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Deposition of platinum group elements and polycyclic aromatic hydrocarbons on ryegrass exposed to vehicular traffic

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Abstract – Along highways, platinum group elements (PGE: Pt, Pd and Rh) and polycyclic aromatic hydrocarbons (PAHs) produced by the vehicle traffic can be deposited on grass and soil, leading to a risk of contamination through the food chain via farm animals. We studied the deposition of platinum group elements and polycyclic aromatic hydrocarbons on ryegrass pots exposed along a highway with 65 000 vehicles per day for 90 days in summer and autumn. After this exposure period, the ryegrass pots were transferred to a remote area for 5 weeks. The Pt, Pd and Rh concentrations were determined in ryegrass samples using inductively coupled plasma mass spectrometry (ICP-MS) and PAHs were quantified by high-pressure liquid chromatography (HPLC). Our results show that Pd and Rh concentrations increased with time of exposure. Pd concentrations ranged from 1.5 to 6.8 ng/g dry weight (d.w.), Rh from 1.5 to 3 ng/g d.w. and Pt from 0.3 to 1.4 ng/g d.w. The total PAH concentrations varied from 6 to 175 ng/g d.w. PGE and PAH deposition on ryegrass near the highway occurred mainly for the first weeks of exposure. During the 5 weeks following the exposure, the levels and profiles of PAHs and PGE (except Rh) in highway grasses transferred to a remote area did not significantly decrease, suggesting a potential risk of contamination for dairy ruminants.

Pt / Pd / Rh / PAHs / ryegrass / deposition / vehicular traffic

1. INTRODUCTION

Road traffic is responsible for metallic and organic pollutant emissions that are likely to contaminate the environmental matrices (Colvile et al., 2001). Among these pollutants, this study focused on platinum group elements and polycyclic aromatic hydrocarbons. Platinum group elements are produced from the abrasion of the automotive catalyst washcoat. Platinum (Pt), palladium (Pd) and rhodium (Rh) are used as catalysts to convert toxic gases (carbon monoxide, hydrocarbons and oxides of nitrogen) into water, carbon dioxide and nitrogen (Barefoot, 1999). Several authors have observed a link between the increase in catalyst used in the vehicle industry and the increase in platinum group element concentrations in the environment (Barbante et al., 2001; Djingova et al., 2003). Moreover, Pt concentrations ranging from 1.2 to 30 ng/g have been detected in grass samples collected in the vicinity of highways (Ely et al., 2001; Djingova et al., 2003), whereas the Pt concentration in grass collected far away from all contamination sources was lower than 0.03 ng/g dry weight (Helmers and Mergel, 1998). Studies have demonstrated that plants are able to assimilate Pt, Pd and Rh (Pallas and Jones, 1978; Schäfer et al., 1998). Some platinum compounds are known to be cytotoxic and may have mutagenic and carcinogenic effects (Gebel et al., 1997). Pd and its compounds are known to be potentially allergenic (Castelain and Castelain, 1987). Pd and Rh are known to

be less cytotoxic than Pt (2 times and 30 times, respectively) and have a smaller mutagenic potential (Bunger et al., 1996).

PAHs are produced by incomplete combustion of organic matter at high temperatures (Shabad et al., 1980). Road traffic is one of the main PAH emission sources (Hautela et al., 1995; Duran et al., 2001). Several studies have detected total PAH concentrations ranging from 120 to 950 ng/g in grass samples collected in the vicinity of roads and highways (Bryselbout et al., 2000; Crépineau-Ducoulombier et al., 2003). PAHs are potentially mutagenic and persistent compounds in the environment (Shabad et al., 1980). PGE and PAH emissions due to road traffic represent a potential source of fodder contamination. These compounds may enter into the food chain by massive ingestion of contaminated fodder by dairy ruminants. Indeed, PGE (Crépineau-Ducoulombier et al., 2005) and PAHs (Grova et al., 2000, 2006) may be detected in milk after oral administration of these compounds. The objective of this study is to determine Pt, Pd, Rh and PAH deposition on fodder (ryegrass). Ryegrass pots were exposed along a highway to determine the effects of the exposure duration and the evolution of the pollutant concentrations in the plants after removal from the highway.

2. MATERIALS AND METHODS

2.1. Cultivation of ryegrass plants

To limit the variability of in situ sampling, we used and adapted the biomonitoring method (German VDI 3957)

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based on the monocotyledon *Lolium perenne* (Garrec and Van Haluwyn, 2002) which is currently used to assess organic (Crépineau-Ducoulombier et al., 2004) and metallic (Klump et al., 2002) contaminations in fodder. Furthermore, ryegrass is a fodder widely used in ruminant rations. In a climate chamber maintained under standardized conditions of temperature (15 °C), luminosity (14 h-day/10 h-night) and humidity (60 to 80%), the ryegrass was cultivated in ninety-one pots of 22.5 cm in diameter. Each pot was filled with 5 kg of soil (Castorama, NF U 44-571, 23% organic matter), seeded with 1.35 g of seeds (34 g/m²) and watered with 250 mL of tap water three times a week directly on the soil to avoid any washing off of the grass. One month after seeding, the ryegrass was cut every five days with washed scissors, 8 cm from the soil to stimulate growth. When exposed, the pots were put at ground level to simulate grass in pastures. The ryegrass was 25 cm above the bottom; this was enough to avoid splashes of local soil. Moreover, to avoid any contact with local grass, all vegetation had been previously removed around the pots and was regularly cut during the experiments. Pt, Pd and Rh concentrations in the soil were determined because platinum group element transfer from the soil toward the plant has previously been suspected (Schäfer et al., 1998). The concentrations were 0.25, 2.1 and 18.3 ng/g dry weight, respectively, for Pt, Rh and Pd, which are usual concentrations in soil. For PAHs, soil concentrations were not established because PAH transfer from soil toward the plant is known to be negligible (Wild et al., 1992; Kipopoulou et al., 1999).

2.2. Sampling sites

Two sites were chosen in the same area, “the Plateau Lorrain” (East of France), and were less than 20 km apart: a road site (highway A33) where the daily average traffic was 65 000 vehicles and a remote site: a pasture at the experimental farm of the laboratory (Bouzule) located 700 m from the nearest road.

2.3. Exposure of ryegrass pots on sites

After 4 weeks of cultivation under controlled conditions in a climate chamber, the 84 pots of ryegrass were placed randomly at a distance of 5 m from the verge of the highway A33 on the side of the prevailing wind for 90 days. 7 Pots were not exposed and were used as control and placed in a greenhouse. The sampling frequency was every 15 days (grass was collected from 7 pots). After the 90th day of exposure, the 35 remaining pots of ryegrass were transferred to the remote site (Bouzule) for a further period of 5 weeks (week 1 to week 5). The sampling was then carried out every week (7 pots each week). The grass samples were cut with washed scissors (using distilled water, acetone and hexane) 6 cm from the soil in order to avoid all telluric interference and were immediately wrapped in aluminium paper. Pt, Pd, Rh and PAH concentrations were determined in all exposed ryegrass samples as well as in the control pots of ryegrass.

2.4. Pt, Pd, Rh and PAH analysis in ryegrass

Grass samples were dried for 5 days at 30 °C, in a climate chamber, and ground (Retsch grinding) using a 1.5-mm sieve.

Pt, Pd and Rh extraction and analysis. The extraction and analysis of platinum group elements was performed by the “Laboratoire des Pyrénées” (Lagor, France) using the norm NF EN ISO 17294-2. The dried grass samples were mineralized using a Thermo Electron ETHOS 1600 microwave. 1 g was placed in the digestion container. 2.5 mL of nitric acid 69%, 1 mL of oxygenated water 30% and 5 mL of deionized water were added. After agitation, the mixture was left to rest for about 12 hours. After launching of the digestion program, a progressive rise in temperature up to 160 °C was applied to obtain a landing of mineralization. This landing was maintained for 15 minutes. At the end of the digestion, the mineralized matter was placed in a vial.

Analysis of platinum group elements was done by inductively coupled plasma mass spectrometry (ICP/MS) using a VARIAN 810 UltraMass using internal standards: Iridium 194 for Pt (194–195), and Inadium 115 for Pd (106–108) and for Rh (104–105). The evaluation of the method was carried out following the norm XPT 90–210. For the precision of the analysis, five standard curves were used, validated by a Fisher test. Quantification limits are 0.25 ng/g dry weight for Pt and Rh, and 0.5 ng/g dry weight for Pd.

PAH extraction and analysis. PAH extraction and analysis was performed at UR AFPA-INPL (Nancy, France). PAH extraction was carried out on dried grass using the procedure described by Dugay et al. (2002). Two grams of grass were mixed with 50 mL toluene and placed in an ultrasound bath (SM35E-DTH, Branson) for 2 hours. After filtration (Cat No 1820150, Whatman), the solution was evaporated to dryness at 40 °C, and the residue saponified for 30 min at 60 °C with 50 mL of an 80% (v/v) methanol-water solution containing 1M KOH. The saponified solution was cleaned up by solid-phase extraction using OASIS HLB cartridges. After the solution was deposited, the cartridges were washed with 10 mL of water and the PAHs were eluted with 8 mL of methylene chloride. The extract containing PAHs was dried with anhydrous Na₂SO₄ and evaporated to dryness under nitrogen using a dry bath (DB 3 Techne), and the residue was dissolved in 1 mL acetonitrile. The solution was stored overnight at –20 °C, before HPLC analysis.

The HPLC system included a controller (Waters 600), an autosampler (Waters 717 plus) equipped with a 20-μL injection loop, a column oven (600 treater), a fluorescence detector (Waters 2475) and data-processing software (Millennium 32). HPLC analysis was carried out using a Vydac 201 TP 54 column (250 × 4.6 mm ID, particle size 5 μm) with a gradient elution program using H₂O and CH₃CN. Detection was performed by fluorimetry. All solvents were analytical- or HPLC-grade reagents. The KOH and anhydrous Na₂SO₄ were p.a. grade. Glassware was baked out at 500 °C or solvent rinsed with toluene prior to use. The PAH 610 polynuclear aromatic hydrocarbons mix was obtained from Interchim (M-610-QC).

The mobile phase comprised acetonitrile (A) and ultrapure water (B). Gradient elution conditions were as

follows: 0–20 min, linear gradient 40% A–80% A; 20–30 min, 80% A isocratic; 30–40 min, linear gradient 80% A–95% A; 40–50 min, 95% A isocratic. The flow rate was 1 mL·min⁻¹. The excitation and emission wavelength program for PAH detection varied, respectively, from 250 to 295 nm and from 355 to 420 nm. PAH calibration curves were performed by diluting a standard mixture (M-610-QC, Interchim) containing the 16 PAHs of the United States Environmental Protection Agency (US-EPA). The extraction yield varied from 35 to 111% according to the PAH. The final result was expressed in ng/g dry weight and the quantification limit was 0.5 ng/g dry weight.

2.5. Statistical analysis

Analysis of variance using the SAS statistical software General Linear Model (GLM) procedure was applied to the PGE and PAH concentrations at the various exposure times at a significance level of 5%. Data is presented as mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1. Platinum group element deposition on ryegrass

Figure 1 shows the Pt, Pd and Rh concentration evolution in the ryegrass during the exposure to the highway, from day 0 to day 90, and after the removal of the pots and placement in the remote pasture, from week 1 to week 5. The exposure of the ryegrass pots to the highway led to an increase in all studied compounds. Pd and Rh concentrations were found to increase significantly with time of exposure. Pd concentrations were also higher than those of Rh and Pt. Pd concentrations ranged from 1.5 to 6.8 ng/g dry weight while Rh concentrations were found between 1.6 and 3 ng/g dry weight and Pt concentrations ranged from 0.3 to 1.4 ng/g dry weight.

The concentrations of Pd and Pt shown in Figure 1a, c suggest a two-step deposition process. During a first step from day 0 to day 8, Pd and Pt concentrations increased rapidly by a factor of 3; then during a second step from day 8 to day 90, concentrations varied by a factor lower than 2. For the Rh concentrations shown in Figure 1b the variation factor between the exposed and unexposed ryegrass was low (<2). Figures 1a, c also indicate an absence of decrease in Pd and Pt concentrations when the pots were placed at the remote site. For Rh, concentrations were found to be reduced when placed at the remote site (Fig. 1b). This observation suggests that these compounds are not only found on grass surfaces but probably are adsorbed in the cuticular waxes or penetrate the plant's internal tissues. Pt and Pd adhesion to plant tissue appears to be strong, although their behavior is still unknown. In order to determine the way the PGE were fixed to the plant tissues, we treated contaminated ryegrass plants with chloroform, according to Garrec and Renard (1996). After this treatment, more than 50% of PGE remained fixed to the ryegrass. This result contributes to explain why the concentrations did not decrease

considerably even when the contaminated ryegrass samples were placed far away from the highway.

The platinum group element distribution was found to be different between exposed ryegrass and unexposed ryegrass. In unexposed ryegrass at day 0 the Pd concentration of 1.5 ng/g d.w., and that of Rh of 1.6 ng/g d.w. were similar, and higher than the Pt concentration of 0.3 ng/g d.w. In the exposed ryegrass from day 8 to day 90, Pd concentrations increased from 4.5 to 6.8 ng/g d.w. These Pd concentrations were found to be much higher than Rh or Pt concentrations, which also increased from, respectively, 1.6 to 3.1 ng/g d.w. and 0.7 to 1.5 ng/g d.w. in the same period. Thus, platinum group element distribution changed greatly during exposure: when compared with Pt and Rh, Pd turned out to be the major compound and represented about 60% at day 90. At the remote site, from week 1 to week 5, the Pd concentrations of 4 to 4.7 ng/g d.w. always remained higher than Pt concentrations (0.6 to 1.1 ng/g d.w.) and Rh concentrations (0.7 to 1 ng/g d.w.) which were both similar. These results revealed a higher accumulation of Pd than Pt or Rh. This higher Pd concentration did not follow the tendency of PGE emissions by vehicles. Indeed, some studies (Moldovan et al., 1999; Rauch et al., 2002) showed that vehicles emitted more Pt than Pd on roads. For example, emission values for a diesel vehicle were: 223 ng Pt/km, 75.8 ng Pd/km and 33.7 ng Rh/km (Rauch et al., 2002). The highest Pd concentrations could be explained by the properties of the Pd compounds which are more soluble and more mobile in the environment than Pt and Rh compounds (Moldovan et al., 2001) and therefore more bioavailable for plants.

The results of platinum group element analysis also showed a non-negligible concentration of Pt, Pd and Rh in unexposed ryegrass, suggesting plant contamination by soil. Platinum group element transfer from soil towards the plant was previously observed by Schäfer et al. (1998) on various plants (spinach, cress, phacelia and stinging nettle). When applying the platinum group element soil to plant transfer coefficient suggested by Schäfer et al. (1998), we concluded that this transfer seems to be negligible in our study.

3.2. PAH deposition on ryegrass

The total PAH concentrations varied from 6 ng/g d.w. at day 0 to 175 ng/g d.w. at day 90 (Fig. 2). Two phases could be distinguished: a first phase corresponding to the first two weeks of exposure with concentrations increasing significantly by a factor of 23; and a second phase from day 15 to day 90 with the concentrations remaining similar. This result suggests that ryegrass behaves like a bioaccumulator species. Indeed, when ryegrass is exposed to a pollutant, the concentration increases until the air – plant equilibrium is reached (Bakker et al., 2001a; Thomas et al., 1998). For ryegrass (*Lolium perenne*), this study indicates that in situ conditions the air-plant equilibrium seems to be reached after 15 days of exposure for PAHs. We know the time to obtain equilibrium varies according to the plant species: 24 to 240 seconds for *Citrus* and 58 to 580 days for *Ilex* (Bakker et al., 2001a). The total

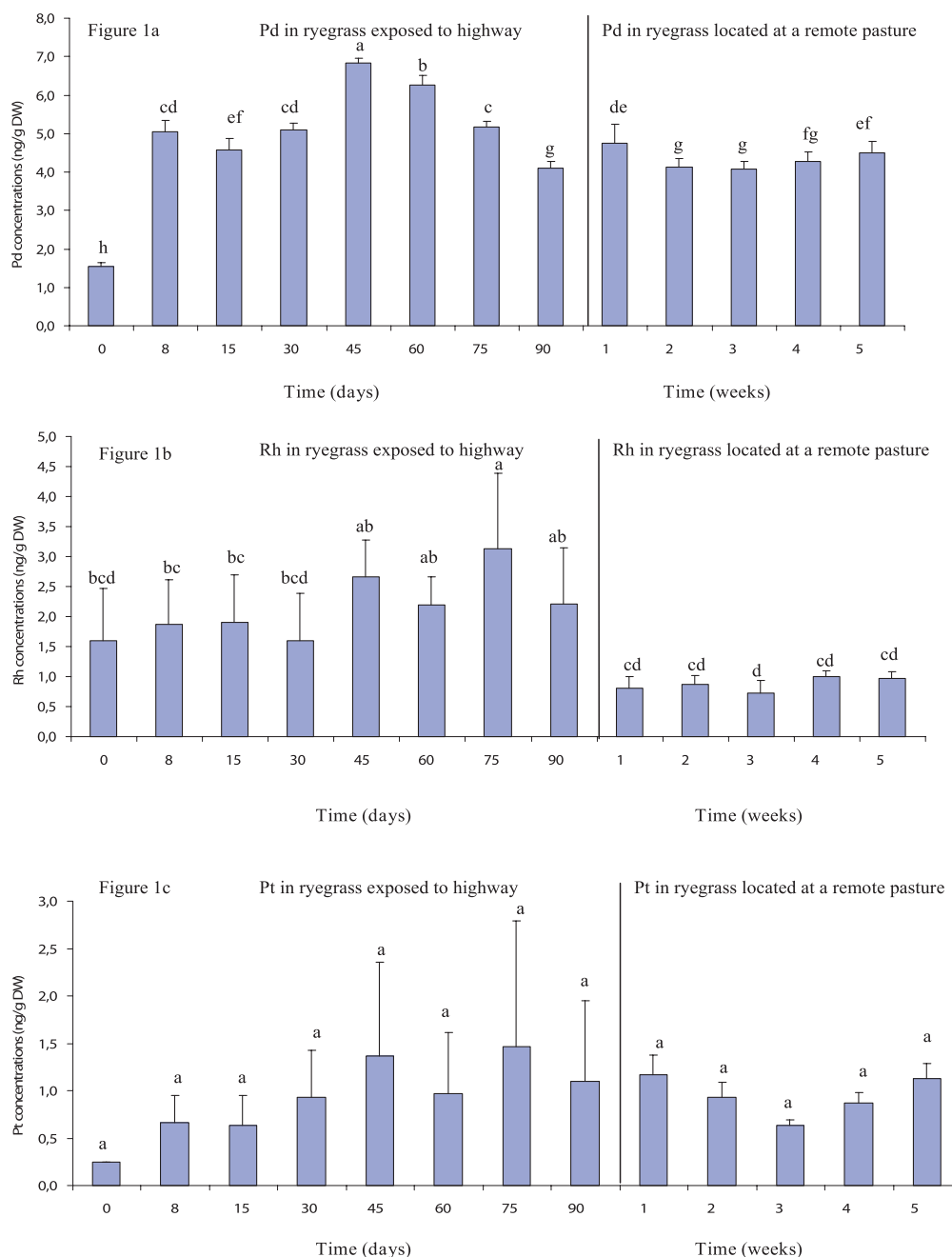


Figure 1. Pd, Rh and Pt concentration evolution in ryegrass exposed along the highway (day 0 to day 90) and in the remote pasture (week 1 to week 5). DW: dry weight; Pd: (a–h), Rh: (a–d), Pt: (a) for each figure, mean results without a common letter differ significantly, $P < 0.05$; the vertical bars correspond to the standard deviation.

PAH concentrations at the end of the exposure period (day 90) were similar to the total PAH concentrations detected at the remote site (week 1 to week 5). This absence of decrease shows that PAHs are strongly fixed to the ryegrass. This result is in accordance with Bakker et al. (2001b), who demonstrated on two species, *Plantago major* and *Plantago media*, that PAHs are fixed in cuticular waxes and in the internal tissues of the plant leaves.

The PAH distributions shown in Figure 3 were different between the unexposed (day 0) and exposed ryegrass (day 8 to day 90). Only 3 compounds were detected in the unexposed ryegrass: phenanthrene, fluoranthene and pyrene, whereas 12 PAHs were detected in the exposed one. The concentrations of these 3 compounds were found to be lower in the unexposed ryegrass compared with the exposed ryegrass; because of their ubiquitous character and their strong volatility, they

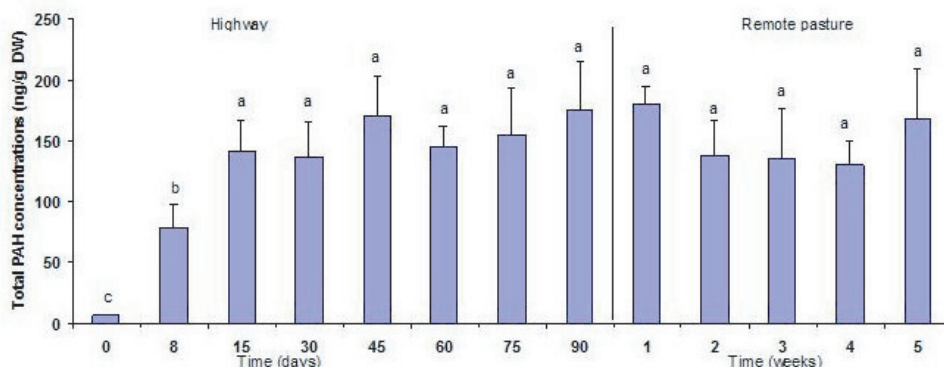


Figure 2. Total PAH concentration evolution in ryegrass exposed along the highway (day 0 to day 90) and in a remote pasture (week 1 to week 5). DW: dry weight; (a, b, c); mean results without a common letter differ significantly, $P < 0.05$; the vertical bars correspond to the standard deviation.

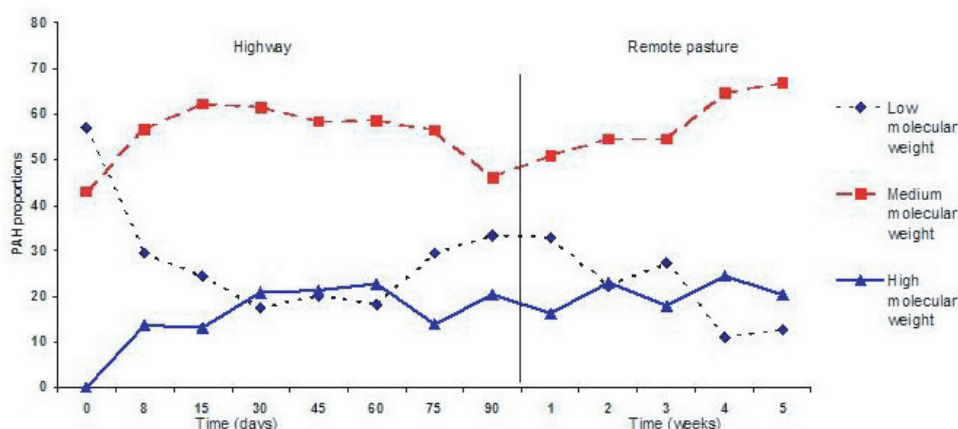


Figure 3. PAH distribution evolution in the ryegrass exposed along the highway (day 0 to day 90) and in a remote pasture (week 1 to week 5). Low molecular weight (PAHs of 2 to 3 rings: acenaphthene, phenanthrene, anthracene); medium molecular weight (PAHs of 4 rings: fluoranthene, pyrene, benzo(a)anthracene, chrysene); high molecular weight (PAHs of 5 rings or more: benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene).

have even been detected in plants cultivated inside greenhouses, but their concentrations were quite low (Bakker et al., 2001b; Crépineau-Ducoulombier et al., 2004). For each time of exposure, these 3 PAHs were the main compounds of all ryegrass samples.

Eight days after the exposure, the high molecular weight PAHs (5 and 6 rings) were detected and represented almost 23% of the total PAH concentrations (Fig. 3). For the whole exposure period (day 8 to day 90), PAH profiles were similar: 15 to 20% for high molecular weight PAHs (5 and 6 rings), 17 to 33% for low molecular PAHs (2 to 3 rings) and between 46 and 62% for intermediate PAHs (4 rings). At the remote site, the ryegrass presented the same PAH profile from the first to the fifth week. This profile was not different from the PAH profile during the exposure to the highway. The detection of high molecular weight PAHs, potentially more toxic, in exposed ryegrass at a non-negligible level demonstrates a possible contamination of fodder produced close to highways (Bryselbout et al., 2000; Crépineau-Ducoulombier et al., 2004).

4. CONCLUSION

Platinum group element and PAH deposition on ryegrass in the vicinity of a highway indicated that deposition mainly occurs for the first weeks of exposure to the average values of 8 ng/g for platinum group elements and 175 ng/g for PAHs. The platinum group element profile changed greatly during exposure: when compared with Pt and Rh, Pd turned out to be the major compound and represented about 60% at day 90. For PAHs, the compounds including 5 rings or more (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene), and potentially more harmful, were detected only in exposed ryegrass. The absence of decrease during the 5 weeks following exposure revealed that the contaminants are strongly fixed to the ryegrass. Further investigations are necessary to characterize this fixing phenomenon better. The rapid contamination of the ryegrass during its exposure to the highway as well as the maintenance of platinum group elements and PAHs after its removal and placement in the remote pasture show the

exposure risk of fodder cultivated near roads. Considering that the dairy ruminant does not graze exclusively near highways, we will carry out further studies to determine the Pt, Pd, Rh and PAH contamination levels on a whole fodder plot scale.

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