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Original article

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Abstract – The contamination of pastures by polycyclic aromatic hydrocarbons (PAHs) from vehicular emissions is the first step of the contamination of the food chain including the grazing ruminants producing dairy food. In this study, we measured PAH concentrations in grass exposed for 30 days and for 75 days using a standardised culture of *Lolium perenne*. This method allowed the comparison of the grass contamination between two sites of different daily traffic (highway and rural road) and two control sites: isolated pasture and climate chamber. The results showed that total PAH concentrations ranged from 18 ng/g DW to 414 ng/g DW (DW: dry weight). The highest concentration was detected along the rural road and appeared not to be directly linked to the vehicular flow but probably to the driving cycles of the vehicles. The PAH concentrations were not found to be time-dependent as the values remained similar for the ryegrass exposed for 30 days or 75 days.

Polycyclic Aromatic Hydrocarbons / pasture / *Lolium Perenne* / vehicular traffic

1. INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are known to be potentially mutagenic compounds and are widely present in ecosystems [17]. They are produced by incomplete combustion and one of the main emission sources is vehicular exhausts, which are a major contributor to air pollution [6, 9, 10]. The Commission of the European Communities proposed in 2003 a directive providing the framework of legislation on air quality (COM(2003)423). PAHs are part of this document but the Commission underlines the lack of comparable and consistent references.

The increase in road traffic in Eastern Europe has raised concerns about an increase in air pollution, and consequently we need investigations on the contamination of grass growing in pastures located by roads. Lactating ruminants may graze this potentially contaminated grass, thus PAHs could be transferred through the food chain towards humans via dairy products [8]. Several authors have reported PAH contamination in the vicinity of roads in different matrices (various vegetation and soils) [3, 5, 19] but little is known about the effect of traffic density and of long-term seasonal exposure on deposition and accumulation of PAHs into fodder.

The objective of our work is to assess contamination levels of pastures by PAHs. The grass contamination occurs by the deposition of these pollutants on surface grass in an atmospheric way: the particulate or gaseous phase. These compounds enter the food chain fodder-ruminant-milk by the massive ingestion of the animals: a lactating cow ingests 15 kg of grass (dry weight) a day. This ingestion shows that in terms of food safety the level of concentration in PAHs in the pastures is a real concern since the lipophilic characteristics of the compounds lead them to accumulate in milk. In this study we are focusing on the variation factors of this pollution and especially the source effect on pollutant deposition. A bio-monitoring method (German VDI 3957) with the bioaccumulator ryegrass (*Lolium perenne)* was used to simulate the growing ryegrass seeded in pastures along roads. Our purpose is therefore to characterise the contamination of a typical fodder exposed to PAH emission sources. In this study, the bio-monitoring method limits the inter-species variability of the sampling of the in situ growing grass and allows more comparison between sites. Since the deposition pathway is a function of the ambient temperature, the compounds' volatility and plant characteristics [1, 18] our aim is to identify an in situ contamination with plants never exposed to any atmospheric pollution.

2. MATERIALS AND METHODS

To limit the variability of the in situ sampling in pastures, we used and adapted to our aims the biomonitoring method (German VDI 3957) based on the monocotyledon *Lolium perenne* [7]. Forty-eight pots of ryegrass were cultivated under standardised conditions of temperature (15 °C), luminosity (14 h-day/10 h-night) and humidity (60%) in a climate chamber. Each pot of ryegrass was filled with 5 kg of soil (NF U 44-571, 23% organic matter), 1.35 g of seeds (34 g/m^2) and was watered with 250 ml of tap water three times a week, directly

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on the soil to avoid any washing off of the grass. Although previous findings have demonstrated that the plant up-take of PAHs from soil is negligible for plant contamination [12] the soil total PAHs was checked before the seeding and revealed usual levels of PAHs [19]. One month after the seeding, the ryegrass was cut every five days with washed scissors 8 cm from the soil to stimulate the growth. When exposed, the pots were put at ground level to simulate the grass in the pastures. However, the ryegrass was 25 cm from the bottom of the pot, which is enough to avoid splashes of local soil. Moreover, to avoid any contact with local grass, all vegetation has been previously removed around the pots and was regularly cut during the experiments.

2.1. Sampling sites

Four sites were chosen in the East of France (Lorraine) for the exposure of the ryegrass pots. Two roads were selected according to the monitored traffic: a rural highway (A31) where the daily average traffic is 57 000 vehicles and a major rural road (N74) where the daily average traffic is 7 000 vehicles. No industrial or urban sites are located less than 10 km from the chosen sites, limiting the pollutant deposition from other sources than the roads. To limit the variability in the climatic measurements or the environmental parameters, the two sites were chosen in the same area ("Plateau Lorrain") and are less than 10 km apart. The environmental local conditions were checked before the exposure: temperature, rainfall and hygrometry were similar. Two control sites were also selected: an isolated pasture located 700 m away from any road and the climate chamber where the ryegrass was seeded, and which is a hermetically closed place. The outside ambient air is filtered before entering the chambers. Twelve pots were placed downwind in March 5 m from the verge of each road, directly on the soil to simulate conditions of pasture. Twelve pots were, respectively, placed at the control sites in the pasture and in the climate chamber.

2.2. Time of exposure

The first sampling was carried out in April, 30 days after exposure (six pots at each site). This period corresponds to the first period of grazing for lactating cows in spring. The second sampling was carried out at the end of May (six pots at each site) and corresponds to the hay-making season. The harvest was carried out two days after the last rainfall to reduce immediate effects of this parameter. The grass was cut with prewashed scissors (using distilled water, acetone and hexane) 6 cm from the soil. The samples from each site were pooled to obtain two representative samples for each site and for each time of exposure. After sampling, the cut grass was dried for 5 days in a climate chamber (20 °C, 60–80% hygrometry) before being ground.

2.3. PAH analysis

Detailed extraction procedures are described in the NF ISO 15302 method and were conducted by Micropolluant Technologie Ltd (Thionville, France). PAHs were quantified by gas chromatography coupled to mass spectrometry in the single ion monitoring mode, using internal deuterated standards: naphthalene-d_8 (*m/z* 136) for naphthalene, acenaphtene-d_{10} (*m/z* 164) for acenaphthene, acenaphtylene and fluorene, phenanthrene-d_{10} (m/z 188) for phenanthrene, anthracene, fluoranthene and pyrene, chrysene-d_{12} (*m/z* 240) for chrysene and benzo(a)anthracene and perylene-d_{12} (*m/z* 264) for perylene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene. The analytical detection level was 0.5 ng PAH g^{-1} grass. Losses of analytes were quantified by scientists from Micropolluant Technologie: 40–70% for acenaphtylene to fluorene and 0–30% for the heaviest compounds.

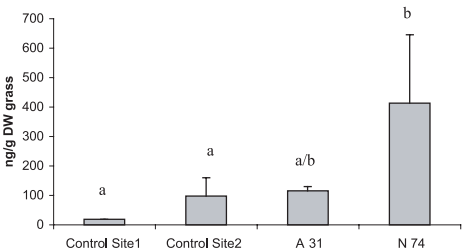

Figure 1. Total PAHs concentrations in *Lolium perenne* according to the different sites of exposure (control site 1: climate chamber, control site 2: remote pasture, A31: highway, N74: rural road). DW: dry weight. (a,b): mean values with a different letter differ signifcantly ($P > 0.05$).

2.4. Statistical analysis

Analysis of variance using the SAS statistical software General Linear Model (GLM) procedure was applied to the total PAH concentrations at the various sites. The student's t-test was used for comparison of the means at a significant level of 5%. Data are presented as mean + standard deviation.

3. RESULTS AND DISCUSSION

3.1. Total PAH concentrations at the various sites

The statistical analyses demonstrated that a significant difference exists between PAH concentrations from the control site 1 (climate chamber), the control site 2 (isolated pasture) and the verge of the N74 road. The total concentration ranged from 18 ng/g DW to 414 ng/g DW (Fig. 1). These findings complete previous studies of local grass growing along major roads [14]. The concentration in the pots of ryegrass located at the highway site (A31) was not different from the control sites, or the road site, suggesting that the increase in traffic is not the main factor affecting the deposition of PAHs on pastures. The most polluted grass was located along the verge of the rural road (N74) with 7 000 vehicles per day, versus 57 000 vehicles per day on the highway. Nevertheless, when the pots were away from any road contamination, the PAH compounds were poorly detected in the vegetal matrix. The difference between the concentration in the grass from the climate chamber and the grass from the N74 site is a factor of 26. This confirms that PAH contamination of fodder occurs when it is located near roads [5, 10, 16,

Table I. PAH profiles in *Lolium perenne* according to the different sites: A31: highway, N74: rural road, Control site 2: remote pasture, control site 1: climate chamber. PAH concentrations are given as mean values ± standard deviations and expressed in ng/g (dry matter); *nd: not detected.

Compounds	Aromatic rings	A31	N74	Control site 2	Control site 1
Naphtalene	2	8.68 ± 7.7	18.81 ± 33.6	10.70 ± 10.6	6.09
Acenaphtylene	3	nd*	1.26 ± 2.5	nd	nd
Acenaphtene	3	nd	1.10 ± 1.3	nd	nd
Fluorene	3	0.91 ± 1.8	3.28 ± 2.3	nd	nd
Phenanthrene	3	30.59 ± 10.5	47.79 ± 26.3	34.54 ± 20.8	7.52
Antracene	3	1.89 ± 2.2	1.85 ± 2.3	2.15 ± 3	nd
Fluoranthene	4	21.61 ± 4.1	63.13 ± 31.6	18.22 ± 9.9	nd
Pyrene	4	29.65 ± 13.8	74.23 ± 44.8	31.62 ± 24.1	5.32
Benzo(a)antracene	4	2.09 ± 1.5	14.25 ± 9.3	nd	nd
Chrysene	5	5.31 ± 3.6	22.21 ± 11	nd	nd
Benzo(b)fluoranthene	5	nd	44.20 ± 51.1	nd	nd
Benzo(k)fluoranthene	5	nd	17.02 ± 21	nd	nd
Benzo(e)pyrene	5	nd	12.66 ± 16.9	nd	nd
Benzo(a)pyrene	5	nd	22.59 ± 33.2	nd	nd
Indenol(1,2,3-cd)pyrene	5	4.27 ± 4	31.83 ± 29.8	nd	nd
Dibenzo(a,h)antracene	5	nd	2.64 ± 5.3	nd	nd
Benzo(g,h,i)perylene	6	10.65 ± 7.9	34.77 ± 25.3	nd	nd

17] and that it is not related to the number of vehicles but probably linked to the properties of the vehicular emissions and especially the driving cycles (speed, hot or cold start of the vehicles). Previous studies have reported that the PAH concentration was found to decrease as cylinder exhausts' temperature increases [11] and that the driving cycle has a significant impact on the distribution of the emitted polycyclic aromatic hydrocarbons. The PAH emissions are lowered when the cars have a higher speed [4].

3.2. Total PAH concentrations according to the time of exposure

The pots of ryegrass exposed for 75 days did not present a significantly higher total PAH concentration than the pots exposed for 30 days. At the highway site and at the control site 1 the concentrations remained similar at 30 days and 75 days; respectively, 103 ng/g DW and 128 ng/g DW and 19 and 18 ng/g DW. At the rural road and the control site 2 the increase was not significant; respectively, 366 to 430 ng/g DW and 53 to 141 ng/g DW.

The contamination of pastures is constant during a period of one and a half months, during the hay-making season. This result is of great concern for assessing pasture contamination and risks of transfer of PAHs through the food chain via grazing lactating cows. This suggests that the environmental variables such as vapour pressure, temperature and relative humidity, responsible for deposition and the loss processes (revolatilisation, biodegradation, photodegradation, wash-off and windblow) were established before the end of the first month. We need further investigations to know if this total concentration varies significantly during a shorter time of exposure, to assess when the ruminants are the most exposed to this contamination.

3.3. PAHs at the various sites

The analyses of ryegrass revealed different profiles at the various sites, leading to a difference in contamination according to the vehicular emissions. At the two control sites no high molecular weight compounds were detected in the ryegrass, nor any compounds with more than four aromatic rings (Tab. I). The carcinogenic compounds (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene and indenol[1,2,3,c-d]pyrene, according to the US-EPA) were not detected in this matrix. This result is in agreement with other studies using samples composed of local grass. They demonstrated that high molecular weight compounds are associated with particulate matter and are not transported long distances away from the sources [3]. Moreover, the contamination of ryegrass at the control sites was mainly due to four compounds: naphtalene, phenanthrene, fluoranthene and pyrene, which are known to present a low toxicity (TEF of 0.001) [15] and to be stable under photodegradation [13]. The profiles detected at the road site were not similar. Every high molecular weight and five-ring compounds were detected in concentrations ranging from about 3 ng/g DW (dibenzo(a,h)anthracene) to 44 ng/g DW (benzo(b)fluoranthene). These compounds represented about 50% of the total PAH concentration. At the highway site, only four compounds of more than four rings were detected (benzo(a)anthracene, chrysene, indenol[1,2,3-c]pyrene and benzo(g,h,i)perylene) and they represented only 5% of the total concentration. The

detection of these compounds was explained by their behaviour in ambient air: they are mainly detected in the particular phase. As we know that plant uptake of the PAHs is mainly controlled by equilibrium partitioning or particle-bound deposition, this result is in agreement with other findings [2]. The profiles detected in the ryegrass revealed that the contamination of fodder is not similar according to the sites. This result suggests a different level of contaminants ingested by dairy cows grazing on pastures located in these different kinds of sites. The contamination of fodder is traffic-related: the levels of contamination at the control sites are very low, but it is not proportional to the increase in traffic. The compounds detected at the road site (lower traffic than at the highway site) are numerous and present higher risks of toxicity, carcinogenic compounds being detected in the ryegrass pots.

3.4. PAHs according to the time of exposure

Our study did not show any significant result for the evolution of the compound concentrations. Nevertheless, some results can be outlined before carrying out further inquiries. At each site, anthracene and fluorene were no longer detected after 75 days of exposure. The high molecular weight compounds were still detected at the N74 site with a slight decrease in concentrations, while naphtalene, phenanthrene, pyrene and fluoranthene showed a slight increase in concentrations. During the exposure, no rain was registered, so we can suggest that the decrease in the compounds is due mainly to wind or sunlight. Pyrene, fluoranthene and phenanthrene are known to remain stable under photodegradation while benzo(a)pyrene, benzo(e)pyrene and indenol[1,2,3-c]pyrene are degraded more rapidly by sunlight [13].

4. CONCLUSION

The use of *Lolium perenne* appears to be suitable for simulating PAH contaminations of pastures. Indeed, this method allows a real comparison of grass collected at different sites. This study demonstrates that PAH contamination of grass from pastures can be detected with a value of 400 ng/g DW in the vicinity of a typical rural road (daily traffic of 7 000 vehicles). This result means that a grazing ruminant could ingest about 6–8 mg PAHs per day. Since such an exposure level is not negligible, it would be of great interest to analyse the "feed to milk" transfer ratio of these pollutants. This study also reveals a lower impact of the highway on grass pollution when compared with the contamination levels observed near the rural road. The explanations of this apparently contradictory result is probably related to the properties of the PAH vehicular emissions. Concerning the time of exposure, PAH concentrations in ryegrass did not increase. Thus PAH levels are not time-dependent and seem to reach a saturation level. Further investigations are necessary to better characterise these phenomena and to be more representative by increasing the number of repetitions.

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