DEPOSITION OF SELECTED NDL-PCB AND PBDE CONGENERS ON *ZEA MAIS* LEAVES INTENDED FOR SILAGE PRODUCTION: MODELLED CONTRIBUTION TO CONTAMINATION LEVELS IN DAIRY PRODUCTS

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Introduction

Persistent organic pollutants deposition on leaves of high-stem fodders intended for animal nutrition may contribute to raise contamination levels in food of animal origin, above the background levels[1,2]. *Zea mais* cultivars harvested for silage production for dairy animal nutrition, mainly acknowledge such mechanical contamination from air deposition: the 40-50 cm height cut from the soil surface minimizes the potential role of top soil contribution (Figure 1), reported of major relevance for grass pasture and hay[3]. To this purpose, the wide surface of *Zea mais* leaves able to cover in the last two months of the vegetation cycle almost all the agriculture field, may act as deposimeter for the evaluation of the environmental quality parameters of air within a rural context. Along to the leaves contamination, the corn silage-based dietary regimen and the related milk yields of dairy animals, and the Carry-Over Rates (COR) from forage to milk, represent the main determinants to be accounted for the environmental and food safety assessment. Within this frame, Non Dioxin Like – PolyChloroByphenil (NDL-PCB $_{TOT}$ = Σ30 congeners #18, 28, 31, 33, 49, 52, 66, 70, 74, 91, 95, 99, 101, 110, 128, 138, 141, 146, 149, 151, 153, 170, 174, 177, 180, 183, 187, 194, 196, and 203); ICES Σ6 congeners #28, 52, 101, 138, 153, and 180) and selected PolyBromoDiphenylEther congeners (Σ8 PBDE #28, 47, 99, 100, 153, 154, 183 and 209) indicated to monitor contamination levels in food by the European Food Safety Authority[4,5] were analyzed on *Zea mais* leaves sampled from impacted and not impacted agriculture areas, in Italy.

Figure 1. Harvesting technique of corn intended for silage production

Materials and methods

Sampling: Twenty-eight corn fields were selected accounting for the presence/absence of industrial (steel and non-ferrous smelter plants), anthropogenic (municipal waste incinerator, 8-lanes highway, a civil and military airport), emission point sources. The rural background area was selected in the Southern Italy, following the results of an extensive environment&health study on soil, air, water, dairy milk and biomonitoring [6, 7]. From each field, *Zea mais* leaves were collected from plants cultivated in 1 ha, according to a X scheme. Sampling was scheduled on the harvesting time (late August 2012) of corn for silage production. Each fresh leaf was weighted and measured to calculate the surface area, that was then corrected for its projection to the soil.

Plant leaves representative of each field were then rolled to prevent the loss of deposition and placed in a 2.5 L glass jars, previously decontaminated by mean of organic solvents washes. The determination of selected contaminants was performed according to an already described procedure based on the isotope dilution method [5], with small adaptations. After the addition of internal standards (Wellington Labs), depositions were removed from leaves surface by the mean of 3 x 1,000 mL mixture of n Hexane,/Dichloromethane washings, After the acidic attack on H_2SO_4 impregnated Extrelut™ columns, n-Hexane eluates (final volume 1 mL) were purified on 1 g activated basic alumina glass column, by a 2 mL elution with Dichlorometane. After the addition of the keeper, extracts were then reduced to a minimum volume, and redissolved with the injection standard in Nonane (final volume 100 µL). Selected PCBs were quantified by HRGC-LRMS/MS (Thermofisher TSQ Quantum XLS™), on a J&W 60m DB5MS GC capillary column; PBDEs in HRGC-HRMS (Thermofisher DFS™) on a Varian, VF-1 15m GC column. Results were expressed both as ng/g (DM) and on ng/m^2 (LOQs <0.01 ng/g on dry matter (DM) basis for each of the selected congeners). To this purpose, an averaged 30° angle between the corn stem and each leaf was observed, and computed to transpose the absolute leaf surface into its soil projection. Corn silage intake in dairy cow, buffalo, and sheep, and related milk yields recorded at farm level are shown in Table 1. Forage-to-milk Carry Over Rates (COR) of 0.47 for NDL-PCBs and of 0.30 for PBDE # 47 and 99 were derived from the available literature [9, 10, 11]. In this way, estimates of milk contamination for the aforesaid contaminants (ng/g fat basis) were modeled from silage intakes and levels compared with available statistical occurrence descriptors derived from the European Food Safety Authority (EFSA) opinions [4,5], and when available, with occurrence data from dairy farms settled in rural and urban/rural areas.

Table 1. Averaged dairy animals corn silage daily intake and related milk yields

Species	Silage intake kg DM/ head/day	Milk Yield kg/head/day	Fat %	Amount of fat excreted g/day
Dairy cow	8.2	27	3.6	972
Buffalo	5.5	9.5	10	950
Sheep	0.5	1.5	6.0	90

Results and discussion

Statistical Descriptors of the results of NDL-PCB as Σ30 congeners of environmental and as Σ6 congeners of food safety relevance, and of selected Σ7PBDE congeners (ng/g fat basis) on the 28 different corn leaves samples are reported in Table 2. In Table 3, the modelled levels in dairy products recovered from data shown in Tables 1 and 2 are presented, accounting for the above reported forages-to-milk CORs. As term of comparison, the occurrence values inventoried by EFSA are reported.

Table 2. Statistical descriptors (aritmetic mean, 50P, minimum, and maximum) of measured contamination both on ng/g DM and on ng/m^2 basis. on 28 corn leaves samples . Σ6 NDL-PCB values compared with EFSA inventory referred to feed materials of plant origin and with the legislative maximum limit (ML) in place within the EU (EU Regulation 277/2012 /EC), referred to 12% of moisture.

Analytes	Experimental data, N = 28								EFSA		EU
	mean		50P		min		max		mean	50P	ML
	ng/g	ng/m^2	ng/g	ng/m^2	ng/g	ng/m^2	ng/g	ng/m^2	ng/g (12% moisture)		
Σ30 NDL-PCB	1.06	188	0.61	87.3	0.22	36.4	6.64	1,292			
Σ6 NDL-PCB	0.41	72.2	0.23	34.1	0.08	13.4	2.52	491	0.53	0.40	10.0
Ratio% Σ6/ Σ30	38.7	38.4	37.7	39.1	38.6	36.8	37.9	38.0			
Σ8 PBDE	1.97	310	1.50	386	0.06	8.34	6.45	696			
Σ7 PBDE*	0.13	22.0	0.06	8.00	0.02	4.00	0.97	180			
PBDE #47	0.04	7.40	0.02	2.70	<0.01	1.04	0.34	68.2			
PBDE #99	0.06	9.34	0.02	2.92	<0.01	1.34	0.59	85.0			
PBDE #209	1.84	287	1.47	379	0.03	3.41	6.38	689			

*Sum of PBDE congeners #28, 47, 99, 100, 153, 154, and 183.

Uncertainties: the following uncertainties can be considered for discussion. The sampling timing is referred to the end of a very dry summer period, in 2012, in Italy. This probably increased the amount of deposition on corn leaves, due to the scarcity of rainfalls able to wash-out particulate at least from the surface of apical leaves (Figure 1). No occasional sources of emission (i.e. fires, back-yard burning ,...) were recorded around the sampling areas considered. The presence of occasional sources may be of major relevance for leaves contamination, due to the absence of abatment devices for such emissions in presence of POPs ontaminated/generating wastes, and the limited dilution in the air. Analysis: Leaves surface was washed with organic solvents: the extraction was not extended also to the potential contaminants systematically uptaken fom soil and present in the inner part of leaves. Under the study design, the systematic uptake and transfer of considered contaminants to the epigeal part of corn has been considered of negligible relevance. Dairy animals intake: Silage intake and related animal production data were recovered from those farms settled in the sampling area. Seasonal variation (winter/summer) of ± 20% in silage consumption, and milk and fat yields were described: usually, the lower silage intake corresponds to a lower milk yield, thus not affecting in a relevant way the overall mass balance. An averaged COR of 0.47 for NDL-PCB was considered, even single congeners may show differences in the bio-availability. Because of COR rates were recovered from studies carried out on a limited number of animals, an average value was assumed as more robust. Anyway, possible variation could occurr in the forage-to milk transfer, accounting for relevant differences in the PCB profile of the deposition (not considered in this paper). In presence of discrepancies about PBDE 99 COR, under a conservative approach, we assumed the highest reported value of 0.30, that seemed also consistent with the frequency of the determination of such PBDE in milk. To this purpose, PBDE congeners # 47 and 99 were considered only for modeled concentration in milk because their higher bio-availability with respect to other PBDEs, and for the presence of a congener specific occurrence database in dairy milk not heavily affected by the presence of left-censored data.

Table 3. Modeled levels (ng/g fat basis) for Σ6 NDL-PCB and PBDE #47 and 99 in dairy products, under the minimum, median and maximum con silage contamination from deposition, and comparison with reported occurrence data from EFSA

	Cow milk **Min; 50P; Max**	**Buffalo milk** **Min; 50P; Max**	**Sheep milk** **Min; 50P; Max**	**EFSA* Dairy** **50P; mean; 95P**
Σ6 NDL-PCB	0.5; 2.2; 11;	0.3; 1.4; 6.9;	0.3; 1.4; 6.6;	2.6**; 6.7**; 16**
PBDE #47	<0.1; <0.1; 1.4;	<0.1; <0.1; 0.9;	<0.1;<0.1; 0.9;	na; 0.20; na
PBDE #99	<0.1; <0.1; 2.4;	<0.1; <0.1; 1.6;	<0.1; <0.1; 1.5;	na; 0.17; na

*Upper Bound values; ** referred to milk at farm; na = not available

From Table 2, the computed median always is lower than the mean value for the selected contaminants, with the exception of PBDE #209, that represents the most contributing xcongener to the Σ8 PBDE sum. The NDL-PCB and the Σ7 PBDE data clearly indicate the presence of a skewed distribution where the maximum levels recorded belong to corn fields impacted from depositions of a wide industrial/urban settlement, in the Northern Italy (Figure 2). All the four samples drawn from such areas were characterized by values always above the mean contamination. According to the modelled values (Table 3), such urban/rural depositions, alone, are able to raise the background NDL-PCB, and selected PBDE #47 and 99 levels in cow milk well above the 50P and median values of the occurrence distribution reported by EFSA[4,5]. Such estimates do not account for the potential aggregate contribution from top soil and sediments as those arising from the grass/hay intake and from surface water (ponds, lakes, rivers) drinking. Such top soil and water contribution may be of more relevancethan that from silage in free grazing/ extensive farmed herds, such as buffalo and sheep, where silage consumption is reduced, as consequence of the broader time spent on pasture. Owing to the above, the available Σ6 NDL-PCBs and PBDE #47 and 99 background values recorded in buffalo milk (N = 4; <0.25, 0.03 and 0.04 ng/g fat basis, respectively), in sheep milk (N = 5; <0.25, 0.07, and 0.10) from the corresponding corn leaves sampling areas are in good agreement with the modeled values reported in Table 3, thus indicating the overall good environmental status of soil, water and air in the rural area. Such environmental quality is proposed as a target, to support both food safety and food security issues of local production. Because Σ6 NDL-PCB deposition as ng/m^2/day was measured occasionally under air quality monitoring plan, it seemed worthy to convert observed leaves contamination results on gravimetric basis, also in term of ng/m^2. Available data referred to the air quality in the urban/rural impacted area considered (Figure 2), reported NDL-PCB deposition in the range 3 - 14 ng/m^2/day, during the late spring, summer season period that overlapped *Zea mais* cultivation, in the 2012. Such environmental values, under the assumption of a full vegetation cycle of the plant of 60 days, and of a 30% of deposition retained on corn leaves surface, would lead to 60 – 280 ng/m^2 contamination on leaves, in substantial agreement with the found values (Table 2).

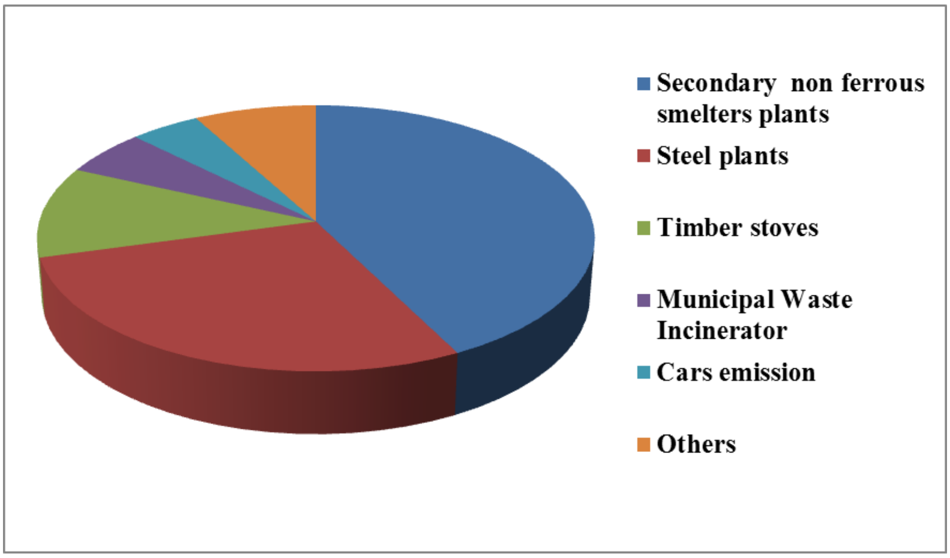

Figure 2. Main sources of micro-pollutants emission in air and their relative contribution inventoried in the impacted urban/rural area considered as worst case for corn leaves sampling.

To conclude, corn leaves may be considered as a bio-deposimeter, of potential relevance for food safety in dairy animals fed on corn silage. The occurrence levels in products of animal origin from impacted area may lead to an increase of the dietary intake of persistent organic pollutants in the urban/rural population. The indication of environmental quality standards able to support local and family farms activities may be envisaged, within the frame of a wider environment&health approach.

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