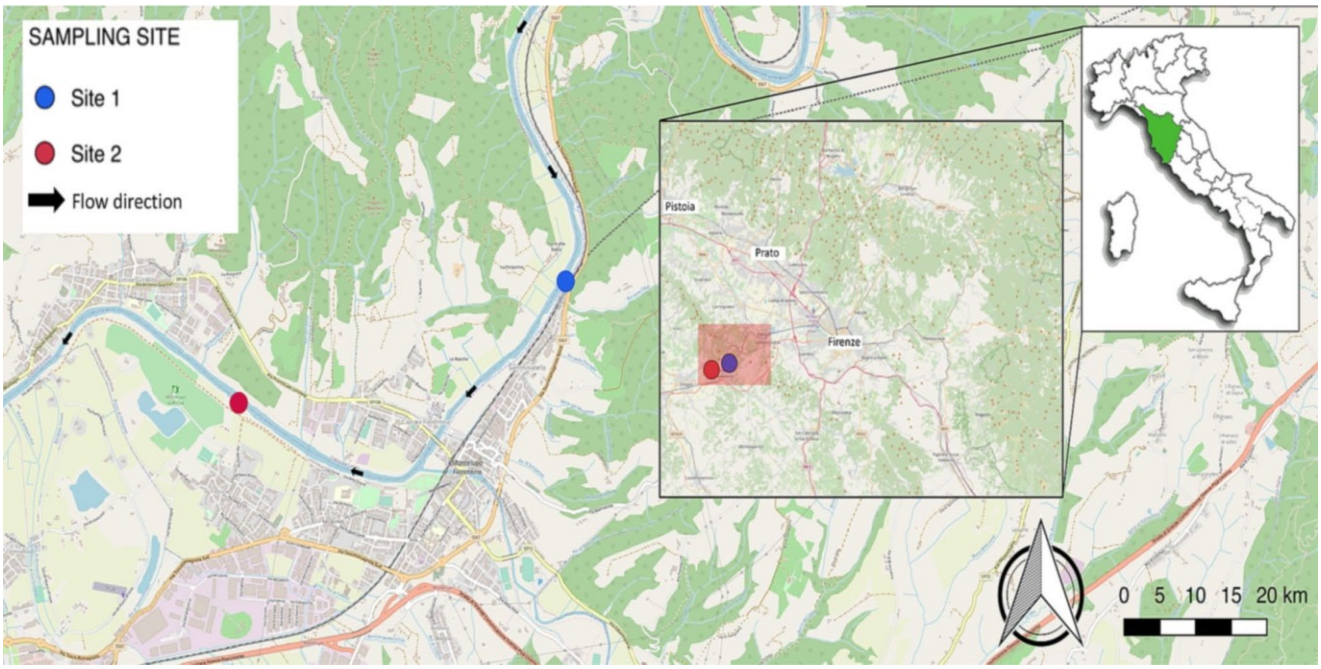

Fig. 1 Location of sampling sites (site 1: Samminiatello; site 2: Fibbiana), Tuscany, central Italy. The study sites are shown in the insert

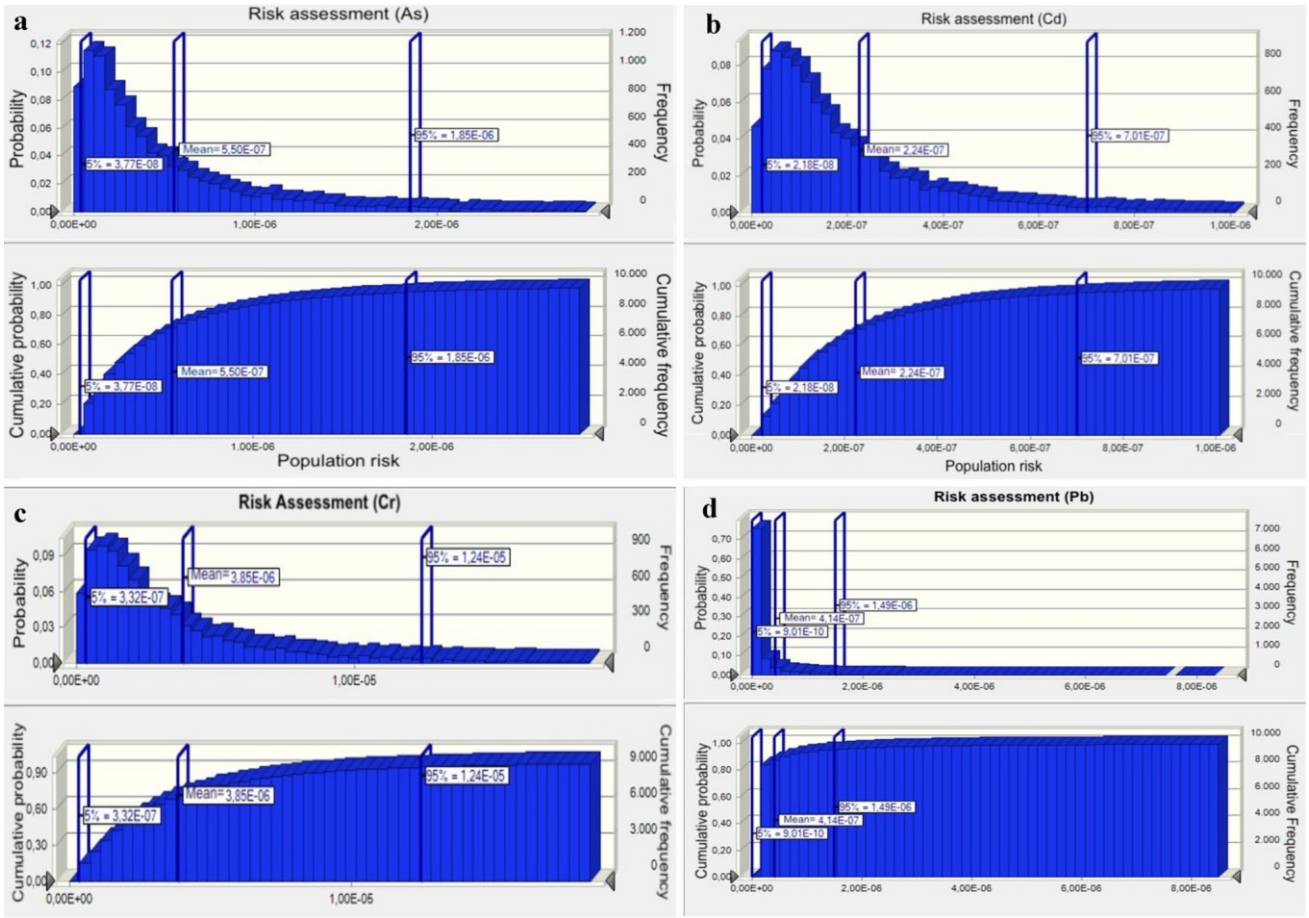

Fig. 2 Probability and frequency plot of cancer risk due to *Sinotaia quadrata* consumption: **a** arsenic (As), **b** cadmium (Cd), **c** chromium (Cr), **d** lead (Pb)

Table 1 Potentially toxic element concentrations (μg g^{-1}) in *Sinotaia quadrata* from Samniniatello (site 1) and Fibbiana (site 2)

Site	As	Cd	Cr	Cu	Hg	Pb	Zn
1	0.112 ± 0.18	0.001 ± 0.006	0.009 ± 0.007	1.89 ± 0.40	0.045 ± 1.88	0.113 ± 0.05	3.55 ± 0.55
2	0.685 ± 0.89	0.002 ± 0.011	0.021 ± 90.018	2.39 ± 1.21	0.060 ± 2.50	0.141 ± 0.02	3.68 ± 1.81

Plus-minus values are the mean ± standard deviation

Table 2 Concentration of the six indicators of NDL-PCBs (28, 52, 101, 138, 153, 180) in *Sinotaia quadrata* from Samminiatello (site 1) and Fibbiana (site 2)

Site	Lipid (%)	PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180	Σ_6 PCB
1	32	< LOQ	< LOQ	< LOQ	1.64 ± 0.08	1.43 ± 0.10	< LOQ	3.07
2	32	< LOQ	1.77 ± 0.05	< LOQ	2.17 ± 0.07	1.77 ± 0.12	1.61 ± 0.07	7.32

Data are reported as the mean of three pools (n = 10); < LOQ = concentration below limit of quantification (1.2 ng g^{-1} w.w.). Plus-minus values are the mean ± standard deviation

Table 3 Concentration (μg kg^{-1} w.w.) of benzo[a]pyrene (B[a]P), benzo[a]anthracene (B[a]A), chrysene (Chr), and benzo[b]fluoranthene (B[b]FL) detected in *Sinotaia quadrata* from Samminiatello (site 1) and Fibbiana (site 2); < LOQ = concentration below the limit of quantification (0.5 μg k g^{-1} w.w.)

Site	B[a]P	B[a]A	Chr	B[b]FL
1	< (LOQ)	0.5 ± 0.02	< LOQ	< LOQ
2	< (LOQ)	0.7 ± 0.02	< LOQ	< LOQ

Plus-minus values are the mean ± standard deviation

Table 4 Microplastics detected in *Sinotaia quadrata* at the two sites (n = 5 per site) are reported as total items, items/ animal ± standard deviation (SD), mean size (μm) ± SD, shape, color, and chemical type

Site	Total items	Items/animal	SD	Mean (μm)	Size	SD	Shape	Color	Chemical type
1	4	0.8	1.30	784.6	627.61		Fiber	Black	PP
							Fiber	Blue	nylon
							Fragment	Green	PET
							Fragment	Green	PET
2	5	1	0.37	136.98	45.78		Fiber	Tan	PET
							Fiber	Tan	PET
							Fiber	Tan	PET
							Fiber	Blue	nylon
							Fragment	Green	PET

PET polyethylene terephthalate, *PP* polypropylene

Table 5 Estimated weekly intake (EDI; μg kg^{-1} day^{-1}), target hazard quotient (THQ), and total target hazard quotient (TTHQ; sum of each THQ obtained from each element) by the consumption of *Sinotaia quadrata* in an average and a high-level mollusk consumer

Index	Consumption	As	Cd	Cr	Cu	Hg	Pb	Zn	TTHQ
EDI	Average	0.10	0.0003	0.004	0.54	0.01	0.03	0.92	–
	High	0.20	0.0007	0.008	1.09	0.03	0.06	1.84	–
THQ	Average	0.002	0.000001	0.000000003	0.00001	0.00002	0.000009	0.000003	0.0026
	High	0.004	0.0000003	0.000000005	0.00003	0.00004	0.00002	0.000006	0.0051

Table 6 Average daily intake (ADI; mg k g^{-1} day^{-1}) and incremental lifetime cancer risk (ILCR) associated with the consumption of *Sinotaia quadrata* in an average and a high-level mollusk consumer

Index	Consumption level	As	Cd	Cr	Pb
ADI	Average	0.00000013	0.00000033	0.00000388	0.00003246
	High	0.00000025	0.00000066	0.00000776	0.00006488
ILCR	Average	1.90E−07	1.26E−07	1.94E−06	2.76E−07
	High	3.81E−07	2.51E−07	3.88E−06	5.511E−07

Table 7 Average daily dose exposure (ADD; ng kg^{-1} day^{-1}) and hazard quotient (HQ) of NDL-PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180) for an average and a high-level mollusk consumer

Index	Consumption	PCB-28	PCB-52	PCB-101	PCB-138	PCB-153	PCB-180	Σ6 PCB	HI
ADD	Average	0.31	0.38	0.31	0.49	0.41	0.36	2.24	–
	High	0.61	0.76	0.61	0.97	0.82	0.72	4.49	–
HQ	Average	0.03	0.04	0.03	0.05	0.04	0.04	–	0.22
	High	0.06	0.08	0.06	0.09	0.08	0.07	–	0.45

Table 8 Dietary daily intake (DDI) (μg kg^{-1} day^{-1} b.w.) of benzo[a]pyrene (B[a]P), benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b] fluoranthene (B[b]FL), and PAH4 index (sum of B[a]P, B[a]A, Chr, B[b]FL) for an average and a high-level mollusk consumer

Index	Consumption	B[a]P	B[a]A	Chr	B[b]FL	PAH4
DDI	Average	0.0001	0.0002	0.0001	0.0001	0.0005
	High	0.0003	0.0004	0.0003	0.0003	0.0011

Supplementary files

Table S1. Limit of Detection (LOD) (μg g^{-1}), reference material values and percentages (%) of recovery for each element.

Element	LOD	Reference Material	% Recovery
As	0.021	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	98
Cd	0.0015	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	103
Cr	0.0012	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	97
Cu	0.0003	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	99
Hg	0.0004	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	97
Pb	0.013	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	94
Zn	0.00072	HISS-1 Marine Sediment Reference Materials for Trace Metals and other Constituents	113

Table S2. MS transitions used for PCBs detection.

PCB	Quantification Ion (m/z)	Qualification Ions (m/z)
28	256	186 258
52	292	220 290
155 (IS)	360	290 362
101	326	254 328
153	360	290 325
138	360	290 325
180	394	324 396
198 (IS)	430	358 393

Table S3. Exposure parameters used to calculate the human health risk by the consumption of *Sinotaia quadrata*.

Exposure parameters	Value(s)
Consumption rate (Cr)	7.86 for average mollusk consumers; 35.70 for high-level mollusk consu
Average human body weight (ABW)	70 (adults)
Exposure frequency (EF)	365
Exposure duration (ED)	70
Oral reference dose (RfD)	Cr: 1500; Cu: 40; Pb: 3.5, Zn: 300, Cd: 1.0; Hg: 0.7; As: 50
Carcinogenic slope factor (SF)	As: 1.5, Cd: 0.38, Cr: 0.5, Pb: 8.5×10^{-3}

Table S4. Summary of the Monte Carlo simulation.

Element	5%	Mean	95%
As	3.77E-8	5.50E-7	1.85E-6
Cd	2.18E-8	2.24E-7	7.01E-7
Cr	3.32E-7	3.85E-6	1.24E-5
Pb	9.01E-10	4.14E-7	1.49E-6

Table S5. Sensitivity chart obtained from Monte Carlo simulation (MCS); CPF= Cancer potency factor.

Element	Variable	Contribution to variance (in percentage; %)
As	Element concentration	21.7
	CPF	53.4
	Ingestion rate	23.9
	Adult Body Weight	-1.0
Cd	Element concentration	1.5
	CPF	67.9
	Ingestion rate	28.9
	Adult Body Weight	-1.7
Cr	Element concentration	8.4
	CPF	63.2
	Ingestion rate	26.8
	Adult Body Weight	-1.6
Pb	Element concentration	0.9
	CPF	93.4
	Ingestion rate	5.3
	Adult Body Weight	-0.4