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Research Article

$PM_{2.5}$-bound polyhalogenated carbazoles (PHCZs) in urban Beijing, China: Occurrence and the source implication

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ABSTRACT

Polyhalogenated carbazoles (PHCZs) are recently raising much attention due to their toxicity and ubiquitous environmental distribution. However, little knowledge is known about their ambient occurrences and the potential source. In this study, we developed an analytical method based on GC-MS/MS to simultaneously determine 11 PHCZs in $PM_{2.5}$ from urban Beijing, China. The optimized method provided low method limit of quantifications (MLOQs, 1.45–7.39 fg/m^3) and satisfied recoveries (73.4%–109.5%). This method was applied to analyze the PHCZs in the outdoor $PM_{2.5}$ ($n = 46$) and fly ash ($n = 6$) collected from 3 kinds of surrounding incinerator plants (steel plant, medical waste incinerator and domestic waste incinerator). The levels of Σ_{11}PHCZs in $PM_{2.5}$ ranged from 0.117 to 5.54 pg/m^3 (median 1.18 pg/m^3). 3-chloro-9H-carbazole (3-CCZ), 3-bromo-9H-carbazole (3-BCZ), and 3,6-dichloro-9H-carbazole (36-CCZ) were the dominant compounds, accounting for 93%. 3-CCZ and 3-BCZ were significantly higher in winter due to the high $PM_{2.5}$ concentration, while 36-CCZ was higher in spring, which may be related to the resuspending of surface soil. Furthermore, the levels of Σ_{11}PHCZs in fly ash ranged from 338 to 6101 pg/g. 3-CCZ, 3-BCZ and 36-CCZ accounted for 86.0%. The congener profiles of PHCZs between fly ash and $PM_{2.5}$ were highly similar, indicating that combustion process could be an important source of ambient PHCZs. To the best of our knowledge, this is the first research providing the occurrences of PHCZs in outdoor $PM_{2.5}$.

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Introduction

Polyhalogenated carbazoles (PHCZs) are a group of emerging contaminants, which have similar structure to polychlorinated dibenzofurans (PCDFs). PHCZs were reported to exhibit similar aryl hydrocarbon receptor (AhR) toxicity as dioxin (Riddell et al., 2015), and the toxic equivalency factors (TEFs) of PHCZs were around 1.3×10^{-4} to 9.7×10^{-3} fold of the dioxin by CYP1B1 gene expression and 1.3×10^{-5} to 6.6×10^{-4} fold by CYP1A1. Among them, 2,3,7,8-tetrachlorocarbazole was one of the most toxic PHCZ congeners, like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Several PHCZ congeners may disrupt the endocrine system and interfere with steroidogenesis (Yue et al., 2020). In addition, 2,7-dibromo-9H-carbazole (27-BCZ), 3-bromo-9H-carbazole (3-BCZ), 3,6-dibromo-9H-carbazole (36-BCZ) showed estrogen receptor agonistic effect and estrogen-like effects (increased uterus weights and epithelial cell heights).

Extensive occurrences of PHCZs were found in the environment (Ji et al., 2021; Lin et al., 2016). PHCZs were widely detected in soil (Liu et al., 2021; Tao et al., 2020; Mumbo et al., 2016), sediment (Guo et al., 2017; Wu et al., 2016; Zhou et al., 2019a), biological tissue (Wu et al., 2018; Wu et al., 2017) and water (Li et al., 2020; Wang et al., 2021). In 1984, 1,3,6,8-tetrachlorocarbazole was detected in the sediment of Buffalo River, NY, USA and the concentration was even higher than polycyclic aromatic hydrocarbons (PAHs) (Kuehl et al., 1984). The levels of Σ_{26}PHCZs in sediments of Upper Great Lakes were slightly higher than BDE209 (Decabromodiphenylether) and Σ_{39}PCBs (Polychlorinated biphenyls) (Guo et al., 2017). The occurrence of 14 PHCZs in PM_{10} in indoor residences was investigated in Germany at low level, with median congener value of several pg/m^3 (Fromme et al., 2018). Even though, the occurrences of PHCZs in air were rarely reported.

PHCZs could be emitted through both various anthropogenic emissions and natural processes. Many studies have pointed out that the indigo dyes manufacture may be closely related to the formation of PHCZs (Parette et al., 2015). PHCZs can also be generated by the halogenation of carbazole, which is additive in the electric devices, such as light emitting diodes (LED) (Morin et al., 2005), combustion products of biomass (Glarborg et al., 2003) and petroleum, component of bitumen (Lauby-Secretan et al., 2011). In aquatic environment, PHCZs may be generated in the disinfected water as aqueous chlorination of residual carbazole under bromide condition (Wang et al., 2019). Some natural sources were found by the marine organism through enzymatic synthesis (Chen et al., 2018). However, at present, our understanding towards the origin of PHCZs is limited. The known origins can't explain the high level in the sediment (Guo et al., 2017). Recently, a latest study found the existence of PHCZs in the fly ash and stack flue gases of an e-waste incinerator (Zhou et al., 2021), which provided the possibility of the atmospheric existence of PHCZs.

Beijing is a big city with 21.5 million permanent populations in 2019 (http://nj.tjj.beijing.gov.cn/nj/main/2020-tjnj/zk/indexch.htm). The North Plain of China, including Beijing, experienced serious air pollution during the last few years (Huang et al., 2014; Lang et al., 2017), and was one of the most polluted areas (Lelieveld et al., 2015). $PM_{2.5}$ (particulate matter with aerodynamic diameter less than 2.5 μm) is a serious environmental problem all over the world, especially in developing countries. And ambient $PM_{2.5}$ was the fifth-ranking mortality risk factor in 2015, and caused 4.2 million mortality in 2015 (Cohen et al., 2017; Forouzanfar et al., 2016). Increased $PM_{2.5}$ concentrations induce the disease risk of respiratory system, cardiovascular, etc. In the past few years, persistent organic pollutants (POPs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), PCDFs, polychlorinated naphthalenes (PCNs) were detected in $PM_{2.5}$ samples of Beijing (Zhu et al., 2016; Cao et al., 2018). Besides, non-target analysis has identified thousands of organic chemicals in $PM_{2.5}$ (Lin et al., 2019), however, many kinds of the contaminants are not quantified yet and their inhalation risks need further evaluation. Considering the wide environmental distribution and potential dioxin-like toxicology, the occurrence and health risk of $PM_{2.5}$-bound PHCZs require detailed investigation.

In addition, gas chromatograph coupled with triple quadrupole mass spectrometer (GC-MS/MS) has been widely applied to analyze the trace contaminants in complex matrices with high selectivity and sensitivity (Ayala-Cabrera et al., 2021; Rivera-Austrui et al., 2017). Although some methods have been developed to investigate the occurrence of PHCZs in the environment, the complex environmental media and low environmental residue make it difficult to identify and quantify the PHCZs (Ji et al., 2021; Sun et al., 2022a). And some of the current methods consumed plenty of solvent for cleanup, which was not eco-friendly. Thus, an optimized method was preferred to analyze the atmospheric samples.

The aim of this study is to (1) develop a sensitive and quick method to analyze the PHCZs in atmospheric samples; (2) investigate the occurrences and variations with related factors of $PM_{2.5}$-bound PHCZs; (3) explore the potential existence of PHCZs in fly ash and elucidate the combustion source of $PM_{2.5}$-bound PHCZs.

1. Materials and methods

1.1. *Chemicals and materials*

Dichloromethane (DCM) and *n*-hexane (*n*-Hex) were pesticide grade from J. T. Baker (Phillipsburg, NJ, USA). Mixture standard solution of 3-chloro-9H-carbazole (3-CCZ), 3,6-dichloro-9H-carbazole (36-CCZ), 1,3,6,8-tetrachloro-9H-carbazole (1368-CCZ), 2,3,6,7-tetrachloro-9H-carbazole (2367-CCZ), 3-bromo-9H-carbazole (3-BCZ), 2,7-dibromo-9H-carbazole (27-BCZ), 3,6-dibromo-9H-carbazole (36-BCZ), 1,3,6-tribromo-9H-carbazole (136-BCZ), 1,3,6,8-tetrabromo-9H-carbazole (1368-BCZ), 1-bromo-3,6-dichloro-9H-carbazole (1-B-36-CCZ) and 1,8-dibromo-3,6-dichloro-9H-carbazole (18-B-36-CCZ), as well as the surrogate mixture of ^{13}C-labelled 3,6-dichloro-9H-carbazole (^{13}C-36-CCZ), ^{13}C-labelled 1,3,6,8-tetrachloro-9H-carbazole (^{13}C-1368-CCZ) and internal standard $^{13}C_{10}$-1,2,3,4,5,7-hexachloronaphthalene (^{13}C-PCN-IS) were purchased from Wellington Laboratories, Canada.

Silica gel (0.063–0.1 mm) and florisil solid phase extraction (1 g, 6 mL, Supelco) were purchased from Merck, USA.

1.2. Sample collection and preparation

A high-volume air sampler (HV-1000R, SIBATA, Japan) was employed to collect $PM_{2.5}$ samples in Beijing Urban Ecosystem Research Station (116°21′2″E, 40°0′51″N) from September 2019 to August 2020. The sampler was located on a roof (10 m above the ground) of a building which was surrounded by residences and campuses. The sampling flowrate was 1000 L/min and the total volume of each sample was 1379.9 m^3. The duration time was 23 hr every week. A total of 46 $PM_{2.5}$ samples were collected on quartz fiber filters (MK360, Ahlstrom, Sweden) and were weighted by a precision balance (MS204TS, Mettler Toledo, Switzerland). The flowrate, temperature and atmospheric pressure were calibrated regularly.

Fly ash samples were collected in 3 kinds of plants from 2 steel plants (SP), 1 medical waste incinerator (MWI) and 3 domestic waste incinerators (DWI) around Beijing (Appendix A Fig. S1).

The samples were stored at -20°C until further processing and analysis. The silica gel and quartz fiber filters were prebaked at 550°C for 6 hr before using. All the glassware was prewashed by a laboratory dishwasher (SCD 1160, Salvislab, Switzerland). Before using, the glassware was washed by acetone, DCM, and n-Hex sequentially.

A quarter of the $PM_{2.5}$ filters (approximately 345 m^3) were cut for analysis. The filters were cut into small pieces, added with 1 ng ^{13}C-36-CCZ and ^{13}C-1368-CCZ, and extracted using hexane and dichloromethane (1:1, *V/V*) by accelerated solvent extraction (ASE 300, Dionex, USA). The ASE extraction condition is 100°C, 5 min preheat, 10 min static, 120 sec purge, 3 cycles. Around 0.5–1 g fly ash was weighted and extracted by Soxhlet extraction in toluene for 20 hr. Then the extracts were concentrated to around 2 mL by a rotary evaporation concentrator (Hei-VAP Expert, Heidolph, Germany). Then the concentrates were cleaned up by 5 g silica gel topped with 5 g anhydrous sodium sulfate, followed by 1 g florisil SPE (Supelco, USA). The elution condition was optimized based on the previous method (Wu et al., 2018), and the results were shown in Appendix A Fig. S4. Briefly, 30 mL hexane prewashed the 5 g silica gel and 5 g anhydrous sodium sulfate column, after adding concentrates, the target compounds were eluted by 30 mL hexane and dichloromethane (3:2, *V/V*) and concentrated. The concentrates were further purified by 1 g florisil. 6 mL hexane was used to precondition the florisil column. The target compounds were eluted by 12 mL hexane and dichloromethane (2:1, *V/V*) and concentrated with gentle nitrogen to around 15 µL. Before instrumental analysis, the samples were added with 1 ng $^{13}C_{10}$-1,2,3,4,5,7-hexachloronaphthalene. The detailed information was provided in Appendix A.

1.3. Instrument analysis

The pretreated samples were analyzed by gas chromatograph coupled with triple quadrupole mass spectrometer (TQ8050, SHIMADZU, Japan). The GC oven was equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 µm, J&W Scientific, USA). The GC oven temperature program was set as follows: the initial oven temperature was held at 100°C for 5 min, and increased to 310°C at 10°C/min, held for 10 min. The temperatures of the injector and transfer line were kept at 250 and 280°C, respectively. One microliter of the sample was injected with splitless mode and the carrier gas (helium, purity > 99.999%) flow was 1 mL/min. The MS/MS source temperature was held at 280°C. Electron ionization (EI) mode was set at 70 eV and the filament current was set as 150 µA. The MS/MS was operated in Multiple Reaction Monitoring (MRM) mode (Appendix A Table S3). The best impact voltage was confirmed by selecting the highest response among different impact voltage (Appendix A Fig. S3). 3,6-dichloro-9H-$^{13}C_{12}$carbazole was used for quantifying 3-CCZ, 3-BCZ, 36-CCZ, while 1,3,6,8-tetrachloro-9H-$^{13}C_{12}$carbazole was used for the other PHCZs. The target compounds were identified by the retention time and ion ratio of characteristic ions.

1.4. Air quality and meteorological parameters

The data of air quality including $PM_{2.5}$, PM_{10}, SO_2, NO_2, O_3, CO and AQI (Air Quality Index, a composite index of the former six parameters) were obtained from the nearest station of the National Air Quality Ground Observation System (https://quotsoft.net/air/). The meteorological parameters including temperature and RH (relative humidity) were obtained from the National Weather Service (http://data.cma.cn/). The air quality and meteorological parameters during the sampling periods were summarized in Appendix A Table S4.

1.5. Quality control and quality assurance

The filters were wrapped by aluminum foil in ziplock bag before and after sampling. A field blank was simultaneously pretreated and analyzed for each batch of samples. No target compounds were detected in field blanks. The measured recoveries of surrogate standards were 66% ± 13% (^{13}C-36-CCZ) and 79% ± 14% (^{13}C-1368-CCZ). The method limit of quantification (MLOQ) was evaluated by replicate analyses ($n = 7$) of blank filters spiked with 50 pg each of the target PHCZs. The MLOQs for individual PHCZs were calculated by multiplying the standard deviations generated from replicate analyses with a Student's *t*-value appropriate for a 99% confidence level (Wu et al., 2016). The MLOQs of the target compounds ranged from 1.45 (3-BCZ) to 7.39 fg/m^3 (18-B-36-CCZ). The recovery test was performed by adding 1 ng PHCZs to the blank filters, pretreating the sample with the same method, and the recovery rate ranged from 73.4% (3-CCZ) to 109.5% (2367-CCZ).

1.6. Exposure and risk assessment

The dioxin-like relative effect potencies (REPs) of PHCZs were estimated based on their induction of CYP1A1 gene expression in breast cancer cells, and the values of REPs for the detected PHCZs ranged from 0.000013 to 0.00066 (Riddell et al., 2015). The Toxic Equivalent Quantity (TEQ, fg TEQ/m^3) was used to estimate the toxicity of 11 PHCZs relative to the most toxic dioxin, 2,3,7,8-TCDD.

$$TEQ = \sum (C_i \times REP_i) \times 1000$$

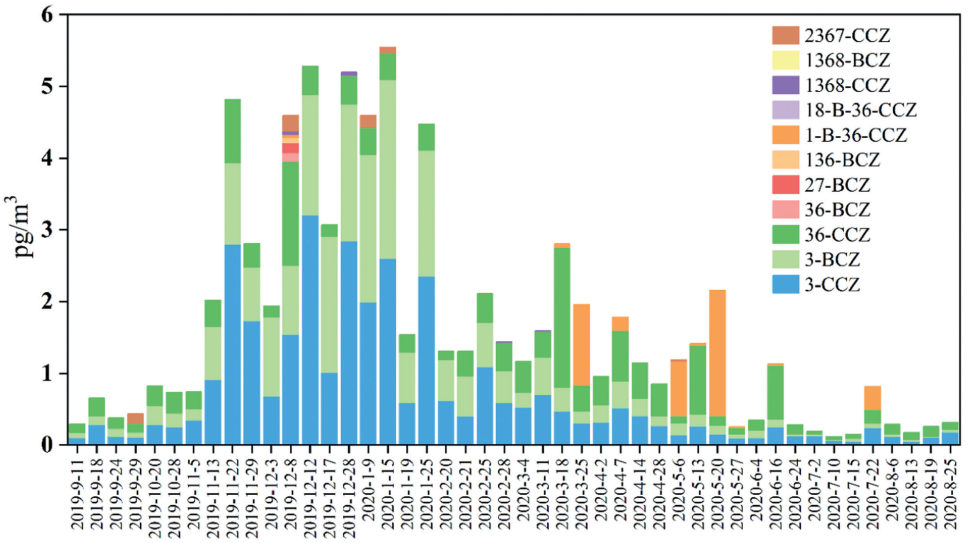

Fig. 1 – The temporal variation of the concentration of 11 PHCZs in one-year duration.

Where C_i (pg/m^3) is the concentration of each PHCZ detected in outdoor $PM_{2.5}$; REP_i is the relative dioxin-like toxic effect potency of the corresponding PHCZ congener.

To evaluate the dioxin-like toxic risk of the detected PHCZs in outdoor $PM_{2.5}$, the Estimated Daily Intake (EDI, fg TEQ/kg bw/day) of PHCZs through the exposure of outdoor $PM_{2.5}$ was calculated as follows:

$$EDI = \sum (C_i \times REP_i) \times IR \times ET/(1.44 \times BW)$$

Where IR (m^3/day) is the inhalation rate; ET (min) is the outdoor exposure time; BW (kg) is the body weight. The detailed information was provided in Appendix A Tables S5 and S6.

2. Results and discussion

2.1. *Occurrence of PHCZs in $PM_{2.5}$*

2.1.1. *Level and congener profiles of PHCZ in $PM_{2.5}$*

Among 11 PHCZs, 3-CCZ, 3-BCZ and 36-CCZ were detected in all outdoor $PM_{2.5}$ samples. The other 8 PHCZs were detected at low frequency, accounting for 34.8%, expressed as Σ_8PHCZ. The 11 PHCZs in $PM_{2.5}$ were at low level, from 0.117 to 5.54 pg/m^3, with a median value of 1.18 pg/m^3 (Fig. 1). As a contrast, the previous research of indoor PM_{10} and dust (Fromme et al., 2018) showed higher level and diversity congeners composition, which detected 13 PHCZ congeners with median value from 0.9 to 6.1 pg/m^3. The difference may result from the different source between indoor and outdoor environment.

The congener profile in various matrices was related to the emission characteristics and environmental behavior. For example, the proportion of 3-CCZ, 36-CCZ and 3-BCZ in $PM_{2.5}$ accounted for 7%–63% (mean 38%), 5%–70% (mean 30%), 6%–62% (mean 25%), respectively. This congener profile in $PM_{2.5}$ was mainly lower halogenated, which was similar to that in soil and water. And 36-CCZ was the predominant congener in soil, followed by 3-CCZ and 3-BCZ (Liu et al., 2021; Tao et al., 2020; Mumbo et al., 2016). This occurrence resulted from both anthropogenic emissions (coal mining, domestic sewage discharge, chemical production) and natural formation. In the drinking water of Wuhan, 36-CCZ and 3-CCZ were the dominant congeners, resulting from chlorinated disinfection (Wang et al., 2019) as well as anthropogenic emission (Wang et al., 2021). While in sediments and fish, the proportion of higher halogenated carbazoles increased. In sediments, PHCZs were dominated by 1368-BCZ, 36-BCZ and 36-CCZ (Guo et al., 2017; Wu et al., 2017; Guo et al., 2014). The decreasing trend of some congeners (e.g., 1368-CCZ) after 1900 indicated dominantly natural source. Despite this, 1368-CCZ was also regarded as the production of indigo dye (Parette et al., 2015). In Great Lake fish, PHCZs enriched mainly in 36-CCZ, 136-BCZ and 1368-CCZ, whose logK_{ow} were in the range of chemicals tending to bioaccumulate and biomagnify (Wu et al., 2018; Wu et al., 2017).

Comparison with similar structure of chemicals to dioxin, PCN and PAHs was also conducted (Appendix A Table S7). The $PM_{2.5}$-bound PHCZs were around the same level with PCN in Beijing, ranging from 0.12 to 2.51 pg/m^3 (Zhu et al., 2016). The dioxin level was at the same level or slightly higher than PHCZs (Hao et al., 2018; Kim et al., 2021). The PAHs level was 10^3 to 10^5 orders of magnitude higher than PHCZs. Besides, the dominant PHCZs in $PM_{2.5}$ were lower halogenated congeners, such as 3-CCZ, 3-BCZ and 36-CCZ, which were different from PCN and PCDD/Fs.

2.1.2. *Seasonal trend of PHCZs*

For the 11 PHCZs, obvious seasonal variation was observed among the sampling period (Figs. 1 and 2). Briefly, the level decreased in the following order: winter (1.17–5.54 pg/m^3, median 2.81 pg/m^3) > spring (0.85–2.80 pg/m^3, median 1.30 pg/m^3) > autumn (0.30–2.02 pg/m^3, median 0.69 pg/m^3) > summer (0.12–2.15 pg/m^3, median 0.28 pg/m^3). Overall, the level of Σ_{11}PHCZs was significantly higher in winter than in the other seasons ($p < 0.001$). For the congeners, the levels of 3-CCZ and 3-BCZ were significantly higher in winter than in

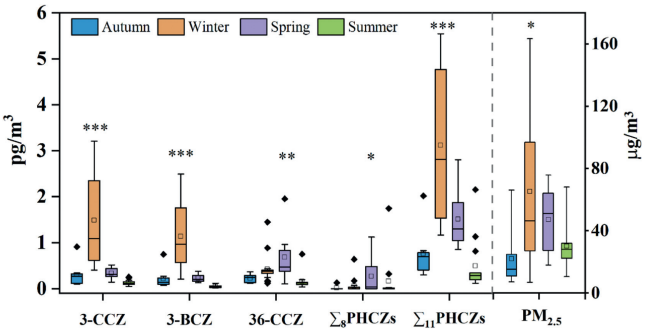

Fig. 2 – The seasonal trend of PHCZs and $PM_{2.5}$ concentration. The 8 PHCZs, except for 3-CCZ, 3-BCZ and 36-CCZ, were detected in low level and low frequency, thus were expressed as Σ_8PHCZs. The asterisks indicate the significant level of the season than the other seasons. * represents $p < 0.001$; ** represents $p < 0.01$; * represents $p < 0.05$.**

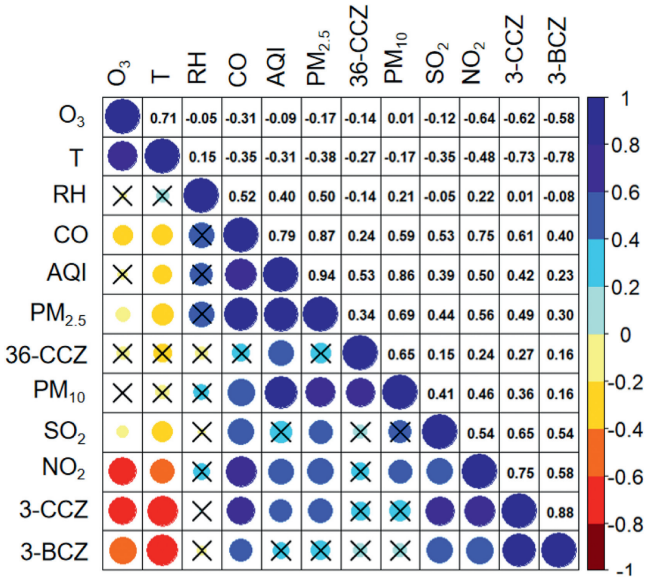

Fig. 3 – Pearson correlation of target PHCZs, corresponding air quality and meteorological parameters. AQI is a comprehensive index, including $PM_{2.5}$, PM_{10}, O_3, CO, SO_2 and NO_2. Meteorological parameters refer to the corresponding average temperature (T) and relative humidity (RH). The significant relation ($p < 0.05$) was shown as circles, while the insignificant relation ($p > 0.05$) was shown with circles under cross symbols. The upper right number is corresponding to the Pearson Correlation Coefficient R.

other seasons ($p < 0.001$). The high concentration of the above congeners in winter was partly related to the high concentration of $PM_{2.5}$ in this period ($p = 0.037$) (Fig. 2). Similar trends were also observed for similar structure compounds, such as PCDD/Fs (Zhao et al., 2018) and PAHs (Ma et al., 2020), due to the high concentration of $PM_{2.5}$ in winter.

The levels of 36-CCZ and Σ_8PHCZs were significantly higher in spring than in other seasons ($p = 0.0068$ and $p = 0.048$, respectively). 36-CCZ was also reported as the dominant congener in soils, which accounted for 67% near Erhai Lake (Tao et al., 2020), 51% in Taizhou (Zhou et al., 2019b), 40.67% in farmland of northeast China (Liu et al., 2021). The soil dust contributed highest to $PM_{2.5}$ in the spring of Beijing (23%) than the other seasons (3%–18%) (Zhang et al., 2013). In recent years, the annual contribution of dust to $PM_{2.5}$ in Beijing was 6.82% in 2019 (Park et al., 2022), 12% from 2018 to 2019 (Luo et al., 2022), 8.5%–14.4% in 2018 (Huang et al., 2021). The highest level of $PM_{2.5}$-bound 36-CCZ in spring indicated ambient PHCZs may partly stem from the suspended particles from the surface soil.

2.1.3. *The related factors of PHCZs in $PM_{2.5}$*

Pearson correlation was conducted to explore the effect of the air quality parameters and meteorological parameters on 3 dominant PHCZ congeners' level (Fig. 3). 3-CCZ and 3-BCZ showed a strong correlation ($r = 0.868$, $p < 0.001$), indicating their coexistence in the $PM_{2.5}$ samples. Besides, the level of 3-CCZ and 3-BCZ are positively correlated with SO_2 and NO_2, but negatively correlated with the level of O_3 and temperature. Since SO_2 and NO_2 were considered as the typical combustion products of industries and vehicle emission, the positive correlation of 3-CCZ and 3-BCZ with SO_2 and NO_2 may indicate their co-emissions. The half-life of 3-CCZ and 3-BCZ is relatively short according to EPI Suite 4.1 (2.09 hr and 2.10 hr, respectively, Appendix A Table S1), their actual behavior in the atmosphere is rarely known. The low level of temperature and O_3 in winter may provide relatively stable ambient environment, and may result in the accumulation of 3-CCZ and 3-BCZ. 36-CCZ is positively related with PM_{10} and AQI. PM_{10} was considered as greatly influenced by dust, such as surface soil, sands, road dust and construction dust. The correlation between PHCZs and PM_{10} supports the inference in Section 2.1.2 that ambient PHCZs may partly stem from surface soil. Overall, the 3 dominant congeners showed increasing trend with the PM concentration.

2.2. *Source implication of PHCZs in $PM_{2.5}$*

We collected 6 fly ash samples from 3 different kinds of plants around Beijing. The concentration of PHCZs in fly ash samples ranged from 338 to 6101 pg/g (Fig. 4). The level of this study is around an order of magnitude lower than that found in the e-waste disposal plant, with the concentration of 88200 pg/g (Zhou et al., 2021). Several factors including the raw material (Shen et al., 2021b), combustion condition, and purification process (Hsu et al., 2021; Sun et al., 2020) could influence the emission characteristics of POPs. The PHCZ levels of 2 steel plant fly ash samples were even slightly higher than the PCDDs (average: 195–1130 pg/g) and PCN (average: 2400 pg/g) of steel making plant (Shen et al., 2021a). To date, the risk of the industrial combustion-derived PHCZs is still not clear. The PHCZs concentration of 2 SP and 1 MWI was higher than that of 3 DWI. The relative contribution was as follows: 3-CCZ (9.8%–48.7%) > 36-CCZ (15.3%–41.5%) > 3-BCZ (5.8%–34.6%) > Σ_8PHCZs (n.d. –42.9%). It's noteworthy that the congener profiles of PHCZs are similar among fly ash and outdoor $PM_{2.5}$ in

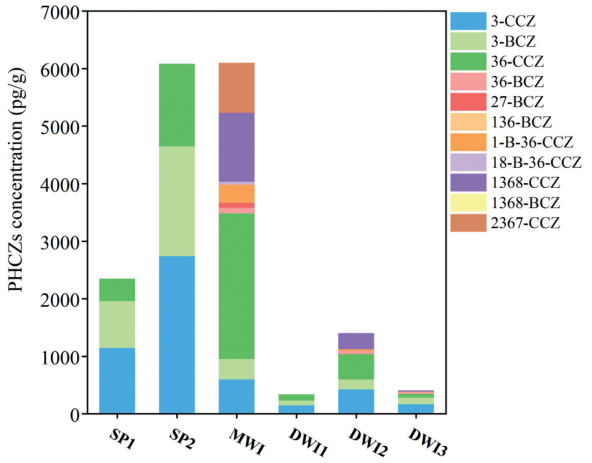

Fig. 4 – PHCZs concentration in fly ash. SP: steel plant, MWI: medical waste incinerator, DWI: domestic waste incinerator.

this study (Appendix A Fig. S5). Thus, the industrial combustion process may be an important source of ambient PHCZs.

Most of the existing studies focus on the aquatic origin of PHCZs. There are few studies on their ambient origin. Some studies pointed out that the combustion process may produce PHCZs. 2,4,6-Tribromoaniline (TBA), a kind of widely used compound as flame retardant, could generate bromocarbazole at a residence of several seconds at around 550°C (Bindra and Narang, 1995). There are only 3 field investigations on the industrial emission of PHCZs, we summarized the level in Table 1. In an e-waste incinerator, the level of 11 PHCZs was 4.6 times that of 11 PBDEs in fly ash and 21 times that of 17 PCDD/Fs in flue stack gas (Zhou et al., 2021). Another study investigated chemical bottom liquid from manufacturing plants and fly ash from a medical waste incinerator (Sun et al., 2022b). And the level of 11 PHCZs in our study (6101 pg/g) was comparable with this study (6970 pg/g) in fly ash from different medical waste incinerators. Based on the current results, PHCZs have been identified through various matrices (fly ash, flue gas and chemical bottom liquid) from several kinds of industrial plants at comparable level with PCDD, proving that industrial process is an important source of PHCZs. And further detailed research on the emission characteristics, generation and removal of PHCZs during the industrial combustion process is needed.

2.3. *Exposure and risk assessment*

The estimated TEQ of 11 PHCZs ranged from 0.006 to 0.284 fg TEQ/m^3 (median 0.059 fg TEQ/m^3), far below Japanese ambient air standard of 600 fg TEQ/m^3 for dioxin (MOE, 1999). The TEQ in this study is also far below the TEQ of $PM_{2.5}$-bound PCDD/Fs in Beijing (70 fg TEQ/m^3), and Southeast Asia (23–565 fg TEQ/m^3) (Zain et al., 2021).

The calculated EDI ranged from 1.97×10^{-4} to 9.05×10^{-3} fg TEQ/kg bw/day, and 1.74×10^{-4} to 7.98×10^{-3} fg TEQ/kg bw/day for adults and children, respectively, far lower than the U.S. EPA reference dose (RfD) 700 fg TEQ/kg bw/day (EPA 2012). The PHCZ EDI of drinking water in Wuhan, China was 1 fg TEQ/kg bw/day to 90 fg TEQ/kg bw/day (Wang et al., 2021),

Table 1 – The level of PHCZs emitted from industrial process.

Plant[a]	3-CCZ	3-BCZ	36-CCZ	1368-CCZ	1-B-36-CCZ	27-BCZ	36-BCZ	18-B-36-CCZ	136-BCZ	1368-BCZ	2367-CCZ	Sum	Reference
SP[b]	1145–2739	813–1909	392–1433	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2350–6081	this study
MWI[b]	599	355	2530	1198	314	86	97	50	n.d.	n.d.	872	6101	
DWI[b]	149–425	81–173	62–437	n.d.–283	n.d.–30	n.d.	n.d.–57	n.d.	n.d.	n.d.	n.d.	338–1405	
EWI[b]	6417	308	28398	50950	448	255	162	230	56.0	60.9	961	88246	
													Zhou et al., 2021
EWI[c]	1469–1612	228–21058	309–440	18.8–58.0	n.d.–36	62.6–141	41.8–1101	n.d.–17	n.d.	n.d.–11	n.d.–58.8	2539–24125	
MWI[b]	4300	20	1200	630	720	n.d.	n.d.	n.d.	n.d.	n.d.	100	6970	Sun et al., 2022b
MP[d]	97.57	<MDL	52.37	6.98	<MDL	<MDL	<MDL	n.d.	n.d.	n.d.	<MDL	156.92	

[a] abbreviations. SP: steel plant; MWI: medical waste incinerator; DWI: domestic waste incinerator; EWI: e-waste incinerator; MP: manufacturing plant.
b, c, d: the corresponding sample matrices are fly ash (pg/g), stack flue gas (pg/m^3), and chemical bottom liquid (ng/mL).

much higher than the EDI through inhalation. Considering the high concentration PHCZ in fishes (Guo et al., 2017; Wu et al., 2018) through food consumption, the inhalation exposure risk of PHCZ is negligible compared with drinking water and food consumption.

3. Conclusion

In this research, we investigated the occurrence and source of $PM_{2.5}$-bound polyhalogenated carbazoles in urban Beijing. The level of the 11 target PHCZ congeners was very low (several pg/m^3), comparable to PCNs, resulting in negligible inhalation risk. The level of PHCZs showed seasonal trend, which was controlled by the emission characteristics and atmospheric conditions. Besides, PHCZs were detected in fly ash samples of various incinerators, which may contribute to the ambient PHCZs. The diverse detection of PHCZs in different incinerators indicates the thermal generation of PHCZs through diverse combustion processes, which expands the present knowledge of PHCZs' origin.

Nevertheless, there are still some questions to be solved in the future. (1) Limited standards restrict the comprehensive detection and risk assessment of PHCZ. The future research shall identify more congeners in the environment. (2) Quicker and more efficient pretreatment methods are needed for the low-level detection in complex environmental matrices. (3) According to our research, the combustion process is an important source of atmospheric PHCZs, more studies are needed on the emission intensity under different procedure parameters, control measures and the impact on atmospheric environment.

Declaration of Competing Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.10.048.

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