Environmental status of an Italian site highly polluted by illegal dumping of industrial wastes: the situation 15 years after the judicial intervention

Matteo Vitali[1]*, Federica Castellani[1,2], Giorgia Fragassi[3], Alfonso Mascitelli[3], Cecilia Martellucci[4], Gianfranco Diletti[5], Emanuela Scamosci[6], Maria Luisa Astolfi[7], Leila Fabiani[8], Riccardo Mastrantonio[8], Carmela Protano[1], Vincenzo Romano Spica[9], Lamberto Manzoli[10]

[1] Department of Public Health and Infectious Diseases, University of Rome La Sapienza, P.le Aldo Moro, 5, 00185 Rome, Italy - matteo.vitali@uniroma1.it; federica.castellani@uniroma1.it; carmela.protano@uniroma1.it

[2] Department of Ecological and Biological Sciences, Tuscia University, Largo dell'Università snc, 01100 Viterbo, Italy

[3] Regional Healthcare Agency of Abruzzo, Via Attilio Monti 9, 65127 Pescara (PE), Italy - fragassi.giorgia1@gmail.com; mascite@libero.it

[4] Department of Biomedical Sciences and Public Health, University of the Marche Region, Via Tronto 10/a, 60020 Torrette di Ancona (AN), Italy - ceciliamartellucci@gmail.com

[5] Istituto Zooprofilattico Sperimentale of Abruzzo and Molise "Giuseppe Caporale", via Campo Boario, 64100 Teramo (TE), Italy - g.diletti@izs.it

[6] Environmental Protection Regional Agency of Abruzzo, via Marconi 49, 65126, Pescara (PE), Italy - e.scamosci@artaabruzzo.it

[7] Department of Chemistry, University of Rome La Sapienza, P.le Aldo Moro, 5, 00185 Rome, Italy – marialuisa.astolfi@uniroma1.it

[8] Department of Life, Health & Environmental Sciences - University of L'Aquila, P.le Salvatore Tommasi 1, 67100 Coppito, L'Aquila, Italy; leila.fabiani@univaq.it; riccardo.mastrantonio@graduate.univaq.it

[9] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Piazza Lauro De Bosis 6, 00135 Rome, Italy – vincenzo.romanospica@uniroma4.it

[10] Department of Medical Sciences, University of Ferrara, Via Fossato di Mortara 64B, 44121 Ferrara (FE), Italy - lmanzoli@post.harvard.edu

*Corresponding author

S1. Maps of the 13 sheep farms, three goat farms and 16 chicken farms.

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S1. Maps of the 13 sheep farms, three goat farms and 17 chicken farms.

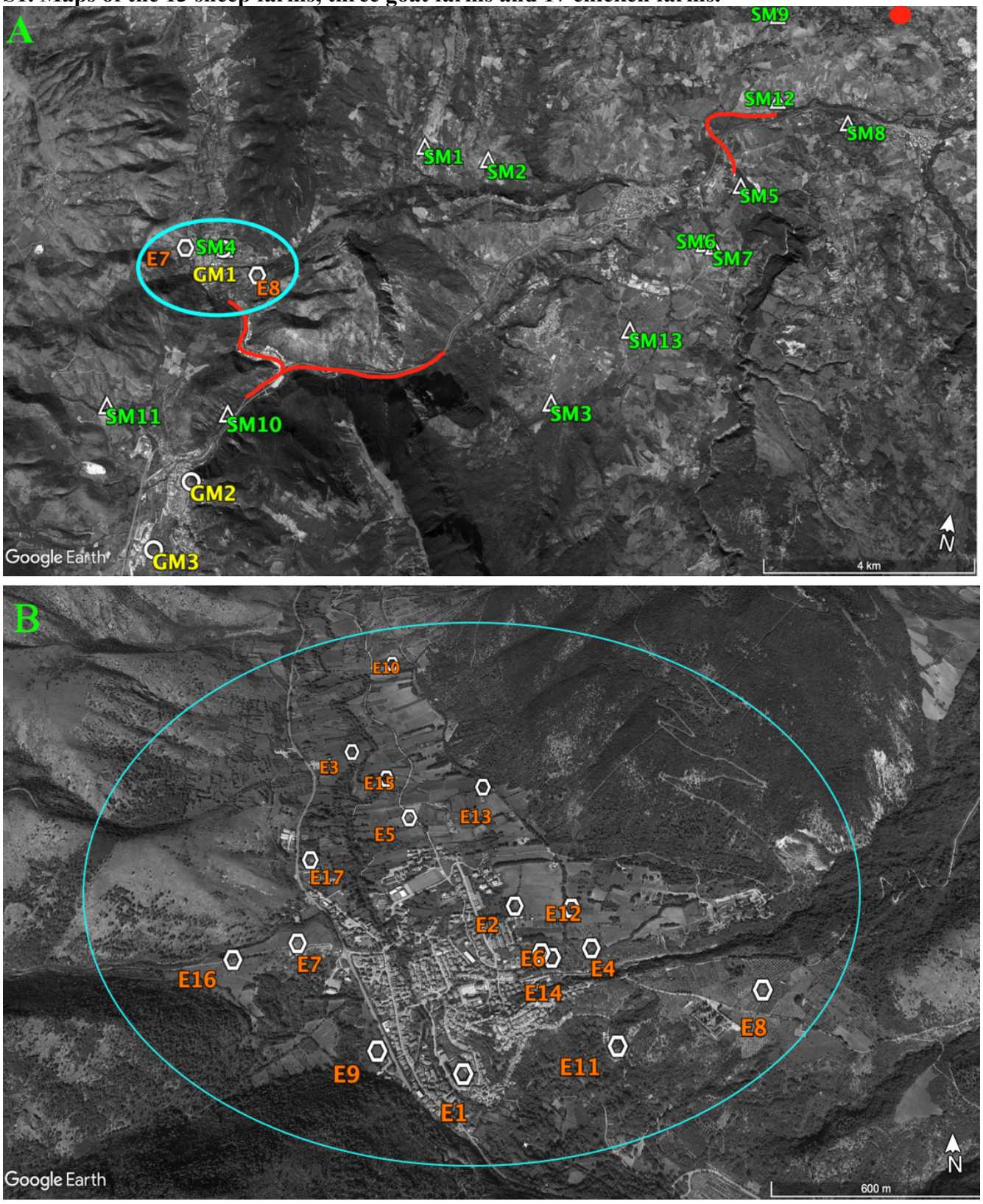

Figure S1. Maps of the 13 sheep farms (SM1, SM2, SM3, SM4, SM5, SM6, SM7, SM8, SM9, SM10, SM11, SM12, SM13), three goat farms (GM1, GM2, GM3; panel A) and 17 chicken farms

(E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, E11, E12, E13, E14, E15, E16, E17; panel B). The panel B is the expansion of the highlighted area with a light blue circle in panel A. All the farms are located no more than 5 km from the perimeter of the Site of National Interest (SNI) of Bussi sul Tirino (red areas; panel A) (Google Earth).

S2. Maps of the 11 air sampler monitoring sites

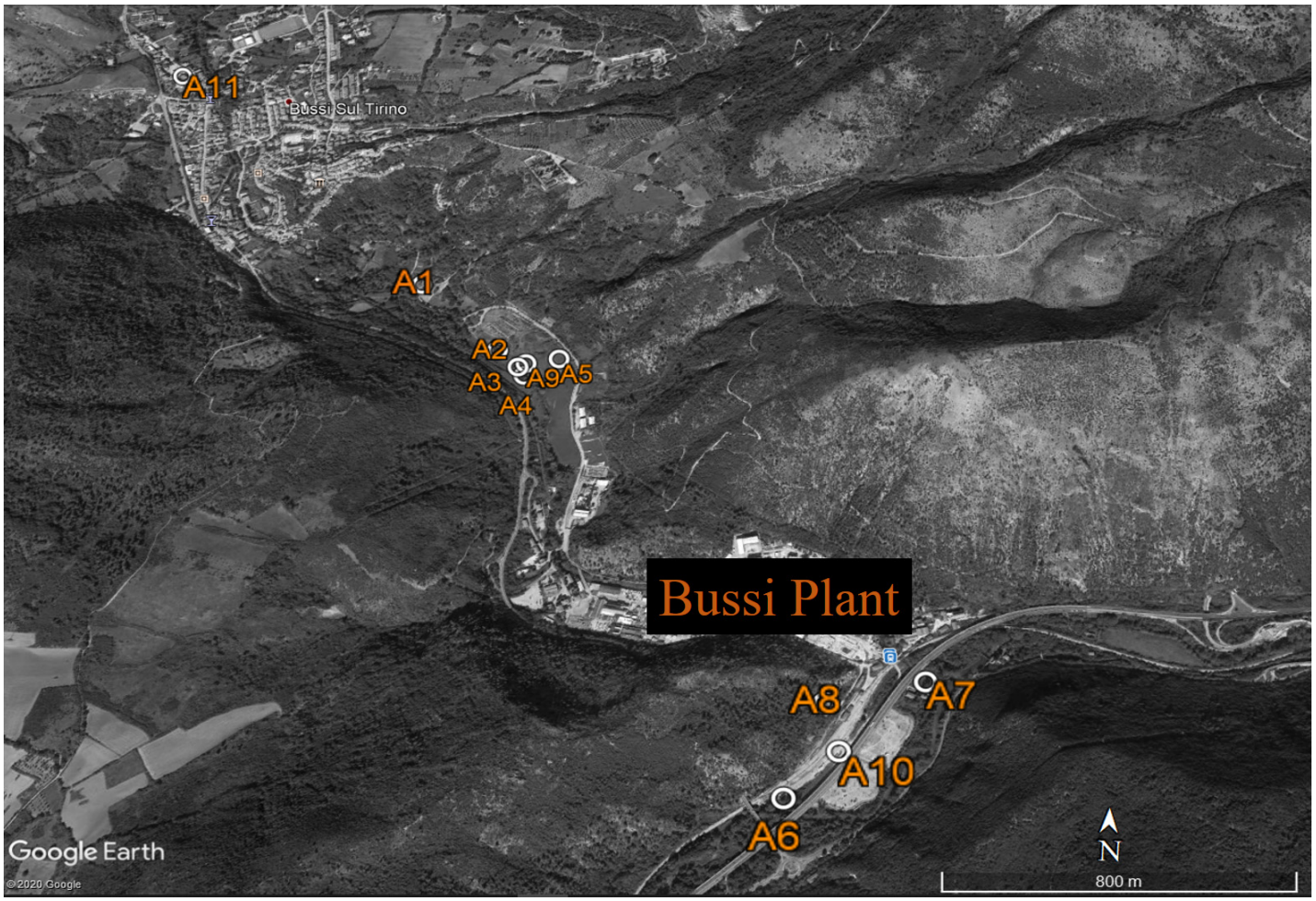

Figure S2. Maps of the 11 air sampling monitoring sites (A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11) in Bussi city. (Google Earth).

S3. Maps of the five drinking water collection sites and the 4 river water collection sites

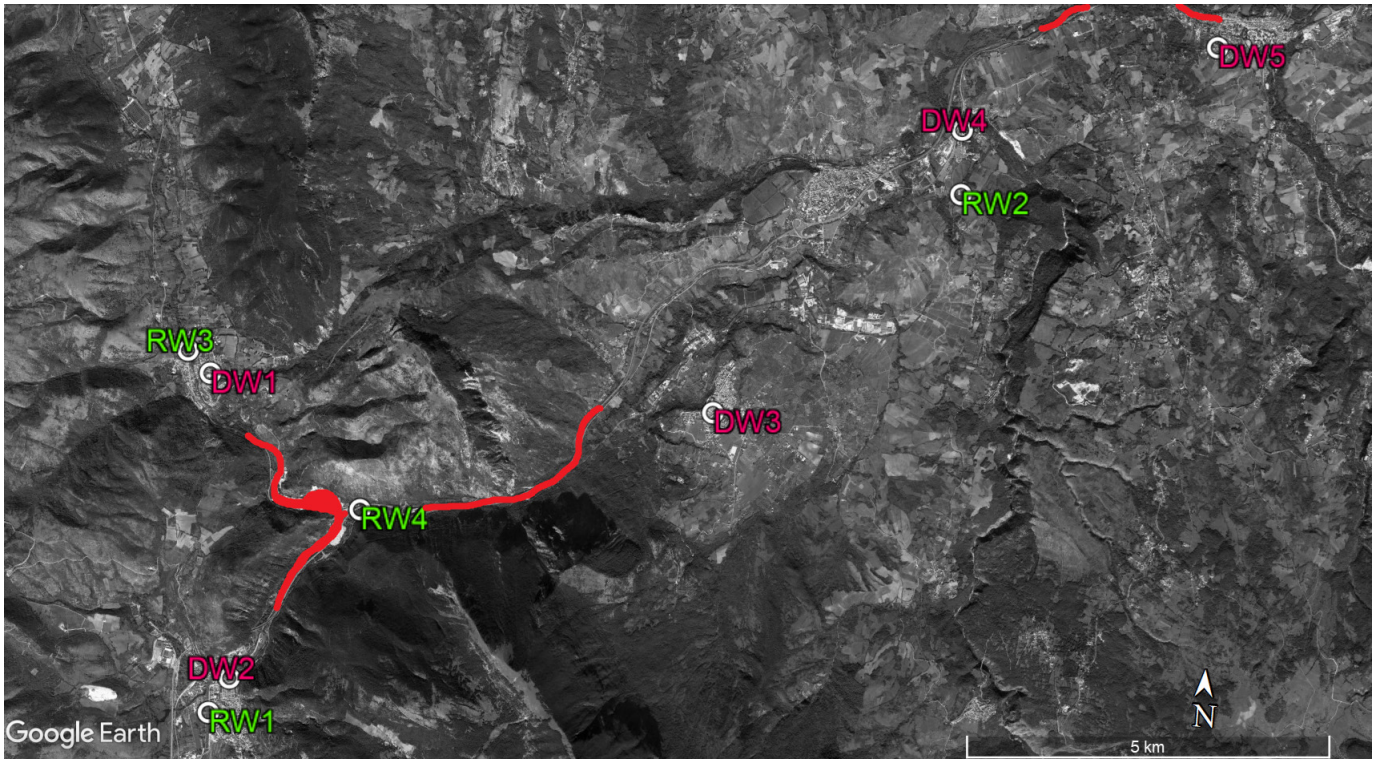

Figure S3. Maps of the five drinking water collection sites (DW1, DW2, DW3, DW4, DW5) and the four river water collection sites (RW1, RW2, RW3, RW4). All the drinking water collection sites are located no more than 5 km from the perimeter of the SNI (red areas).

S4. Maps of the ten collection sites in which wild edible mushroom samples were collected

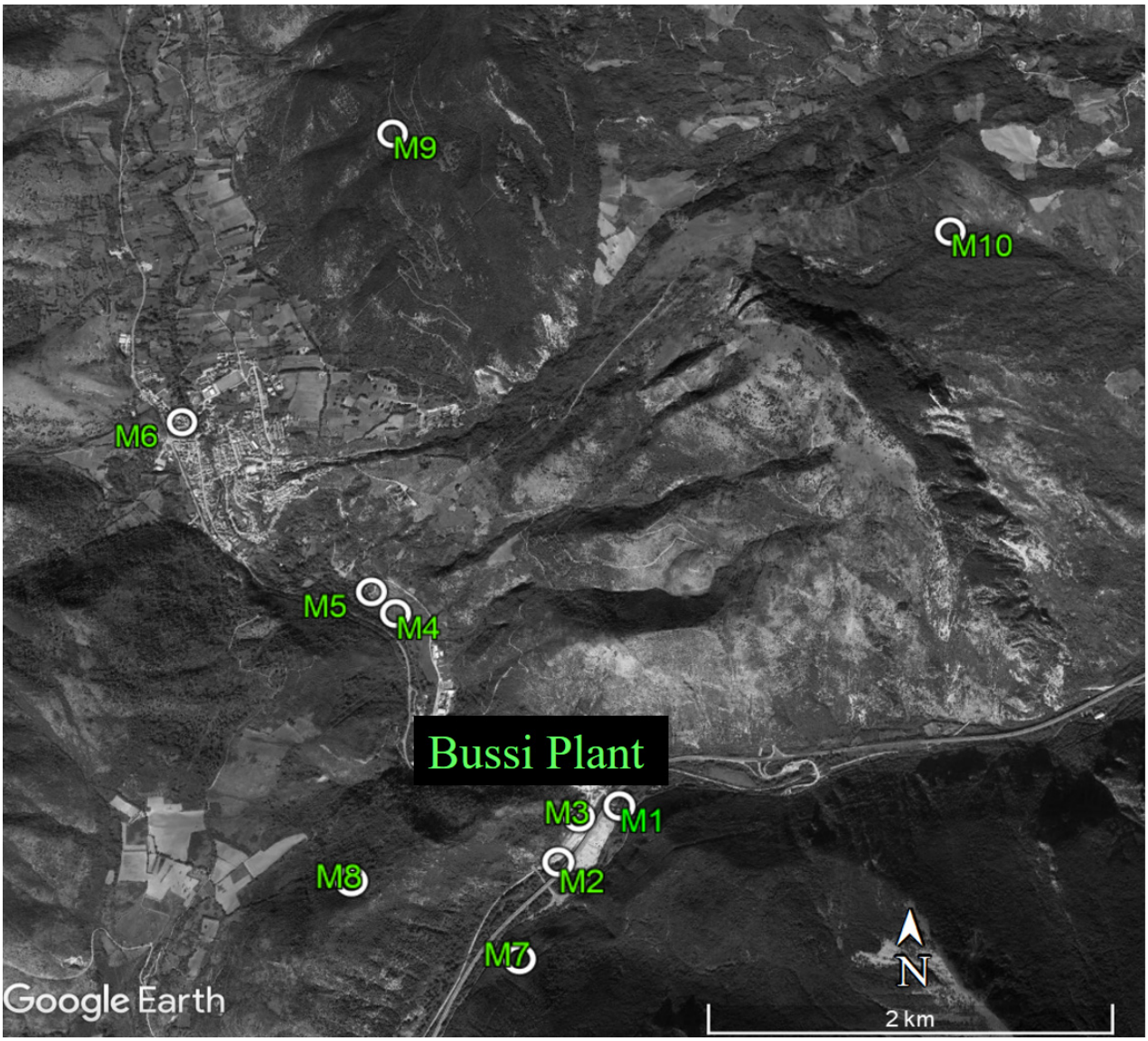

Figure S4. Maps of the ten sites where wild edible mushroom samples were collected (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10). All the sites are located no more than 5 km from the Bussi plant (Google Earth).

S5. Sampling Procedures

S5.1 Milk and eggs collection

Milk samples were collected by the local veterinary unit from 16 small farms (Figure S1A), between September 2017 and February 2018. Farms were all characterized by extensive or free-range farming conditions. All the considered animals were mestizo breed, healthy and of similar age (with an average age of four years). As regarding their dieting habits, all the animals usually grazed liberally around the farm, but they also have available locally produced alfalfa hay to supplement their diet. Each collected milk sample (in total 16 milk samples) was an aliquot (about 1 L) of all milk harvested from the same hand milking, in order to obtain a sample representative of all the rearing. Immediately after the collection, the milk was stored in polyethylene containers, refrigerated on ice and transported to the laboratory for pretreatment and analytical determinations.

Egg samples of hens (Gallus gallus) were collected between September 2017 and January 2018 in 16 home-producing farms (Figure S1B). The hens were living on free-range farms and their diet has been integrated with the chicken feed. In order to obtain a representative sampling, from each chicken farms six eggs were collected. Also in this case, after collection, the samples were kept in a cool-bag and transported to the laboratory. The analytical determinations were conducted using the aggregate hen egg samples consisting of six eggs for each sampling site.

S5.2 Air collection

Air sampling campaign was performed between August 2018 and November 2018 by exposing six passive Radiello® samplers to outdoor air for a period of two weeks (Figure S2). The six samplers were placed in the vicinity of the most contaminated areas. In order to obtain a representative sampling, in all sampling sites the Radiello® samplers were placed 2 m above ground level, in a shaded place to be protected from the direct sun light. At the end of the two-week sampling period, Radiello® samplers were collected, transported to the laboratory and stored at -20°C until the analysis.

S5.3 River waters collection

Water samples were collected from Tirino and Pescara rivers, water bodies which flow in the SNI area. In particular, four and two different sampling sites have been identified along the Pescara and Tirino river, respectively (Figure S3). The samplings were performed in the period between September 2017 and November 2018 on a quarterly basis (60 samplings in total). Whenever possible all the water samplings were performed in the middle of the watercourse (from a bridge), or from the shore. Samples were collected below the water surface (about 30 cm) by using methanol pre-cleaned polypropylene plastic bottles (1 L). After filling, the bottles were stored at 4 °C until the analysis.

S5.4 Drinking waters collection

Samples were collected between September 2017 and November 2018 from drinking water fountains within the perimeter of the SNI (Figure S3). Samples (5 L for each sample) were collected from water fountains in pre-cleaned amber glass bottles. All samples were stored at 4 °C until analysis for a maximum 24 h.

S5.5 Mushrooms collection

During August 2017 and November 2018, 30 samples of the fruit bodies of three wild edible mushroom species were collected: *Cantharellus cibarius* (N=10), *Boletus edulis* (N=10) and *Macrolepiota procera* (N=10). Mushroom samples were collected by the Local Health Authority of Pescara in 12 sampling sites located no more than 5 km from the perimeter of the SNI (Figure S4). After the collection, the samples were stored in polyethylene bags and immediately transported to the laboratory for the analysis.

S5.6 Urine collection

Urine samples were collected from 44 males and 54 females in June 2018 with no occupational exposure to Pb and Hg and resident for at least ten years within 5 km of the SIN perimeter. The ages of the donors ranged from 30 to 89 years with an average age of 65 years. Before the collection, all the donors had been informed on the purpose of this research and had signed the consent forms. The samples were stored in a polypropylene tube and immediately frozen at −20°C until the analysis.

S6. Analytical Procedures

S6.1 Analysis of PCDD/Fs and PCBs in food matrices

PCDD/Fs and PCBs analysis in egg and milk samples were carried out by the accredited laboratory of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (Experimental Zooprophylactic Institute of Abruzzo and Molise) in accordance with US EPA method 1613B and US EPA 1668B, respectively (U.S. EPA, 1994; U.S. EPA, 2008). The quantifications of 17 PCDD/Fs and 12 PCBs, were performed by using a gas chromatograph coupled to high resolution double-focusing mass spectrometer.

After the homogenization, 25 g of egg and 100 ml of milk samples were added with 50 µl of $^{13}C_{12}$-labelled PCBs surrogates standards solution at 10 ng mL^{-1} in 2,2,4-trimethylpentane (PCB-28, PCB-52, PCB-77, PCB-81, PCB-101, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-169, PCB-180, PCB-189) and 50 µl of $^{13}C_{12}$-labelled dioxins/furans surrogates standards solution at 10 ng mL^{-1} in 2,2,4-trimethylpentane (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF). After the isotopic enrichment and the denaturation of proteins whit ethanol and ammonia, the samples were added with petroleum ether and diethyl ether (1:1) to separate the two layers (aqueous and organic) and to collect the organic phase. After the collection,

the organic phase was filtered and concentrated to dryness by using rotary evaporator. The gravimetric determination of total lipid content was then assessed on all samples; after that, the extract was resuspended in 10 mL of hexane and processed by double liquid-liquid extraction (first with sulfuric acid and then with aqueous solution of KOH) to remove the lipidic components.

Due to the complexity of the extracted matrix, a subsequent purification by multiple columns was required to minimize interferences. The extracted volume (about 20 mL) was passed through a multilayer column (silica, acidic silica, and basic silica). The column was eluted with 70 ml of hexane and the eluate was concentrated by using rotary evaporator to about 2 ml, which was quantitatively transferred to the top of the second column packed with alumina and activated carbon. This column was washed with 10 mL of hexane (not collected) and eluted with 40 mL of n-hexane-dichloromethane mixture (50:1) to collect PCBs and 60 mL of n-hexane-dichloromethane mixture (50:50) to collect PCDD/Fs. Both the eluates were evaporated to dryness under a gently nitrogen flux and, finally, recovered with 50 µL of 2,2,4-trimethylpentane.

S6.2 Analysis of PAHs in food matrices

Four PAHs (Benzo[a]anthracene, (BaA); Chrysene, (CHR); Benzo[b]fluoranthene, (BbFA); Benzo[a]pyrene, (BaP)) were determined in accordance with CE 333/2007 method for the determination of BaP in food matrices.

After the homogenization, 2 g of food matrices (egg or milk) were added with 50 µL of $^{13}C_{12}$-labelled PAHs surrogate standards solution at 10 ng mL^{-1} in acetonitrile. The sample was then added with 10 mL of water and extracted in the dark with acetonitrile by using magnetic stirrer. After the addition of magnesium sulfate and sodium chloride (ORIGINAL QuEChERS), the mixture is centrifuged (4500 rpm for 10 minutes at 4 °C) and the organic phase is removed. The extract was then purified by solid phase extraction and then concentrated to dryness by using gentle flow of nitrogen gas. The extract was then recovered with 20 µL of toluene, filtered and then

analyzed by using gas chromatography coupled with triple quadrupole tandem mass spectrometry (GC-MS/MS).

S6.3 Analysis of mercury and lead in food matrices

Metals analysis in egg and milk samples were carried out in accordance with UNI EN 13804 method for the sample preparation, with UNI EN 13805 method for the sample digestion and UNI EN 15763 for the inductively coupled plasma mass spectrometry (ICP-MS) analysis. Briefly, 2 g of milk and 1 g of egg were mineralized in a microwave oven (Start D - Microwave Digestion System, Milestone, Bergamo, Italy) by using 5mL of ultrapure concentrated HNO_3 (67%; Promochem, LGC Standards GmbH, Wesel, Germany) and 2mL of H_2O_2 (30%; Promochem, LGC Standards GmbH, Wesel, Germany). The concentration of lead and mercury in the mineralized matrices was determined by using inductively coupled plasma mass spectrometry (ICP-MS, Bruker 820-MS, Billerica, MA, USA). The quantification of the analytes was performed by standard addition methods using ^{187}Re, ^{206}Pb and ^{199}Hg.

S6.4 Analysis of VOCs in air samples

Radiello® samplers were placed into a glass amber vial and added with 100 µL of internal standard solution. The air samples were extracted for 30 minutes in an ultrasonic bath by using 2 mL of carbon disulfide (CS_2). The extract was then analyzed by using gas chromatography coupled with triple quadrupole tandem mass spectrometry (GC-MS/MS).

S6.5 Determination of VOCs in water samples

The analysis of four VOCs (carbon tetrachloride, trichloroethylene, tetrachloroethylene and hexachloroethane) in water samples was carried out by using Stratum Purge and Trap Concentrator equipped with a VOCARB 3000 trap used to transfer VOCs into the GC/MS.

Briefly, 5 mL of unprocessed water samples was purged by nitrogen gas at 40 ml min^{-1} for 15 min at room temperature. Then the analytes concentrated in the trap were desorbed at 400 ml min^{-1} for 3 min at 250 °C. After desorption, the trap was baked at 300 °C for 10 min. Chromatographic separation was performed on a SPB-624 capillary column (30 m × 0.25 mm × 1.4 μm film thickness; Supelco) employing helium as carrier gas. Xcalibur software (Thermo Fisher Scientific) was used for data acquisition/processing and instrument management.

S6.6 Determination of PAHs in water samples

The analysis of four PAHs (BaA; Chr; BbF; BaP) in water samples were carried out by accredited laboratory of Abruzzo Regional Agency for the Protection of the Environment in accordance with Method ISS.CAB.039 rev.00/2007. The determination of target analytes was performed by using a gas chromatograph coupled to high resolution magnetic sector mass spectrometry (DFS Magnetic Sector GC-HRMS) (Thermo Scientific, Bremen, Germany). Briefly, 1 L of water sample was added with 50 μL of $^{13}C_{12}$-labelled PAHs surrogate standards solution at 10 ng mL^{-1} and extracted by liquid-liquid extraction (LLE) by using 50 mL of dichloromethane (DCM). The LLE procedure was repeated for 3 times, collecting every time the organic phase. About 150 mL of DCM were then concentrated by using rotary evaporator to about 5 mL. The extract was then purified by multiple columns and the eluate was evaporated to dryness and recovered with 25 μL of DCM. The triple quadrupole operated in MS/MS-EI (electron ionization) Multiple Reaction Monitoring (MRM) mode. Quantification was performed by isotopic dilution technique applied to the two most abundant product ions for native and $^{13}C_{12}$-labelled standards.

S6.7 Analysis of Pb and Hg in waters

Lead and mercury analysis in water samples were carried out by accredited laboratory of Abruzzo Regional Agency for the Protection of the Environment in accordance with the Italian Institute of Health, Method ISS.DBA.035 rev.00/2007 and ISS.DAB.013 rev.00/2007, respectively.

Briefly, for mercury analysis 45 ml of water samples were mineralized in a microwave oven (Start D - Microwave Digestion System, Milestone, Bergamo, Italy) by using 5 mL of ultrapure concentrated HNO3 (67%; Promochem, LGC Standards GmbH, Wesel, Germany) and 2 mL of H_2O_2 (30%; Promochem, LGC Standards GmbH, Wesel, Germany). The mercury concentration in the mineralized matrices was determined by using cold vapour atomic absorption spectroscopy.

For lead analysis, the acidified water samples were analyzed by using electrothermal atomisation atomic absorption spectroscopy.

S6.8 Analysis of Pb and Hg in mushroom samples

The analysis of Pb and Hg in wild edible mushroom samples were performed as described in Sarikurkcu et al. (2011) with some modifications. Briefly, all the mushroom samples were cleaned, cut and dried at 40 °C until constant weight. Dried samples were homogenized by using a Teflon ball mill. About 0.5 g of dried material was digested with 4 mL HNO_3 (65%) and 2 mL of H_2O_2 (30%) and, after the digestion, diluted to 50 mL with deionizer water. All the samples were then analyzed by using inductively coupled plasma mass spectrometry (ICP-MS). Lead and mercury concentrations were determined on a dry weight basis. Limit of detection (LOD) is defined as the concentration corresponding to three times the standard deviation obtained by injecting 10 times the reactive blank.

S6.9 Analysis of metals (Hg and Pb) in urine samples

Lead analysis in urine samples were carried out in accordance with 6020B (SW-846) method for the quantitative determination by using inductively coupled plasma mass spectrometry (ICP-MS) analysis. The data were collected according to a previously reported method (Astolfi et al., 2020). The sample pretreatment was previously described in Protano et al. (2016).

Mercury analysis in urine samples was carried out according to the US EPA 7473 method (US EPA 2007) by using an Advanced Mercury Analyzer (AMA-254, Altec Ltd., Prague, Czech Republic).

The instrumental conditions for AMA analysis were described in a previous study in detail (Astolfi et al., 2019).

The limits of detection (LODs) of the analytical procedures described in S6.1 – S6.9, calculated as the concentration at which signal to noise ratio (S/N) of each compound is > 3, are reported in Table 1.

Table 1. Limits of detection for all the investigated analytes.

Matrices	**pg g^{-1} fat**		**ng g^{-1} fat**	**µg kg^{-1}**	**ng g^{-1} µg l^{-1} for water and urine samples mg kg^{-1} d.w. for mushroom samples**		**µg m^{-3} for air samples; µg l^{-1} for water samples**
	PCDD/Fs (min-max)	**DL-PCBs (min-max)**	**NDL-PCBs (min-max)**	**PAHs (min-max)**	**Pb**	**Hg**	**c-VOCs (min-max)**
Milk	0.0084-0.017	0.053-0.072	0.041-0.082	0.12-0.31	2.3	0.7	-
Eggs	0.008-0.017	0.05-0.07	0.04-0.08	0.12-0.24	5.7	2	-
Air	-	-	-	-	-	-	0.12-0.21
River Waters	-	-	-	-	5	5	0.013-0.75
Drinking Waters	-	-	-	-	5	5	0.013-0.75
Mushrooms	-	-	-	-	0.4	0.1	-
Urine	-	-	-	-	0.3	0.03	-

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