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Novel method for *in situ* investigation into graphene quantum dots effects on the adsorption of nitrated polycyclic aromatic hydrocarbons by crop leaf surfaces

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ABSTRACT

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are PAH derivatives with more toxic effects to ecosystem, and the partitioning of NPAHs in crop system constitutes the potential exposure to human health through the dietary pathway. In the present study, a novel method for *in situ* detection of 9-nitroanthracene (9-NAnt) and 3-nitrofluoranthene (3-NFla) adsorbed onto the leaf surfaces of living soybean and maize seedlings was established based on the fiber-optic fluorimetry combined with graphene quantum dots (GQDs) as a fluorescent probe. The detection limits for the *in situ* quantification of the two adsorbed NPAHs ranged from 0.8 to 1.6 ng/spot (spot represents determination unit, 0.28 cm^2 per spot). Using the novel method, the effects of GQDs on the adsorption of individual 9-NAnt and 3-NFla by the living soybean and maize leaf surfaces were *in situ* investigated. The presence of GQDs altered the adsorption mechanism from the sole film diffusion to the combination of film diffusion and intra-particle diffusion, and shortened the time required to achieving adsorption equilibrium by 15.8–21.7%. Significant inter-species and inter-chemical variability existed in terms of the equilibrated adsorption capacity (q_e) with the sequence of soybean > maize and 3-NFla > 9-NAnt. The occurrence of GQDs enlarged the q_e values of 9-NAnt and 3-NFla by 22.8% *versus* 28.7% for soybean, and 16.2% *versus* 20.3% for maize, respectively, which was largely attributed to GQDs-induced expansion to the surface area for adsorbing NPHAs and the stronger electrostatic interaction between the -NO_2 of NPAH molecules and the functional groups (*e.g.,* -COOH, -OH) of GQDs outer surfaces. And, the varied enhancement degrees in the order of 3-NFla > 9-NAnt might be explained by the steric effects that resulted in the easier accessibility of -NO_2 of 3-NFla to the outer surface of GQDs. Summarily, the GQDs increased the retention of NPAHs on crop leaf surfaces, potentially threatening the crop security.

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are strong environmental mutagens and carcinogens originating from both primary emissions and secondary reactions in the atmosphere (Sun et al., 2017). Like parent PAHs (PPAHs), NPAHs are reported to be a class of semi-volatile compounds (SOCs) (Yang et al., 2017), and NPAHs have been detected in both the particulate and gaseous phases of combustion emissions and the ambient atmosphere. More remarkably, studies have shown that the occurrence of NPAHs in ambient air is 10–100 times lower than those of their PPAHs, but their toxicity is 10–100,000 times higher than that of PPAHs (Fujii et al., 2017; Gao et al., 2018). Living plant cuticles can enrich hydrophobic SOCs mainly from the atmosphere due to the presence of hydrophobic wax layer, which strongly affect the fates of SOCs (Barber et al., 2002; Su and Wania, 2005; Li et al., 2009). Meanwhile, the SOCs adsorption by crop or vegetation leaf cuticles also constitutes a direct exposure pathway to human health (Desalme et al., 2013). Recently, we *in situ* investigated the uptake of parent and alkylated PAHs by crop leaf surfaces, as well as the effects of surfactants (Sun et al., 2018a). NPAHs have been detected in plant materials, such as tree barks, tea leaves, fresh parsley, *etc.* However, few studies have delved into the adsorption of gaseous NPAHs by living crop leaf surfaces.

As a matter of zero-dimensional luminescent carbon-based nanomaterials, graphene quantum dots (GQDs) can be viewed as a scaled down version of graphene oxide (GO), and also as an enlargement of

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benzene-based molecules, *i.e.,* PAHs (Li et al., 2013, 2017). Emerging as superior functional materials, GQDs have received extensive attention in various studying areas, including biological, environmental and energy-related applications (Zhang et al., 2012; Sun et al., 2014; Sahub et al., 2018). With such promising potential for considerable applications, GQDs would be released into the environment and transported between environmental media inevitably, which may trigger uncertain ecological effects (Lin et al., 2014). Recently, we observed that the presence of graphene (GNS) and GO nanosheets inhibited the total depuration of PPAHs from the living spinach leaf surfaces (Sun et al., 2018b). However, the effects of GQDs on the adsorption of gaseous NPAHs by living crop leaf surfaces are poorly understood.

A variety of analytical methods have been established for the measurement of NPAHs levels in environmental samples, such as GC/MS, LC/ECD, GC/TOFMS, *etc.* (Domeño et al., 2012; Fujii et al., 2017; Li et al., 2016). As is well known, these methods are accompanied with destructive pretreatments (extraction and separation), which might destroy the original forms and eliminate the spatial distributions of NPAHs in/on plant tissues in unknown ways. Therefore, the development of *in situ* method for direct determination of NPAHs on crop leaf surfaces is of great necessarily. By far, some *in situ* approach have been established to determine the PPAHs levels adsorbed on the plant leaf surfaces, such as solid surface fluorescence, laser-induced nanosecond time-resolved fluorescence, and fiber-optic fluorimetry (FOF) (Wang et al., 2008; Sun et al., 2016), *etc.* Although fluorescent response of NPAHs is relatively lower than that of PPAHs due to the high electron-withdrawing nitro-group, Guiñez et al. (2017) established a novel method for the quantification of 9-nitroanthracene (9-NAnt), and 3-nitrofluoranthene (3-NFla) in water samples based on molecular fluorescence detection. Therefore, the suitability of existing methods for the *in situ* determination of NPAHs on crop leaf surfaces is worthy of attention. Additionally, due to the high fluorescence yield, GQDs have attracted considerable attention in the determination of biological and chemical substances (Liu et al., 2017; Achadu and Nyokong, 2017; Luo et al., 2018). Recently, a novel fluorescence quenching method for *in vivo* quantification of N/O/S-containing PAHs (dibenzothiophene, carbazole and dibenzofuran) retained on the mangrove root epidermis using GQDs as a fluorescent probe (Li et al., 2018). However, the question of whether it could realize the *in situ* quantification of NPAHs adsorbed onto the living crop leaf surfaces using GQDs as a fluorescent probe remain unclear.

In this work, 9-NAnt and 3-NFla were selected as the model components of NPAHs owing to their relative prevalence in gas phase. Soybean and maize were chosen as the studying focuses due to the most commonly consumed crops around the world. The overall objectives of the present study were to develop a novel method for *in situ* determination of NPAHs adsorbed onto the living soybean and maize leaf surfaces based on GQDs as a fluorescent probe, and to *in situ* determine the effects of GQDs on the adsorption kinetics of gaseous NPAHs by the two crop leaf surfaces.

2. Materials and methods

2.1. Apparatus and reagents

A standard sample of 9-NAnt (purity > 95%) was supplied from Sigma-Aldrich Co. Ltd. (UK) and 3-NFla (purity > 95%) from AccuStandard Inc. (USA). In this study, The GQDs with the diameter ~3 nm were purchased from J&K Scientific Co. Ltd. (USA). All of the other chemicals (A.R.) were obtained from Shanghai Trustin Chemical Co., Ltd (China). Stock solutions of NPAHs at 500 μg/L were prepared in methanol and stored at − 20 °C before use. GQDs solutions dispersed in methanol were prepared and then sonicated for 10 min, followed by incubation on the bench overnight. The dispersions of GQDs in methanol were monitored using the zeta potential, and the value was smaller than − 45 mV, indicating that GQDs colloidal particles had good stability and carried negative charge.

Fluorescence spectra of NPAHs and GQDs were all *in situ* recorded on a Cary Eclipse fluorescence spectrophotometer equipped with fiber-optic accessories of 2-m length and 150-W Xenon lamp (Agilent, USA) (Fig. S1). During the measurement, the excitation and emission slits were fixed at 10 nm and 5 nm, respectively; PMT voltage was 600 V and the scan rate was set as 12,000 nm/min; an angle between the fiber-optic probe and the tested crop leaves was kept at 45° to avoid the interference from the scattered light (Fig. S1). 10-μL flat head micro-injections (Shanghai Medical Laser Instrument Plant, China) that used for introducing NPAHs and GQDs solution onto the soybean and maize leaf surfaces. The stomatal conductance of two crop leaves with and without GQDs pressure was measured using a Delta-T AP4 leaf porometer (Barber et al., 2002).

2.2. Preparation and pretreatment of living crop seedlings

The plants were cultured for 21 days in a controlled climate chamber (MGC-450HP, Shanghai Yiheng Science Instruments Co. Ltd., China): light irradiation was set for 12 h light (18,000 lx) and 12 h dark, and temperatures were set at 24 °C during light period and 22 °C during dark regime, with relative humidity of 55%. Then, the seedlings with similar growing stage (5–6 leaves) and biomass were obtained from the cultured plants for the following adsorption experiments. Next, the silts adhered onto the leaf surfaces were washed off successively with tap water and Milli-Q water three times. Finally, the determination areas evenly distributed on the front, middle and nether of leaf surfaces were produced using a large circle end of 5-mL pipette based on the method as recorded by Sun et al. (2016). The area of the circle (0.28 cm^2, r ≈ 0.30 cm) was marked as a unit of 'spot', basically having the same area as the fiber-optic probe, which has been displayed visually in Fig. S1.

2.3. Quantification of NPAHs adsorbed onto the crop leaf surfaces

For the seedlings without GQDs treatment, the fluorescence spectra of 9-NAnt and 3-NFla with five concentration gradients ranging from 0 to 400 ng/spot and 0–500 ng/spot, respectively, were directly obtained using the FOF method, and the operations were consistent with what were recorded in our previous studies (Sun et al., 2016, 2018a). For the GQDs-treated seedlings, the GQDs solutions were introduced onto the 'spots' to produce the dosages of 100 ng/spot. Two hours later, respective 9-NAnt and 3-NFla solutions with five concentration gradients ranging from 0 to 400 ng/spot and 0–300 ng/spot were introduced onto these 'spots' interacted with GQDs. After the complete evaporation of methanol, the fluorescence spectra of GQDs were acquired between 380 and 550 nm wavelength range at the excitation wavelength (λ_{ex}) of 325 nm using the FOF method.

2.4. Adsorption experiment

In the present study, 'seedlings without GQDs treatment' and 'seedlings treated with GQDs' referred to the control and experimental group, respectively. For each item, three replicate seedlings and three replicate leaves (one per seedling) were selected for the adsorption experiment. Firstly, 100 ng/spot dosages of GQDs were introduced onto the 'spots' on the leaf surfaces of living soybean and maize seedlings. Two hours later, the seedlings treated with/without GQDs were all transferred into a 125-L exposure chamber to perform adsorption experiment. The controlled exposure chamber was basically the same as that used in our previous study (Sun et al., 2018a) with minor modifications. Briefly, the key parameters were set as follows: light/dark regime, 16/8 h; temperature: (23 ± 2) °C; relative humidity: (40 ± 3)%; illumination intensity: 18000 lx. The contaminated air was produced based on the method reported by Sun et al. (2016): approximately 10 mg of individual 9-NAnt and 3-NFla were dissolved in