UNIVERSITÀ DEGLI STUDI DI NAPOLI
FEDERICO II

Ph.D. in Chemical Sciences

XXXI Cycle

Assessment of dietary exposure to inorganic and persistent organic contaminants in relation to environmental pollution in the Campania region

Dr. Sara Lambiase

Tutors

Prof. Marco Trifuoggi

Dr. Mauro Esposito

Supervisor

Prof. Piero Pucci

Ph.D. Coordinator

Prof. Luigi Paduano

2015-2018

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ABSTRACT

The project is aimed at studying the links between environmental pollution and the contamination levels of inorganic and persistent organic pollutants (POPs) in food. In order to achieve the purpose of the study, eggs from free-range hens, of *Gallus gallus domesticus* species, were used as bioindicators of the PCDD/F and PCB contamination levels of the environment where the hens live. In addition to food of animal origin, foods of plant origin, collected in the same areas of the eggs, were also analysed. In parallel, an *in vivo* study designed to investigate how and to what extent PCDD/Fs and PCBs are transferred from exposure sources, precisely soil and to a lesser extent feed, to hen eggs was carried out.

All analysed samples were compliant with the maximum limits fixed by the Regulations (EU, 1259/2011; EU, 277/2012; Ministry for the Environment, Land and Sea, 2006) and with the action levels (EU, 663/2014), except for the eggs collected in the Area 2, that exceeded the maximum permitted limit set for PCDD/Fs and the eggs collected from the Area 1, that exceeded the action level set for DL-PCBs. The most abundant congeners found in egg samples were OCDD (51.0%), 1,2,3,4,6,7,8-HpCDD (13.3%) and 2,3,7,8-TCDF (8.4%) for PCDD/Fs, PCB-118 (52.8%) and PCB-105 (21.8%) for DL-PCBs and PCB-138 (25.8%) and PCB-153 (37.0%) for NDL-PCBs. Regarding vegetable and feed samples, the PCDD/F congeners above the LOQs were only 25% and 12%, respectively; concerning PCBs, the most abundant congeners were PCB-118 (50.7%), PCB-105 (23.1%), PCB-28 (19.7%), PCB-101 (18.6%), PCB-153 (18.8%) and PCB-180 (19.4%) for vegetables and PCB-118 (61.1%), PCB-105 (15.8%), PCB-28 (24.0%), PCB-101 (23.8%) and PCB-52 (20.9%) for feed. Finally, in soil samples the most abundant congeners found were OCDD (78.5%), 1,2,3,4,6,7,8-HpCDD (10.1%), PCB-118 (43.5%), PCB-105 (24.7%), PCB-138 (30.9%) and PCB-153 (34.8%). From the analysis of PCDD/F and PCB congeners, it was found that eggs and soil showed a very similar profile as well as vegetables and feed and, in particular,

PCB-153 and PCB-138 predominated in soil and eggs, while PCB-28 and PCB-52 were also found in vegetables and feed. Regarding the *in vivo* study, the concentrations of PCDD/Fs and PCBs found in the eggs, after the transfer in the Area 2, were progressively increased until reaching the maximum concentrations at 27^{th} and 45^{th} week, but the increase did not follow a linear trend and this probably occurred because the contaminants occurring in the soil of the Area 2 were less available for absorption by the hens. It was also observed that the trend of PCDD/F and PCB concentrations in the eggs could also be due to the seasonal variability of these contaminants in the environment. The concentrations of PCDD/Fs and PCBs found in the eggs during the *in vivo* study were used to calculate the biotransfer factors (BTFs) for each of the 35 congeners measured. The analysis of the BTFs calculated showed that the lower chlorinated congeners had higher BTFs than other congeners and, consequently, this meant that they were absorbed and transferred into the eggs more than others. Regarding NDL-PCBs, on the contrary, the lower chlorinated congeners showed lower BTFs.

Moreover, a preliminary study concerning persistent inorganic environmental pollutants was also carried out. The analysis revealed low TE levels both in eggs and in vegetable samples. However, a greater accumulation of Fe and Zn was observed in eggs compared to vegetables with the highest concentrations of TEs found in lettuce samples. Among REEs, the only elements detected were: Th found in all samples; Sc and Ce found in vegetables; La, Pr, Nd, Sm and Eu found only in lettuce samples.

Chapter I

FOREWORD

In recent years, Institutions and Scientific Community in the Campania region have shown a great interest in monitoring environmental pollutants, especially those considered very toxic for human health, in food and environmental matrices. The interest in the study of environmental pollutants is due to the concern of the serious toxic effects that these substances, even at low concentrations, may cause to human health. In particular, this interest originated as a consequence of two dramatic events made known by the communication organs such as:

– Dioxin emergency in the territory of the Campania region
– "Land of Fires" emergency

The dioxin emergency in the Campania region began in the spring 2002, when the Ministry of Health carried out a Surveillance Plan that covered the whole national territory, in compliance with the Council Directive 96/23, aimed at investigating the presence of certain substances and their residues in animals and animal products. During the monitoring plan, the concentrations of dioxins and furans exceeded the maximum levels set by the Council Regulation (EC) 2375/2001 in two samples of sheep milk collected by the competent local health authorities in two farms located in Villa Literno (CE) and Mariglianella (NA), respectively (Diletti et al., 2003). Due to these exceedances of the maximum permitted limits, a further 15 bulk milk samples were collected from farms located between the provinces of Caserta and Naples. Thirteen of these samples showed dioxins and furans concentrations higher than the maximum levels allowed. Therefore, the Regional Council approved an intervention plan to face the dioxin emergency that was divided into 2 phases. In the first phase, which began in 2003, circular areas, called *red areas*, with a radius of 1 km around the farms that were contaminated with dioxins, were identified. Milk samples collected from all farms in these areas were analysed eventually leading to the killing of 10,000 sheep, 2,776 cattle, 145

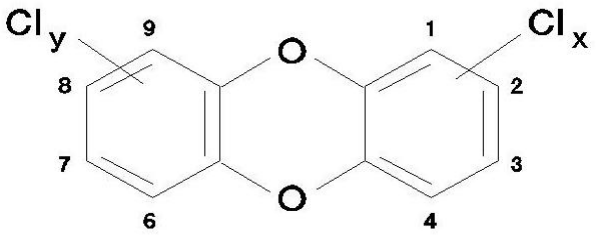
buffaloes and 45 goats and the destruction of about 8,000 tons of milk (APAT, 2007). In the second phase, started in 2004, milk samples were collected from farms located throughout the region and mainly in the areas not investigated during the first phase. During this monitoring, only 5 samples, 2 in the province of Naples and 3 in the provinces of Avellino and Salerno, exceeded the maximum permitted levels of dioxins. Simultaneously, investigations of environmental matrix samples also began.

The emergency known as "*Land of Fires*" represents a series of critical issues of the Campania region concerning waste disposal. In particular, it concerns the illegal disposal of urban and industrial waste, almost always both toxic to humans and to the environment, which are dumped on soils for urban or agricultural use, thus causing soil and often also aquifer pollution. The term was used for the first time in the Ecomafie Report (2003) by Legambiente in reference to the practice of burning the waste with consequent release of toxic fumes into the atmosphere. The investigations began in 1991 and continue until today. According to the data reported in the Legambiente dossier (2015), at least 10 million tons of toxic waste, from various parts of Italy, have been spilled in Campania, including aluminium processing waste, industrial purifiers, liquid waste containing heavy metals, waste containing aminated, paintsludge, polluting earth from reclamation works. Currently, the area affected by pollution, known generically as Land of Fires, includes 57 municipalities: 33 municipalities are located in the province of Naples and 24 municipalities are located in the province of Caserta.

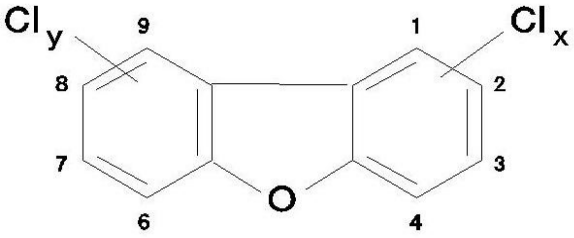
1. INTRODUCTION

1.1. DIOXINS AND FURANS

The generic term “dioxins” refers to a group of 210 tricyclic polychlorinated chemical substances which have very similar physical and chemical properties. Dioxins are divided into two different classes: 75 polychlorinated dibenzo-*p*-dioxins (PCDDs) and 135 polychlorinated dibenzofurans (PCDFs). The different congeners vary for the number and position of the chlorine atoms, that can be between 1 and 8. The general structures are given in Figure 1.



(a) polychlorinated dibenzo-*p*-dioxins (PCDDs)



(b) polychlorinated dibenzofurans (PCDFs)

Fig. 1. General structures of PCDDs (a) and PCDFs (b).

1.1.1. PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties of PCDD/Fs, as well as for any toxic substance, are important in understanding their analytical, physiological, and environmental properties and vary according to the degree and position of chlorine substitution.

In general, PCDD/Fs are hydrophobic substances that have very low water solubilities and high octanol-water partition coefficients (K_{ow}) (EHC 88, 1989; IARC, 1997; Trinh and Chang, 2018; Table 1). The octanol-water partition coefficient measures how hydrophilic or hydrophobic a chemical substance is. It is an important factor for predicting the distribution of organic chemicals between lipid and water and to evaluate the bioconcentration potential of a toxicant. Sometimes this parameter has been effectively used as an indicator of the preferred degradative *in vivo* pathways. These properties suggest that PCDD/Fs are very lipophilic substances that are more likely to be distributed in lipids where they can bioaccumulate.

Table 1
Water solubilities and octanol/water partition coefficients of PCDD/Fs.

Compounds	S^a (mg L^{-1} at 25 °C)	Log K_{ow} [a]
2,3,7,8-TCDD	0.0158	7.06
1,2,3,7,8-PeCDD	4.00×10^{-3}	7.55
1,2,3,4,7,8-HxCDD	1.15×10^{-3}	7.94
1,2,3,6,7,8-HxCDD	1.10×10^{-3}	7.94
1,2,3,7,8,9-HxCDD	1.10×10^{-3}	7.92
1,2,3,4,6,7,8-HpCDD	3.15×10^{-4}	8.27
OCDD	1.03×10^{-4}	8.48
2,3,7,8-TCDF	0.0243	6.13
1,2,3,7,8-PeCDF	0.0679	6.47
2,3,4,7,8-PeCDF	0.00481	6.56
1,2,3,4,7,8-HxCDF	1.64×10^{-3}	6.92
1,2,3,6,7,8-HxCDF	1.56×10^{-3}	6.93
1,2,3,7,8,9-HxCDF	1.24×10^{-3}	7.01
2,3,4,6,7,8-HxCDF	1.06×10^{-3}	7.07
1,2,3,4,6,7,8-HpCDF	4.92×10^{-4}	7.37
1,2,3,4,7,8,9-HpCDF	2.83×10^{-4}	7.60
OCDF	1.22×10^{-4}	8.03

[a]S and log K_{ow} from Wang and Wong (2002, 2003).

PCDD/Fs have low vapour pressures (P_v) and high octanol-air partition coefficients (K_{oa}) (Trinh and Chang, 2018; Table 2). These parameters indicate their gas/particle partitioning in ambient air.

Table 2
Vapour pressures and octanol/air partition coefficients of PCDD/Fs.

Compounds	P_v[a] (Pa at 25 °C)	Log K_{oa}[b] at 25 °C
2,3,7,8-TCDD	2.57×10^{-5}	10.0
1,2,3,7,8-PeCDD	4.17×10^{-6}	10.6
1,2,3,4,7,8-HxCDD	8.91×10^{-7}	11.1
1,2,3,6,7,8-HxCDD	8.51×10^{-7}	12.2
1,2,3,7,8,9-HxCDD	8.51×10^{-7}	12.3
1,2,3,4,6,7,8-HpCDD	2.04×10^{-7}	11.4
OCDD	6.61×10^{-8}	13.0
2,3,7,8-TCDF	8.91×10^{-5}	10.0
1,2,3,7,8-PeCDF	1.70×10^{-5}	11.4
2,3,4,7,8-PeCDF	1.15×10^{-5}	11.5
1,2,3,4,7,8-HxCDF	3.24×10^{-6}	11.9
1,2,3,6,7,8-HxCDF	3.09×10^{-6}	12.0
1,2,3,7,8,9-HxCDF	2.45×10^{-6}	12.1
2,3,4,6,7,8-HxCDF	2.09×10^{-6}	12.2
1,2,3,4,6,7,8-HpCDF	9.33×10^{-7}	12.2
1,2,3,4,7,8,9-HpCDF	5.75×10^{-7}	12.3
OCDF	2.88×10^{-7}	12.8

[a]P_v from Wang and Wong (2002, 2003).

[b]Log K_{oa} from Harner et al. (2000).

These pollutants are very resistant to biodegradation and stable compounds even at high temperatures. The 2,3,7,8-TCDD, indeed, degrades to temperatures above 750 °C; however, it undergoes substitution reactions, as well as photochemical dechlorination. On the contrary, OCDD is completely destroyed by treatment with hot alkali (EHC 88, 1989).

1.1.2. SOURCES OF ENVIRONMENTAL POLLUTION

PCDDs and PCDFs are not produced commercially; indeed, there is no known technical use for these compounds. These toxicants occur as undesired by-products during combustion and some industrial processes, manufacture of other chemicals and metal production (Conesa et al., 2016; Li et al., 2019).

The formation of PCDD/Fs during combustion reactions occurs through a succession of destruction and formation pathways. The formation can occur through simultaneous oxidation and chlorination of a carbonaceous matrix, at temperatures between 200 °C and 400 °C in the presence of oxygen (Stieglitz, 1998). PCDD/Fs formation can also take place when a precursors enter in the combustion process by a catalytic-assisted coupling mechanism at a temperatures between 200 °C and 400 °C (Altarawneh et al., 2009). The precursors include PCP/PCP-Na (Pentachlorophenol), PCBs, chloroparaffins in waste oils, inorganic chlorine and thermoplastics. These chemical compounds are used for the production of wood preservatives, pesticides and in leather and plastics industry.

The combustion processes, that lead to the formation of PCDD/Fs, include incineration of municipal, hospital, hazardous waste and sewage sludge; fuel combustion in metal and metal treatment industry; fuel combustion in cement production processes; combustions for energy production like combustions of fuels containing chlorinated compounds used in transports, burning of treated wood and oil combustions; accidental and outdoor fires of heterogeneous materials, such as municipal waste, tires, plastics and waste containing chlorinated compounds; forest fires in presence of chlorinated compounds for the combustion of lignin and cellulose and volcanic eruptions with a mechanism of production of dioxins analogous to forest fires (Tuppurainen et al., 1998; Anderson and Fisher, 2002; Kim et al., 2003; Chang et al., 2004; APAT, 2006; Ooi and Lu, 2011; Zhou et al., 2015; Hahladakis et al., 2018).

PCDD/Fs are formed during the production of some chemicals such as vinyl chloride monomer (VCM), polystyrene and dichloro ethylene that are used in the plastics industry.

In the past, these compounds were also formed during the synthesis of some chemicals that are now banned such as chlorinated phenols (2,4-dichloro-, 2,4,6-trichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol) and their derivatives, chlorinated diphenyl ether herbicides, hexachlorobenzene, polychlorinated biphenyls (PCBs) and ethylene glycol (EHC 88, 1989; Hue et al., 2014).

1.2. POLYCHLORINATED BIPHENYLS

Polychlorinated biphenyls (PCBs) are a group of 209 chlorinated aromatic compounds consisting of a biphenyl whose hydrogen atoms are replaced by 1 to 10 chlorine atoms. The basic structure is given in Figure 2.

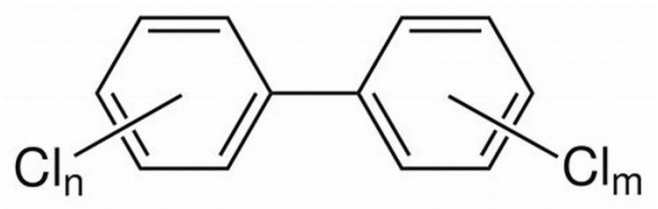


Fig. 2. General structures of polychlorinated biphenyls.

In terms of structure and toxicity, PCBs can be divided into two distinct categories: *dioxin-like* PCBs (DL-PCBs), 12 congeners (non-ortho and mono-ortho chloro substituted) that exhibit toxicological properties similar to dioxins; *non dioxin-like* PCBs (NDL-PCBs), consisting of the remaining 197 congeners. DL-PCBs are often also referred to as coplanar PCBs, since, having at most one chlorine atom in the ortho position, the two aromatic rings can rotate into the same plane.

1.2.1. PHYSICAL AND CHEMICAL PROPERTIES

PCBs, as PCDD/Fs, have a very low water solubility but are soluble in organic compounds and hydrocarbons. They are very lipophilic substances with high octanol/water partition coefficient (log K_{ow}) values, that range from 4.46 to 8.18 for all 209 PCB congeners. They have low vapour pressures (P_v) and high octanol/air partition coefficients (log K_{oa}). The physical and chemical properties of 12 DL-PCBs are reported in Tables 3 and 4 (Trinh and Chang, 2018).

Table 3
Water solubilities and octanol/water partition coefficients of DL-PCBs.

COMPOUNDS		S[a] (mg L^{-1}, 25 °C)	Log K_{ow}[b]
PCB-77	3,3',4,4'-TCB	2.70×10^{-3}	6.50
PCB-81	3,4,4',5-TCB	3.13×10^{-3}	6.53
PCB-105	2,3,3',4,4'-PeCB	1.66×10^{-3}	6.61
PCB-114	2,3,4,4',5-PeCB	2.63×10^{-3}	6.47
PCB-118	2,3',4,4',5-PeCB	2.07×10^{-3}	6.49
PCB-123	2',3,4,4',5-PeCB	8.99×10^{-4}	6.50
PCB-126	3,3',4,4',5-PeCB	1.33×10^{-3}	6.56
PCB-156	2,3,3',4,4',5'-HxCB	1.10×10^{-3}	6.75
PCB-157	2,3,3',4,4',5'-HxCB	2.96×10^{-4}	6.73
PCB-167	2,3',4,4',5,5'-HxCB	1.07×10^{-4}	6.82
PCB-169	3,3',4,4',5,5'-HxCB	1.30×10^{-4}	7.01
PCB-189	2,2',3,3',4,4',5-HpCB	6.30×10^{-5}	7.15

Notes:
[a]S from Huang and Hong (2002).
[b]Log K_{ow} from Yeh and Hong (2002).

Table 4
Vapour pressures and octanol/air partition coefficients of DL-PCBs.

COMPOUNDS		Pv^a (Pa at 25 °C)	Log K_{oa}[b] at 25 °C
PCB-77	3,3',4,4'-TCB	0.002	9.70
PCB-81	3,4,4',5-TCB	3×10^{-3}	9.88
PCB-105	2,3,3',4,4'-PeCB	8.85×10^{-4}	10.2
PCB-114	2,3,4,4',5-PeCB	1.24×10^{-3}	9.62
PCB-118	2,3',4,4',5-PeCB	1.23×10^{-3}	9.86
PCB-123	2',3,4,4',5-PeCB	1.3×10^{-3}	9.83
PCB-126	3,3',4,4',5-PeCB	4.84×10^{-4}	10.6
PCB-156	2,3,3',4,4',5'-HxCB	2.1×10^{-4}	10.4
PCB-157	2,3,3',4,4',5'-HxCB	2.02×10^{-4}	10.6
PCB-167	2,3',4,4',5,5'-HxCB	2.8×10^{-4}	10.6
PCB-169	3,3',4,4',5,5'-HxCB	6.88×10^{-3}	11.3
PCB-189	2,2',3,3',4,4',5-HpCB	4.77×10^{-3}	11.2

Notes:
[a]P_V from Foreman and Bidleman (1985).
[b]Log K_{oa} from Chen et al. (2002).

Due to their physical and chemical properties, PCBs, as PCDD/Fs, are very stable substances that resist to degradation processes for long periods. They remain chemically unchanged, also in the presence of oxidising agents and other chemicals and at high temperatures (up to 170 °C) (Zeng et al., 2013; Tang et al., 2015; Reddy et al., 2019). For these reasons, they are considered persistent pollutants both in biotic and abiotic environments. In addition to these characteristics, PCBs have specific properties as a high boiling point, non-flammability, dielectric properties, poor electrical conductivity and high heat capacity; due to these advantages, they have been utilised in numerous industrial applications.

1.2.2. SOURCES OF ENVIRONMENTAL POLLUTION

The global production of PCBs is estimated to be at least 1.3 million tons (Breivik et al., 2002), 10% of which accumulate in the environment (Reddy et al., 2019). Nowadays, their production is banned in most countries as they are persistent organic pollutants (POPs).

PCBs have been synthesized by chlorination of a biphenyl in presence of a catalyst such as iron, iodine, aluminium chlorides, tin, and antimony. They have never been produced as a single congener but as mixtures that generally consist of about 100 to 140 PCB congeners, with mono- and non-ortho substituted PCBs as minor or trace constituents (Frame et al., 1996a, b). Some of their commercial names are Aroclor, Clophen, Delor, Fenclor, Kanechlor, Phenochlor and Pyralene. When commercial PCB mixtures are produced, it is well known that they inevitably contain some impurities consisting of polychlorinated naphthalenes and PCDFs, while the presence of PCDDs has not been reported (IARC, 1978; EHC 88, 1989).

The main applications for PCBs were in electric products such as dielectric fluids, capacitors, transformers, voltage regulators, bushings, electromagnets, cable insulations and florescent bulbs. They have also been used in construction materials as plasticizers, such as window glazing, sealants, flooring material, caulking and grout in floor and joints and in industrial products such as paints, printing inks, carbonless paper, clothing pigments and dyes, pesticides, plastic water and baby bottles (Reddy et al., 2019).

1.3. DISTRIBUTION IN THE ENVIRONMENT AND BIOTA

PCDD/Fs and PCBs are found worldwide and in all environmental media (soils, sediments, water and air). Soil is considered a natural sink for persistent and lipophilic compounds such as PCDD/Fs and PCBs; in particular, highly contaminated soil may represent a secondary source of these contaminants. Once released into the soil, PCDD/Fs and PCBs are absorbed by the organic carbon and consequently their mobility and bioavailability decrease whereas their persistence increases (Buckley-Golder, 1999). PCDD/Fs and PCBs enter the soil through different pathways: atmospheric deposition, industrial productions, application of sewage sludge, use and disposal of PCDD/Fs and PCB containing products, accidental releases and releases from nearby contaminated areas. Air is a major pathway for PCDD/F and PCB migration; their transport can occur in either the vapour phase or particulate-bound and then, through a deposition mechanism, they are accumulated on the surface of vegetation, soil and sediments that become a reservoir of these contaminants. Although PCDD/Fs and PCBs are lipophilic and slightly soluble in water, water is another important pathway for migration of these pollutants both in solution and particulate-bound.

PCDD/Fs and PCBs from soil, sediment, air and water enter the food chain by uptake and bioaccumulation in plants and animal fats. Plants, and therefore also fruits and vegetables, receive these environmental contaminants mainly through gas-phase deposition, particulate wet and dry deposition, while the uptake through the roots is considered almost non-relevant (Lovett et al. 1997; Schuhmacher et al., 2006; Ben et al, 2017). Concerning animal exposures, PCDD/Fs and PCBs uptake occurs mainly through ingestion of contaminated feed, vegetables and soil. Then, being substances with a high affinity for lipids and resistance to metabolic degradation, they can accumulate in adipose tissues of animals and bio-concentrate further up the food chain. Consequently, 90% of human exposures to these toxicants occurs through food, in particular the consumption of food of animal

origin (fish, meat, eggs, milk and dairy products) that contain higher levels of these contaminants than food of plant origin, contributing for about 80% (WHO, 2010; EFSA, 2012)

1.4. EFFECTS ON HUMAN HEALTH

Dioxins and dioxin-like compounds are extremely toxic and bioaccumulative substances that, even at very low concentrations, can be very dangerous for biota and can exert adverse effects on human health. The biological effects of these substances are tissue specific and can be classified into three categories: altered homeostasis resulting from changes in hormones and their receptors; altered metabolism resulting from changes in enzyme levels; altered growth and differentiation resulting from changes in growth factors and their receptors (Birnbaum, 1994).

All these effects implicate the aryl hydrocarbon receptor (AhR), an intracellular receptor, found in most tissues (Carlstedt-Duke, 1979), that is involved in the expression of a large number of genes and, consequently, in regulation of numerous physiological processes. The AhR is a tetrameric complex, consisting of two molecules of hsp90, the ligand binding subunit and a molecule of p50, that functions as a transcriptional enhancer. The mechanism of TCDD action is similar to that exhibited by a hormone binding to its specific receptor and involves three phases: recognition of the signal, transduction of the signal and response. In the first phase, TCDD binds to the Ah receptor by a highly specific interaction and this bond represents the signal recognition. This interaction depends on the number and position of lateral halogenation, the polarizability, the planarity and the stacking interactions of molecules (Romkes et al.,1987; McKinney, 1989). The compounds with four chlorine atoms in the positions 2,3,7 and 8 are the most toxic congeners, because the others show weak or no binding to the AhR and/or are metabolised much faster than the 2,3,7,8-congeners. In fact, each chlorine atom further decreases the toxicity but the effects continue to be the same (Van den Berg et al.,

2006). Regarding PCBs, only congeners able to assume a planar position can bind to the receptor. These PCBs are the non-ortho substituted compounds which are free to rotate along the C-C bridge and can assume the planar conformation similar to PCDD/Fs. Among these, the 3,3',4,4',5-PeCB (PCB-126) is the most toxic congener. Each chlorine atom in the ortho position makes rotation and formation of the planar conformation more difficult. As a consequence, the eight mono-ortho substituted congeners show effects about thirty thousand times weaker than 2,3,7,8 TCDD and all other PCBs do not seem to show dioxin-like effects (Van den Berg et al., 2006; Lindén et al., 2010).

The binding of TCDD to the AhR causes the release of the hsp90, a heat shock protein with inhibitory regulatory functions, from the receptor and the migration of the complex into the nucleus. Then, the ligand-AhR complex undergoes a dimerization with the nuclear protein ARNT (AhR Nuclear Translocator) and this process converts the AhR into a high affinity binding DNA form. The complex TCDD-AhR-ARNT binds to a specific site on DNA, called DRE (Dioxin Responsive Enhancer) and stimulates the transcription of specific genes with subsequent production of mRNA and therefore enzymatic induction. In humans and other vertebrates, the response and biological effects, obtained when dioxins and dioxin-like compounds interact with the AhR, depend upon the environment of each cell and tissue and result in several clinical manifestations including risk factors for cancer, immune deficiency, reproductive and developmental abnormalities, central and peripheral nervous system pathology, endocrine disruption including diabetes and thyroid disorders, decreased pulmonary functions and bronchitis, altered serum testosterone level, eyelid pathology including meibomian gland hypersecretion and hyperpigmented conjunctivae, gum pigmentation, nausea, vomiting, loss of appetite, skin rashes including chloracne and skin hyperpigmentation, hypertrichosis, liver damage, elevated serum cholesterol and triglycerides, enamel hypomineralization of permanent first molars in children, increased risk of mortality associated with high levels of occupational

exposure to dioxins with acute ischemic cardiovascular events. Transient acute health effects including headache, inability to have erections or ejaculations, pruritis, personality changes, irritability, pain in the abdomen or extremities, diarrhoea, insomnia and fatigue have also been described (Schecter et al., 2006).
In addition to these effects, some authors showed that dioxins and dioxin-like compounds can induce oxidative stress both *in vivo* and *in vitro* (Yoshida and Ogawa, 2000; Liu et al., 2016).

1.5. TOXIC EQUIVALENCY FACTORS

PCDD/Fs, as well as DL-PCBs, are present in environmental media and in food as complex mixtures. However, the different congeners do not show all the same toxicity and when their concentration is determined in a sample, the analytical concentration is expressed using an additive toxic equivalency (TEQ) approach based on the toxic equivalency factors (TEFs).
TEQ concept, developed during the mid 1980's (Safe et al., 1985; Van den Berg et al., 2006), is a toxicological approach that expresses the concentration of a harmful compound in relation to another substance, taken as a reference, capable of generating the same toxic effects. The criteria for inclusion of a compound in the TEF concept are: a structural relationship to the PCDD/Fs, binding to the AhR, induction of AhR-mediated biochemical and toxic responses and persistency and accumulation in the food chain (Van den Berg et al., 2006). For PCDD/Fs and DL-PCBs, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), considered the most toxic dioxin, is usually taken as the reference compound.
The total TEQ is calculated by the sum of the products of the concentration of each compound multiplied by its TEF value and is an evaluation of the total 2,3,7,8-TCDD–like activity of the mixture. The equation used for the TEQ calculation is:

$$TEQ = \sum (C_i \cdot TEF_i)$$

TEFs are obtained by comparing the binding affinity of halogenated organic compounds for the AhR receptor, with the affinity of TCDD, considered the most potent inducer and therefore the most toxic compound.

There are two different classifications of TEFs; the international TEFs (I-TEFs 1988), established by the NATO Committee on the Challenges of Modern Society (NATO/CCMS, NATO Report No. 178, 1988), are used for the expression of the total concentration of PCDD/Fs in environmental samples; the World Health Organization TEFs (WHO-TEFs 2005, Van den Berg et al., 2006) are used to express the total PCDD/Fs and DL-PCBs concentrations in food samples and for DL-PCBs in environmental samples. In this research project, the WHO-TEF 2005 (Table 5) was used both for food and soil samples in order to compare the results and to calculate the transfer factors.

Table 5
Summary of WHO 2005 TEF values.

CONGENER	TEF	CONGENER	TEF
Dibenzo-p-dioxin (PCDD)		**DL-PCBs: non-ortho chlorosubstituted**	
2,3,7,8-TCDD	1	**PCB 77**	0.0001
1,2,3,7,8-PeCDD	1	**PCB 81**	0.0003
1,2,3,6,7,8-HxCDD	0.1	**PCB 126**	0.1
1,2,3,6,7,8-HxCDD	0.1	**PCB 169**	0.3
1,2,3,7,8,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	0.01		
OCDD	0.0003		
Dibenzofurans (PCDF)		**DL-PCBs: mono-ortho chlorosubstituted**	
2,3,7,8-TCDF	0.1	**PCB 105**	0.00003
1,2,3,7,8-PeCDF	0.03	**PCB 114**	0.00003
2,3,4,7,8-PeCDF	0.3	**PCB 118**	0.00003
1,2,3,4,7,8-HxCDF	0.1	**PCB 123**	0.00003
1,2,3,6,7,8-HxCDF	0.1	**PCB 156**	0.00003
1,2,3,7,8,9-HxCDF	0.1	**PCB 157**	0.00003
2,3,4,6,7,8-HxCDF	0.1	**PCB 167**	0.00003
1,2,3,4,6,7,8-HpCDF	0.01	**PCB 189**	0.00003
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0003		

1.6. CONTAMINATION OF EGGS

Research among laying hen farms in EU countries has shown that hens which spend a lot of time outdoors, such as free-range and organic eggs, contain more PCDD/Fs than conventional ones (De Vries et al., 2006). Also data collected by the European Food Safety Authority (EFSA) on PCDD/F and PCB concentrations in egg samples from different EU countries, showed an increase in the levels of these contaminants in free range eggs compared to those of conventional farms (EFSA, 2012).

The deposition of PCDD/F emissions leads to an increase in the environmental levels of these contaminants, in fact higher levels of PCDD/Fs and PCBs are found in soil, vegetation and sediments close to emission sources than to elsewhere. Consequently, as reported in many studies, higher levels of contaminants are also found in foods of animal origin such as cow milk, beef and mutton and eggs coming from these sites. Lovett et al. (1998) found higher PCDD/Fs levels in poultry products (meat and eggs) from a site close to an incinerator than those found elsewhere demonstrating that contamination levels depend on the environment where the animals live.

As in other animals, intake of pollutants in hens takes place from the environment where they live and eggs, having a high lipid content of about 10%, tend to accumulate lipophilic environmental contaminants such as PCDD/Fs and PCBs. Therefore, hens raised in free range or organic systems, that spend more time outdoors than those raised indoors, are more likely to bioaccumulate environmental contaminants.

The transfer mechanism of PCDD/Fs and PCBs from the environment to eggs is certainly the oral route. These contaminants are ingested by hens and partly transferred into the fat component of the egg yolk.

Out of a number of exposure sources, commercial feed and non-commercial feed are the main sources followed by soil, plants, insects and worms for animals raised outdoors. (Kijlstra, 2004).

The most used commercial feeds for hens contain cereals in grain (wheat, oats, barley, corn, sorghum, millet, panic, rye, rice, spelled, quinoa) to which an amounts of fat, food of animal origin and various additives according to the specific animal diets are added. Some farmers, alongside commercial feed, offer their hens also non-commercial feed mostly consisting of vegetable and garden scraps. These alternative feed have a very variable composition and, therefore, it is difficult to quantify their content of pollutants. However, although some authors (Brandsma et al., 2004) have found a connection between the high levels of PCDD/Fs in eggs and feeding with vegetable and garden scraps, it is not possible to assert that these scraps represent a source of contamination. Instead, it is possible to speculate that the time spent outside to eat the scraps leads an increase in the ingestion of soil, insects and worms.

In addition to feed, soil is another important source of PCDD/F and PCB contamination in animals; animals foraging on contaminated soil accumulate high levels of these pollutants in their tissues.

Data found in various studies show that soil is the most important source of PCDD/Fs intake (Petreas et al., 1996; Schuler et al., 1997; Fiedler et al., 2000; Kijlstra, 2004) followed by insects and worms that hens accidentally ingest when they scratch the yard in search of food (Kijlstra, 2004; Table 6).

Table 6
Source of PCDD/F and PCB contamination in free-range hens assuming a 25% transfer of the PCDD/F intake (Kijlstra, 2004).

Source	Low estimate (pg g^{-1})	High estimate (pg g^{-1})
Regular feed	0.05	1.25
Worms and insects	0.25	1.5
Herbs and grass	0.25	0.5
Soil	0.25	2.5
Total	0.8	5.75

Note:
Data represent the contribution of the various sources to the egg PCDD/Fs level per gram fat.

Considering that hens living outdoor spend about 50% of their time scratching the ground in search of food (Bestman and Wagenaar, 2002), it was estimated that during the scratching they can ingest about 4 g of animal protein and ingest between 2 to 10 g of soil per day (De Vries et al., 2006). Fiedler et al. (2000) found that the contents and congener profiles of the PCDD/Fs in the eggs were similar to those found in the soil on which the hens lived. Furthermore, Brandsma et al. (2004) found a correlation between the PCDD/Fs levels in the eggs and those found in the soil ($p < 0.10$); they also found that the congener pattern in earthworms was similar to that found in the eggs. However, the nature of this ingestion is not known; it could be accidental or for self-medication because the soil could have a therapeutic function (Wakibara et al., 2001; De Vries et al., 2006).
Regarding accumulation and elimination half-lives of PCDD/Fs and PCBs in hens, there are few data in literature. However, studies conducted analysing other animals show that their half-lives are congener and tissue dependent. In rats, as well as for guinea pigs, half-life is between 30 to 31 days and it is 14.8 days in hamsters; in humans, the half-life increased with increasing percentage of body fat (PEF) and decreased with increasing relative changes in PEF and with age (De Vries et al., 2006). According to Zabik et al. (1998), at low doses and concomitant

low residue levels, the half-life of PCDD/Fs in chickens is between that of the guinea pig and the hamster.

When the hens are exposed to PCDD/Fs and PCBs through ingestion of contaminated feed, soil, plants, insects and worms, the distribution of these toxicants is congener dependent and is different in the various tissues. Stephens et al. (1995) found that between 5 to 30% of the intake is excreted in eggs, 7 to 54% in animal fat and less than 1% in liver. Ikeda et al. (2004) found that the level of PCDD/Fs in eggs is dependent on the hens' intake as well as the concentration found in the edible tissues of the chickens. Furthermore, they suppose that adsorbed PCDD/Fs are first stored in fat tissue and then transferred to the eggs. Precisely, PCDD/Fs are incorporated in very low-density lipoproteins (VLDL), the major lipoproteins in chicken plasma that plays a crucial role in the development of the yolk. Their research also showed that exposure of layers to PCDD/Fs can induce a reversible inhibition of egg laying. Schwetz et al. (1973) found that the chicken embryo is highly sensitive to dioxins. Chickens exposed to low dioxin doses did not show effects on their health, unlike high doses (1000 ng TEQ per day) that can cause about 80% mortality. Petreas et al. (1996) confirmed that PCDD/Fs accumulate less in female than in male chickens and, therefore, eggs represent a way of elimination of PCDD/Fs as it happens with milk for cows. Furthermore, in a study conducted by Tlustos et al. (2004), it was found that levels of PCDD/Fs in eggs are also closely related to the age of the hens.

1.7. AIM OF THE PROJECT

The general objective of this research project is the investigation of the impact that environmental pollution has had on the quality of food products, one of the most relevant issues that has been affected the Campania region in recent years having a serious impact on the agro-food sector of this territory. Precisely, the project aims to evaluate the impact that the environmental pollution, often caused by the illegal dumping of urban and industrial waste and their fires, has had on the quality of Campania food products and, therefore, to determine the exposure level of the population to such contaminants.

This objective was pursued through the determination of organic pollutants, mainly in food matrices of animal and vegetable origin, as well as in environmental matrices. Samples were collected both from the areas classified at greater risk, because they are closer to the sources of pollution, and from geographical areas not included in the so-called "Land of Fires".

Studies among outdoor-reared farm animals showed that animals which spend a long time outdoors, such as free-range hens may bioaccumulate lipophilic organohalogen environmental contaminants in their tissues and organs and, consequently, also in their derivate products (milk and eggs) more than those raised indoors (Fernandes et al., 2011). Therefore, animals with outdoor access may represent a good model for studying the quality of the ecosystem (Stephense et al., 1995, Fournier et al., 2012). In order to study the links between environmental pollution and the contamination levels of persistent organic pollutants (POPs) in food of animal origin, eggs from free-range hens, of *Gallus gallus domestics* species, were used as bioindicators of the PCDD/F and PCB contamination levels of the environment where the hens live.

Eggs of hens raised outdoors were collected from different areas of the Campania region and were analysed for the background investigation of PCDD/F and PCB contamination levels of the areas.

In addition to food of animal origin, food of plant origin, collected in the same areas of eggs, was also analysed to assess the contamination levels present in vegetable matrices and to investigate the differences that exist in pollutant bioaccumulation in these products compared to food of animal origin. In particular, lettuce, courgettes, aubergines and potatoes were investigated for the purpose of the project. In general leaf vegetables, such as *Lactuca sativa L.*, present, in edible parts, concentrations of pollutants higher than other species, because the part exposed to atmospheric deposition is large. Other studies showed that absorption of organic contaminants from soil through the root system is not significant except for the *Cucurbitaceae* family; while for vegetables of the *Solanum tuberosum L.*, species absorption occurs by contact with the soil and, in this case, the contaminant is mainly confined to the peel (Grassi et al., 2010; Esposito et al., 2015).

Moreover, in order to assess the contamination sources and the fraction of PCDD/Fs and PCBs transferred from feed and soil to hens and from soil to vegetable, feed and soil samples were also collected and analysed.

An important phase of the project was to conduct an *in vivo* study using the hens, designed to investigate the transfer of PCDD/Fs and PCBs from the exposure sources, particulary soil and to a lesser extent feeds, to hen eggs and thus their bioavailability. Studying the assimilation and biotransfer factors of PCDD/Fs and PCBs in animals used for food is a basic topic to predicting and controlling human exposure to these contaminants. The knowledge of the transfer factors is important because the different congeners of PCDD/Fs and PCBs are assimilated by animals in different ways and therefore there are differences in their bioaccumulation in animal tissues. Consequently, the study of the transfer mechanisms of PCDD/Fs and PCBs from the environment to the animals, and transfer from feed to the animals enables us to define which congeners are most absorbed and their bioaccumulation rates in animals. This aspect is very important considering that the various congeners present a different human toxicity.

The transfer of PCDD/Fs and PCBs from environment to plants and animals is demonstrated by many authors. However, it is well known that not all the amount of contaminants present in the soil can actually move into plants and animals. Consequently, evaluation of their concentration in soil is not enough but it is essential to assess their mobility and bioavailability. Bioavailability is the fraction of a chemical, with respect to its total amount, which is able to penetrate actively or passively in living organisms and which is available for biological processes (Peijnenburg et al., 1997, 2007). Therefore, knowing the bioavailability of a pollutant is an important information to correctly assess the risk and the negative effects that these substances can cause in humans and in the environment.

Bioavailability of environmental contaminants can be determined using chemical methods that involve the selective extraction of the investigated contaminant or using biological methods through the direct measurement of bioaccumulation of the contaminants in the tissues of a living organism used as a sentinel (bioindicator). Generally, when biological methods are used, artificial matrices, spiked with the contaminants whose bioavailability has to be studied, are prepared and are put in contact with the living organism chosen as bioindicator. In this project, a soil naturally contaminated with PCDD/Fs and PCBs was used and the uptake of contaminants was studied by changing the environment where the hens lived. The bioavailability for all 35 PCDD/F and PCB toxic congeners was determined by direct measurement of residues in eggs. Through the analysis of the results obtained, it was possible to calculate the biotransfer factors for each PCDD/F and PCB congener and to develop a predictive model concerning the transfer of pollutants from the environment to food.

During the project, an analytical method for the determination of PCDD/Fs and DL-PCBs in egg samples was validated according to the Commission Regulation (EU) 589/2014 (current Comm. Reg. EU 644/2017). Moreover, the method was compliant with the EN ISO/IEC 17025 standards and was accredited by the Italian Accreditation Body Accredia.

2. MATERIALS AND METHODS

2.1. SAMPLES

For the aim of the project, 10 sampling areas, located in the five provinces of the Campania region and precisely 2 in each province, were investigated. The sampling method applied, for egg and vegetable samples, was that established by the Commission Regulation (EU) 644/2017. Specifically, with regard to vegetables, they were considered products traded in bulk consignments. The crops from which the samples were collected were very small, being destined for self-consumption, therefore it was not necessary to subdivide the lots into sublots. The aggregate samples collected had a weight of 1 kg and consisted of at least 3 incremental samples of similar weight and at least 100 grams each. The incremental vegetable samples were taken directly from plants and at various points in the field. The sampling points were chosen randomly in order to make the sample as representative as possible of the lot from which it came, because only in this way the result of the analysis were traceable to the food subject to control. The aggregate sample was obtained by uniting the incremental samples.

Concerning hens' eggs, the aggregate sample size was at least of 12 eggs as established by the Regulation EU 644/2017.

Each sample was placed in a clean and dry container, made of inert material that protected it from any contamination, from the loss of the investigated analytes due to absorption by the walls of the container and from any damage that could occur during transportation. In this specific case, bags for food use were used, which were carefully closed and identified in a unique way.

Regarding feed, sampling was carried out following the requirements set by the Commission Regulation (EU) 709/2014. Incremental samples were randomly collected from the package using a steel shovel. The incremental samples had a similar weight and were united to form a single aggregate sample of 4 kg. The single sample was carefully mixed to obtain a homogenised sample that then was

reduced to 2 kg (reduced sample) by the quartering method. The final samples were obtained from the reduced sample and their weight were 500 g.

Concerning soil, it is not homogeneous matrix, indeed, it presents a great variability both on surface and in depth and this variability is also found in small spaces such as in the same field. This variability can be found in the weaving, in the type of structure and in the content of the different nutrients and the organic substance. Even environmental pollutants are obviously distributed unevenly. Consequently, establishing a good sampling plan was a fundamental operation to obtain samples that were representative of the area from which they were taken. Therefore, it was important to choose the sampling method, the points to be collected and the number of sampling operations to be made. For the aim of this project, soil samples were collected at a depth between 0 and 20 cm following a X non-systematic sampling pattern (Fig. 3).

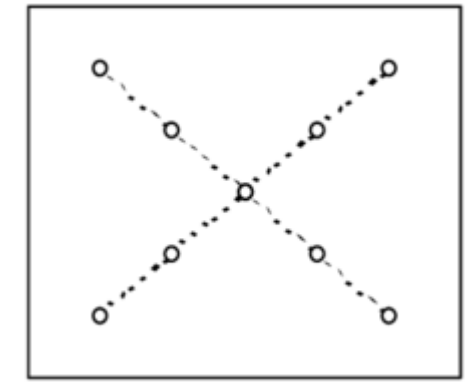


Fig. 3. X non-systematic sampling pattern.

The incremental samples were collected in 5 points along the X pattern using a steel shovel. The incremental samples had a similar weight and were united to form a single aggregate sample of 5 kg. The single aggregate sample was carefully

mixed to obtain a homogenised sample that then was reduced to 1 kg (reduced sample) by the quartering method.
After collecting, the samples were transported to the laboratory and kept in a freezer at - 20 °C until the analysis.

2.2. *IN VIVO* STUDY

An *in vivo* study designed to investigate how and to what extent PCDD/Fs and PCBs are transferred from exposure sources, precisely soil, to hen eggs, was started in September 2016. A group of 9 laying hens (*Gallus gallus domesticus*), aged around 2 years, was selected for the study. The hens were raised outdoors, as free-range according to conventional animal husbandry practice, in a private garden situated in the Area 1. An area of 20 x 10 meters, bounded by a fence made of wire mesh, was intended to the experiment. Inside the area the hens had available a hen house for shelter from the weather, equipped with feeders, drinking troughs and nests where to lay eggs. In addition to the shelter, the hens had a large open area where to scratch and a small open area where they could shelter from the sun during the hottest hours of the day.
The hens were fed with a feed made from corn grains that, in order to not discourage them from scratching, was provided in the mangers at half-day and before dusk. During the day, the moments of rest were alternated with the constant search for food and most of the daily food intake was the result of soil scratching, where hens could find various spontaneous grasses and plants but also small insects, earthworms and worms. For the study, the hens'eggs were collected every 4 weeks for two months and were analysed for PCDDs, PCDFs and PCBs. Samples of feed and soil were also collected and analysed in order to calculate the biotransfer factors.
Then, the hens were transferred to another free-range system, similar to that located in the Area 1, situated in a garden in the Area 2. After the transfer, the hens were continually fed with a conventional maize silage feed that was also this time

analysed for the research of PCDD/Fs and PCBs. The eggs were collected every 2 weeks during the first four months and every 4 weeks thereafter and were analysed. At the same time, another group of hens, consisting of 10 animals, was used as a control group. The hens of the control group were raised indoors, in an closed area with a cement floor covered with straw and wood shavings. They were fed with the same corn feed used for the other hens. The control group hens could not go outside and therefore never had contact with the environment and the soil.

2.3. ANALYTICAL METHODS

The analytical methods used for the identification and quantification of PCDDs, PCDFs and PCBs in eggs, vegetables, feed and soil are based on the United States Environmental Protection Agency (US EPA) methods and precisely the US EPA method 1613 revision B for determination of tetra- through octa-chlorinated dibenzo-*p*-dioxin and dibenzofuran congeners and the US EPA method 1668 revision C for determination of chlorinated biphenyl congeners. In both methods, instrumental analyses are performed by high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS).

In brief, samples were homogenized, freeze-dried, spiked with a known amount of $^{13}C_{12}$-isotope labelled internal standards and extracted using an Accelerated Solvent Extraction (ASE) system. Then, for PCDD/F and DL-PCB analysis, extracts were cleaned up with a multilayer acid column followed by an automated cleanup using a PowerPrep system; for NDL-PCB analysis, extracts were cleaned up by an acidified diatomaceous columns and a florisil cartridges. Analysis were performed by a high resolution gas chromatograph coupled with a high resolution mass spectrometer with a double focusing system (DFS Magnetic Sector GC-HRMS system, Thermo Fisher Scientific) operating at a resolution of 10000.

2.3.1. SAMPLE PRETREATMENT

2.3.1.1. EGG SAMPLES

For analysis, samples, each consist of 12 eggs, were first defrosted at room temperature, then were deprived of the egg shell and homogenized, combining the albumen and the yolk, using a high-speed knife mill/blender (Retsch Grindomix GM 200). For each sample, 50 g of product for PCDD/Fs and DL-PCBs and 20 g for NDL-PCBs were weighed in disposable aluminium trays, which were frozen at -18 °C for 12 hours for the subsequent lyophilization phase.

2.3.1.2. VEGETABLE SAMPLES

Vegetable samples were defrosted, carefully washed, deprived of the not edible parts and homogenized using a high-speed knife mill/blender (Retsch Grindomix GM 200). For each sample, 10 g of product for PCDD/Fs and DL-PCBs and 2 g for NDL-PCBs were weighed in disposable aluminium trays which were frozen at -18 °C for 12 hours and then lyophilized.

2.3.1.3. FEED SAMPLES

Feed samples consisted of grains, therefore it was not necessary to grind and homogenise them further. For feed analysis, 15 g of sample for PCDD/Fs and DL-PCBs and 5 g for NDL-PCBs were weighed and directly extracted with the ASE system. The moisture content was calculated for each sample because, as required by the Regulation EU 277/2012, the total PCDD/F, DL-PCB and NDL-PCB concentrations are expressed at 12% of moisture.

For the determination of moisture content, 5 g of feed sample, ground into particles which pass through a 0.5 mm sieve, were weighed in a container with a lid. The container, without lid, was placed in an oven at 130 °C for 2 hours. Then, it was

closed with the lid, extracted from the oven, left to cool for 45 min in a glass desiccator and weighed. Moisture content is given with the relation:

$$Moisture\,\% = \frac{(m - m_0)}{m} \cdot 100$$

where,

- m is wet weight of sample in grams
- m_0 is dry weight of sample in grams

2.3.1.4. SOIL SAMPLES

Soil samples were first passed through a 2 mm sieve, then 10 g of product for PCDD/F and DL-PCB analysis and 2 g for NDL-PCB analysis were weighed and extracted with the ASE system. Also for soil, the moisture content was calculated because the total PCDD/F, DL-PCB and NDL-PCB concentrations were expressed as dry weight.

2.3.2. LYOPHILIZATION

To perform lyophilization, the samples, eggs and vegetables, were frozen in aluminium tanks at -18 °C for at least 12 hours and transferred to the freeze-dryer (Christ Alpha 1-4 LSC) equipped with an oil-sailed rotary vacuum pump. The whole process took place in three phases. In the first phase, the sample was frozen at a temperature of -56 °C; in the second phase, the sublimation, which occurred by heating the sample up to a temperature of about 20 °C and with a vacuum of 1.030 mbar, began; in the last phase, there was the desorption or secondary sublimation which occurred by further lowering the pressure to remove the water remaining adsorbed on the porous surface of the samples. The samples, after being lyophilized, were weighed and extracted.

2.3.3. EXTRACTION

The analytes of interest were extracted with a solid-liquid extraction technique and precisely using an Accelerated Solvent Extraction (Dionex ASE 350) system. This technique greatly improves efficiency and reproducibility of the extraction, reduces the extraction times and the amount of solvent used and makes possible to automate the entire process. The extraction takes place using closed receptacles (cells) and occurs at high temperature and pressure. The elevated pressure is used to keep the solvents in the liquid state as the temperature is increased above their boiling point. Using the increase in temperature and pressure, the solubility of the analytes is increased and the viscosity of the solvents decreases; hence, the diffusion of the analytes in the solvent is improved, this leads to an increase in extraction efficiency and a reduction in extraction times. For the extraction, the cells were filled with the samples, which were freeze-dried in the case of eggs and vegetables, to which diatomaceous earth was added as dispersing material. Before extraction, the $^{13}C_{12}$-isotope labelled extraction standards were added to each sample. A fiberglass filter and a sodium sulphate spatula were put on the bottom of each cell. A second fiberglass filter was used to close the cell.. The system fills the cell with the extraction solvent consisting of a mixture of hexane-acetone 4:1, heats and pressurizes up to a temperature of 110 °C. The elevated temperature increases the extraction efficiency of analytes of interest from their matrix, and a pressure of about 1600 psi, this is referred to as a static extraction. Once the static extraction is complete, the system pumps press solvent trough the cell and purges with nitrogen. The complete extraction involves three static cycles and lasts 45 minutes. Then, the extracts were dried using a rotary evaporator at 40 °C.

2.3.4. CLEAN UP BY ACID MULTILAYER COLUMN

Clean up by an acid multilayer column is carried out when samples contain large quantities of other organic compounds. For this purification, borosilicate chromatography columns with sintered frit were used. The multilayer acid columns were prepared as follows: filled with n-hexane picograde for residue analysis, then one anhydrous sodium sulphate and two silica spatulas, the stationary phase constituted by 35 g of Extrelut® and 40 g of sulfuric acid 96%, two silica and one anhydrous sodium sulphate spatulas were added in this order. After filling, the columns were carefully packed until a steady and uniform stationary phase was obtained. Then, the extracts, dissolved in a 1:1 n-hexane/dichloromethane mixture, were loaded at the head of the column, eluted with a 8:2 n-hexane/toluene mixture and collected in glass tubes. The eluates were concentrated to a volume of about 0.5 mL using an evaporator TurboVap with a vortex nitrogen flow and a thermostated bath.

2.3.5. CLEAN UP BY POWER-PREP SYSTEM

After the first manual purification by the acid multilayer column, samples were subjected to an additional purification using the PowerPrep®, a totally automated chromatographic system which performs a second purification and also allows to separate samples into two fractions, one containing PCDD/Fs and non-ortho-substituted PCBs, and the other containing mono-ortho-substituted PCBs. The PowerPrep® is equipped with three distinct disposable columns, an acid, basic and neutral silica (ABN = acid-base-neutral), an alumina and a carbon columns that each have a different function and mechanism for separating the analytes. Acid, neutral, and basic silica and alumina columns are used to remove nonpolar and polar interferences and eliminate the organic material still present in samples; carbon column is used to remove nonpolar interferences and to separate PCDD/Fs

and non ortho-substituted PCBs from the mono-ortho-substituted PCBs. Acid, neutral, and basic silica and alumina columns separate molecules on the basis of their polarity, whereas carbon column separates analytes by affinity on the basis of the planarity of molecules. PCDD/Fs and DL-PCBs were eluted from ABN silica and alumina columns with n-hexane and were retained in carbon column. Then, they were differentially eluted by varying the solvents: the mono-ortho-substituted PCBs (PCB 105, 114, 118, 123, 156, 157, 167, and 189) were eluted with n-hexan, and the PCDD/Fs and the non-ortho-substituted PCBs (PCB 77, 81, 126 and 169) with toluene. Both fractions were collected in two glass tubes and concentrated to a final volume of about 150 μL using the TurboVap® device.

2.3.6. HRGC/HRMS ANALYSIS

For instrumental analysis, samples were transferred to glass vials, one for PCDD/Fs and one for DL-PCBs analysis and concentrated using a centrifugal evaporator until all the solvent was removed. Afterwards, the PCDD/Fs and DL-PCBs internal standard solutions were added to the vials. All samples were analysed using the DFS Magnetic Sector HRGC-HRMS system, Thermo Fisher Scientific, a high resolution mass spectrometer coupled with two high resolution gas chromatographs, one used for PCDD/Fs and one for PCBs analysis.

The HRGC-HRMS operates in electron-ionization mode with an ionization energy of -45eV.

The DFS is a double focusing sector instrument that has in series both magnetic and electrostatic sector. Ions, that exit from the EI ionization box, undergo a double focusing; the magnetic sector selects the ions according to their m/z ratio, the electrostatic sector according to their energy. The double focusing greatly increases the resolution of spectrometer, which can even reach up to 100,000.

Double focusing analysers can have different geometries. The DFS Magnetic Sector GC-HRMS system Thermo Fisher Scientific is a double focusing sector

instrument with reverse Nier-Johnson geometry that corresponds to direct Nier-Johnson geometry, consisting of a 90º angle of the electrostatic sector, a long distance of intermediate drift and a 60º angle of the magnetic field with the same direction of curvature, but the order of electrostatic and magnetic sectors are inverted. This means that the ions traverse the separating magnetic field before entering the electric sector (Fig. 4).

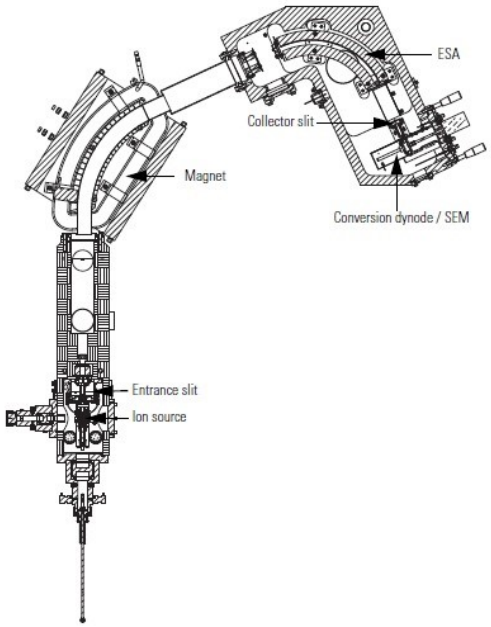


Fig. 4. Schematical view of analyser geometry.

2.3.7. INSTRUMENTAL CONDITIONS

For the analyses, splitless injections were used in the GCs and the temperature of the inlets were maintained to 280 °C. For PCDD/F analysis, the chromatographic separation was performed on a TR-1 (60 m, id 0.25 mm, 0.1 μm, Thermo Fisher Scientific) fused silica capillary column coated with 5% phenyl, 94% methyl, 1% vinyl silicone. The oven temperature program was showed in Table 7.

Table 7
Gas-chromatographic conditions for PCDD/F analysis.

Temperature (°C)	Time (minutes)	Hold (minutes)
100	-	2.0
220	12.0	10.0
235	3.0	7.0
315	4.4	2.0

For PCB analysis, the chromatographic separation was performed on a HT8 (60 m, id 0.25 mm, 0.25 μm, SGE Analytical Science) fused silica capillary column coated with 8% phenylpolycarborane-siloxane. The oven temperature program was showed in Table 8.

Table 8.
Gas-chromatographic conditions for PCB analysis.

Temperature (°C)	Time (minutes)	Hold (minutes)
90	-	1.0
180	22.5	0.0
285	2.8	0.0
320	11.7	0.0

The transfer line temperature was set at 280 °C and the ion source was held at 295 °C.

Mass calibration was achieved used perfluorotributylamine (FC43). The mass spectrometry analysis were carried out using the multiple ion detection (MID) mode monitoring the M^+ and $(M+2)^+$ or $(M+2)^+$ and $(M+4)^+$ ions. During the acquisition, the resolution of the spectrometer was approximately of 10000 (10% peak valley).

2.3.8. STANDARD SOLUTIONS

For the project, certified standard solutions of the Cambridge Isotope Laboratories (CIL) in nonane were used.

2.3.8.1. LABELED-COMPOUND SPIKING SOLUTION

The working solutions of labelled compound spiking solution standards contain $^{13}C_{12}$-isotope labelled PCDD/Fs and PCBs corresponding to the congeners to be measured and are used for internal standardization, calculation of recovery and quantification of samples to be analysed. The standard used for PCDD/Fs was the solution CIL EDF 8999, that contains all the labelled compounds corresponding to those to be analysed except for 1,2,3,7,8,9-HxCDD and OCDF (Appendix I, Table 9). The standards used for PCBs were the solution CIL EC 4977 (Appendix I, Table 10) and the solution CIL EC 4058 (Appendix I, Table 11), that contain 12 labeled DL-PCBs and 6 labeled NDL-PCBs, respectively.

2.3.8.2. INTERNAL STANDARD SOLUTIONS

The internal standard solutions contain $^{13}C_{12}$-isotope labeled PCDD/Fs and PCBs and are used for internal instrumental standardisation and calculation of recovery. The standard used were the solution CIL EDF 5999 for analysis of PCDD/Fs and non-ortho-substituted PCBs (Appendix I, Table 12), the solution CIL EC 4979 for

DL-PCBs analysis (Appendix I, Table 13) and PCB-123 for NDL-PCBs analysis (Appendix I, Table 14).

2.3.8.3. STANDARD SOLUTIONS OF NATIVE COMPOUNDS

The standard solutions of native compounds contain unlabelled PCDD/F and PCB congeners and were used to spike samples at different concentrations for the validation of the method. The standards used were the solution CIL EDF 7999for PCDD/Fs (Appendix I, Table 15), the solution CIL EC 4989 for DL-PCBs (Appendix I, Table 16) and the solution Dr. Eherenstorfer MIX PCB 20030100 for NDL-PCBs (Appendix I, Table 17).

2.3.8.4. CALIBRATION STANDARDS

Calibration standard solutions (CS), which contain the native PCDD/F and DL-PCB congeners and the corresponding $^{13}C_{12}$-isotope labeled congeners, were used for the instrumental calibration. The solutions CIL EDF 9999 (Appendix I, Table 18) for PCDD/F analysis and CIL EC 4976 (Appendix I, Table 19) for DL-PCB analysis were used. For NDL-PCB analysis, the calibration standard solutions, called MR, were prepared using the Dr. Eherenstorfer solution MIX PCB 20030100, the solution CIL EC 4058 and the solution containing PCB-123 (Appendix I, Table 20).

2.3.9. CALIBRATION

Before calibration, the parameters of the gas-chromatograph were set to improve the compound separation and selectivity and of the mass spectrometer to work at a resolution of at least 10,000. These settings were the same as used for sample analysis.

The calibration for PCDD/Fs and DL-PCBs was performed at 5 different concentration levels using the CS solutions containing the native congeners at 5 different concentration levels and the corresponding labeled congeners at a

concentration of 100 ng/mL. These solutions permitted the relative response (RR, labeled to native) and response factor (RF) to be measured as a function of concentration. The calibration for NDL-PCBs was performed at 3 different levels applying the same criteria.

2.3.10. QUALITATIVE DETERMINATION

For the qualitative analysis of PCDD/Fs, PCBs or labeled compounds, all the criteria described in the EPA methods (1613B and 1668C) were verified.

2.3.11. QUANTITATIVE DETERMINATION

The quantitative analysis of PCDD/Fs and PCBs was performed using both the isotope dilution and the internal standard techniques.

2.3.11.1. ISOTOPE DILUTION QUANTITATION

This quantitation was used for the 15 PCDD/Fs substituted by 2,3,7,8 and for PCBs and used the relative response (RR) values calculated during the calibration phase. The concentrations were calculated as follows:

$$C_{ex}(ng/mL) = \frac{(A1_n + A2_n) \cdot C_l}{(A1_l + A2_l) \cdot RR}$$

where,

- C_{ex} is the concentration of the PCDD/Fs and PCBs in the extract.
- A_{1n} and A_{2n} are the areas of the primary and secondary m/z's for the PCDD/Fs and PCBs.
- A_{1l} and A_{2l} are the areas of the primary and secondary m/z's for the labeled compound.
- C_l is the concentration of the labeled compound in the calibration standard.

Due to a potential interference, the labeled analog of OCDF were not added to the sample. Therefore, OCDF was quantitated against labeled OCDD. As a result, the

OCDF concentration was corrected for the recovery of labeled OCDD. Also $^{13}C_{12}$-1,2,3,7,8,9-HxCDD was not added before extraction of the sample because it was used as an instrument internal standard. Therefore, 1,2,3,7,8,9-HxCDD was quantitated using the average response of the labeled analogs of the other two 2,3,7,8-substituted HxCDD's: 1,2,3,4,7,8-HxCDD and 1,2,3,6,7,8-HxCDD. The concentration of 1,2,3,7,8,9-HxCDD was corrected for the average recovery of the other two HxCDD's.

2.3.11.2. INTERNAL STANDARD QUANTITATION

The concentrations of 1,2,3,7,8,9-HxCDD, OCDF were calculated using the response factors (RF) determined during the initial calibration and the following equation:

$$C_{ex}(ng/mL) = \frac{(A1_s + A2_s) \cdot C_{is}}{(A1_{is} + A2_{is}) \cdot RF}$$

where,

- C_{ex} is the concentration of the PCDD/Fs in the extract.
- $A1_s$ and $A2_s$ are the areas of the primary and secondary m/z's for the PCDD/Fs.
- $A1_{is}$ and $A2_{is}$ are the areas of the primary and secondary m/z's for the internal standard.
- C_{is} is the concentration of the internal standard.

2.3.12. METHOD VALIDATION

The method validation for the identification and quantification of 7 PCDDs, 10 PCDFs and 12 DL-PCBs in hen eggs was conducted in accordance with the Commission Regulation (EU) 589/2014 (current Comm. Reg. EU 644/2017). For the tests, blank samples of eggs (Table 22) were spiked with standard solutions containing the PCDD/Fs and PCBs to be measured at concentrations in the range of

0.5 ×, 1 × and 2 × the maximum limits fixed by the Commission Regulation (EU) 1259/2011 (Table 21). The determination of the limit of quantification (LOQ) was performed at a concentration of one fifth of the maximum level (Table 21).

All results obtained were corrected for the blank matrix to obtain accurate results. It was also verified that the results were compliant with the requirements of the Regulation (EU, 589/2014; EU, 644/2017). In particular, the accuracy was evaluated and expressed as trueness and precision as relative standard deviation (RSD_R) calculated under laboratory reproducibility conditions (Table 21).

The methods were compliant with the EN ISO/IEC 17025 standards and was accredited by the Italian Accreditation Body Accredia.

Table 21
Blank samples (eggs) spiked at concentrations in the range of 0.2 ×, 0.5 ×, 1 × and 2 × the maximum level (ML) set by the Regulation EU 1259/2011.

Replicates	PCDD/Fs	REC %	DL-PCBs	REC %	∑ PCDD/Fs + DL-PCBs	REC %
0.2xLM-1(LOQ)	0.45	90	0.58	117	1.04	104
0.2xLM-2(LOQ)	0.43	86	0.48	97	0.92	92
0.2xLM-3(LOQ)	0.54	109	0.48	95	1.02	102
Mean	0.49	98	0.53	106	0.98	98
SD_R	0.06	-	0.06	-	0.07	-
RSD_R%	12.33	-	11.44	-	6.72	-
0.5×LM-1	1.06	85	0.76	61	1.83	73
0.5×LM-2	1.16	93	0.56	45	1.72	69
0.5×LM-3	1.37	109	0.67	54	2.04	81
0.5×LM-4	1.36	109	1.33	107	2.69	108
0.5×LM-5	1.05	84	1.33	107	2.38	95
0.5×LM-6	1.45	116	1.31	105	2.76	110
Mean	1.24	99	1.00	80	2.24	89
SD_R	0.17	-	0.37	-	0.44	-
RSD_R%	13.85	-	36.86	-	19.74	-
1×LM-1	2.24	89	2.32	93	4.55	91
1×LM-2	2.61	104	2.44	98	5.05	101
1×LM-3	1.91	76	2.27	91	4.18	84
1×LM-4	2.39	96	2.60	104	5.00	100
1×LM-5	2.43	97	2.43	97	4.86	97
1×LM-6	2.06	82	2.54	101	4.59	92
1×LM-7	2.16	86	2.58	103	4.74	95
Mean	2.26	90	2.44	97	4.61	92
SD_R	0.19	-	0.08	-	0.13	-
RSD_R%	8.49	-	3.18	-	2.89	-
2×LM-1	4.94	99	4.68	94	9.62	96
2×LM-2	4.81	96	4.61	92	9.42	94
2×LM-3	4.54	91	5.20	104	9.74	97
2×LM-4	4.18	84	5.24	105	9.42	94
2×LM-5	5.78	116	5.17	103	10.95	110
2×LM-6	5.28	106	5.16	103	10.45	104
Mean	4.92	98	5.01	100	9.93	99
SD_R	0.56	-	0.29	-	0.63	-
RSD_R%	11.43	-	5.71	-	6.31	-
ML	2.5	-	-	-	5.0	-

Table 22
PCDD/Fs and DL-PCBs concentrations and standard deviation in conventional hen eggs used as blank samples expressed as pg WHO-TEQ g^{-1} fat using the upper-bound approach.

	Replicates	PCDD/Fs	DL-PCBs	∑ PCDD/Fs + DL-PCBs	Fat (g)
Sample 1	1	0.09	0.11	0.20	5.0
	2	0.09	0.10	0.18	5.2
	Mean	0.09	0.10	0.19	-
	SD	0.01	0.01	0.01	-
Sample 2	1	0.11	0.17	0.28	3.8
	2	0.05	0.15	0.19	5.0
	Mean	0.08	0.16	0.24	-
	SD	0.05	0.01	0.06	-
Sample 3	1	0.05	0.26	0.30	4.7
	2	0.07	0.24	0.31	4.8
	Mean	0.06	0.25	0.31	-
	SD	0.02	0.01	0.005	-
Sample 4	1	0.11	0.13	0.24	5.6
	2	0.04	0.13	0.18	5.5
	Mean	0.08	0.13	0.21	-
	SD	0.05	0.003	0.05	-

3. RESULTS AND DISCUSSION

3.1. DIOXINS AND POLYCHLORINATED BIPHENYLS LEVELS

All samples were analysed for the determination of 7 PCDD, 10 PCDF and 18 PCB (12 DL-PCBs and 6 NDL-PCBs) congeners. The results are shown in Tables 23 and 24. PCDD/F concentrations, represented by the sum of 7 PCDDs and 10 PCDFs, DL-PCB concentrations represented by the sum of 12 DL-PCBs and their total sum were calculated using WHO-TEFs 2005 (Van den Berg et al., 2006; Table 6) and expressed with the upper-bound approach.

A total of 10 egg samples were analysed for determining of PCDD/Fs and PCBs. 9 samples were compliant with the maximum limits fixed by Commission Regulation (EU) 1259/2011, that are 2.5 pg WHO-TEQ g^{-1} fat and 5.0 pg WHO-TEQ g^{-1} fat for PCDD/Fs and the sum of PCDD/Fs and DL-PCBs, respectively and with the action levels fixed by Commission Recommendation (EU) 663/2014, that are 1.75 pg WHO-TEQ g^{-1} fat for both PCDD/Fs and DL-PCBs (Tables 23 and 24). A PCDD/F concentration (2.59 pg WHO-TEQ g^{-1} fat), that exceeded the maximum permitted limit, was found only in the eggs collected in the Area 2. Finally, the eggs collected from the Area 1, although complying with the maximum limits (EU, 2011), exceeded the action level set for DL-PCBs (EU, 2014) with a concentration about 2 times higher than the action level (3.43 pg WHO-TEQ g^{-1} fat) (Table 23 and 24). The concentration of PCDD/Fs found in the eggs ranged from a minimum of 0.20 pg WHO-TEQ g^{-1} fat to a maximum of 2.59 pg WHO-TEQ g^{-1} fat with a mean value of 0.76 pg WHO-TEQ g^{-1} fat, while the concentrations of DL-PCBs varied from a minimum of 0.14 pg WHO-TEQ g^{-1} fat to a maximum of 3.43 pg WHO-TEQ g^{-1} fat with a mean value of 1.11 pg WHO- TEQ g^{-1} fat and their sum from 0.34 pg WHO-TEQ g^{-1} fat to 4.49 pg WHO-TEQ g^{-1} fat with a mean of 1.87 pg WHO-TEQ g^{-1} fat (Table 25).

Table 23
PCDD/F and total PCDD/F and DL-PCB concentrations in samples from the investigated Areas.

Sample area	Sample type	PCDD/Fs[a]	Congeners above the LOQ[b] (%)	∑ PCDD/Fs + DL-PCBs[a]
Area 1	Eggs	1.06	82	4.49
	Feed	0.008	0	0.009
	Courgette	0.004	12	0.007
	Aubergine	0.005	24	0.005
	Potato	0.006	29	0.008
	Lettuce	0.022	18	0.033
	Soil	0.42	88	0.59
Area 2	Eggs[c]	2.59	100	4.24
	Feed	0.003	24	0.007
	Courgette	0.017	18	0.032
	Aubergine	0.015	24	0.019
	Potato	0.034	41	0.048
	Lettuce	0.016	12	0.036
	Soil	5.02	100	6.40
Area 3	Eggs	0.35	59	0.50
Area 4	Eggs	0.49	59	1.77
Area 5	Eggs	0.43	88	1.69
Area 6	Eggs	0.43	82	0.94
Area 7	Eggs	0.20	47	0.34
Area 8	Eggs	0.56	82	1.90
Area 9	Eggs	0.34	76	0.82
	Courgette	0.036	35	0.038
	Potato	0.017	41	0.020
Area 10	Eggs	1.17	88	2.05

Notes:
PCDD/F and total PCDD/F and DL-PCB concentrations expressed as: pg WHO-TEQ g^{-1} fat in egg samples, pg WHO-TEQ g^{-1} wet weight in vegetable samples, ng WHO-TEQ kg^{-1} with a moisture content of 12% in feed samples and pg WHO-TEQ g^{-1} dry weight for soil samples. The concentrations are expressed using the upper-bound approach.
[a] TEQs calculated according to WHO_{2005}-TEFs
[b] Number of congeners above the LOQ out of the 17 PCDD/F congeners analysed.
[c] Eggs collected from experimental breeding of hens at the 53rd week.

The analysis of NDL-PCBs showed that the levels found in the egg samples were below the maximum limit permit by Commission Regulation (EU) 1259/2011 and ranged between 1.33 and 22.57 ng g^{-1} fat with a mean value of 7.53 ng g^{-1} fat (Tables 24 and 25). The mean concentrations of PCDD/Fs, DL-PCBs and NDL-PCBs found in this project were lower than those found in a different study on eggs

from free range hens carried out in the Campania region (0.87 pg WHO-TEQ g^{-1} fat, 1.57 pg WHO-TEQ g^{-1} fat and 11.80 ng g^{-1} fat, respectively) (Lambiase et al., 2017).

Table 24
DL-PCB and NDL-PCB concentrations in samples from the investigated Areas.

Area	Sample type	DL-PCBs	Congeners above the LOQ[a] (%)	NDL-PCBs	Congeners above the LOQ[b] (%)
Area 1	Eggs	3.43	100	22.57	100
	Feed	0.0005	42	0.24	100
	Courgette	0.003	83	0.13	17
	Aubergine	0.0004	58	0.19	17
	Potato	0.002	58	0.14	0
	Lettuce	0.011	92	1.08	100
	Soil	0.17	100	1.11	83
Area 2	Eggs[c]	1.64	100	13.56	100
	Feed	0.004	83	0.17	83
	Courgette	0.015	92	0.25	50
	Aubergine	0.003	83	0.17	33
	Potato	0.013	92	0.18	33
	Lettuce	0.020	83	0.15	67
	Soil	1.38	100	24.43	100
Area 3	Eggs	0.14	100	1.32	100
Area 4	Eggs	1.28	100	3.94	100
Area 5	Eggs	1.26	100	8.18	100
Area 6	Eggs	0.51	100	2.14	100
Area 7	Eggs	0.14	100	1.51	100
Area 8	Eggs	1.34	100	6.86	100
Area 9	Eggs	0.48	100	5.90	100
	Courgette	0.003	83	0.13	33
	Potato	0.004	100	0.16	67
Area 10	Eggs	0.88	100	9.31	100

Notes:
DL-PCBs expressed as: pg WHO-TEQ g^{-1} fat in egg samples, pg WHO-TEQ g^{-1} wet weight in vegetable samples, ng WHO-TEQ kg^{-1} with a moisture content of 12% in feed samples and pg WHO-TEQ g^{-1} dry weight for soil samples. NDL-PCBs expressed as: ng g^{-1} fat in egg samples, ng g^{-1} wet weight in vegetable samples, µg kg^{-1} with a moisture content of 12% in feed samples and ng g^{-1} dry weight for soil samples.
The concentrations are expressed using the upper-bound approach.
[a]Number of congeners above the LOQ out of the 12 DL-PCBs congeners analysed.
[b]Number of congeners above the LOQ out of the 6 NDL-PCBs congeners analysed.
[c]Eggs collected from experimental breeding of hens at the 53rd week.

Table 25
PCDD/F, DL-PCB and NDL-PCB concentrations in eggs and vegetables samples.

Sample type		PCDD/Fs	DL-PCBs	∑PCDD/Fs + DL-PCBs	NDL-PCBs
Eggs n = 10					
	Min	0.20	0.14	0.34	1.33
	Max	2.59	3.43	4.49	22.57
	Mean	0.76	1.11	1.87	7.53
	SD	0.72	0.97	1.44	6.55
Vegetables n = 10					
	Min	0.004	0.0004	0.005	0.13
	Max	0.036	0.020	0.048	1.08
	Mean	0.017	0.007	0.025	0.26
	SD	0.011	0.007	0.015	0.29

Notes:
PCDD/Fs and DL-PCBs expressed as: pg WHO-TEQ g^{-1} fat in egg samples, pg WHO-TEQ g^{-1} wet weight in vegetable samples. NDL-PCBs expressed as: ng g^{-1} fat in egg samples, ng g^{-1} wet weight in vegetable samples.
The concentrations are expressed using the upper-bound approach.

Moreover, the results of this research were also compared with those found in studies carried out in other countries showing generally lower pollutant concentrations. Hoogenboom et al. (2016), in a study performed on home-produced eggs in the Netherlands, found median values of 2.8 pg WHO-TEQ g^{-1} fat for PCDD/Fs, 2.0 pg WHO-TEQ g^{-1} fat for DL-PCBs and 4.8 pg WHO-TEQ g^{-1} fat for the sum of both and 13 ng g^{-1} fat for NDL-PCBs. Rawn et al. (2012) examining Canadian free range eggs, measured levels of contamination ranging between 0.060 and 10.6 pg WHO-TEQ g^{-1} fat for PCDD/Fs, 0.042 and 2.15 pg WHO-TEQ g^{-1} fat for DL-PCBs and 0.193 and 9.33 ng g^{-1} fat for NDL-PCBs.

Feed samples were within the limits set by Commission Regulation (EU) 277/2012 and the maximum concentrations found were 0.008 ng WHO-TEQ kg^{-1} for PCDD/Fs, 0.004 ng WHO-TEQ kg^{-1} for DL-PCBs, 0.009 ng WHO-TEQ kg^{-1} for their sum and 0.24 µg kg^{-1} for NDL-PCBs (Table 22, 23 and 24). These results were lower than data found in other studies (Kijlstra, 2004; Piskorska-Pliszczynska et al., 2014) except for NDL-PCB concentration that was 0.04 µg kg^{-1} in the study by Piskorska-Pliszczynska et al. (2014).

Concentrations found in vegetable samples were well below the action levels set by Commission Recommendation (EU) 663/2014 for both PCDD/Fs and DL-PCBs (Table 23 and 24). Analysis showed concentrations ranging between 0.004 and 0.036 pg WHO-TEQ g^{-1} with a mean value of 0.017 pg WHO-TEQ g^{-1} for PCDD/Fs, between 0.0004 and 0.020 pg WHO-TEQ g^{-1} with a mean value of 0.007 pg WHO-TEQ g^{-1} for DL-PCBs and between 0.13 and 1.08 ng g^{-1} with a mean value of 0.26 ng g^{-1} for NDL-PCBs (Table 25). The levels of PCDD/Fs and PCBs found in this project were similar to those found in other studies (Grassi et al., 2010; Ben et al., 2017).

The sample collected from soil in the experimental Area 1 contained 0.42 pg WHO-TEQ g^{-1} of PCDD/Fs, 0.17 pg WHO-TEQ g^{-1} of DL-PCBs and 1.11 ng g^{-1} of NDL-PCBs; whereas samples from the experimental Area 2 contained 5.02 pg WHO-TEQ g^{-1} of PCDD/Fs, 1.38 pg WHO-TEQ g^{-1} of DL-PCBs and 24.43 ng g^{-1} of NDL-PCBs (Table 23 and 24). These results showed contamination levels below the limit of 10 pg TEQ g^{-1} adopted in Italy in soils for green and residential uses (Ministry for the Environment, Land and Sea, 2006). Moreover, the contamination levels found in soil were comparable with those found in other studies (APAT, 2007; Colombo et al., 2011; Piskorska-Pliszczynska et al., 2014).

3.2. ANALYSIS OF THE CONGENER PROFILE

Each sample analysed showed a characteristic profile of PCDD/F, DL-PCB and NDL-PCB congeners that are summarised in Figure 5. For statistical analysis the concentrations of the congeners below the LOQ were considered equal to 0.5 LOQ.

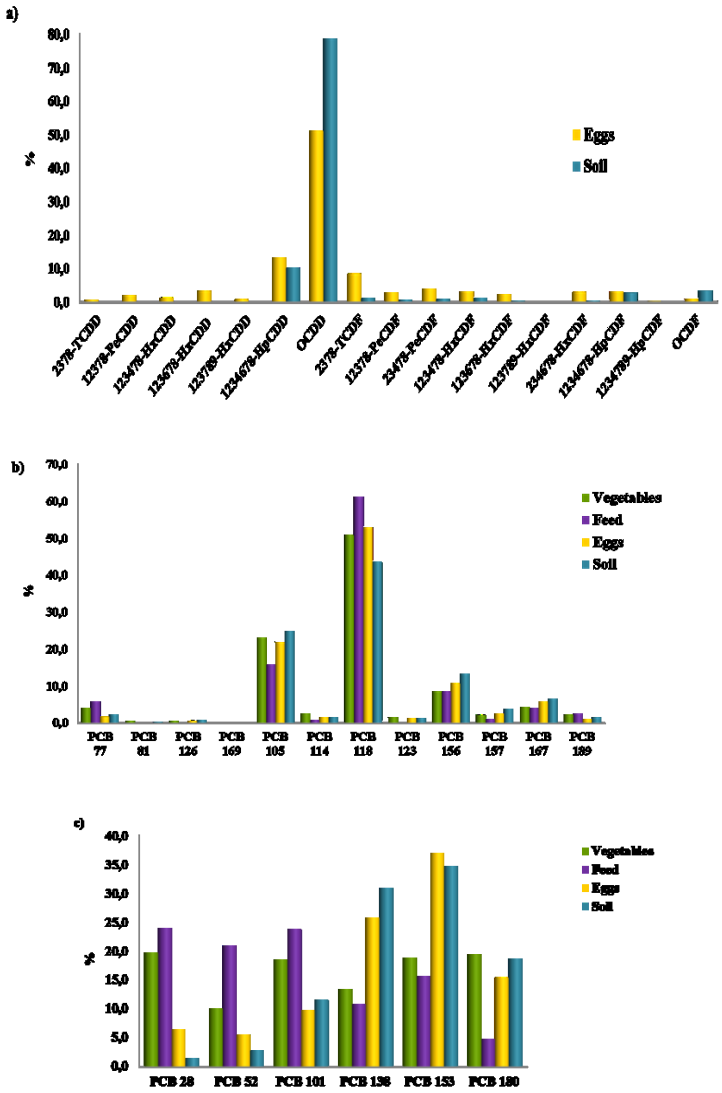


Fig. 5. Mean contribution of a) PCDD/Fs, b) DL-PCBs and c) NDL-PCBs in samples.

The concentrations of the two most toxic congeners, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, both with a TEF value equal to 1, were detected in 70% and 50% of the egg samples analysed, respectively, and ranged from a minimum of 0.003 pg g^{-1} fat (0.5 LOQ) to a maximum 0.21 pg g^{-1} fat with a mean value of 0.06 pg g^{-1} fat for 2,3,7,8-TCDD and from a minimum of 0.03 pg g^{-1} fat (0.5 LOQ) to a maximum of 1.03 pg g^{-1} fat with a mean value of 0.25 pg g^{-1} fat for 1,2,3,7,8-PeCDD. Their mean contributions to the total PCDD/F WHO-TEQ value were 8.8% and 32.0%, respectively. Other noticeable contributors were 2,3,7,8-TCDF (11.7%) and 2,3,4,7,8-PeCDF (17.6%) (Fig. 6).

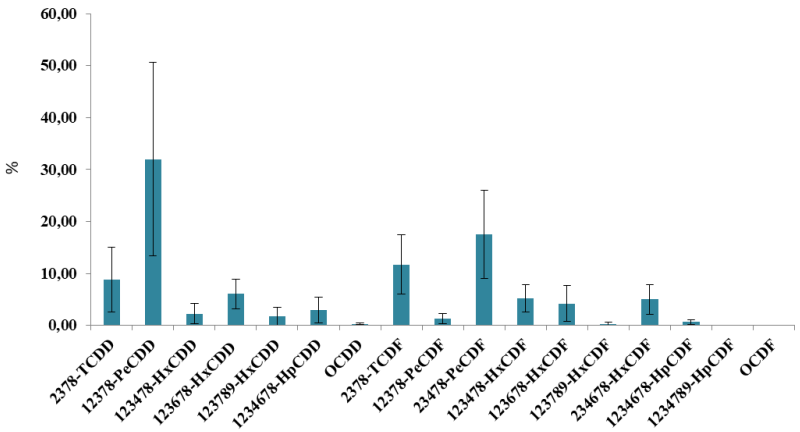


Fig. 6. Mean contribution and standard deviation of PCDD/F congeners in egg samples in total PCDD/Fs WHO-TEQ value.

The most abundant congeners were OCDD, detected in 100% of eggs, 1,2,3,4,6,7,8-HpCDD, detected in 90% of eggs and 2,3,7,8-TCDF detected in 100% of eggs. Their maximum concentrations in eggs were 28.27 pg g^{-1} fat, 10.10 pg g^{-1} fat and 2.47 pg g^{-1} fat, respectively and their mean percentages, in relation to the analytical sum of the 17 PCDD/Fs, were 51.0%, 13.3% and 8.4%, respectively (Fig. 7). Nevertheless, the mean contribution of OCDD and 1,2,3,4,6,7,8-HpCDD

to 17 PCDD/Fs WHO-TEQ sum were very low, 0.3% for OCDD and 3.0% for 1,2,3,4,6,7,8-HpCDD, because they have very low TEF values, 0.0003 and 0.01 respectively (Fig. 6).

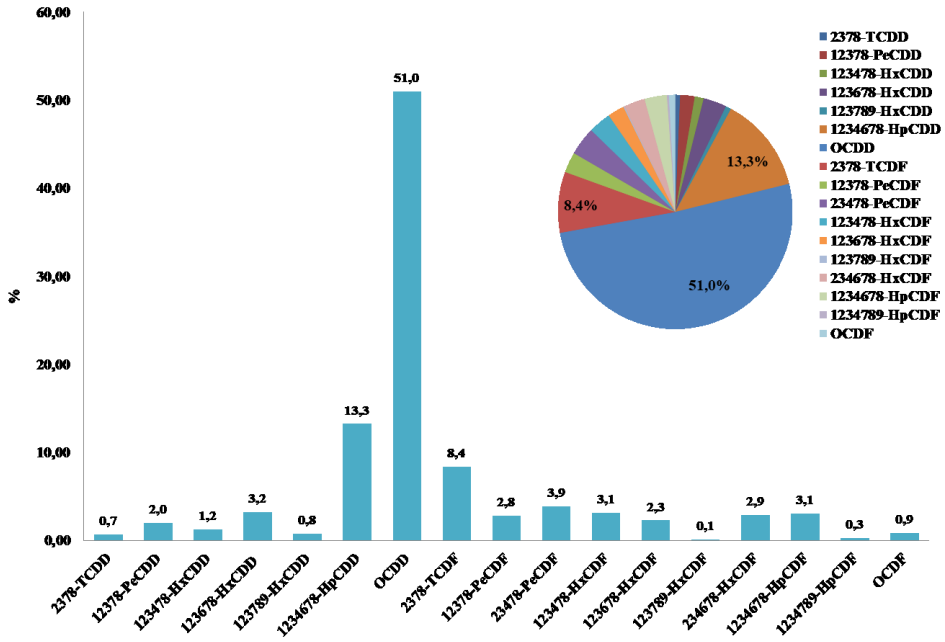


Fig. 7. Mean contribution of PCDD/Fs present in egg samples.

The analysis of PCB in the egg samples showed that the concentrations of all 18 congeners, in all analysed egg samples, were above the LOQs. PCB-126, the most toxic DL-PCB, with a TEF value of 0.1, was detected in all egg samples and its concentration ranged between 1.24 pg g^{-1} fat and 31.56 pg g^{-1} fat with a mean value of 10.21 pg g^{-1} fat. Considering that PCB-126 has a very high TEF value, this PCB is the congener that contributes mainly to 12 DL-PCBs TEQ sum with an average percentage contribution of 92%. Instead, the most abundant DL-PCB congeners, detected in 100% of egg samples, were PCB-118 and PCB-105; their maximum concentration were 2.84 pg g^{-1} fat for PCB-118 with a mean percentage of 52.8% in relation to the analytical sum of the 12 DL-PCBs and 960.87 pg g^{-1} fat for PCB-

105 with a mean percentage of 21.8% (Fig. 8). For NDL-PCBs, the most abundant congeners were PCB-138 and PCB-153; their maximum concentrations were 5.67 ng g^{-1} fat for PCB-138 and 9.96 ng g^{-1} fat for PCB-153 with a mean percentage of 25.8% and 37.0 %, respectively (Fig. 9). Similar profiles, with a predominance of OCDD, 1,2,3,4,6,7,8-HpCDD, PCB-118, PCB-105, PCB-153 and PCB-138 were also found in eggs analysed by other authors (Lovett et al., 1998; Pussemier et al., 2004; van Overmeire et al., 2009; Rawn et al., 2012; Lambiase et al., 2017).

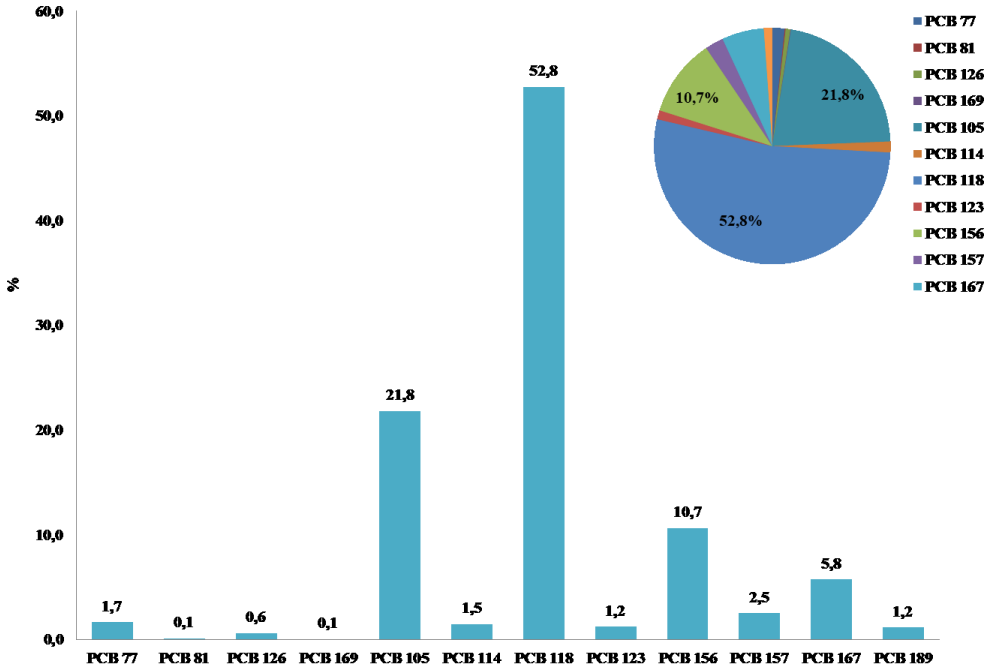


Fig. 8. Mean contribution of DL-PCBs in egg samples.

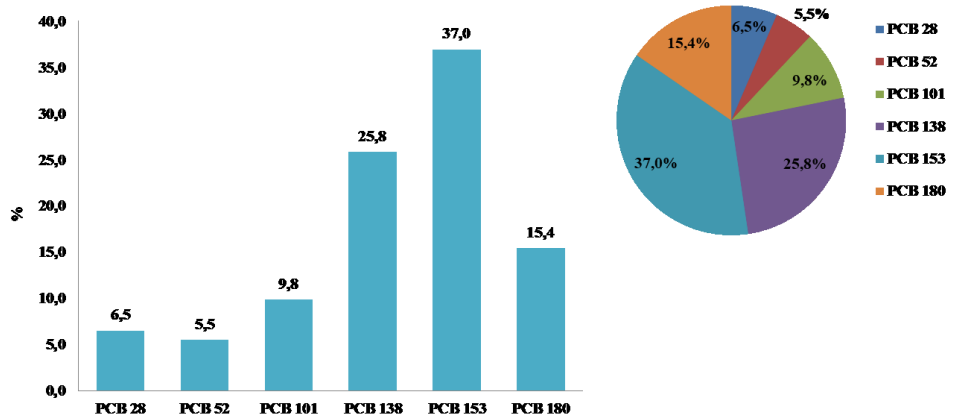


Fig. 9. Mean contribution of NDL-PCBs in egg samples.

In the analysis of vegetable samples, only 25% of PCDD/F congeners were above the LOQs, the remaining 75% was undetectable, making it impossible the definition of a dioxin congener profile. OCDD was the only congener detected in 100% of vegetables. Its maximum concentration was 0.33 pg g^{-1}, about 86 times lower than the concentration of OCDD found in eggs. Concerning PCB, 82% of DL-PCB congeners and 42% of NDL-PCB congeners were above the LOQs. PCB-126 was detected in 80% of samples and its concentration ranged between 0.001 (0.5 LOQ) pg g^{-1} and 0.19 pg g^{-1} with a mean value of 0.07 pg g^{-1} and an average percentage contribution of 0.45%. The most abundant congeners of DL-PCBs were PCB-118 and PCB-105, detected in 100% of vegetable samples; their maximum concentrations were 13.44 pg g^{-1} for PCB-118 with a mean percentage of 50.7% and 15.23 pg g^{-1} for PCB-105 with a mean percentage of 23.1% (Fig. 10). The 6 congeners of NDL-PCBs showed very similar contributions among them; the most abundant congeners were PCB-28, PCB-180, PCB-153 and PCB-101 detected in 30%, 10%, 90% and 10% of samples, respectively. Their maximum concentrations were 0.05 ng g^{-1} for PCB-28 with a mean percentage of 19.7%, 0.22 ng g^{-1} for

PCB-180 with a mean percentage of 19.4%, 0.46 ng g^{-1} for PCB-153 with a mean percentage of 18.8% and 0.07 ng g^{-1} for PCB-101 with a mean percentage of 18.6% (Fig. 11).

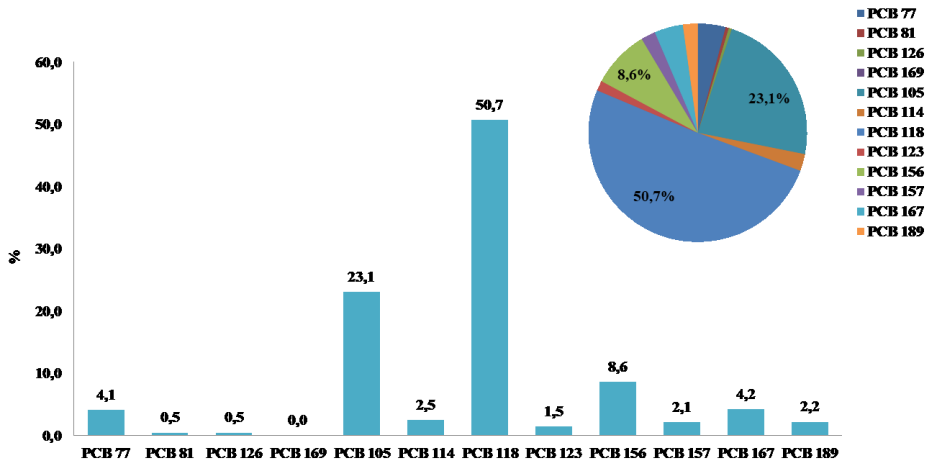


Fig. 10. Mean contribution of DL-PCBs in vegetable samples.

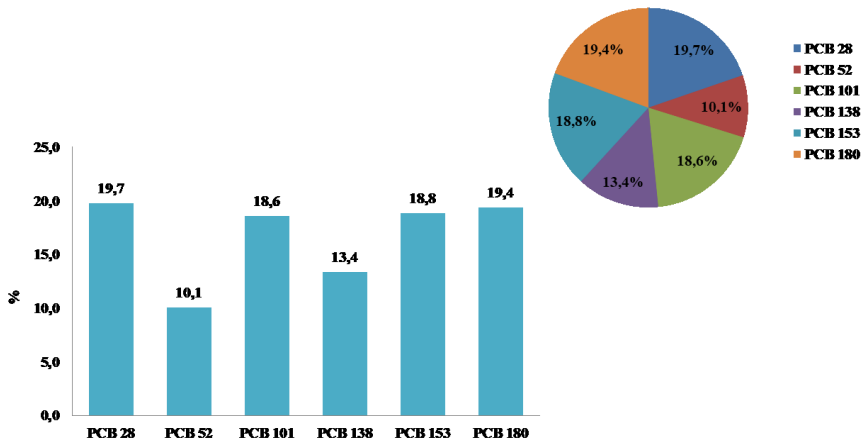


Fig. 11. Mean contribution of NDL-PCBs in vegetable samples.

In feed samples, only 12% of PCDD/F congeners were above the LOQs and then a dioxin congener profile could not be defined as for vegetables. Instead, concerning PCB, 62% of dioxin-like congeners and 92% of non dioxin-like congeners were above the LOQs. PCB-126 was found only in the feed used for the hens raised in the experimental Area 2 and its concentration and mean percentage contribution, in relation to the analytical sum of the 12 DL-PCBs, were 0.04 pg g^{-1} and 0.18%, respectively. The most abundant PCB congeners, detected in both feed samples, were PCB-118 and PCB-105 for DL-PCBs and PCB-28, PCB-101 and PCB-52 for NDL-PCBs. Their maximum concentrations were 7.25 pg g^{-1} for PCB-118 with a mean percentage contribution of 61.1%, 1.71 pg g^{-1} for PCB-105 with a mean percentage contribution of 15.8% (Fig. 12), 0.06 ng g^{-1} for PCB-28 with a mean percentage contribution of 24.0%, 0.05 ng g^{-1} for PCB-101 with a mean percentage of 23.8% and 0.04 ng g^{-1} for PCB-52 with a mean percentage of 20.9% (Fig. 13).

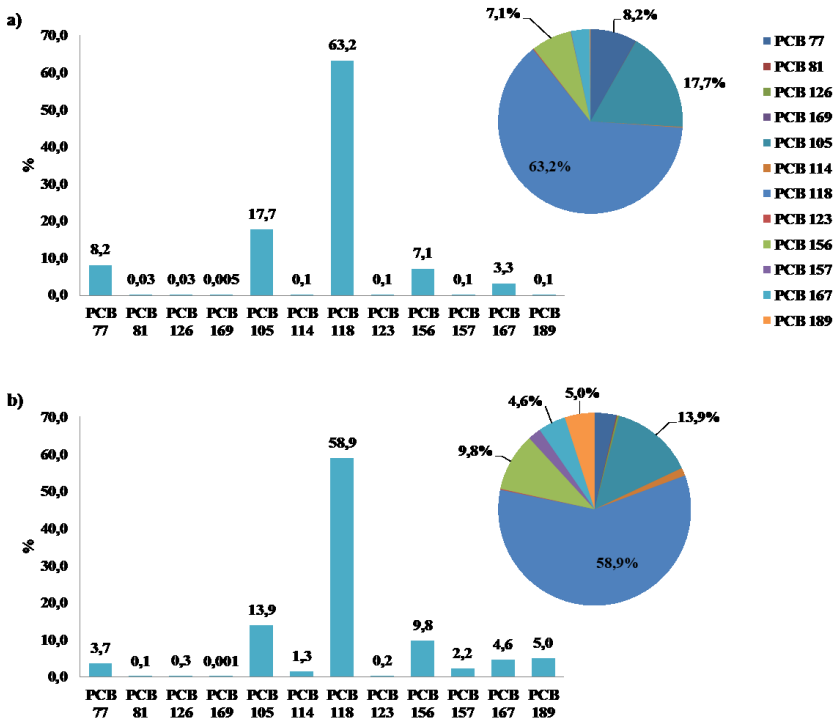


Fig. 12. Mean contribution of DL-PCBs in feed samples: a) from the Area 1 and b) from the Area 2.

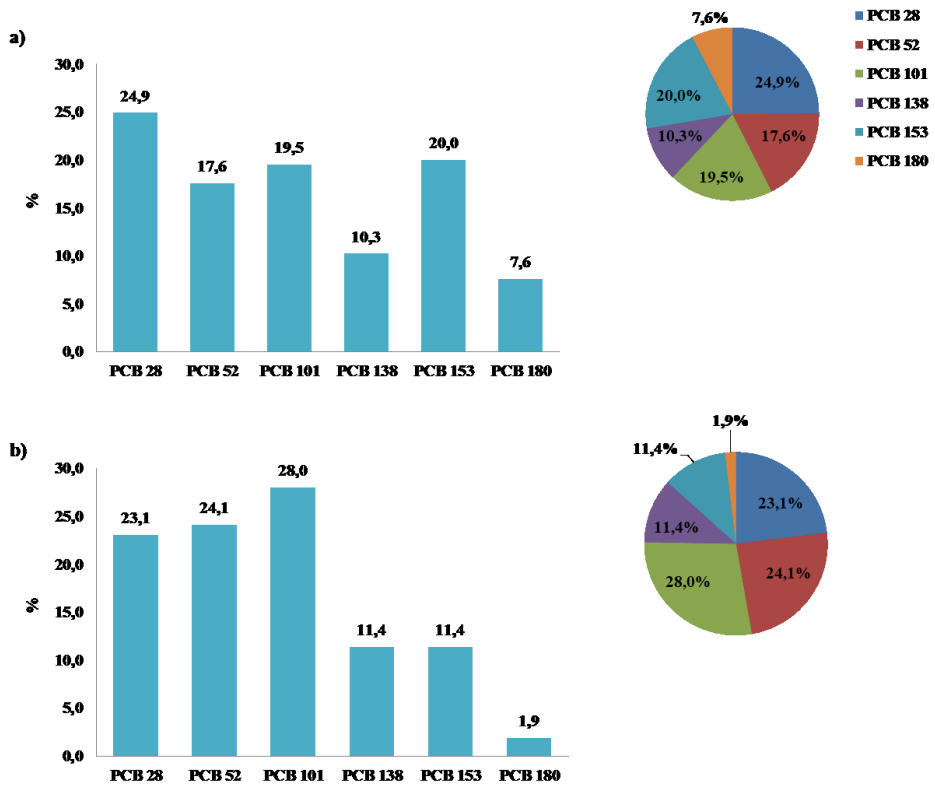


Fig. 13. Mean contribution of NDL-PCBs in feed samples: a) from the Area 1 and b) from the Area 2.

Unlike feed samples, 85% of PCDD/F congeners in soil was above the LOQs. 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD were detected both in the soil of the Area 1 and in that of the Area 2 and their maximum concentrations were 0.25 pg g^{-1} and 0.91 pg g^{-1}, respectively. OCDD and 1,2,3,4,6,7,8-HpCDD were the most abundant congeners detected in both soil samples. Their maximum concentrations were 351.29 pg g^{-1} for OCDD with a mean percentage contribution, in relation to the analytical sum of 17 PCDD/Fs, of 78.5% and 40.14 pg g^{-1} for 1,2,3,4,6,7,8-HpCDD with a mean percentage contribution of 10.1% (Fig. 14). The concentrations of all 18 congeners of PCB were above the LOQs, except for PCB-28 in the soil

collected from the Area 1 which was below the LOQs. The maximum concentration of PCB-126, was 12.81 pg g^{-1} and its mean percentage contribution to the sum of 12 DL-PCBs was 0.9%. The most abundant DL-PCB congeners were PCB-118 with a maximum concentration of 770.56 pg g^{-1} and a mean percentage contribution of 43.5% and PCB-105 with a maximum concentration of 444.20 pg g^{-1} and a mean percentage contribution of 24.7% (Fig. 15); while for the NDL-PCBs, PCB-138 with a maximum concentration of 7.98 ng g^{-1} and a mean percentage contribution of 30.9% and PCB-153 with a maximum concentration of 7.70 ng g^{-1} and a mean percentage contribution of 34.8% were the most abundant congeners detected (Fig. 16).

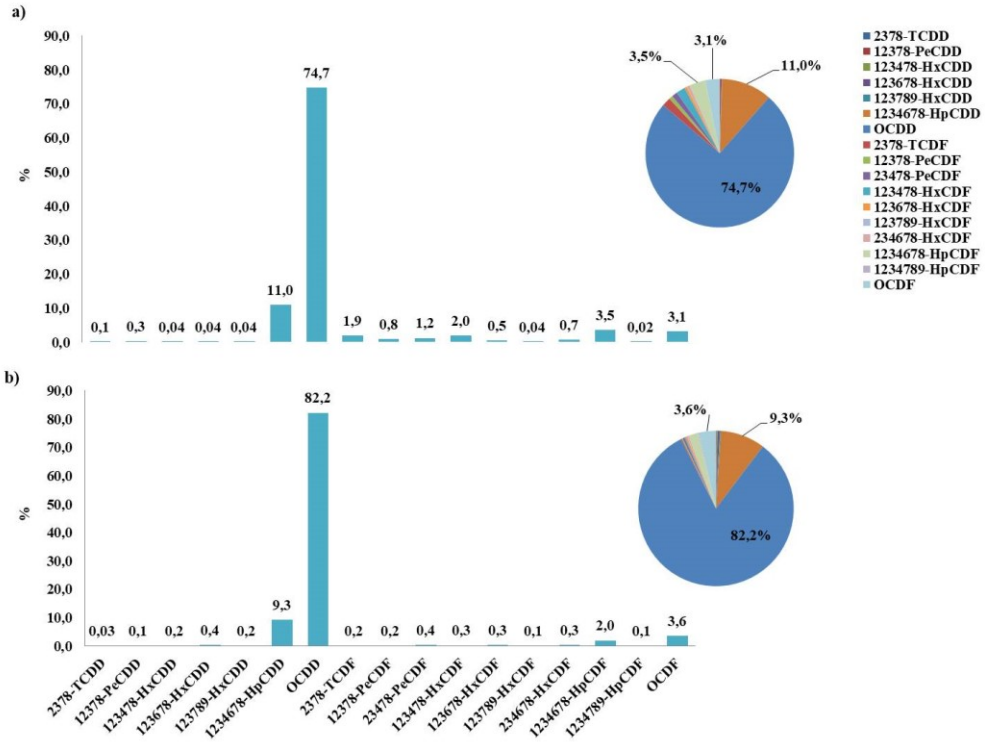


Fig. 14. Mean contribution of PCDD/Fs in soil samples.

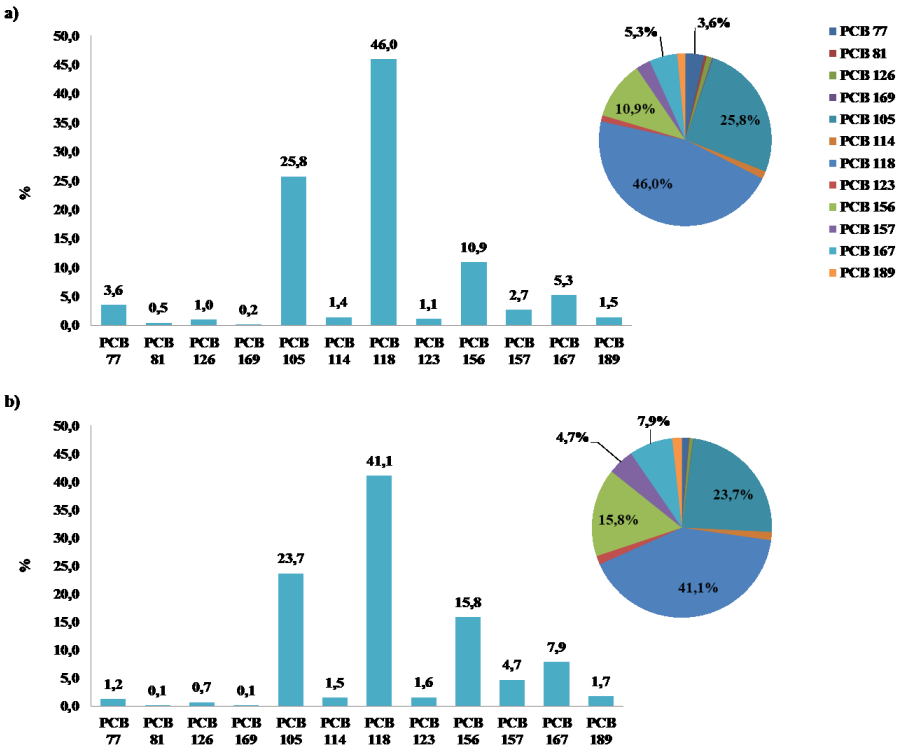


Fig. 15. Mean contribution of DL-PCBs in soil samples.

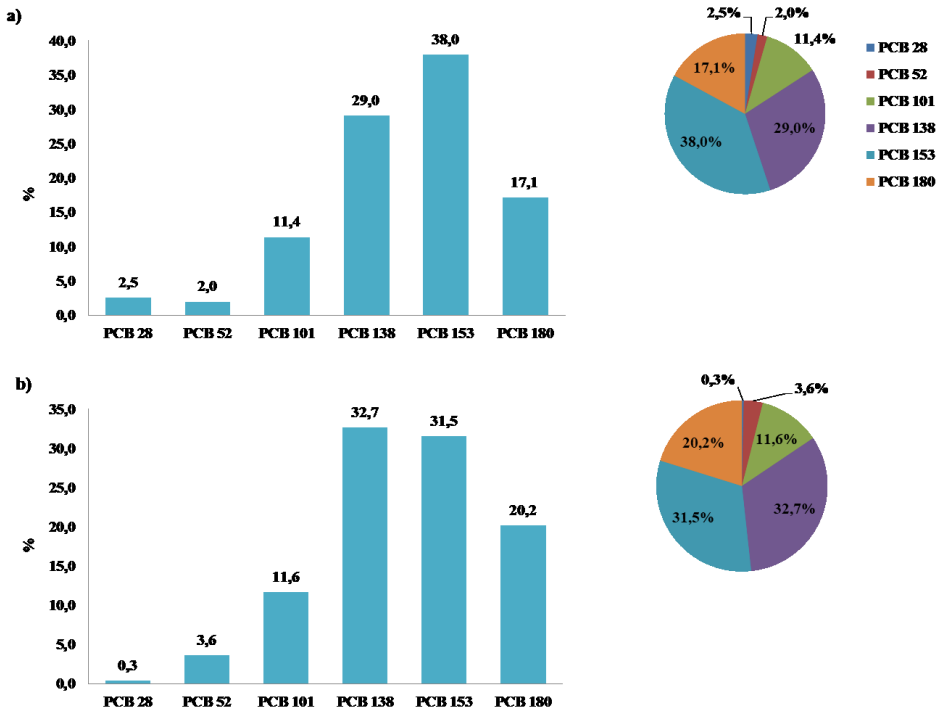


Fig. 16. Mean contribution of NDL-PCBs in soil samples.

The principal component analysis (PCA) was also performed using STATISTICA 5 (StatSoft Inc., Tulsa, OK, USA). Four principal components, accounting for 69% of total variance in the observed variables, were extracted (Table 26). The first principal component explained 26% of total variance and was more heavily affected by 2,3,7,8-TCDF and NDL-PCBs (PCB-28, PCB-138, PCB-153, PCB-180) and to a lesser extent by 1,2,3,7,8-PeCDD and PCB-169. The second main component explained 17% of total variance and essentially consisted of 1,2,3,6,7,8-HxCDD, OCDF and some DL-PCBs (PCB-126, PCB-156, PCB-167). The results of the PCA analysis showed that these congeners were significantly related to each other suggesting that they had a common source of contamination. Therefore, the

analysis of PCDD/F and PCB congener profiles could be used for the identification of contamination source (Masunaga et al., 2003).

Table 26
Loading pattern of the 35 PCDD/F and PCB congeners in eggs on the four main principal components.

	Factor 1	Factor 2	Factor 3	Factor 4
2378-TCDD	-0.57	-0.36	-0.37	0.43
12378-PeCDD	**0.66**	-0.35	0.57	0.29
123478-HxCDD	0.17	-0.21	0.24	0.21
123678-HxCDD	0.06	**0.92**	0.01	0.09
123789-HxCDD	0.41	-0.24	**0.69**	0.07
1234678-HpCDD	-0.22	-0.02	0.02	0.28
OCDD	**-0.60**	-0.38	-0.21	-0.18
2378-TCDF	**0.86**	-0.12	-0.38	-0.10
12378-PeCDF	-0.22	-0.17	-0.34	**0.64**
23478-PeCDF	0.38	-0.25	**-0.84**	-0.01
123478-HxCDF	-0.57	-0.36	-0.37	0.43
123678-HxCDF	0.42	-0.14	**0.68**	0.01
123789-HxCDF	0.42	0.05	**0.62**	0.20
234678-HxCDF	-0.05	-0.11	0.06	**-0.76**
1234678-HpCDF	-0.53	-0.30	-0.15	-0.07
1234789-HpCDF	0.51	-0.35	**0.72**	0.16
OCDF	0.06	**0.92**	0.00	0.09
PCB 77	-0.22	-0.17	-0.34	**0.64**
PCB 81	0.58	-0.13	-0.28	-0.51
PCB 126	-0.06	**0.85**	-0.13	0.34
PCB 169	**0.62**	0.23	**-0.72**	-0.06
PCB 105	-0.53	-0.30	-0.16	-0.07
PCB 114	-0.59	-0.26	-0.10	-0.12
PCB 118	-0.37	-0.23	0.01	**-0.78**
PCB 123	-0.24	-0.42	-0.02	0.42
PCB 156	-0.03	**0.89**	-0.02	0.08
PCB 157	-0.24	-0.22	0.00	**-0.76**
PCB 167	-0.07	**0.84**	-0.04	0.07
PCB 189	-0.33	-0.06	0.10	-0.03
PCB 28	**0.87**	-0.27	-0.21	0.02
PCB 52	0.24	-0.54	0.26	0.52
PCB 101	0.58	-0.28	**-0.68**	0.13
PCB 138	**0.93**	0.05	-0.17	0.02
PCB 153	**0.91**	-0.09	-0.34	-0.05
PCB 180	**0.92**	-0.14	-0.29	-0.01
Initial eigenvalue	8.98	5.90	5.10	4.17
Cumulative eigenvalue	8.98	14.88	19.98	24.15
% total variance	25.66	16.85	14.58	11.91
% cumulative variance	25.66	42.50	57.08	69.00

3.3. *IN VIVO* STUDY

The concentrations of PCDD/Fs and PCBs found in the eggs collected from the hens involved in the *in vivo* experiments are reported in Table 27.

Table 27
PCDD/Fs, DL-PCBs expressed as pg WHO-TEQ g^{-1} fat and NDL-PCBs expressed as ng g^{-1} fat in eggs during the *in vivo* study.

Area	Week of experiment	PCDD/Fs	DL-PCBs	∑ PCDD/Fs + DL-PCBs	NDL-PCBs
Area 1	1 st week	1.06	3.43	4.49	22.57
	5 th week	1.30	3.61	4.91	23.18
	9 th week	1.58	2.68	4.26	25.82
Area 2	23 th week	0.90	1.87	2.77	12.52
	25 th week	3.92	3.32	7.24	20.37
	27 th week	5.51	4.24	9.75	26.32
	29 th week	2.82	3.11	5.93	20.00
	31 th week	3.67	3.82	7.50	22.37
	33 th week	3.20	2.24	5.44	16.78
	35 th week	2.70	2.18	4.88	15.38
	37 th week	2.77	2.49	5.26	18.56
	37 th week[a]	0.16	0.06	0.22	1.055
	41 th week	2.82	2.52	5.34	21.58
	45 th week	3.92	3.21	7.13	30.94
	49 th week	2.61	1.63	4.24	14.64
	53 th week	2.59	1.64	4.24	13.56

[a]Eggs from the hens of the control group.

The hens raised in the Area 1 were monitored for 9 weeks and the eggs were sampled and analysed 3 times. In this period, the contamination levels found did not show significant variation ranging between 1.06 and 1.58 pg WHO-TEQ g^{-1} fat for PCDD/Fs, between 2.68 and 3.61 pg WHO-TEQ g^{-1} fat for DL-PCBs and between 22.57 and 25.82 ng g^{-1} fat for NDL-PCBs.

The most abundant congeners of PCDD/Fs were 2,3,7,8-TCDF, OCDD, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF. Their maximum concentrations were 2.95 pg g^{-1} fat with a mean percentage contribution to the analytical sum of PCDD/Fs of 28.1%

for 2,3,7,8-TCDF, 2.13 pg g^{-1} fat with a mean percentage contribution of 19.7% for OCDD, 1.43 pg g^{-1} fat with a mean percentage contribution of 11.5% for 2,3,4,7,8-PeCDF and 1.05 pg g^{-1} fat with a mean percentage contribution of 8.5% for 1,2,3,7,8-PeCDF (Fig. 17).

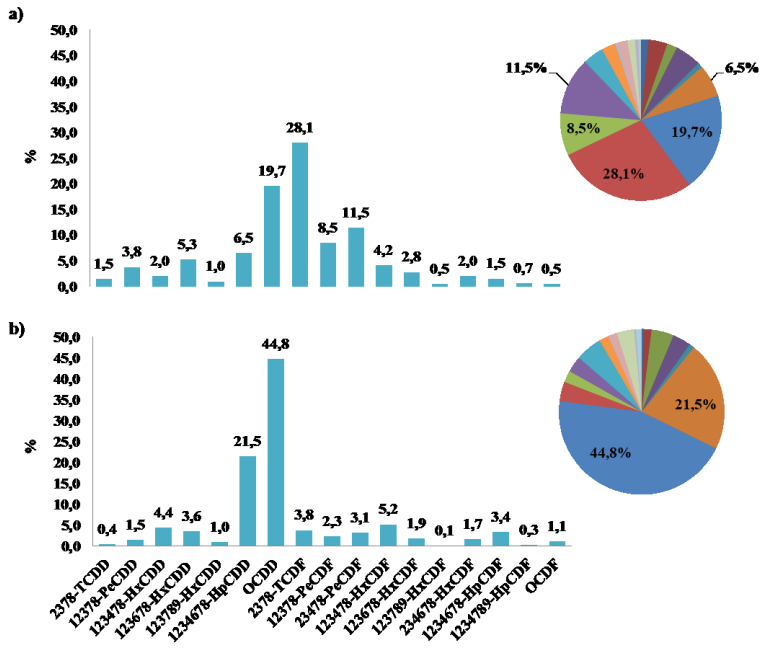


Fig. 17. Mean contribution of PCDD/Fs in egg samples: a) eggs from hens before the transfer to the Area 2, b) eggs from hens after the transfer to the Area 2.

The most abundant congeners of PCBs found in the eggs were PCB-118, PCB-105 and PCB-156 for DL-PCBs and PCB-153, PCB-138 and PCB-180 for NDL-PCBs. Their maximum concentrations were 2837.96 pg g^{-1} fat with a mean percentage contribution to the analytical sum of DL-PCBs of 57.0% for PCB-118, 960.87 pg g^{-1} fat with a mean percentage contribution of 19.2% for PCB-105 and 457.06 pg g^{-1} fat with a mean percentage contribution of 9.4% for PCB-156 (Fig. 18).

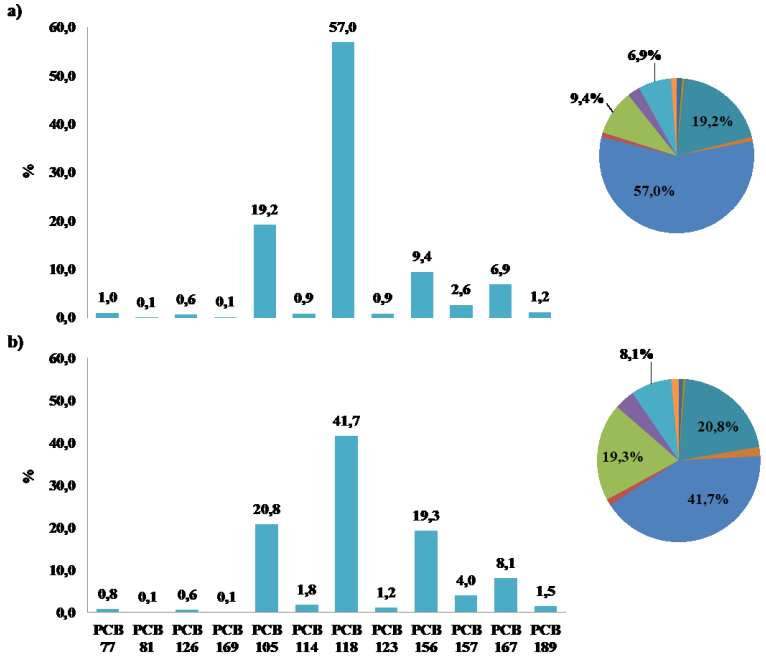


Fig. 18. Mean contribution of DL-PCBs in egg samples: a) eggs from hens before the transfer to the Area 2, b) eggs from hens after the transfer to the Area 2.

Finally, the maximum concentrations of NDL-PCBs were 12.79 ng g^{-1} fat with a mean percentage contribution of 47.5% for PCB-153, 7.81 ng g^{-1} fat with a mean percentage contribution of 29.4% for PCB-138 and 4.34 ng g^{-1} fat with a mean percentage contribution of 16.7% for PCB-180 (Fig. 19).

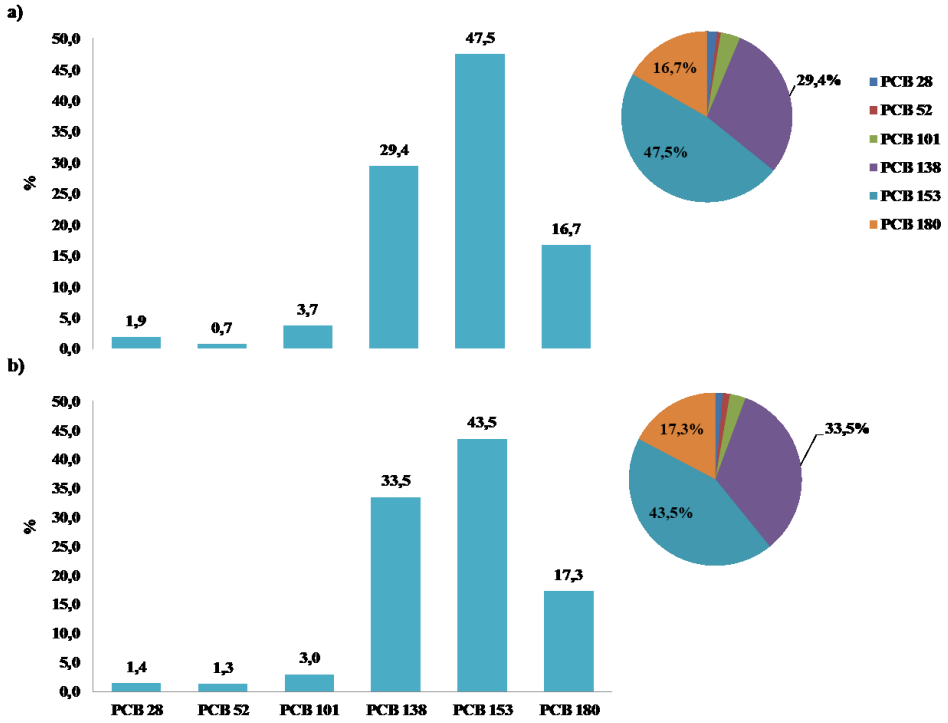


Fig. 19. Mean contribution of NDL-PCBs in egg samples: a) eggs from hens before the transfer to the Area 2, b) eggs from hens after the transfer to the Area 2.

After 9 weeks, the hens were transferred to the experimental Area 2 where they were monitored for further 44 weeks. The concentrations found in the eggs ranged between 0.90 and 5.51 pg WHO-TEQ g^{-1} fat for PCDD/Fs, between 1.63 and 4.24 pg WHO-TEQ g^{-1} fat for DL-PCBs and between 12.52 and 30.94 ng g^{-1} fat for NDL-PCBs (Table 27). Following the transfer, the levels of PCDD/Fs and PCBs found in the eggs were progressively increased until reaching the maximum concentrations at 27th and 45th week. The concentrations measured at 27th and 45th week were significantly higher than those found in the eggs when the hens were raised in the Area 1 (Fig. 20).

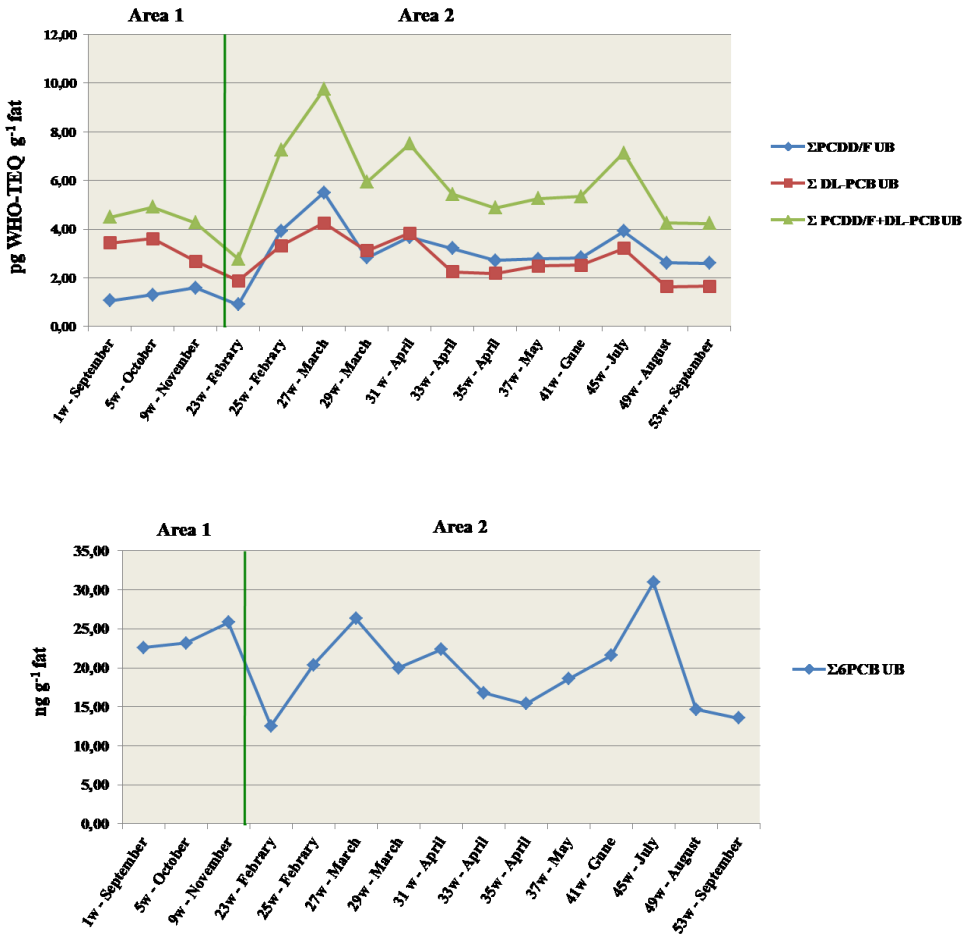


Fig. 20. Transfer trend of PCDD/Fs, DL-PCBs and NDL-PCBs during the 53 weeks of the *in vivo* study.

To investigate the possible contamination sources and the behaviour of PCDD/F and PCB congeners transferred from the environment to eggs, both the feed used for the hens diet and the soils of the yards where the hens lived were analysed (Table 28).

Table 28
PCDD/F, DL-PCB and NDL-PCB levels in feed, soil and eggs of the *in vivo* study.

Area	Sample type	PCDD/Fs	DL-PCBs	∑PCDD/Fs + DL-PCBs	NDL-PCBs
Area 1	Soil	0.42	0.17	0.59	1.11
	Feed	0.009	0.001	0.009	0.24
Area 2	Soil	5.02	1.38	6.40	24.43
	Feed	0.003	0.004	0.007	0.17
	Eggs[a]	2.77	2.49	5.26	18.57
	Eggs[b]	0.16	0.06	0.22	1.06

Notes:
Concentrations of PCDD/Fs and DL-PCBs expressed as: pg WHO-TEQ g^{-1} fat in egg samples, ng WHO-TEQ kg^{-1} with a moisture content of 12% in feed samples and pg WHO-TEQ g^{-1}dry weight for soil samples.
Concentration of NDL-PCBs expressed as: ng g^{-1} fat in egg samples, µg kg^{-1} with a moisture content of 12% in feed samples and ng g^{-1} dry weight for soil samples.
The concentrations are expressed using the upper-bound approach.
[a]Eggs collected at 37th weeks from the hens of the study.
[b]Eggs collected at 37th weeks from the hens of the control group.

The feed collected from the Areas 1 and 2 did not show significantly different concentrations of contaminants indicating that the main contamination source of the eggs could most likely be the soil. In fact, the soil of the Area 2 was significantly more contaminated than that of the Area 1. Nevertheless, it was interesting to notice that the increase of PCDD/F and PCB concentrations found in the eggs did not follow a linear trend, being in some weeks of the study even decreased (Fries et al., 1999). Since the bioavailability of organic pollutants is affected by physical, chemical and biological properties of soil and by soil constituents, it was possible to hypothesize that the contaminants present in the soil of the Area 2, in particular PCBs, were less available for absorption by the hens. The organic fraction of soils is considered the principal sorbent of organic contaminants thus reducing their bioavailability. It is known that the soil organic matter can modifies the absorption of organic contaminants, such as PCDD/Fs and PCBs, in the intestinal lumen of animals (Delannoy et al., 2014; Pu et al., 2006; Saghir et al., 2007). Delannoy et al. (2015), in a study aimed to assess the retention of NDL-PCBs by soil during the digestive processes in piglets, found that high

levels of organic carbon (>100 g kg^{-1}) and black carbon content (3.0 g kg^{-1}) in soil were related to a significant reduction in the bioavailability of contaminants (relative bioavailability approach, RBA), which however remained above 45%.

A further possibility to explain the absence of a linear trend in the concentrations of PCDD/Fs and PCBs in the eggs might be the seasonal variability of these contaminants in the environment, as reported by several authors (Lake et al., 2013; Esposito et al., 2017; Węgiel et al., 2018; Fiorito et al., 2019).

In addition, in order to have a further evidence of the correlation between the contamination found in the eggs and that found in the soil, the eggs sampled at the 37^{th} week were compared to those from the control hens. The eggs from the hens raised outdoors showed much higher concentrations of PCDD/Fs and PCBs than the eggs from control hens (Table 28). Considering that the feed used for the two groups of hens was the same, the only possible contamination source was almost certainly represented by the soil.

Following the hens transfer to Area 2, the congeners profiles also showed a variation. The PCDD/F congeners profile found in the eggs from the Area 2 shown a noticeable change as compared to the profile obtained when the hens lived in the Area 1, with the mean percentage contributions of 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF considerably reduced, while those of OCDD and 1,2,3,4,6,7,8-HpCDD greatly increased (Fig. 17).

Concerning DL-PCBs, the mean percentage contribution of PCB-118 decreased, while that of PCB-156 increased. with the mean percentage of PCB-105 remaining almost constant (Fig. 18).

When the profiles of DL-PCB congeners found in the soil and the feed were considered, it was possible to hypothesize that the variations of the congener profile, that occurred in the eggs after the transfer in the Area 2, was due to contributions coming from both the soil and the feed.

Finally, although the soil of the Area 2 contained a concentration of NDL PCBs about 22 times higher than that found in the soil of the Area 1, the profile of the 6

NDL-PCBs in the two soils completely overlapped. As a consequence, the NDL-PCB profiles found in the eggs when the hens lived in the Area 1 and those found after the transfer were also very similar (Fig. 19). Moreover, it was interesting to notice that the less chlorinated PCBs (PCB-28, PCB-52 and PCB-101), which are also the most volatile, showed a very low bioaccumulation in the eggs.

3.4. BIOTRANSFER FACTORS

The transfer of PCDD/Fs and PCBs from exposure sources to eggs was investigated through the calculation of biotransfer factors (BTFs) that were derived for each of the 35 PCDD/F and PCB congeners analysed. The method used was that proposed by Fernandes et al. (2011) and Rose et al. (2012).

Feed and soil were the two contamination sources considered to estimate the hens' daily intake of contaminants to measure BTFs. For the calculation, the mean daily intake of feed and soil were considered as input fluxes of contaminants for each hen. The mean daily feed intake for each hen was estimated considering the total amount of feed provided each day. The total amount of feed, supplied daily to the 9 studied hens, was 1170 g and therefore it was estimated that each hen consumed about 130 g per day.

The precise daily ingestion of soil by each hen could not be accurately measured because this ingestion is due to a completely accidental and fortuitous mechanisms that happen while the hens scratch in the yard. Therefore, the exposure of hens to PCDD/Fs and PCBs through the ingestion of soil could be only reliable estimated. The input flux due to this contamination source was estimated considering that each hen ingested 6 g per day of soil on average during the scratching (SCAN, 2000).

Specific input fluxes for each PCDD/F and PCB congener and for each considered contamination source (feed and soil) were calculated by multiplying the concentrations found in feed and soil by the corresponding daily consumption. For example, for 2,3,7,8-TCDD in soil of the Area 1 the input flux was:

$$0.010 \text{ ng kg}^{-1} \times 0.006 \text{ kg day}^{-1} = 0.00006 \text{ ng day}^{-1}$$

Then, the BTFs for each PCDD/Fs and PCBs were obtained dividing the concentration of each congener found in eggs by the corresponding total input

fluxes which are given by the sum of the inputs derived from the feed and the soil. Thus, the BTF for 2,3,7,8-TCDD in the eggs of the Area 1 was:

$$\frac{0.2016 \text{ ng kg}^{-1} \text{ fat}}{(3.4125\text{E-}05 \text{ ng day}^{-1} + 0{,}00006 \text{ ng day}^{-1})} = 2155.6 \text{ day kg}^{-1} \text{ fat}$$

Similar calculations were carried out for all 35 PCDD/F and PCB congeners and using concentrations found in eggs from hens reared for the *in vivo* experimental study. Precisely, the BTFs are calculated for eggs collected at the beginning of the experiment (1st week), at 23rd week, 27th week, 37th week and 53rd week (Table 29).

In addition, the BTFs were also calculated for each congener found in the eggs from the hens of the control group by using the concentrations of PCDD/Fs and PCBs detected in the eggs collected at 37th week. The determined BTFs were compared with those derived from the results of the eggs collected in the same period (37th week) from the hens raised outdoors (Table 29).

Table 29
BTFs for hens of the *in vivo* study.

Congener	Area 1	Area 2				
	1st week	23rd week	27th week	37th week[a]	37th week[b]	53rd week
2,3,7,8-TCDD	2155.6	40.4	142.2	247.9	143.0	175.6
1,2,3,7,8-PeCDD	346.5	39.4	193.1	163.2	395.9	238.4
1,2,3,4,7,8-HxCDD	644.7	62.6	997.5	262.9	3905.6	171.8
1,2,3,6,7,8-HxCDD	2600.9	21.4	245.9	134.5	307.6	127.7
1,2,3,7,8,9-HxCDD	29.6	19.5	122.5	74.5	2929.2	69.6
1,2,3,4,6,7,8-HpCDD	39.4	11.8	97.1	28.0	105.8	28.3
OCDD	19.4	1.8	17.0	7.4	11.3	7.1
2,3,7,8-TCDF	1171.2	180.5	330.8	214.6	5695.7	157.6
1,2,3,7,8-PeCDF	528.2	103.5	211.7	166.4	160.4	132.3
2,3,4,7,8-PeCDF	541.4	49.6	245.8	116.2	163.9	79.0
1,2,3,4,7,8-HxCDF	91.0	99.4	947.0	205.5	275.5	134.3
1,2,3,6,7,8-HxCDF	224.8	20.9	213.8	100.5	247.6	75.1
1,2,3,7,8,9-HxCDF	17.2	1.5	84.0	31.9	1394.9	41.5
2,3,4,6,7,8-HxCDF	170.0	14.0	142.1	77.8	2789.7	73.7
1,2,3,4,6,7,8-HpCDF	17.2	8.2	58.6	27.8	48.8	22.3
1,2,3,4,7,8,9-HpCDF	5.6	8.6	72.0	38.4	226.7	37.4
OCDF	22.4	1.9	14.8	5.5	25.5	4.2
PCB 77	603.0	130.5	270.0	180.0	110.7	189.1
PCB 81	1112.1	231.6	417.4	224.0	280.7	179.8
PCB 126	5042.9	271.7	605.9	359.2	112.3	233.2
PCB 169	3654.4	331.7	863.1	434.4	4882.1	348.7
PCB 105	3481.4	216.5	675.0	366.4	231.9	264.8
PCB 114	4720.2	170.9	1036.5	492.9	166.4	319.6
PCB 118	3948.3	311.8	600.3	370.1	132.7	258.5
PCB 123	6114.5	228.0	637.1	348.5	1538.7	260.1
PCB 156	4009.9	195.8	1147.6	464.7	83.3	380.0
PCB 157	7785.3	145.8	657.9	371.5	85.4	311.6
PCB 167	6098.7	237.9	671.1	452.2	73.9	381.3
PCB 189	5511.7	132.3	481.7	265.9	34.5	237.0
PCB 28	7.0	46.1	54.8	46.5	66.1	85.8
PCB 52	2.6	33.4	21.2	14.5	47.9	43.3
PCB 101	12.1	38.0	20.6	3.2	26.2	10.5
PCB 138	45.8	80.2	231.4	161.5	48.1	115.5
PCB 153	33.8	160.8	298.0	226.1	56.9	144.0
PCB 180	38.5	89.0	224.3	140.1	78.2	111.8

Notes:
BTFs expressed as day kg^{-1} fat.
[a]Eggs collected at 37th weeks from the hens of the study.
[b]Eggs collected at 37th weeks from the hens of the control group.

The analysis of the BTFs showed that the lower chlorinated congeners had higher BTFs than other congeners indicating that they were absorbed and transferred into the eggs more than others. This result was in agreement with other authors

reporting that the higher chlorinated congeners showed a lower absorption and a higher excretion into the feces (Fries et al., 1999; Pirard and De Pauw, 2005; Hoogenboom et al., 2006; Traag et al., 2006; Fernandes et al., 2011; Rose et al., 2012). Regarding NDL-PCBs, on the contrary, the lower chlorinated congeners showed lower BTFs because according to De Vos et al. (2005) these PCBs are readily metabolized to a much higher extent than the other NDL-PCBs.

Chapter II

FOREWORD

In order to achieve a complete understanding of the relationships that exist between environmental pollution and pollutant levels in food, a preliminary study concerning persistent inorganic environmental pollutants has also been carried out. The study was designed within the frame of this Ph.D. project concerning the investigation of persistent organic contaminants. During the project, tests to develop and validate an analytical method for determination of trace elements and rare earth elements in egg and vegetable samples were started. The method was developed according to the Commission Regulation (EU) 333/2007 (current Comm. Reg. EU 836/2011) and the requirements set by the EN ISO/IEC 17025.

The elements investigated in this project were Antimony (Sb), Arsenic (As), Barium (Ba), Beryllium (Be), Bismuth (Bi), Boron (B), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Iron (Fe), Manganese (Mn), Mercury (Hg), Nickel (Ni), Lead (Pb), Copper (Cu), Rubidium (Rb), Selenium (Se), Vanadium (V), Zinc (Z), REEs including Lanthanum (La), Cerium (Ce) Praseodymium (Pr), Neodymium (Nd), Samarium (Sm), Europium (Eu), Gadolinium (Gd), Terbium (Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium (Tm), Ytterbium (Yb), Lutetium (Lu) and Scandium (Sc), Thorium (Th) and Yttrium (Y).

4. INTRODUCTION

4.1. TRACE ELEMENTS AND RARE EARTH ELEMENTS

Trace elements (TEs) are present in the environment at low concentrations, with values lower than 10 mg kg^{-1} (Tchounwou P.B. et al., 2012), and therefore considered in trace; both metals and metalloids belong to this group. Many of these elements are called heavy metals because they have an atomic density ranging between 3.5 and 7 g/cm^3 (Duffus, 2002; Hodson, 2004; El-Kady and Abdel-Wahhab, 2018); they are also known as potentially toxic elements (PTEs). The most extensively studied TEs include As, B, Cd, Cr, Co, Fe, Pb, Mn, Ni, Cu, Se and Zn. Some of these, such as B, Fe, Mn, Cu, Zn, at low concentrations, play an important role in the normal growth of plants whereas others, such as Co, Fe, Mn, Cu, Se and Zn are important for the normal growth and health of animals and humans being considered essential to healthy functioning of the biota including humans. However, these same elements, at high concentrations, are considered environmental contaminants which may result in serious toxic effects in humans. Other elements, such as As, Cd, Cr and Pb, show a high toxicity even at low concentrations and, for this reason, they are carefully studied.

Rare earth elements (REEs), including the lanthanides from La to Lu, are also considered trace elements. The elements Sc, Y and Th can also be included in this group because they show similar chemical properties to lanthanides and they are often found in the same mineral aggregates. In nature, REEs are found in mixture rather than in isolation and they occur at low concentrations in environmental matrices. For this reason, many scientists have used REEs in study of geological and geochemical processes (Li et al., 2013). REEs are considered non-essential elements for living systems showing a moderate toxicity. Nevertheless, they can have some beneficial effects on the physiological and biochemical responses of plants and animals and are used as plant growth regulators for crops and as feed additives for livestock, poultry and aquaculture (Jiang et al., 2012).

Conversely to organic pollutants TEs, are naturally present in earth crust, and they do not undergo biodegradation processes. Consequently, once released in the environment, they tend to bioaccumulate in soil, water and vegetation and then can bioconcentrate in biota and consequently in the food chain.

4.2. SOURCES OF TRACE ELEMENTS

TEs can have both natural and anthropogenic origins. They are naturally present in the earth's crust, like aluminium, iron and magnesium which are present with 7.4%, 4.7% and 2.1%, respectively (Manaham, 2000). Consequently, the main natural TEs source of environmental contamination is represented by erosion of rocks that depends on intensity, amount and frequency of rains, presence or absence of vegetation, intensity of wind and physical properties of soil (Rodríguez et al., 2013; El-Kady and Abdel-Wahhab, 2018). In addition to the release of TEs from rocks, other natural sources are volcanic phenomena, forest wildfires and sea salt (Edelstein and Ben-Hur, 2018).

Anthropogenic activities are the main sources of TEs accumulation in the environment, plants, animals and eventually in food chain. Anthropogenic sources include agricultural practices due to the use of fertilisers, organic manures, agrochemicals and composts (Goretti et al., 2016; Islam et al., 2014); activities related to the mining industry, such as smelting and mineral extraction; fuel consumption for example due to vehicle transport; wastewater (El-Kady and Abdel-Wahhab, 2018; Edelstein and Ben-Hur, 2018).

REEs are used in high technology applications, such as flat screen display, optical fibres, alloys, medical imaging, but also in traditional applications, such as agriculture, metallurgy, supermagnets, and petroleum refining (Gwenzi et al., 2018).

4.3 EFFECTS ON HUMAN HEALTH

TEs can induce a series of organ damage and numerous adverse effects on human health, even at low exposure levels. Their toxicity involves several mechanisms of action which are often characteristic and specific for each element and element form; many of these toxic processes are not yet completely known. One of the main toxicity mechanisms, which especially involve redox active metals, such as Co, Cr, Fe, Cu and other metals, concerns the generation of oxidative stress which results in increasing production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), the depletion or degradation of intracellular antioxidants and scavengers of free radicals and the inhibition or reduction of metabolism and enzymes involved in detoxification processes (Gupta et al., 2019). Free radicals formed due to oxidative stress, can exert DNA damage, lipid peroxidation and alteration of calcium and sulphydryl homeostasis (Jomova and Valko, 2011). The redox inactive metals, like Cd, As and Pb induce their toxic effects by binding to sulphydryl groups of proteins and/or by causing glutathione depletion (Jomova and Valko, 2011).

These toxicity mechanisms may result in a number of adverse effects on human health (El-Kady and Abdel-Wahhab, 2018; Gupta et al., 2019) some of which include: abortion, pregnancy hypertension and neonatal death, endocrine disrupting (Rahman et al., 2016) and alteration of the metabolism of nerve-impulse transmitters (Patlolla and Tchounwou, 2005) for As; pulmonary and gastrointestinal irritation, burning sensation, nausea and vomiting, renal dysfunction and bone damage (Satarug et al., 2017), endocrine disrupting (Rahman et al., 2016), gastrointestinal tract erosion, pulmonary, renal injury and coma in acute intoxications for Cd; DNA damage (Hill et al., 2008; Shahid et al., 2017), increasing risk of abortion or miscarriage (Yang et al., 2013), chromosomal abnormalities (Wise et al., 2004) for Cr; nervous system damage (Jones, 2009), alteration of CNS development in the newborn (Manton et al., 2003) retardation in

growth and antisocial behaviours (Litvak et al., 1998; Amodio-Cocchieri et al., 1996) for Pb.

As, Cd, Cr, Hg and Pb are also classified as “known” or “probable” human carcinogens by the United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC).

In the literature there are still few papers concerning toxicity of REEs, which regard only some elements, such as Ce, La and Gd. Nevertheless, it is known that their mechanism of action is similar to those of the other trace elements and involves oxidative stress processes which result in adverse effects on human health, such as growth inhibition, cytogenetic effects and organ-specific toxicity (Pagano et al., 2015a and b; Pagano et al., 2019).

5. MATERIALS AND METHOD

5.1. ANALYTICAL METHOD

The analytical method used for the identification and quantification of 19 TEs, 14 REEs, Sc, Y and Th in hen eggs, vegetables and feed is based on the United States Environmental Protection Agency (US EPA) 3052:1996, US EPA 6010 C:2007, UNI EN 13804:2013 and UNI EN 15763:2010, opportunely adapted. The method allows the determination of 36 elements by microwave assisted acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Briefly, acid digested samples, after being nebulised inside the plasma torch, are dried, atomised and ionised by the high plasma temperature. Then, the ions pass through the sampler and skimmer cones and are transferred to the mass spectrometer where they are separated on the basis of their mass/charged ratio and determined by the detector.

5.2. MICROWAVE ASSISTED ACID DIGESTION

All digestions were performed using an UltraWAVE microwave oven (Milestone, FKV) that has a 1500 W microwave power source.

For digestions, 0.75 g of each homogenized sample were weighed in UltraWAVE reaction tubes equipped with their relative caps and added with 3.0 mL of 70% w/v nitric acid and 1.0 mL of ultrapure water. The following heating program was applied: up to 240 °C in 20 min, hold at 240 °C for 10 min and cooling phase for 15 min until reaching the room temperature. All steps were performed at 1500 W of applied power and the maximum pressure was set at 150 bar. After digestion, all samples were diluted to 25 mL with ultrapure water.

5.3. ICP-MS ANALYSIS

Instrumental analyses were performed by an inductively coupled plasma-mass spectrometry (ICP-MS) Aurora M90 Bruker, US. The conditions of the instrument during the analysis are listed in Table 30.

Table 30
Instrumental conditions of ICP-MS for TEs analysis.

PARAMETER	
RF applied power (kW)	1.4
Plasma gas flow rate (L min^{-1})	16.5
Auxiliary gas flow rate (L min^{-1})	2.00
Sheath gas flow rate (L min^{-1})	0.20
Nebulizer gas flow rate (L min^{-1})	1.00

The isotopes of the elements monitored were: ^{121}Sb, ^{75}As, ^{137}Ba, ^{9}Be, ^{209}Bi, ^{11}B, ^{114}Cd, ^{59}Co, ^{52}Cr, ^{56}Fe, ^{55}Mn, ^{202}Hg, ^{58}Ni, Pb as the sum of ^{206}Pb, ^{207}Pb and ^{208}Pb, ^{65}Cu, ^{85}Rb, ^{78}Se, ^{51}V and ^{66}Zn for TEs, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb and ^{175}Lu for REEs and ^{45}Sc, ^{89}Y and ^{232}Th.

The polyatomic interferences were removed by applying the dynamic reaction cell (DRC) technique, using hydrogen as reaction gas at 50 mL min^{-1}, for each elements except for Hg, REEs, Sc, Y and Th. As internal standards a solution of ^{103}Rh (Rhodium) and ^{175}Lu at 50 µg L^{-1} for TEs and of ^{115}In (Indium) at 1 µg L^{-1} for REEs, Sc, Th and Y were used. For quantitative determination, calibration curves, specific for each elements, were prepared in the concentration ranges between: 0.5 - 100 µg L^{-1} for Be and V; 0.1 - 100 µg L^{-1} for Cr, Mn, Co, Sb and Pb; 1 -100 µg L^{-1} for Ni, Cu and Se; 0.05 - 2.5 µg L^{-1} for Hg; 1 - 100 µg L^{-1} for Bi and Rb; 1 - 1000 µg L^{-1} for Sn and Ba; 10 - 1000 µg L^{-1} for B; 5 - 100 µg L^{-1} for Fe; 0.01 - 5 µg L^{-1}

for REEs and Y; 0.1 - 5 μg L^{-1} for Sc and Th. For each calibration curve, the correlation coefficients were always higher than 0.995. For the QC, two standard reference materials including SRM 1570a (spinach leaves) and 1568b (rice flour) from the National Institute of Science and Technology (NIST, Gaithersburg, MD) and food samples spiked with standard solutions were analysed.

6. RESULTS AND DISCUSSION

The results of the 19 TEs analysed in samples are reported in Tables 31 and 32. For the analyses of the results, the concentrations of the elements below the LOQ were considered to be equal to 0.5 LOQ (Menichini and Viviano, 2004).

Regarding eggs, there are not Regulations that set maximum limits for toxic metals. Among the analysed elements, Fe was the most notable trace elements with a mean value of 19.77 mg kg^{-1} wet weight (ww), followed by Zn (15.78 mg kg^{-1} ww) > Rb (3.07 mg kg^{-1} ww) > Ba (1.83 mg kg^{-1} ww) > Cd (0.67 mg kg^{-1} ww) > Cu (0.49 mg kg^{-1} ww) > Mn (0.16 mg kg^{-1} ww) > Se (0.14 mg kg^{-1} ww) > Bi (0.12 mg kg^{-1} ww) > Cr (0.06 mg kg^{-1} ww) > As (0.04 mg kg^{-1} ww) > Sb (0.02 mg kg^{-1} ww) (Fig. 21). Be was always below the LOQ except for the eggs of the Area 1 which contained 0.03 mg kg^{-1} ww of Be. The elements B, Co, Hg, Ni, Pb and V were below the LOQs in all eggs. Similar concentrations were found by Rubio et al. (2018) in home-grown eggs and in another study carried out in Campania region (Esposito et al., 2016). Conversely, the TE concentrations found in this study were lower than those found by Giannenas et al. (2009) except for As. The concentration of As and Cd found in egg samples could have both a geogenic and anthropogenic origin. According to Paone et al. (2001) and Albanese et al. (2007), highest baseline values of As (between 10.2 and 29.3 mg kg^{-1}) were probably correlated to the presence of pyroclastic deposits that cover a large part of the Campania region. Most of the regional territory shows moderate Cd baseline values below 0.43 mg kg^{-1}, which could be due to the siliciclastic deposits outcropping all over the region (Paone et al., 2001; Albanese et al., 2007). However, anthropogenic activities cannot be excluded as potential contamination sources of these elements.

High levels of Fe and Zn were also found in the feed used for the hens raised in the Area 1. According to Rubio et al. (2018), probably the levels of these elements found in the eggs could be due to the type of feeding of hens. However, in addition to feed, many authors consider soil as the main source of metal contamination for

eggs from hens raised outdoors (Waegeneers et al., 2009b; Vicenvica-Gaileet al., 2013; Domingo, 2014; Grace and MacFarlane, 2016).

Table 31
Concentrations of the studied TEs in egg and feed samples from the investigated areas expressed in mg kg^{-1} as wet weight.

Element	Area 1		Area 3	Area 5	Area 6	Area 7	Area 8	Area 9	Area 10
	Eggs	Feed	Eggs	Eggs	Eggs	Eggs	Eggs	Eggs	Eggs
Sb	0.04	< 0.2	0.02	0.03	0.03	0.02	0.02	< 0.2	0.02
As	0.14	0.01	0.04	0.02	< 0.1	0.02	0.06	0.02	0.03
Ba	4.29	0.90	0.87	3.34	1.57	0.99	1.65	0.70	1.21
Be	0.03	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Bi	0.21	0.03	0.12	0.14	0.11	0.11	0.09	0.10	0.10
B	< 10	238.78	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Cd	0.68	< 0.5	0.72	0.74	0.67	0.66	0.61	0.67	0.60
Co	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Cr	0.09	0.30	0.04	0.10	0.06	0.09	0.06	< 0.5	< 0.5
Fe	17.00	111.07	15.47	22.34	18.43	18.97	21.32	23.47	21.16
Mn	0.16	11.11	0.10	0.16	0.12	0.24	0.11	0.12	0.30
Hg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Ni	< 1	0.76	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Pb	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cu	0.45	4.10	0.40	0.64	0.48	0.51	0.40	0.62	0.42
Rb	5.51	5.76	1.45	2.80	2.78	2.16	5.64	1.91	2.27
Se	< 1	< 1	0.20	0.07	< 1	0.22	< 1	0.33	0.18
V	< 0.5	0.09	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Zn	14.93	39.20	13.12	21.80	17.22	22.04	11.89	14.30	10.91

Table 32
Concentrations of the studied TEs in vegetable samples from the investigated areas expressed in mg kg^{-1} as wet weight.

Element	Area 1				Area 2				Area 9	
	Courgette	Aubergine	Potato	Lettuce	Courgette	Aubergine	Potato	Lettuce	Courgette	Potato
Sb	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
As	0.01	< 0.1	< 0.1	0.02	< 0.1	0.02	< 0.1	< 0.1	< 0.1	< 0.1
Ba	0.36	0.20	0.48	1.68	0.59	0.54	0.49	0.44	0.56	0.88
Be	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Bi	0.04	0.05	0.04	0.06	0.05	0.04	0.04	0.04	0.04	0.04
B	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Cd	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Co	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Cr	0.17	0.22	0.09	0.16	0.13	0.04	0.07	< 0.5	< 0.5	0.06
Fe	4.68	5.57	6.33	44.58	9.06	4.93	5.62	11.65	4.80	9.79
Mn	0.90	1.85	1.79	3.17	1.30	0.90	0.86	0.90	1.04	1.60
Hg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Ni	0.04	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Pb	< 0.1	< 0.1	< 0.1	0.06	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cu	0.80	1.07	1.21	0.95	0.98	0.96	1.80	0.41	0.43	1.54
Rb	7.33	4.13	6.45	18.70	3.45	1.58	1.82	1.37	2.90	3.70
Se	< 1	< 1	0.04	< 1	< 1	< 1	< 1	< 1	< 1	< 1
V	< 0.5	< 0.5	< 0.5	0.10	< 0.5	< 0.5	< 0.5	0.03	< 0.5	< 0.5
Zn	2.03	2.08	3.21	4.49	4.23	1.38	1.44	2.10	1.16	4.08

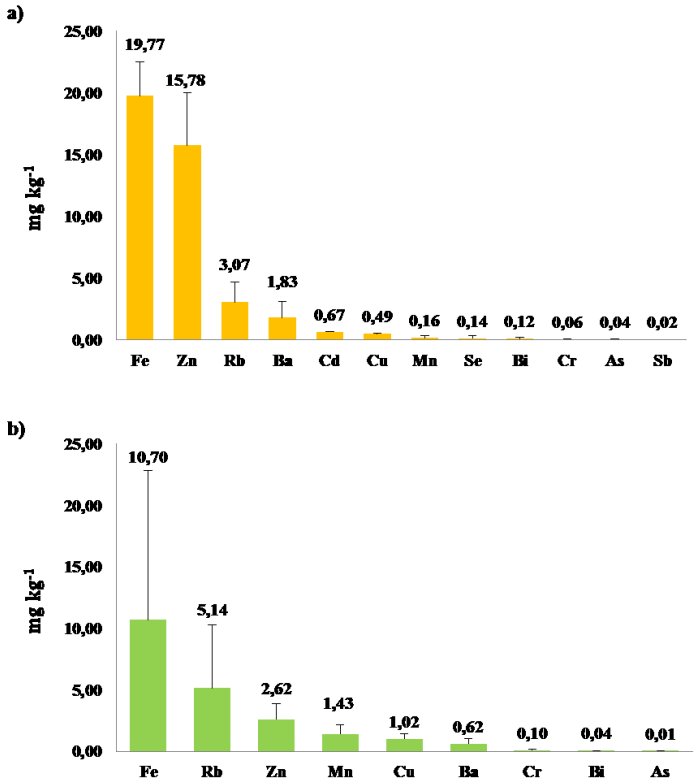


Fig. 21. Mean concentrations and standard deviations of TEs in a) egg samples and b) vegetable samples.

The results of TEs analysis on vegetable samples are reported in Table 32. Unlike eggs, the Commission Regulation (EC) 1881/2006 and the successive amendments, set the maximum limits for Cd and Pb for vegetables. In all samples analysed, the levels of Cd and Pb found were always below the LOQs with the exception of the lettuce from the Area 1 which had a Pb concentration of 0.06 mg kg^{-1} ww that however was complied with the maximum limit (0.30 mg kg^{-1} ww). The most abundant elements found in vegetable samples were Fe with a mean value of 10.70 mg kg^{-1} ww followed by Rb (5.14 mg kg^{-1} ww) > Zn (2.62 mg kg^{-1} ww) > Mn (1.43 mg kg^{-1} ww) > Cu (1.02 mg kg^{-1} ww) > Ba (0.62 mg kg^{-1} ww) > As (0.01 mg kg^{-1} ww). Ni was found only in the courgette of the Area 1 at a concentration of 0.04 mg kg^{-1} ww, Pb only in the lettuce from the

Area 1 at a concentration of 0.06 mg kg^{-1} ww, Se only in the potatoes from the Area 1 at a concentration of 0.04 mg kg^{-1}ww and V was detected only in both lettuce samples from the Area 1 and Area 2 at concentrations of 0.10 mg kg^{-1} ww and 0.03 mg kg^{-1} ww, respectively. Finally, the elements Sb, B, Be, Cd, Co and Hg were below the LOQs in all vegetable samples analysed. The contamination levels of TEs found in vegetable samples were compared with those found by other authors. Hadayat et al., (2018), studying the levels of some heavy metals in 5 different species of vegetables from US, found mean concentrations of As (7.86 µg kg^{-1}), Cr (44.8 µg kg^{-1}), Ba (410 µg kg^{-1}) and Cu (632 µg kg^{-1}) lower than those found in this study; mean concentrations of Cd (9.17 µg kg^{-1}), Pb (12.1 µg kg^{-1}), Co (3.86 µg kg^{-1}) and Ni (58.5 µg kg^{-1}) higher than those found in this study which were below the LOQ; mean concentration of Zn (2528 µg kg^{-1}) comparable to that of this study. In a study of lettuce samples from the Sarno river plain of Campania region, Adamo et al. (2014) found concentrations of Pb (0.05 mg kg^{-1}), and Cr (0.08 mg kg^{-1}) similar to those found in lettuces analysed in this study and concentrations of Cu (1.96 mg kg^{-1}) and Zn (8.45 mg kg^{-1}) higher than those of this study.

The analysis of REEs, Sc, Th and Y in eggs samples showed that all elements were below the LOQs except Th that was found in all eggs with a mean concentration of 0.08 mg kg^{-1} ww and a standard deviation of 0.01. Th is a common element of the lithosphere; its abundance in the earth's crust is about 9.6 mg kg^{-1}, which is slightly less than that of Pb and about 3-4 times higher than that of Uranium (U) (Ragheb, 2016; Soudek et al., 2019). Consequently, the Th levels detected in the analysed egg samples could be due to its natural presence in the environment.

Instead, in the feed sample, the elements Sc, Y, La, Ce, Pr, Nd, Sm and Th were above the LOQs and their concentrations ranged from a minimum of 0.004 mg kg^{-1} ww for Pr and Sm to a maximum of 0.340 mg kg^{-1} ww for Sc (Table 33).

Table 33
Concentrations of REEs, Sc, Th and Y in egg and feed samples from the investigated areas expressed in mg kg^{-1} as wet weight.

Element	Area 1		Area 3	Area 5	Area 6	Area 7	Area 8	Area 9	Area 10
	Eggs	Feed	Eggs	Eggs	Eggs	Eggs	Eggs	Eggs	Eggs
Sc	< 0.20	0.340	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20
Y	< 0.01	0.012	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
La	< 0.01	0.015	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ce	< 0.01	0.038	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pr	< 0.01	0.004	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Nd	< 0.01	0.016	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sm	< 0.01	0.004	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Eu	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gd	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Tb	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dy	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ho	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Er	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Tm	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Yb	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lu	< 0.01	< 0.003	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Th	0.12	0.04	0.09	0.08	0.08	0.08	0.07	0.07	0.08

Finally in vegetables, the elements Sc, Ce and Th were found in all samples with a mean concentration of 0.15 mg kg^{-1} ww, 0.02 mg kg^{-1} ww and 0.04 mg kg^{-1} ww, respectively; while the elements Y, La, Pr, Nd, Sm and Gd were also found in the lettuce samples (Table 34). These results were in agreement with other authors who found higher REE concentrations in food of vegetable origin compared to those found in eggs, with a predominance of the elements Ce, La, Nd, Pr, Sc and Y (Jiang et al., 2012; Li et al., 2013).

Table 34
Concentrations of the REEs, Sc, Th and Y in vegetable samples from the investigated areas expressed in mg kg^{-1} as wet weight.

Element	Area 1				Area 2				Area 9	
	Courgette	Aubergine	Potato	Lettuce	Courgette	Aubergine	Potato	Lettuce	Courgette	Potato
Sc	0.117	0.137	0.156	0.184	0.155	0.120	0.203	0.129	0.164	0.162
Y	< 0.003	< 0.003	< 0.003	0.017	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
La	< 0.003	< 0.003	< 0.003	0.048	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Ce	0.003	0.004	0.004	0.098	0.005	0.003	0.008	0.013	0.005	0.006
Pr	< 0.003	< 0.003	< 0.003	0.010	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Nd	< 0.003	< 0.003	< 0.003	0.035	< 0.003	< 0.003	< 0.003	0.005	< 0.003	< 0.003
Sm	< 0.003	< 0.003	< 0.003	0.008	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Eu	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Gd	< 0.003	< 0.003	< 0.003	0.006	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Tb	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Dy	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Ho	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Er	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Tm	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Yb	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Lu	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Th	0.034	0.037	0.036	0.050	0.039	0.037	0.040	0.039	0.037	0.036

CONCLUSION

The results obtained from this project showed generally low contamination levels of PCDD/Fs and PCBs in eggs and vegetables from the different areas investigated in the Campania region. The congener profiles found in eggs and in soil were very similar and suggesting that the soil should be consider as the main source of the contaminants found in eggs of hens reared outdoors, due to the transfer of pollutants from soil to eggs and hen tissues. This transfer and the subsequent bioaccumulation was caused by the accidental ingestion of soil, plants, insects and worms that occurred when the hens scratched in the yard in search for food. The highest concentrations of PCDD/Fs and PCBs found in the eggs from the Area 1 and Area 2 result from the ingestion of a more contaminated soil. Certainly, the intake of contaminants from soil and their transfer to hens eggs depend on several variables, such as the contamination levels of the soil, the bioavailability of the contaminants and the time spend outdoors by hens pecking in the ground.

Furthermore, the project made it possible to compare the levels of PCDD/Fs and PCBs found in different species of vegetables and in egg samples coming from the same yard. The results obtained showed that, although the vegetables and the eggs were subjected to the same environmental pollution containing the same contaminants at the same concentration and bioavailability, the PCDD/F and PCB levels found in the vegetables were much lower than those found in the eggs. The minor bioaccumulation in the vegetables indicated that the transfer of these contaminants from the soil through the roots and from atmospheric deposition through the leaves is minimal and the exposure time of vegetables is much lower than that of a laying hen.

The study of the congeners profiles showed that the most abundant compounds found in the eggs were 2,3,7,8-TCDF, 1,2,3,4,6,7,8-HpCDD, OCDD, PCB-105, PCB-118, PCB-138, PCB-153, PCB-156, and PCB-180.

OCDD and 1,2,3,4,6,7,8-HpCDD accumulated more in the soil and consequently were found in the eggs. These two congeners were also found in matrices of vegetable origin, with OCDD occurring in 100% of the vegetable samples, and in the feed used for the hens raised in the Area 2. PCB-118, PCB-105 and PCB-156 were the most abundant congeners of PCBs in all the analysed matrices. It should be underlined that the NDL-PCBs congeners pattern found in the eggs and in the soil was very different compared to that found in the vegetables and the feed. PCB-28, PCB-52 and PCB-101, which are the less chlorinated PCBs and the most volatile, predominated in vegetables and in feed, while the eggs and the soil essentially contained PCB-138 and PCB-153.

The *in vivo* study showed that the highest bioaccumulation of PCDD/F and PCB congeners in the eggs was observed for the lower chlorinated PCDD/F congeners and for the higher chlorinated PCB congeners.

The congener 2,3,7,8-TCDD, considered the most toxic dioxin and carcinogenic to humans (Group 1) by the International Agency for Cancer Research (IARC), was found in 37.5% of the samples collected from the different investigated areas and in all egg samples analysed during the *in vivo* study, indicating a high bioaccumulation level.

Moreover, the results of this study also showed that hens raised on soils contaminated by PCDD/Fs and PCBs, even at low concentrations, can accumulate these contaminants above the maximum limits established by the Regulations (EU, 2011) and this could represent a serious risk to human health.

Concerning inorganic contaminants, in general, the results found in this study showed a general low level of TEs in both eggs and vegetable samples with a greater accumulation of Fe and Zn in eggs compared to vegetables. Among vegetables, leaf species, such as lettuce, contained higher TE concentrations than other species. The REE levels found in eggs were always below the LOQs with Th as the only element revealed; while in vegetables, low levels of the elements Sc, Ce and Th were found with elements La, Pr, Nd, Sm and Eu

present only in the lettuce. These results indicate low levels of TEs pollution and no or minimal pollution of REEs in the studied areas.

Appendix I

Table 9
Concentrations of the $^{13}C_{12}$-isotope labelled PCDD/Fs standards.

LABELED COMPOUNDS	CONC. (ng/mL)
$^{13}C_{12}$ 2,3,7,8-TCDD	100
$^{13}C_{12}$ 2,3,7,8-TCDF	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDD	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	100
$^{13}C_{12}$ 2,3,4,7,8-PeCDF	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDD	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDD	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,7,8,9-HxCDF	100
$^{13}C_{12}$ 2,3,4,6,7,8-HxCDF	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDD	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDF	100
$^{13}C_{12}$ 1,2,3,4,7,8,9-HpCDF	100
$^{13}C_{12}$ OCDD	200

Table 10
Concentrations of the $^{13}C_{12}$-isotope labelled DL-PCBs standards.

LABELED COMPOUNDS		CONC. (ng/mL)
PCB-77	$^{13}C_{12}$ 3,3',4,4'-TCB	1.0
PCB-81	$^{13}C_{12}$ 3,4,4',5-TCB	1.0
PCB-105	$^{13}C_{12}$ 2,3,3',4,4'-PeCB	1.0
PCB-114	$^{13}C_{12}$ 2,3,4,4',5-PeCB	1.0
PCB-118	$^{13}C_{12}$ 2,3',4,4',5-PeCB	1.0
PCB-123	$^{13}C_{12}$ 2',3,4,4',5-PeCB	1.0
PCB-126	$^{13}C_{12}$ 3,3',4,4',5-PeCB	1.0
PCB-156	$^{13}C_{12}$ 2,3,3',4,4',5'-HxCB	1.0
PCB-157	$^{13}C_{12}$ 2,3,3',4,4',5'-HxCB	1.0
PCB-167	$^{13}C_{12}$ 2,3',4,4',5,5'-HxCB	1.0
PCB-169	$^{13}C_{12}$ 3,3',4,4',5,5'-HxCB	1.0
PCB-189	$^{13}C_{12}$ 2,2',3,3',4,4',5-HpCB	1.0

Table 11
Concentrations of the $^{13}C_{12}$-isotope labelled NDL-PCBs standards.

LABELED COMPOUNDS		CONC. (µg/mL)
PCL-28	$^{13}C_{12}$ 2,4,4'-TCB	5.0
PCL-52	$^{13}C_{12}$ 2,2',5,5'-TeCB	5.0
PCL-101	$^{13}C_{12}$ 2,2'4,5,5'-PeCB	5.0
PCL-138	$^{13}C_{12}$ 2,2',3,4,4',5'-HeCB	5.0
PCL-153	$^{13}C_{12}$ 2,2',4,4',5,5'-HxCB	5.0
PCL-180	$^{13}C_{12}$ 2.2',3,4,4',5,5'-HpCB	5.0

Table 12
Concentrations of the internal standard solution used for PCDD/Fs and non ortho substituted PCBs analysis.

INTERNAL STANDARD	CONC. (ng/mL)
$^{13}C_{12}$ 1,2,2,4-TCDD	200
$^{13}C_{12}$ 1,2,3,7,8,9-HxCDD	200

Table 13
Concentrations of the internal standard solution used for mono-ortho-substituted PCBs analysis.

INTERNAL STANDARD		CONC. (ng/mL)
PCB-101	$^{13}C_{12}$ 2,2',4,5,5'-PeCB	5.0
PCB-138	$^{13}C_{12}$ 2,2',3,4,4',5'-HxCB	5.0
PCB-194	$^{13}C_{12}$ 2,2',3,3'4,4',5,5'-OCB	5.0

Table 14
Concentrations of the internal standard solution used for NDL-PCBs analysis.

INTERNAL STANDARD		CONC. (ng/mL)
PCB-123	$^{13}C_{12}$ 2',3,4,4',5-PeCB	40

Table 15
Concentrations of PCDD/F standard.

NATIVE COMPOUND	CONC. (ng/mL)
2,3,7,8-TCDD	40
2,3,7,8-TCDF	40
1,2,3,7,8-PeCDD	200
1,2,3,7,8-PeCDF	200
2,3,4,7,8-PeCDF	200
1,2,3,4,7,8-HxCDD	200
1,2,3,6,7,8-HxCDD	200
1,2,3,7,8,9-HxCDD	200
1,2,3,4,7,8-HxCDF	200
1,2,3,6,7,8-HxCDF	200
1,2,3,7,8,9-HxCDF	200
2,3,4,6,7,8-HxCDF	200
1,2,3,4,6,7,8-HpCDD	200
1,2,3,4,6,7,8-HpCDF	200
1,2,3,4,7,8,9-HpCDF	200
OCDD	400
OCDF	400

Table 16
Concentrations of DL-PCB standard.

NATIVE COMPOUND		CONC. (µg/mL)
PCB-77	3,3',4,4'-TCB	2.0
PCB-81	3,4,4',5-TCB	2.0
PCB-105	2,3,3',4,4'-PeCB	2.0
PCB-114	2,3,4,4',5-PeCB	2.0
PCB-118	2,3',4,4',5-PeCB	2.0
PCB-123	2',3,4,4',5-PeCB	2.0
PCB-126	3,3',4,4',5-PeCB	2.0
PCB-156	2,3,3',4,4',5'-HxCB	2.0
PCB-157	2,3,3',4,4',5'-HxCB	2.0
PCB-167	2,3',4,4',5,5'-HxCB	2.0
PCB-169	3,3',4,4',5,5'-HxCB	2.0

Table 17
Concentrations of NDL-PCB standard.

NATIVE COMPOUND		CONC. (µg/mL)
PCB-28	2,4,4'-TCB	10
PCB-52	2,2',5,5'-TeCB	10
PCB-101	2,2'4,5,5'-PeCB	10
PCB-138	2,2',3,4,4',5'-HeCB	10
PCB-153	2,2',4,4',5,5'-HxCB	10
PCB-180	2.2',3,4,4',5,5'-HpCB	10

Table 18
Concentrations in ng/mL of native and labeled PCDD/Fs in the calibration solution.

NATIVE COMPOUNDS	CS 0.2	CS 0.5	CS 1	CS 2	CS 3
2,3,7,8-TCDD	0.1	0.25	0.5	2.0	10.0
2,3,7,8-TCDF	0.1	0.25	0.5	2.0	10.0
1,2,3,7,8-PeCDD	0.5	1.25	2.5	10.0	50.0
1,2,3,7,8-PeCDF	0.5	1.25	2.5	10.0	50.0
2,3,4,7,8-PeCDF	0.5	1.25	2.5	10.0	50.0
1,2,3,4,7,8-HxCDD	0.5	1.25	2.5	10.0	50.0
1,2,3,6,7,8-HxCDD	0.5	1.25	2.5	10.0	50.0
1,2,3,7,8,9-HxCDD	0.5	1.25	2.5	10.0	50.0
1,2,3,4,7,8-HxCDF	0.5	1.25	2.5	10.0	50.0
1,2,3,6,7,8-HxCDF	0.5	1.25	2.5	10.0	50.0
1,2,3,7,8,9-HxCDF	0.5	1.25	2.5	10.0	50.0
2,3,4,6,7,8-HxCDF	0.5	1.25	2.5	10.0	50.0
1,2,3,4,6,7,8-HpCDD	0.5	1.25	2.5	10.0	50.0
1,2,3,4,6,7,8-HpCDF	0.5	1.25	2.5	10.0	50.0
1,2,3,4,7,8,9-HpCDF	0.5	1.25	2.5	10.0	50.0
OCDD	1	2.5	5.0	20.0	100.0
OCDF	1	2.5	5.0	20.0	100.0
LABELED COMPOUNDS	**CS 0.2**	**CS 0.5**	**CS 1**	**CS 2**	**CS 3**
$^{13}C_{12}$ 1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$ 2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$ 2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$ 2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,7,8,9-HxCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$ 2,3,4,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$ OCDD	200	200	200	200	200

Table 19
Concentrations in ng/mL of native and labeled DL-PCBs in the calibration solution.

n.	NATIVE COMPOUNDS	CS 1	CS 2	CS 3	CS 4	CS 5
PCB-77	3,3',4,4'-TCB	1	5	50	400	2000
PCB-81	3,4,4',5-TCB	1	5	50	400	2000
PCB-105	2,3,3',4,4'-PeCB	1	5	50	400	2000
PCB-114	2,3,4,4',5-PeCB	1	5	50	400	2000
PCB-118	2,3',4,4',5-PeCB	1	5	50	400	2000
PCB-123	2',3,4,5',5-PeCB	1	5	50	400	2000
PCB-126	3,3',4,4',5-PeCB	1	5	50	400	2000
PCB-156	2,3,3',4,4',5-HxCB	1	5	50	400	2000
PCB-157	2,3,3',4,4',5'-HxCB	1	5	50	400	2000
PCB-167	2,3',4,4',5,5'-HxCB	1	5	50	400	2000
PCB-169	3,3,4,4',5,5'-HxCB	1	5	50	400	2000
PCB-189	2,2',3,3',4,4',5-HpCB	1	5	50	400	2000
n.	**LABELED COMPOUNDS**	**CS 1**	**CS 2**	**CS 3**	**CS 4**	**CS 5**
PCB-77	$^{13}C_{12}$ 3,3',4,4'-TCB	100	100	100	100	100
PCB-81	$^{13}C_{12}$ 3,4,4',5-TCB	100	100	100	100	100
PCB-105	$^{13}C_{12}$ 2,3,3',4,4'-PeCB	100	100	100	100	100
PCB-114	$^{13}C_{12}$ 2,3,4,4',5-PeCB	100	100	100	100	100
PCB-118	$^{13}C_{12}$ 2,3',4,4',5-PeCB	100	100	100	100	100
PCB-123	$^{13}C_{12}$ 2',3,4,4',5-PeCB	100	100	100	100	100
PCB-126	$^{13}C_{12}$ 3,3',4,4',5-PeCB	100	100	100	100	100
PCB-156	$^{13}C_{12}$ 2,3,3',4,4',5-HxCB	100	100	100	100	100
PCB-157	$^{13}C_{12}$ 2,3,3',4,4',5-HxCB	100	100	100	100	100
PCB-167	$^{13}C_{12}$ 2,3',4,4',5,5'-HxCB	100	100	100	100	100
PCB-169	$^{13}C_{12}$ 3,3',4,4',5,5'-HxCB	100	100	100	100	100
PCB-189	$^{13}C_{12}$ 2,2',3,3',4,4',5-HpCB	100	100	100	100	100

Table 20
Concentrations in ng/mL of native and labeled NDL-PCBs in the calibration solution.

n.	NATIVE COMPOUNDS	MR 1	MR 5	MR 20
PCB-28	2,4,4'-TCB	1	5	20
PCB-52	2,2',5,5'-TeCB	1	5	20
PCB-101	2,2'4,5,5'-PeCB	1	5	20
PCB-138	2,2',3,4,4',5'-HeCB	1	5	20
PCB-153	2,2',4,4',5,5'-HxCB	1	5	20
PCB-180	2.2',3,4,4',5,5'-HpCB	1	5	20
n.	**LABELED COMPOUNDS**	**MR 1**	**MR 5**	**MR 20**
PCB-28	$^{13}C_{12}$ 2,4,4'-TCB	100	100	100
PCB-52	$^{13}C_{12}$ 2,2',5,5'-TeCB	100	100	100
PCB-101	$^{13}C_{12}$ 2,2'4,5,5'-PeCB	100	100	100
PCB-138	$^{13}C_{12}$ 2,2',3,4,4',5'-HeCB	100	100	100
PCB-153	$^{13}C_{12}$ 2,2',4,4',5,5'-HxCB	100	100	100
PCB-180	$^{13}C_{12}$ 2.2',3,4,4',5,5'-HpCB	100	100	100
PCB-123	$^{13}C_{12}$ 2',3,4,4',5-PeCB	100	100	100

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ATTENDED CONGRESSES/WORKSHOPS/SUMMERS SCHOOL/CONTRIBUTION

Fiorito, F., Amoroso, M.G., **Lambiase, S.**, Serpe, F.P., Cioffi, B., Bruno, T., La Nucara, R., Capozzo, D., Maglio, P., Scaramuzzo, A., Galiero, G., Esposito, M., Fusco, G. Correlazione tra contaminanti ambientali e virus enterici in cozze (Mytilus galloprovincialis). VII Convegno Nazionale Società Italiana di Ricerca Applicata alla Molluschicoltura - SIRAM. Portici (NA), 16 - 17 Novembre 2018.

Fiorito, F., Amoroso, M.G., **Lambiase, S.**, Serpe, F.P., Cioffi, B., Bruno, T., La Nucara, R., Capozzo, D., Maglio, P., Scaramuzzo, A., Galiero, G., Esposito, M., Fusco, G. Determinazione di differenti contaminanti ambientali in ostriche (Crassostrea gigas) allevate in stazioni sperimentali della regione Campania. VII Convegno Nazionale Società Italiana di Ricerca Applicata alla Molluschicoltura - SIRAM. Portici (NA), 16 - 17 Novembre 2018

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squalo volpe (Alopias Vulpinus) rinvenuto nel porto di Napoli. XVII Congresso Nazionale della Società Italiana di Diagnostica di Laboratorio Veterinaria - S.I.Di.L.V. 2018. Perugia (PG), 7 - 8 - 9 Novembre 2018

Lambiase, S., Fiorito, F., Serpe, F.P., Maglio, P., Scaramuzzo, A., Trifuoggi, M., Esposito, M. Determination of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in hens eggs. 4th MS Envi Day - Società Chimica Italiana, Divisione di Spettrometria di Massa. Napoli, 1 - 3 Ottobre 2018

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