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Analysis of fullerenes in soils samples collected in The Netherlands[☆]



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ABSTRACT

Fullerenes are carbon based nanoparticles that may enter the environment as a consequence of both natural processes and human activities. Although little is known about the presence of these chemicals in the environment, recent studies suggested that soil may act as a sink. The aim of the present work was to investigate the presence of fullerenes in soils collected in The Netherlands. Samples ($n = 91$) were taken from 6 locations and analyzed using a new developed LC-QTOF-MS method. The locations included highly trafficked and industrialized as well as urban and natural areas. In general, C₆₀ was the most abundant fullerene found in the environment, detected in almost a half of the samples and at concentrations in the range of ng/kg. Other fullerenes such as C₇₀ and an unknown structure containing a C₆₀ cage were detected to a lower extent. The highest concentrations were found in the proximity of combustion sites such as a coal power plant and an incinerator, suggesting that the nanoparticles were unintentionally produced during combustions processes and reached the soil through atmospheric deposition. Consistent with other recent studies, these results show that fullerenes are widely present in the environment and that the main route for their entrance may be due to human activities. These data will be helpful in the understanding of the distribution of fullerenes in the environment and for the study of their behavior and fate in soil.

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1. Introduction

Fullerenes are carbon based nanomaterials widely researched due to their unique properties that make them useful for a large number of applications, including electronics and optics as well as medicine and personal care products (Murayama et al., 2004; Mauter and Elimelech, 2008; Osawa, 2002). Moreover, the possibility to derivatize the closed cage carbon structure, with consequent production of novel nanomaterials, widens the range of uses of these chemicals (Hummelen et al., 1995). Although the annual production is estimated to be tens of tons per year, a large increase is expected in the near future (Piccinno et al., 2012; Hendren et al., 2011), which is likely to imply higher emissions into the

environment. Thus, fullerenes are nowadays referred to as emerging contaminants and there is concern about the environmental and health implications that may arise. In particular, fullerenes may be released directly during production or they may leach from materials as a consequence of their use and disposal. Furthermore, they may be produced in combustion processes and therefore associated with both natural phenomena (e.g. forest fires, volcanic eruptions; Heymann et al., 1994; Howard et al., 1991) and human activities such as industrial processes (e.g. as a byproduct during combustion of carbonaceous materials) and transport (e.g. fuel combustion; Murr et al., 2004).

Little is known about the fate of these chemicals once they end up in the environment. Although some studies suggested that fullerenes may be degraded due to both biotic and abiotic processes (e.g. Tiwari et al., 2014), others highlighted the stability of C₆₀ that may result in their accumulation in the environment (Jehlicka et al., 2005; Parthasarathy et al., 2008; Avanasi et al., 2014; Navarro et al., 2016). However, fullerenes have been shown to affect soil organisms (e.g. earthworms, Van der Ploeg et al., 2011), hence,

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monitoring studies are crucial in order to provide valuable information about the occurrence of these chemicals and to determine the potential environmental risk associated with them.

In general, pristine structures such as C₆₀ and C₇₀ are expected to be more abundant in the environment because of the higher production volumes and their higher natural occurrence. Furthermore, they could result from the degradation of functionalized structures such as methanofullerenes and fullerol (e.g. due to fullerol mineralization, Navarro et al., 2016). Indeed, several studies reported the presence of C₆₀ and C₇₀ fullerenes in environmental matrices (Sanchis et al., 2012, 2013; 2015; Núñez et al., 2012; Astefanei et al., 2014), whereas functionalized structures (i.e. engineered nanomaterials) were seldom detected (Astefanei et al., 2014).

Soil has been predicted to be a sink for fullerenes (Gottschalk et al., 2009) that may enter the terrestrial environment via atmospheric deposition, direct release, water transport and amendment of soils (Sanchis et al., 2012; Navarro et al., 2013). However, most of the research so far has been focused on water and wastewater samples (Farré et al., 2010; Astefanei et al., 2014; Emke et al., 2015; Núñez et al., 2012; Van Wezel et al., 2011; Kolkman et al., 2013) and data regarding the occurrence of fullerenes in soil are limited to a few studies. In particular, Sanchis et al. (2013) reported the presence of fullerenes in soils from Saudi Arabia associated with the petroleum refinery activities in the area. In their study, C₆₀ was the only compound detected, and was found in 19% of the samples at concentrations in the low µg/kg range. Fullerenes were detected to a larger extent (67.6% of the agricultural soils and 91% of the urban soils) during a monitoring study in Brazil where both C₆₀ and C₇₀ were found at concentrations up to 154 ng/kg (Sanchis et al., 2015). In this case, their presence was also correlated to combustion activities, particularly the deposition of atmospheric particulate resulting from combustion processes. Fullerenes were also found in aerosol particulates (Sanchis et al., 2012) and urban atmosphere (Laitinen et al., 2014) as well as resulting from coal combustion (Utsunomiya et al., 2002), suggesting that atmospheric deposition could represent a main route for their entrance in the terrestrial environment.

In the present work, an environmental survey was carried out with the aim of investigating the presence of fullerenes in soil samples collected in The Netherlands. For this purpose, a method employing Ultra High Performance Liquid Chromatography (UHPLC) coupled with High Resolution Mass Spectrometric (HRMS) detection was used (Carboni et al., 2016). The main goal was to investigate the presence of C₆₀ and C₇₀ in highly trafficked and industrialized areas, i.e. those areas where the generation of fullerenes may be related to human activities, in order to assess the influence that these possible sources may have in the occurrence of these chemicals in the environment. The selection of the samples was done in order to represent diverse situations. Thus, soil samples were collected in proximity of expected sources such as a coal power plant (Utsunomiya et al., 2002) as well as in potentially uncontaminated areas (i.e. natural park).

2. Materials and methods

2.1. Reagents and chemicals

Toluene, methanol, isopropanol and acetonitrile (Biosolve, Valkenswaard, The Netherlands) were all analytical grade (LC-MS). Fullerenes C₆₀ (CAS: 99685-96-8) and C₇₀ (CAS: 115383-22-7) standards were purchased by Sigma-Aldrich (Steinheim, Germany). Stock solutions of the individual fullerenes, at the concentration of 5 mg/L, were prepared in toluene and placed on an orbital shaker (Laboshake orbital shaker, Gerhardt, Königswinter, Germany) in the

dark overnight. Further solutions needed for the experiments, were obtained by dilution of the stock solutions, and stored at 4 °C in the dark and sonicated for 2 min before use (Bransonic 12, Branson, Danbury CT, United States).

2.2. Sample collection and treatment

The surface soil samples (top 5 cm, at least 125 g each) were collected in 250 ml amber glass bottles. Each sample consisted of the composite of five sub-samples (25–30 g each) collected at the corners and centre of a 1 m² squared area. A total of 91 surface soils were sampled in 6 locations in The Netherlands:

Location A was an urban area in proximity of a coal power plant in Amsterdam (15 samples, labelled A_01 - A_15); Location B was a rural and urban area nearby the AVR incinerator in Duiven (16 samples, labelled B_01 – B_16); Location C was an area surrounding the Amsterdam's "Ring" highway (26 samples, labelled C_01 – C_25); Location D was in green areas (e.g. parks, flower beds) in the city of Amsterdam (16 samples, labelled D_01 – D_16); Location E was a natural park situated near Castricum (6 samples, labelled E_01 – E_06) whereas Location F samples were collected in proximity of a runway at the Eindhoven airport (12 samples, labelled F_01 – F_12). Additional information regarding the distance of the sampling locations from the expected sources and their coordinates are reported in the [Supplementary information](#). The wind direction was assumed as the main wind direction recorded during the three days before the sampling was performed. The samples were placed in a freezer at –20 °C overnight and lyophilized with a Scanvac Coolsafe freeze-dryer (Labogene, Lyngø, Denmark) in order to remove traces of water. The dried samples were ground and homogenized with an agate mortar and sieved before undergoing extraction with the protocol already reported by our group (Carboni et al., 2013) with minor modifications, i.e. the temperature was set at 20 ± 2 °C as recommended by Perez et al. (2013) and the extraction was performed in 40 ml custom made glass vial in order to allow the extraction of larger volumes. Briefly, circa 25 g of each sample underwent two cycles of extraction with toluene with each cycle consisting of a combination of sonication for 20 min with a Bransonic 12 ultrasonic bath (Branson, Danbury CT, United States) and shaking for 2 h (Laboshake orbital shaker, Gerhardt, Königswinter, Germany). Negative controls consisted of silicon dioxide samples (25 g each) extracted and analyzed with the same procedure. The extracts were stored in a refrigerator at 4 °C in the dark and sonicated for 30 s before to be analyzed.

2.3. Sample analysis

The analyses were performed with the method recently developed in our research group for the identification of fullerenes in soil and sediment samples as described previously (Carboni et al., 2016), using an UHPLC system (Nexera, Shimadzu, Den Bosch, The Netherlands) equipped with a binary pump, autosampler and column oven. Retention of the chemicals was achieved with a core-shell Kinetex 2.6 µm biphenyl 100 Å chromatographic column (Phenomenex, Utrecht, the Netherlands) consisting of a biphenyl stationary phase. The UHPLC system was coupled to a high resolution Quadrupole-Time of Flight mass spectrometer (Q-TOF; maXis 4G, Bruker Daltonics, Wormer, The Netherlands) equipped with an Ion Booster electro spray (IB-ESI) ionization source (Bruker Daltonics, Wormer, The Netherlands) as described elsewhere (Carboni et al., 2016). High purity nitrogen was used as collision gas (Avilo, Dirksland, The Netherlands). Automatic mass calibration was performed for each sample by injecting a 2 mM sodium acetate solution at the beginning of the chromatographic run. Additional information regarding the analytical set up and

operational parameters are reported in the [Supplementary information](#). In addition to the pristine fullerenes structures C₆₀ and C₇₀, the samples were screened for the presence of six methanofullerenes, such as the [6,6]-phenyl-C61-butyric acid methyl ester ([60]PCBM), already investigated by our group (Carboni et al., 2016). The criteria used for the identification of the analytes were: 1) the expected chromatographic retention time (± 0.2 min), 2) a mass accuracy threshold (≤ 5 ppm) and 3) an isotopic pattern fit threshold (≤ 50 mSigma), where mSigma represents the goodness of fit (the smaller the better) between the measured and theoretical isotopic pattern in both MS and MS/MS modes (Carboni et al., 2016). The analyses were performed with an injection volume of 10 μ l after adding methanol (25% in volume) to each sample prior to injection.

External calibration curves were obtained after analyzing standard solutions at concentrations ranging from 500 ng/L to 1 ng/L and quantification was based on chromatographic peak areas. The Bruker Compass 1.7 software was employed in both the data collection and data processing. The method allowed the determination of C₆₀ and C₇₀ fullerenes in the samples with method limit of detection (MLOD) of 67 and 134 pg/kg for C₆₀ and C₇₀, respectively, and limits of quantification (MLOQ) of 223 and 446 pg/kg for C₆₀ and C₇₀, respectively. The matrix effect, i.e. the suppression or enhancement of the signal due to the presence of co-extractants in the samples, was investigated by the addition of a C₆₀ and C₇₀ standard to the extracts at the concentration of 25 ng/l ([Supplementary information](#)).

In order to study the unknown C₆₀-like fullerene, a larger amount (100 g) of the samples from the incinerator area (location B) were extracted to increase its concentration. These samples were additionally analyzed with a modified UHPLC-IB-ESI-QTOF method ([Supplementary information](#)).

For statistical analysis, a linear model was used that considered the relationship between the concentration of the samples and the distance from the possible source. Further statistical analyses were conducted in which samples collected only upwind or downwind from the expected source were taken into account.

3. Results

The high accuracy (< 5 ppm), resolution (80,000 FWHM) and clarity of the MS/MS spectra allowed the unambiguous determination of the fullerenes in the soil samples. The UHPLC chromatograms of the soil extracts ([Fig. 1b](#)) resembled those obtained with standard solutions ([Fig. 1a](#)) with slightly higher background noise at the beginning of the chromatograms. An example of the UHPLC chromatograms obtained in the present study is provided in the [Supplementary information](#) ([Figs. S1 and S2](#)). Fullerenes were found to be present in almost half of the soil samples analyzed (48%) and C₆₀ was the most detected species. C₇₀ fullerene was detected less frequently (7% of the samples) and mostly associated with the presence of C₆₀ whereas methanofullerenes structures (e.g. [60]PCBM) were not detected in any of the samples under investigation.

An additional C₆₀ peak was found in some of the samples ([Figs. 1c and S3](#) in the [supplementary information](#)) that showed mass, isotopic pattern and MS/MS spectra similar to those of the C₆₀ standard. This suggested the presence of other fullerenes species in the extracts. Since the chemical structure has not been unequivocally elucidated, we will refer to this compound as “unknown”. Specifically, the unknown was found only in samples collected in the incinerator area and only when C₆₀ was also present. The unknown had an accurate mass corresponding to C₆₀ but eluted earlier in UHPLC (at a retention time of 3.1 min) suggesting the presence of a C₆₀-like species (e.g. a functionalized fullerene) that,

although separated chromatographically, underwent in-source fragmentation during the ionization process (i.e. the loss of the external functionalization) with formation of pristine C₆₀. In this context, it must be noted that in-source fragmentation of fullerenes has already been reported to occur during MS analysis (e.g. during fullerol analysis, (Chao et al., 2011)). As shown in [Fig. 1c](#), the addition of C₆₀ standard to the original samples resulted in the enhancement of the C₆₀ peak area (at retention time 4 min) but had no effect to the additional peak (at retention time 3.1 min), confirming that the peak at 3.1 min is due to the presence of another C₆₀-like species in the samples. Modification of the analytical method with regard to the chromatography as well as the mass spectrometric method (i.e. by decreasing the temperature and/or the capillary voltage) were not able to prevent the fragmentation of the unknown and had the only effect of reducing the ionization efficiency. Eventually, a larger amount sample intake was extracted (see Materials and Methods section) in order to increase the concentration of the fullerenes in the extracts, i.e. the abundance of the unknown species and its possible precursors. However, these further analyses did not show any presence of precursors and in general did not lead to the identification of the unknown.

Samples collected close to the coal power plant (location A) revealed the presence of fullerenes in 53% of the extracts. As shown in [Fig. 2](#), C₆₀ was the compound detected most often at concentrations ranging from MLOD to 1.44 ng/kg whereas C₇₀ was found at similar concentrations but only in two of the samples. A larger occurrence was found downwind of the powerplant (samples A_7-A_15) at distance up to 1.8 km from the chimney whereas no fullerenes were detected upwind in proximity of the powerplant (samples A_1-A_3). However, the highest concentrations of both C₆₀ and C₇₀ was found close to a coal storage site (sample A_6).

In the samples collected in the incinerator area (Location B), C₆₀ was detected in the majority of the samples (69%) at concentrations ranging from 0.15 to 3.12 ng/kg ([Fig. 3](#)). Although C₇₀ was not found in any of the samples under investigation, an additional peak representing a C₆₀-like structure (the unknown product discussed above) was detected in 44% of the samples and was always accompanied by the presence of C₆₀. In the case of location B, the highest concentrations were found in proximity of the incinerator (sample A_1-A_3), whereas lower concentrations were found mostly downwind in the surrounding area. Similar to what was observed in the power plant location ([Fig. 2](#)), samples collected immediately upwind of the chimney did not show any presence of fullerenes (samples B_7-B_8).

Fullerenes were found in 62% of the samples collected in proximity of the highway in Amsterdam (Location C, see [Fig. 4](#)). C₆₀ was the most abundant species also in this case (in 62% of the samples at concentrations between MLOD and 0.77 ng/kg), whereas C₇₀ was found in 8% of the samples. In the soils collected in the Amsterdam urban area (location D), C₆₀ was the only fullerene found in 18% of the samples at concentrations ranging from 0.43 to 0.87 ng/kg. In contrast, no fullerenes were detected in the samples collected in the natural area in Castricum (Location E) whereas samples collected close to the runway of Eindhoven Airport (Location F) showed the presence of fullerenes in 33% of the soils at concentrations usually below the MLOQ. However, in this case the only quantifiable samples had the relatively high concentrations of 2.14 and 2.17 ng/kg for C₆₀ and C₇₀, respectively.

4. Discussion

Fullerenes were present in almost a half of the surface soils sampled in the present study and C₆₀ was the species detected most often (48% of the samples) in comparison with C₇₀ (7%). However, it must be noted that method detection limits of C₇₀ were higher

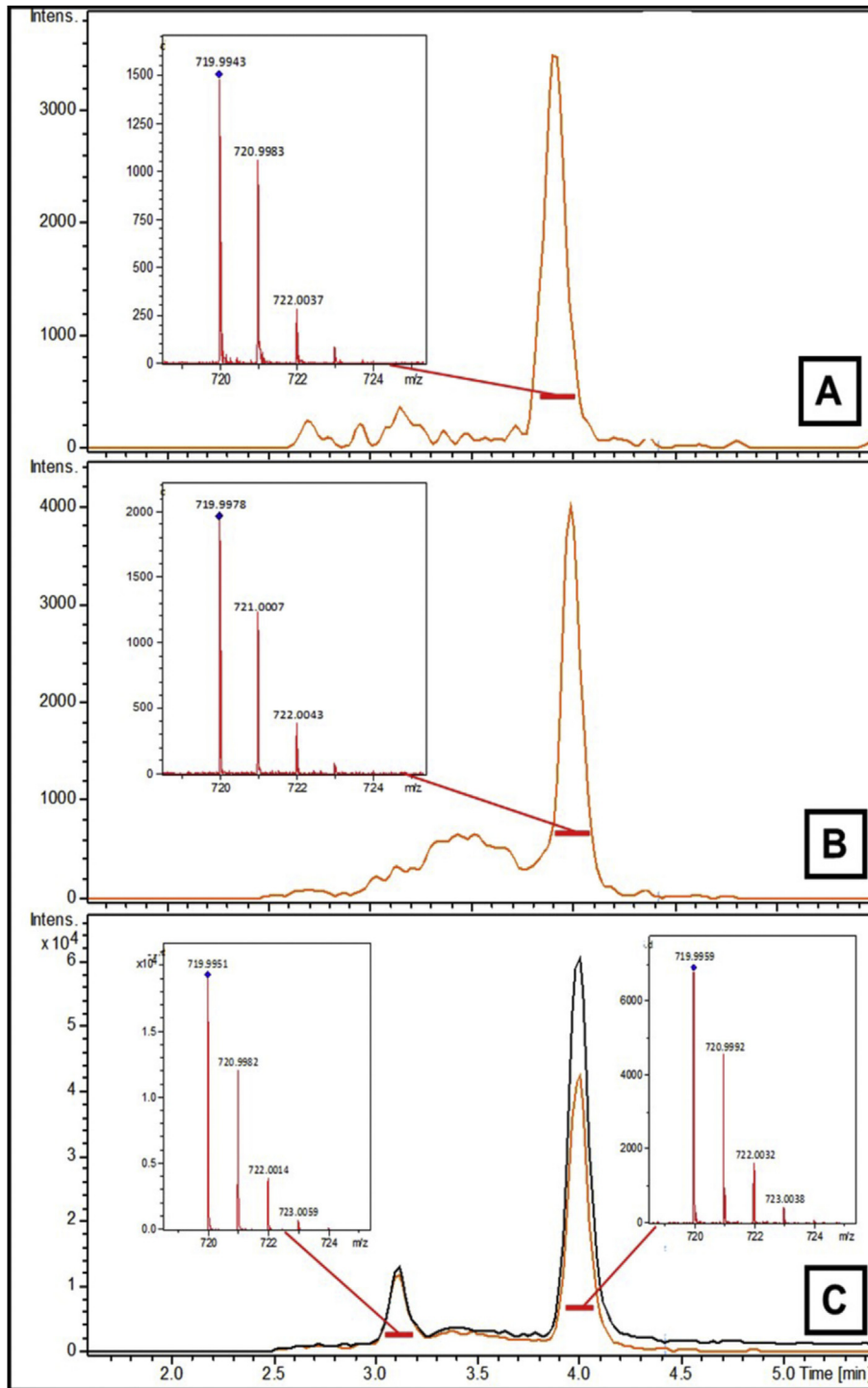


Fig. 1. Chromatographic peak and mass spectra of A) a C_{60} standard at concentration 8 ng/L, B) a soil sample extract from the highway (location C) and C) a sample from the incinerator area (location B) presenting an additional C_{60} peak at retention time 3.1 min. The black line represents the signal of the sample spiked with standard.

compared to C_{60} , which may have hindered the detection of lower concentrations of C_{70} in the samples. Furthermore, methanofullerenes were not detected in any of the samples, indicating that these species were either not released into the soil, or were present at levels below the MLOD or were converted to the non-functionalized C_{60} and C_{70} structures in the environment. The average concentrations, excluding the samples at concentrations below the MLOQ, were 0.7 and 0.5 ng/kg for C_{60} and C_{70} , respectively.

In general, a higher occurrence of fullerenes was observed in intensively trafficked and industrialized areas (e.g. power plant, incinerator and in proximity of the highway) where fullerenes were found in 61% of the samples in average. The incinerator was the most contaminated area with regard to both the occurrence (69% of the samples) and concentration (1.2 ng/kg of C_{60} in average) whereas lower concentrations (<1 ng/kg in average) were found in the other locations sampled. Along the airport's runway, the highest occurrence of C_{60} was found in coincidence with the touch-

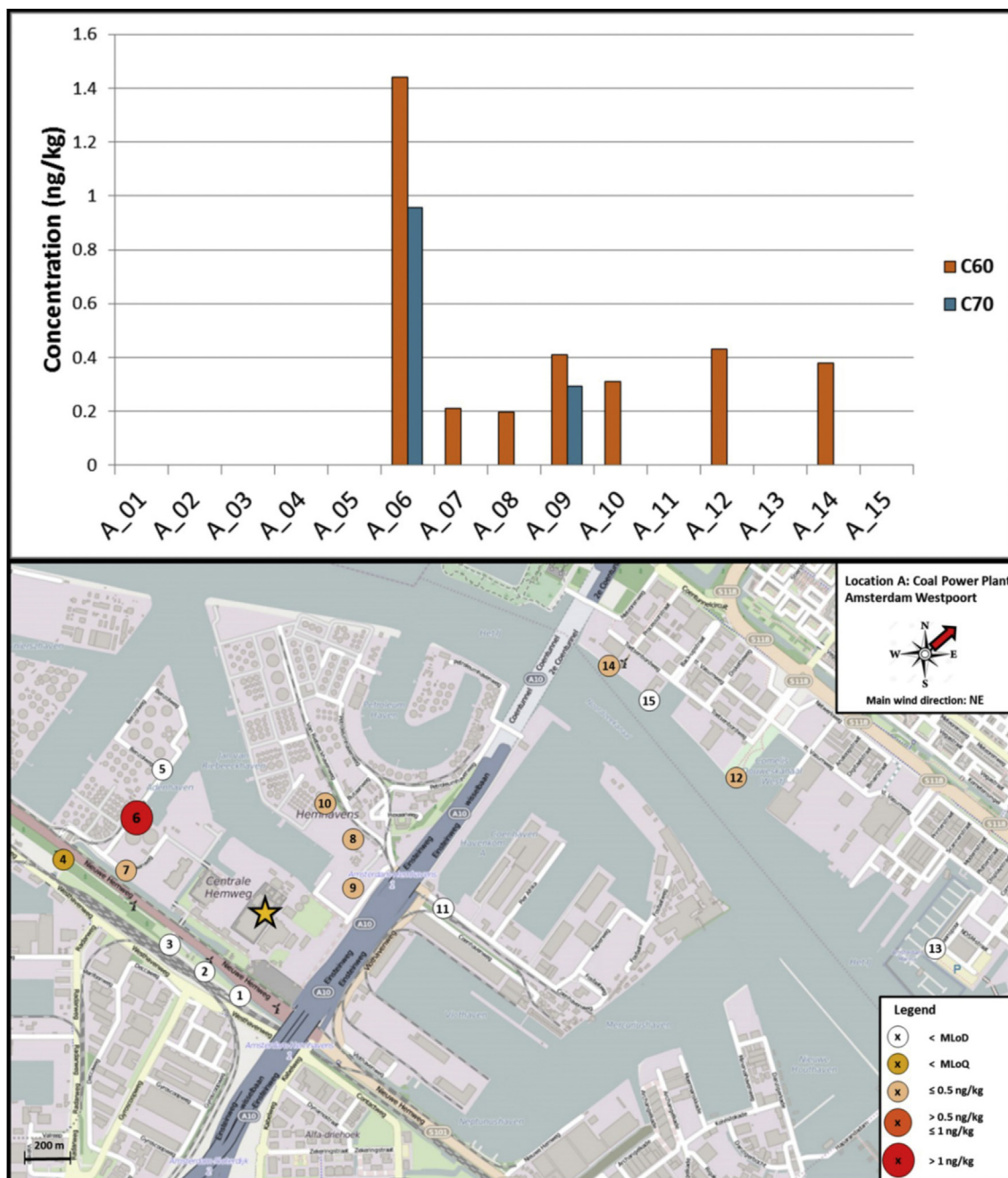


Fig. 2. Concentrations of fullerenes in the coal power plant area (location A) in Amsterdam. Top: concentration of C₆₀ (orange) and C₇₀ (blue) in the samples. Bottom: sampling locations and range of concentrations of C₆₀. The star represents the powerplant's main chimney. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

down spot, thus suggesting that the fullerenes may result from the contact of tires with the runway during landing. In particular, the fullerenes may be directly released from the tires or produced during the landing due to the high temperatures.

In general, our results are consistent with previous observations and modeled data (Gottschalk et al., 2009) and suggest that C₆₀ is the most abundant fullerene species found in the environment at concentrations in the range of ng/kg in soil. In particular, Sanchis et al. recently reported similar results, with regard to both concentration and occurrence (Sanchis et al., 2015), when monitoring trafficked and industrialized areas and suggested that the more frequent occurrence of C₆₀, at relatively higher concentrations, may

be due to its higher stability in the environment in comparison with other pristine and functionalized structures (Sanchis et al., 2013).

It must be noted that, to the best of our knowledge, fullerenes are not intentionally produced close to the sampled areas and that no products containing fullerenes are intensively used or disposed in those areas. Furthermore, no natural sources (e.g. forest fires) are known to the authors that occurred during last years in the sampling locations and that may account for the presence of fullerenes in the surrounding soils. Therefore, the presence of the fullerenes is likely due to human activities and particularly the result of combustion processes involving waste, coal and other fossil fuels or, in the case of the power plant, associated with the presence of coal

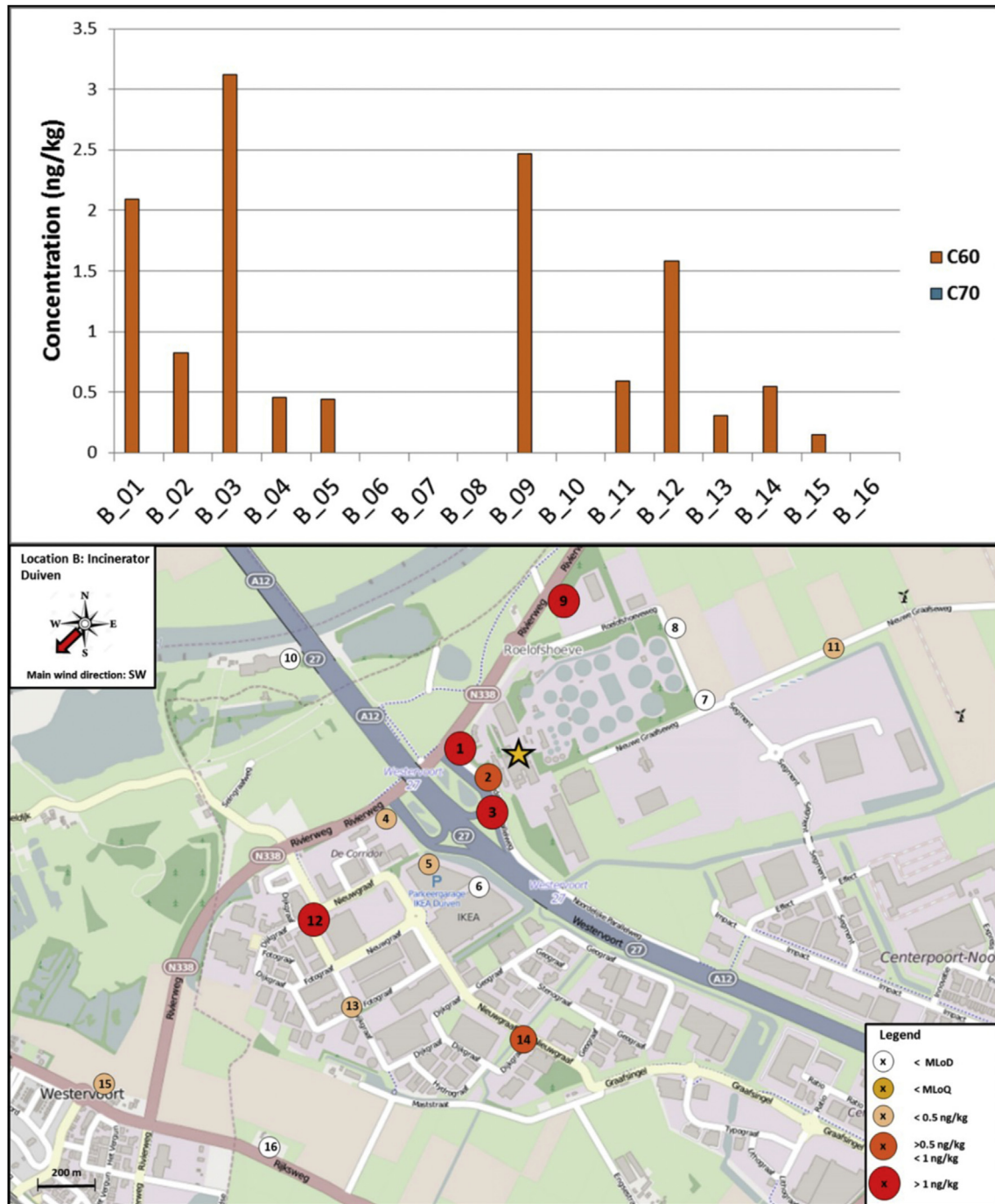


Fig. 3. Concentrations of fullerenes in the incinerator area (location B) in Duiven. Top: concentration of C₆₀ (orange) in the samples, C₇₀ was not detected in any sample. Bottom: sampling locations and range of concentrations of C₆₀. The star represents the powerplant's main chimney. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stocks on the soil. However, although it is not possible to exclude an engineered origin, the fullerenes found in the present work were likely unintentionally produced i.e. not manufactured nano-materials. Although higher concentrations and occurrence were usually found downwind and close to the expected sources (e.g. incinerator or power plant chimney), statistical analysis did not highlight either a significant correlation between the concentration and the distance or a role of the wind in the distribution of the fullerenes ($P > 0.05$). However, it must be noticed that, in the locations sampled, an assessment of the fullerenes origin is

complicated by the presence of multiple sources.

To the best of our knowledge no analytical method currently available is able to discriminate between the several sources of the fullerenes detected in the environment. In this context the ratio between fullerene species could be helpful in understanding the origins, as different sources (e.g. coal or fossil fuel combustion) may produce different ratios. However, such strategy has not been investigated yet during environmental surveys or laboratory controlled experiments. In the present work, the $[C_{70}]/[C_{60}]$ ratio in the samples was relatively constant in both the location A and C,

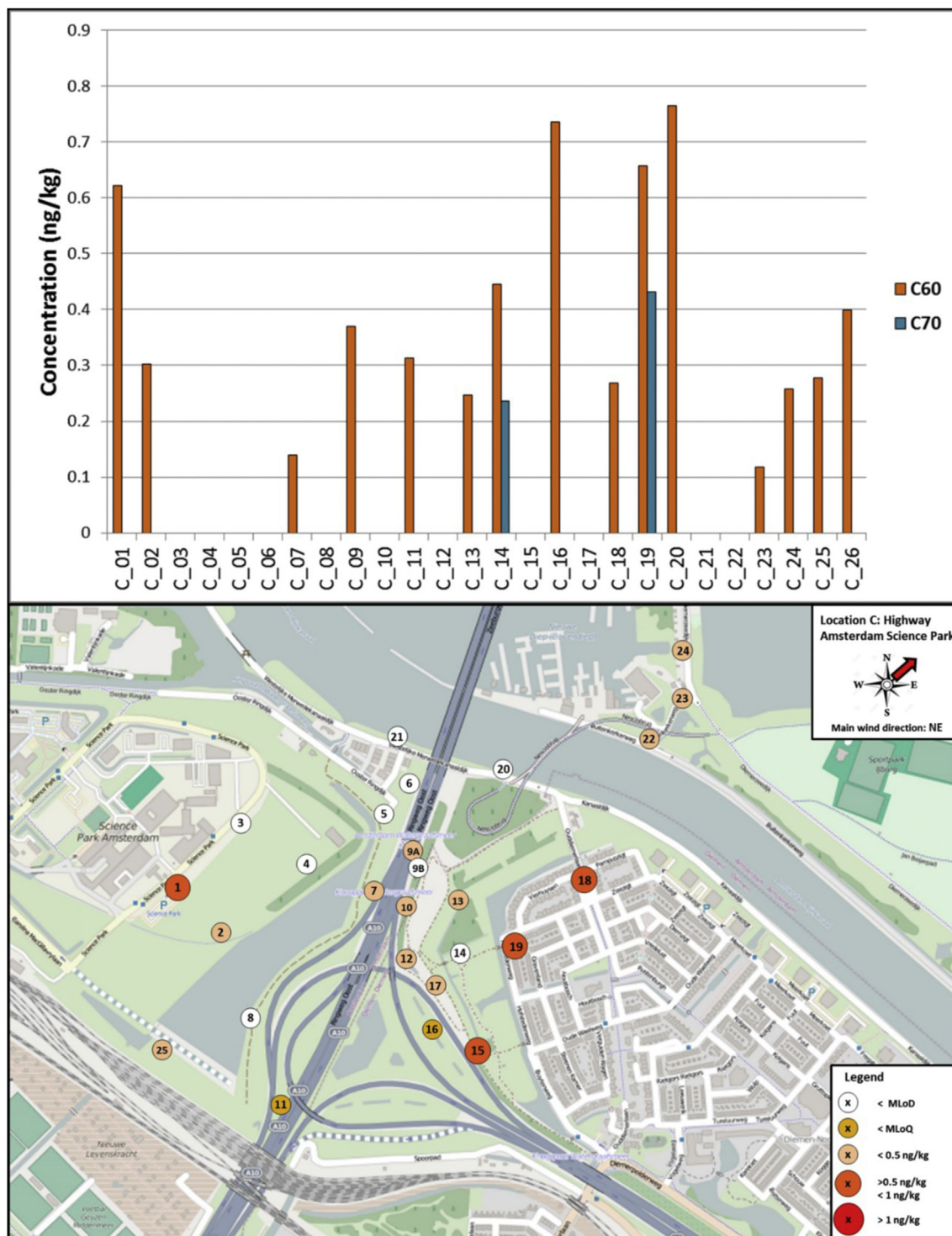


Fig. 4. Concentrations of fullerenes in the highway area (location C) in Amsterdam. Top: concentration of C₆₀ (orange) and C₇₀ (blue) in the samples. Bottom: sampling locations and range of concentrations of C₆₀. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with ratios of 68.9 ± 3.6 and 59.4 ± 8.8 percent, respectively. However, the limited number of samples containing C₇₀ did not allow a more comprehensive comparison.

The C₆₀-like unknown specie was found in samples collected in the incinerator area only and may originate from either specific combustion processes involving diverse materials (e.g. waste combustion) or from changes in the chemical composition of pristine C₆₀ after release in the environment. Although

characterization of the unknown was not possible in the present study, the presence of a specific C₆₀ chromatographic peak at a retention time different than that of the pristine C₆₀, suggests the presence of a functionalized-C₆₀ structure that underwent an in-source fragmentation during the ionization process. Furthermore, the unknown product is apparently more polar than C₆₀ as it eluted earlier than C₆₀ in reverse-phase chromatography. Since the functionalized fullerenes (i.e. the methanofullerene [60]PCBM) do not

undergo in-source fragmentation when analyzed with the same method (Carboni et al., 2016), the energy associated with the bonding of the external group in the unknown compound is probably lower than that generally observed in the commercially available functionalized fullerene derivatives. These observations suggest that the unknown product may be a functionalized fullerene such as the oxides recently characterized by Tiwari et al. (2014) during the ozonation of C₆₀ aerosol.

Finally, although the recovery of pristine C₆₀ during the analysis may be useful for the identification of unknown species in environmental samples, an alternative method should be tested for their characterization. In particular, atmospheric pressure ionization methods such as APPI may provide similar ionization efficiency for the fullerenes (Emke et al., 2015), but preventing or minimizing the in-source fragmentation of the compounds.

5. Conclusions

The present study demonstrates the presence of fullerenes in the terrestrial environment and especially in locations close to highly industrialized and trafficked areas in Netherlands. Similarly to what has been reported by previous studies in different locations, C₆₀ was the most abundant fullerene and was present in higher concentrations in comparison with other species. However, the presence of an unknown C₆₀-like structure(s), which may be linked to either the unintentional release of different fullerene species or the modification of pristine fullerenes in the environment, highlights both the complexity in the environmental study of these chemicals and the need for novel analytical strategies. Although an assessment of the origin of the fullerenes is not possible with the current methodologies, we found no indications that the level of soil contamination with fullerenes is related to the intentional production and/or use of engineered nanomaterials at the sites. Our results rather indicate that fullerenes are unintentionally released, enter the environment particularly by combustion processes and were likely deposited on the soil from the atmosphere. These results will be helpful in the understanding of fullerenes occurrence and behavior in the environment and provide useful data for both modelling studies and for the planning of forthcoming monitoring campaigns.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.09.034>.

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