



Concentrations of heavy metals and polycyclic aromatic hydrocarbons in needles of Masson pine (*Pinus massoniana* L.) growing nearby different industrial sources

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Abstract

Emissions from industrial activities pose a serious threat to human health and impose the need for monitoring both inorganic and organic pollutants in industrial areas. We selected Masson pine (*Pinus massoniana* L.) as potential biomonitor and collected the current (C) and previous year (C+1) needles from three industrial sites dominated by petrochemical, ceramics manufacturing, and iron and steel smelting plants and one remote site to determine heavy metals (Cu, Cd, Pb, Zn, Cr, Ni and Co) and polycyclic aromatic hydrocarbons (PAHs) in unwashed and water-washed needles. Both unwashed and washed C+1 needles showed generally higher concentrations of heavy metals and PAHs than C needles, although the washed needles more clearly spotlighted the accumulation effect of PAHs over exposure time. Water-washing resulted in a significant decrease in needle PAH concentrations with more significant effects shown in C needles. By contrast, needle heavy metal concentrations were much less affected by washing. Although heavy metals and PAHs might differ in adsorption and uptake strategies, their higher concentrations in the needles at the industrial sites indicated conspicuous contamination due to industrial emissions there. The PAH distribution patterns in pine needles accorded with the real types of energy consumption in the study sites and were efficiently used for pinpointing local pollutant sources.

Key words: biomonitoring; needle age; polycyclic aromatic hydrocarbons; water-washing

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Introduction

As human population, urbanization, and industrialization continue to expand, emissions of inorganic heavy metals and organic polycyclic aromatic hydrocarbons (PAHs) into the atmosphere and their hazards on human life are of great concern. When present in excessive amounts, heavy metals are exceptionally toxic due to their non-biodegradable nature and long biological half-life for elimination from the body (Raghunath et al., 1999). PAHs, consisting of two or more fused aromatic rings, arise mainly from incomplete combustion of fossil fuels and pyrolysis of organic materials (Hwang et al., 2003). Most of these organic xenobiotics may pose threats to human beings because of their persistence in the environment, low aqueous solubility, and carcinogenic, mutagenic, teratogenic, and immunosuppressive properties (Hwang et al., 2003; Lehndorff and Schwark, 2004; Migaszewski et al., 2009).

Such pollutants are transported through air mass move-

ments, deposited by wet and dry deposition, and accumulated in vegetation (De Nicola et al., 2008). Plants can uptake heavy metals from the air directly via the stomata or via deposition on the foliage surfaces. PAHs, dependent on their state existing in the atmosphere, can also reach foliage surfaces as vapor and/or particulate and enter into cuticular waxes or by stomatal uptake (Paterson et al., 1991; Simonich and Hites, 1994). Concentrations of target compounds in plants can represent the integrated levels of these pollutants in surrounding air over time, and has been employed as a useful method to determine regional and global contamination patterns and to identify point sources of pollution (Holoubek et al., 2000), although difficulties exist in establishing the clear-cut quantitative relationship between air and leaf pollutant concentrations (Alfani et al., 2001; Hwang et al., 2003; Prajapati and Tripathi, 2008; Tremolada et al., 1996). To date, biomonitoring of pollutant depositions is receiving increasing favor as an alternative to conventional methods, particularly in areas where complex and expensive air sampling devices are unavailable. Conifer species including Scots pine (*Pinus*

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sylvestris L.), Norway Spruce (*Picea abies* (L.) Karst.) have been widely served as good biomonitors for atmospheric pollutants due to their epicuticular waxy layer, wide distribution and easy identification (Hwang et al., 2003; Liu et al., 2006; Tremolada et al., 1996).

Located in the subtropical region of China, Guangdong Province has undergone a fast process of urbanization and industrialization during the last three decades since China's reform and opening-up policy was implemented in the late 1970s. Industrial activities have considerably increased emissions and are considered as key contributors to air contamination and potential threats to human life in this region (Fu et al., 2003). The presence and distribution of heavy metals (Wong et al., 2002) and PAHs (Fu et al., 2003) have been reported in various environmental media including atmosphere (Li et al., 2006), soil (Li et al., 2007, 2008), sediment (Liu et al., 2005; Luo et al., 2008), water (Mai et al., 2002) and mosses (Liu et al., 2005), however, research on the presence and patterns of both organic and inorganic pollutants in plants is limited, particularly at different types of industrial sites.

In this study, we selected Masson pine (*Pinus massoniana* L.), a widespread species in South China, as privileged samplers for contaminants, owing to its accumulating capacity of pollutants (Sun et al., 2009; Tian et al., 2008) and easy age-identification. Needles were collected from three industrial sites and one remote site, and used to analyze the concentrations of heavy metals (Cu, Cd, Pb, Zn, Cr, Ni, and Co) and 16 PAHs listed as priority pollutants by the US Environmental Protection Agency (USEPA). The objective of this study was to investigate the contamination level in the industrial areas in Guangdong by comparing heavy metals and PAH concentrations present in pine needles around industrial sites and the remote site as well as relating them to possible sources. The effects of needle age and water-washing on the concentrations of the studied pollutants were also examined.

1 Materials and methods

1.1 Site description

This study was carried out in Guangdong Province, South China. Guangdong has a population of 91.9 million and a total area of 1.78×10^5 km² (Guangdong Provincial Statistical Bureau, 2005). The region is characterized by a subtropical monsoon climate with southwest prevailing wind in summer and north wind in winter. The mean annual temperature is 21–23°C and ranges from 13–15°C in January to 28–29°C in July. The annual precipitation fluctuates between 1500 and 1800 mm with about 80% of which falls in the wet season from April to September (Editorial Committee of Forests of Guangdong, 2005).

To avoid direct impact of vehicle emission, four sampling sites located in local forests or parks were selected. Danshuikeng forest park (DSK) at Guangzhou, Xiqiaoshan forest park (XQS) at Foshan, and Shanzibei forest (SGS) at Shaoguan were selected as representatives of the industrial emission-impacted areas, while Nankunshan natural



Fig. 1 Location of the studied sites in Guangdong, China. DSK, near a petrochemical complex; XQS, around heavy oil-fired ceramic manufactories; SGS, adjacent to coal-fueled iron and steel plants; NKS, a remote site with no industrial activity nearby.

reserve (NKS) at Huizhou was chosen as the remote clear site. Specifically, DSK is near a petrochemical complex, which was commissioned during the 1960s and has become one of the largest chemical/petrochemical industrial complexes of Guangzhou. The petrochemical refineries produce a wide variety of petroleum and chemical products such as fuels, lubricant oils, natural gas, petroleum coke and asphalt, with the capacity to process up to 13 million tons of crude oil, and produce 200,000 tons of ethylene per year (Li et al., 2009). XQS, located in Nanhai District of Foshan where the ceramic industry consuming heavy oil as fuel has flourished since the late 1980s. XQS is located less than 5 km from the large ceramic manufacturers (Wang et al., 2002). SGS is adjacent to the largest iron and steel company in Guangdong Province, where sintering, coking, iron making and steel making plants are situated and coal is used as the primary fuel. NKS is situated in Huizhou, with no industry established nearby.

1.2 Sampling and preparation

At each site, five mature trees of similar size and age without apparent injury and at least 100 m away from each other were selected for sampling in July 2008. Small branches located at the outer part of the middle canopy at the four orientations (north, south, east and west) were cut by pruning shears. Adequate current (C) and previous year (C+1) needles were collected from the branches and pooled by needle age for each tree, taking care to minimize contact with the needle surface. Detached needles were immediately sealed in polyethylene bags, stored in a home-made cryogenic storage container and carried back to the laboratory.

In the laboratory, the sampled needles from the SGS site were divided into two halves. One half was retained as an unwashed sample. The second half was gently washed with tap water for 1 min and rinsed with deionized water for 2 times (each lasting 30 sec) at room temperature, to remove the fraction deposited on needle surfaces. The

entirety of the needle samples collected from all other sites was washed according to this method. All the needles were then treated separately for heavy metals and PAHs analyses. Samples for heavy metal analyses were oven-dried for 48 hr at 60°C and ground to pass a 0.1-mm sieve; those for PAHs determination were cut into 1-cm-long sections, freeze-dried, and kept frozen at -20°C until analysis.

1.3 Heavy metal analysis

About 0.1500 g of dried needle powder was placed in an acid-washed 50 mL borosilicate glass tube, then 5 mL concentrated nitric acid (16 mol/L) and 1 mL concentrated perchloric acid (12 mol/L) were added. The mixed solution was digested overnight at room temperature, then at about 100°C for 2 hr and at higher temperature (150–190°C) until clear. The solution obtained was diluted to a final volume of 50 mL with distilled-deionized water after cooling. Metal concentrations (Cu, Cd, Pb, Zn, Cr, Ni and Co) were undertaken using inductively coupled plasma atomic emission spectrometry (ICP/AES, Optimal 2000, USA) and expressed as mg/kg on a dry mass basis. Data quality was controlled by the national standard reference material (GSV-3) supplied by China National Environmental Monitoring Center. Analyses of standards and duplicates indicate a relative error of < 5% for most elements.

1.4 PAHs extraction and analysis

The extraction and determination of PAHs in pine needles was conducted at Guangdong Institute of Eco-environment and Soil Sciences. Briefly, 5 g needle samples were mixed with 5 g anhydrous sodium sulfate (analytical grade, Nanjing Chemical Reagent Co., China), added to a mixture (200 µg/L, 5 µL) of acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂ (North Kingston, Ultra Scientific Inc., USA), and Soxhlet-extracted with dichloromethane for 48 hr. In order to eliminate the disturbance of elemental sulfur, 5 g activated copper granules were added to the collection flasks. Rotary evaporated extracts were loaded on a multiple layer chromatographic column packed with silica/alumina (2:1, V/V) and anhydrous sodium sulfate, and then eluted with 60 mL dichloromethane. The eluent was volume reduced on an evaporator, and the solvent exchanged to 1 mL hexane. A gel permeation chromatography (GPC) column (10 mm inner diameter, Accustandard Co., USA) was used to eliminate lipids. The GPC column was eluted with 80 mL of a mixture dichloromethane and *n*-hexane (1:1, V/V). Then the eluted solvent was vacuum-evaporated to 1 mL and finally concentrated to 0.2 mL. For each sample, 1 µg hexamethylbenzene (Aldrich Chemicals, Gillingham, UK) was added as internal standard prior to instrumental analysis.

The following 16 PAHs were analyzed: naphthalene (Nap), acenaphthene (Ace), acenaphthylene (DiH), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluo), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF),

benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-c,d]pyrene (Ind), dibenzo[a,h]anthracene (DiB), and benzo[g,h,i]perylene (BghiP). Total PAHs (Σ PAHs) was defined as the sum of the above components. PAHs compounds were analyzed by GC-MS (Thermo Trace DSQ, USA) in selected ion monitoring (SIM) mode. Samples (1 µL) were injected with a 15-min solvent delay time. The column temperature was initially set at 60°C for 3 min, raised to 200°C at a rate of 20°C/min, increased to 260°C at 4°C/min, and then reached 270°C at 2°C/min (10 min hold time). Helium with high purity was the carrier gas at a flow of 1.2 mL/min and a linear velocity of 25.4 cm/sec at 300°C. Data acquisition and processing were controlled by a HP ChemStation data system. Data quality was ensured using laboratory blanks and recovery standards (SRM 1649A standard reference material, NIST, Gaithersburg, USA). The blanks did not show any detectable PAHs. The mean recoveries for acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂ were 60.5%, 91.7%, 96.8% and 90.3%, respectively. The recovery percentage of deuterated PAHs was taken into account in quantifying all the investigated PAHs. Each sample was analyzed three times and expressed as the mean.

1.5 Statistical analyses

SPSS 15.0 for Windows was used for the data analysis with $\alpha = 0.05$. The statistical difference in mean concentration of a given pollutant among the four sites was evaluated by one-way ANOVA or Kruskal-Wallis ANOVA on ranks, depending on the distribution type of the data set. Paired *t*-test was performed to compare the difference of a given pollutant between C and C+1 needles and that between washed and unwashed needles. Correlation analyses were performed by Pearson or Spearman test, respectively for normally or non-normally distributed data.

2 Results

2.1 Heavy metal and PAH concentrations in pine needles of different age

As shown in Table 1, both the washed and unwashed C+1 needles recorded higher concentrations of heavy metals than C needles with significant differences in Cd, Cr, Pb and Zn, except for Ni presenting a reverse trend. Similarly, PAHs also revealed generally higher levels in the C+1 needles (Fig. 2). For unwashed needles, significantly higher concentrations in C+1 needles were observed for Ace, Flu, Phe, Pyr and Σ PAHs. After washing, differences of Flu, Phe, Ant, Fluo, Pyr, BaA, BbF, BkF, Ind, BghiP and Σ PAHs between C and C+1 needles were significant.

2.2 Heavy metal and PAH concentrations in washed and unwashed needles

At SGS, the unwashed C and C+1 needles showed higher PAH concentrations than washed needles (Fig. 2). The washing effect was especially significant in C needles for all PAHs except for Nap, Ace, DiH, Ant, and Chr.

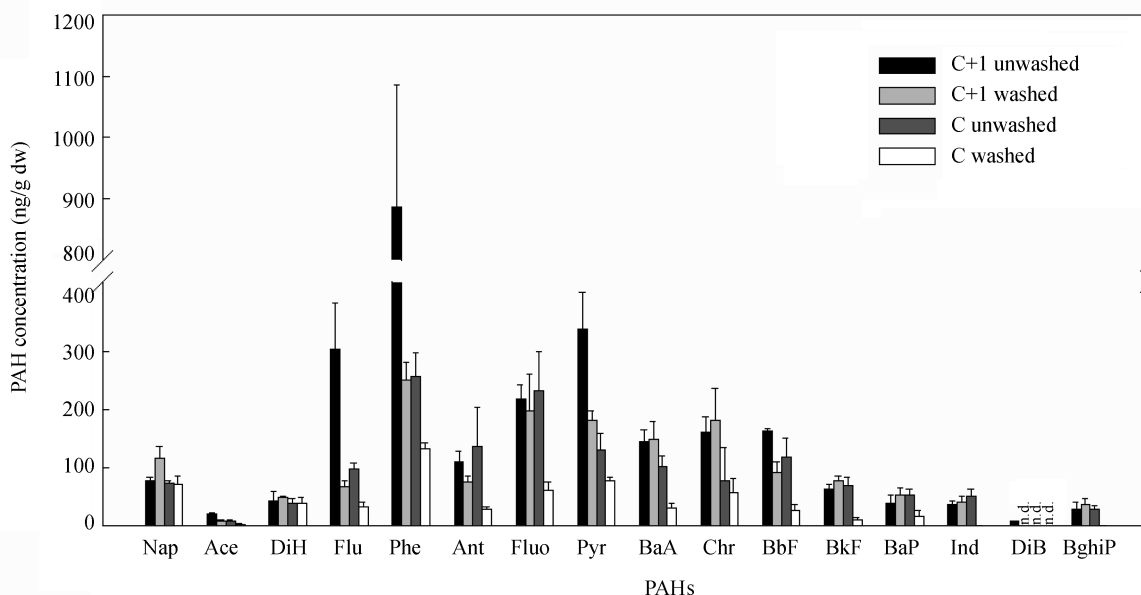


Fig. 2 Concentrations of individual PAH compounds in the washed and unwashed needles of C and C+1 ages sampled from the steel and iron industrial site SGS.

Table 1 Mean concentrations (mg/kg dw \pm SE, $n = 5$) of heavy metals in unwashed and washed Masson pine needles of C and C+1 age at the industrial site SGS

Heavy metal	C+1 needles		C needles	
	Unwashed	Washed	Unwashed	Washed
Cu	7.49 \pm 2.92	6.07 \pm 0.97	5.88 \pm 1.44	6.35 \pm 0.56
Cd	0.99 \pm 0.16	0.92 \pm 0.07	0.51 \pm 0.08	0.54 \pm 0.08
Pb	41.10 \pm 4.20	37.96 \pm 5.43	13.74 \pm 2.29	14.44 \pm 1.69
Zn	64.78 \pm 10.40	57.75 \pm 3.11	34.58 \pm 5.33	34.27 \pm 2.46
Cr	1.84 \pm 0.50	0.84 \pm 0.20	1.12 \pm 0.42	0.36 \pm 0.09
Ni	1.02 \pm 0.30	1.07 \pm 0.16	2.46 \pm 0.60	2.96 \pm 0.41
Co	0.76 \pm 0.14	0.73 \pm 0.11	0.48 \pm 0.06	0.55 \pm 0.10

However, significant differences of PAHs in washed and unwashed C+1 needles were only found for Ace, Flu, Phe, BbF and Σ PAHs. By contrast, water-washing did not significantly affect needle heavy metal concentrations (Table 1).

2.3 Spatial variations of pollutants in washed C+1 needles

In the present study, the washed C+1 needles were used to compare the differences of pollutants among the sites (Table 2). All the heavy metals and most PAHs in pine needles were lower at NKS than at the industrial sites. The highest needle Cu, Ni and Zn concentrations were recorded at XQS, whereas Cd and Pb concentrations peaked at SGS. The largest difference of needle heavy metals between industrial sites and NKS was found for Pb, showing 13 times higher at SGS than at NKS. Total PAHs were the highest at XQS around the ceramic manufacturers (1927.20 ng/g), then decreased at the steel and iron industrial site SGS (1564.70 ng/g), the petrochemical zone DSK (1143.86 ng/g), and NKS (1044.78 ng/g). In terms of individual compounds, Phe, Pyr, Fluo and Chr were highly abundant, particularly at XQS and/or SGS, while Ace, DiH, Ant, BkF, BaP, Ind, BghiP, and DiB concentrations were significantly low in all needles. Moreover, the BbF and BkF concentrations were significantly high at the

industrial sites, whereas Nap and Ace were high at the remote site NKS.

The PAH compounds were grouped into classes according to the number of rings in their structures (Fig. 3). Four-ring PAHs were dominant at all the industrial sites and accounted for 45%–50% of Σ PAHs, while low molecular weight (LMW) PAH compounds (2- and 3-rings) especially Nap were prominent at NKS. Furthermore, needles from SGS exhibited a higher fraction of high molecular weight (HMW) PAHs (5- to 6-ring PAHs) whereas needles from DSK showed higher 2-ring PAH relative to other industrial sites.

To discriminate PAH sources, two diagnostic PAH distribution ratios were plotted, as presented in Fig. 4. The ratio of Σ COMB (sum of major combustion specific compounds including Fluo, Pyr, BaA, Chr, BbF, BkF, BaP,

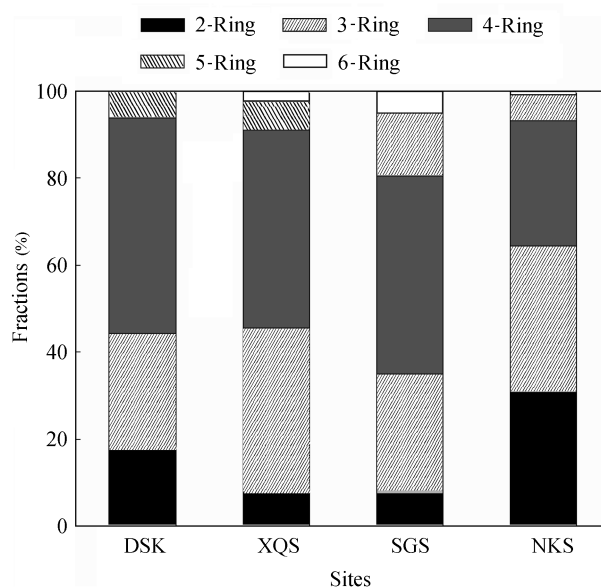


Fig. 3 Fraction of 2-, 3-, 4-, 5-, 6-ring PAHs in Masson pine needles at the sites.

Table 2 Mean concentrations of heavy metals and PAHs in the C+1 needles at the four sites ($n = 5$)

	DSK	XQS	SGS	NKS
Heavy metals (mg/kg dw)				
Cd	0.28 ± 0.05 c	0.58 ± 0.08 b	0.92 ± 0.07 a	0.22 ± 0.03 c
Co	1.56 ± 0.33	1.33 ± 0.31	0.73 ± 0.11	0.62 ± 0.15
Cr	2.33 ± 0.59 a	2.09 ± 0.25 a	0.67 ± 0.08 b	0.62 ± 0.40 b
Cu	7.27 ± 0.96 a	8.56 ± 1.15 a	6.07 ± 0.97 ab	2.92 ± 0.22 b
Ni	1.02 ± 0.21 a	1.26 ± 0.15 a	1.07 ± 0.16 a	0.47 ± 0.10 b
Pb	7.05 ± 1.00 b	25.31 ± 2.86 a	37.96 ± 5.43 a	2.90 ± 0.32 c
Zn	52.69 ± 9.84 b	90.58 ± 12.18 a	57.75 ± 3.11 b	42.55 ± 4.97 b
PAHs (ng/g dw)				
Nap	198.90 ± 77.54 ab	141.52 ± 34.41 b	116.05 ± 21.59 b	320.10 ± 30.26 a
Ace	31.77 ± 3.34 ab	21.90 ± 0.23 ab	9.23 ± 1.82 b	33.10 ± 3.63 a
DiH	49.78 ± 11.35	46.76 ± 7.89	50.75 ± 0.45	55.35 ± 6.53
Flu	45.92 ± 24.70	143.46 ± 82.59	69.35 ± 8.33	36.63 ± 8.35
Phe	157.52 ± 59.15	467.52 ± 246.92	251.97 ± 29.89	203.86 ± 32.83
Ant	35.14 ± 11.08	70.20 ± 35.85	76.90 ± 8.94	49.10 ± 11.38
Fluo	134.74 ± 70.28	207.66 ± 100.78	198.50 ± 62.32	79.34 ± 18.43
Pyr	265.60 ± 114.66	366.66 ± 172.51	182.18 ± 15.47	94.74 ± 27.36
BaA	116.12 ± 76.53	93.28 ± 49.04	150.12 ± 30.39	81.06 ± 30.29
Chr	61.53 ± 33.16	287.15 ± 152.07	182.62 ± 54.28	57.18 ± 21.51
BbF	83.80 ± 28.80 a	111.35 ± 3.65 a	93.70 ± 17.45 a	25.02 ± 6.10 b
BkF	73.70 ± 9.60 a	69.50 ± 13.10 a	78.98 ± 7.61 a	33.88 ± 6.97 b
BaP	44.00 ± 10.07	72.55 ± 22.55	53.52 ± 12.08	21.20 ± 11.80
Ind	n.d.	47.60 ± 43.60	40.92 ± 10.77	10.90 ± 3.51
DiB	n.d.	126.90 ± 20.11	n.d.	n.d.
BghiP	n.d.	61.35 ± 4.35	37.72 ± 9.72	39.30 ± 5.47
Total PAHs	1143.86 ± 483.61	1927.20 ± 955.47	1564.70 ± 91.32	1044.78 ± 114.78

Different letters indicate significant differences ($P < 0.05$) in needles among the sites. n.d.: not determined.

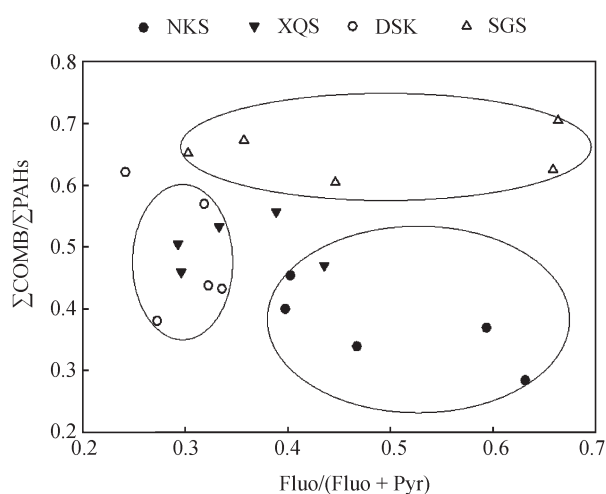


Fig. 4 Bivariate plots of $\Sigma\text{COMB}/\Sigma\text{PAHs}$ versus $\text{Fluo}/(\text{Fluo}+\text{Pyr})$ in washed C+1 needles.

Ind and BghiP) to ΣPAHs , namely $\Sigma\text{COMB}/\Sigma\text{PAHs}$, was higher in washed C+1 needles from industrial sites (0.65 at SGS, 0.50 at XQS and 0.49 at DSK) than at the remote site NKS (0.34). The $\text{Fluo}/(\text{Fluo}+\text{Pyr})$ ratio in needles displayed the sequence of NKS, SGS, XQS and DSK, with means of 0.50, 0.49, 0.35, and 0.30, respectively (Fig. 4).

3 Discussion

3.1 Effects of needle age

Higher concentrations of inorganic heavy metals and organic PAHs in C+1 needles than in C needles demonstrate that these pollutants accumulate over the increasing needle longevity and exposure time (Kuang et al., 2007;

Sun et al., 2009). Ni was the exception, showing higher levels in younger needles, a trend that has been previously observed (Giertych et al., 1997; Sun et al., 2009) and might be ascribed to the resorption of Ni from the older needles and the decreased foliar nutrient uptake capacity with age (Marschner, 1995; Rautio and Huttunen, 2003). The washed needles more clearly spotlighted the accumulation of most PAHs over exposure time than the unwashed ones. All the results suggested that age effects of needles should be considered in biomonitoring heavy metals and PAHs, and the effectiveness were dependent on characteristics of the needles and chemical properties of the studied pollutants.

3.2 Effects of washing before chemical analysis

The water-washing procedure was expected to mechanically remove much of the particulates deposited on the needle surface, including PAHs and heavy metals bound to particles and water-soluble aerosols (De Nicola et al., 2008). Our results showed noticeable effects of water-washing on organic PAHs but minimal ones on inorganic heavy metals, indicating their different chemical properties and adsorption and uptake strategies that might be partially supported by failure correlations between PAHs and heavy metal concentrations in the needles (data not shown). Indeed, gaseous deposition from the air to the waxy layer in plant was reported as a major uptake process for lipophilic organic contaminants (McLachlan et al., 1999), whereas root uptake and translocation might be a more important pathway for some heavy metals to reach the needles than for PAHs (De Nicola et al., 2008).

Moreover, the washing effects even differed among different PAH species. For example, the LMW PAHs

were noticeably reduced by water-washing in both C and C+1 needles, probably due to their higher water-solubility, which increased with the decrease in molecular weight of individual PAHs (De Nicola et al., 2008). According to Simonich and Hites (1995), when PAHs reached the needles by diffusion or by wet and dry depositions they were rapidly sorbed to the surface compartment (the waxy cuticle layer) and then diffused slowly into the inner compartment (cellular lipophilic components). In particular, particle-bound PAH very likely lingered in the surface compartment and became easily exposed to the effects of water during primary deposition while the overwhelming portion could be incorporated into the interior of pine needle although it was estimated to take some months (Lehndorff and Schwark, 2004; Piccardo et al., 2005). This could explain more significant water-washing effect on particle-bound HMW PAHs found in C needles compared with C+1 needles, probably because there was not enough time for PAHs on the surfaces of young needles to diffuse into the inner compartment (Piccardo et al., 2005). By contrast, more PAHs might have been incorporated into the inner compartment of C+1 needles where they were physically protected and thus difficult to be removed by water washing.

On the other hand, the concentrations of all the studied heavy metals were not significantly altered by water-washing, owing to their being trapped in the wax layer and/or their rapid transfer from the foliar surface to interior (Viksna et al., 1999). For example, it was reported that zinc could penetrate quickly into the leaves (Oliva and Valdés, 2004). Similar results were reported by Swaileh et al. (2004) who found that concentrations of Cu, Cd, Zn, Cr and Ni between washed and unwashed leaves of *Inula viscosa* remained the same even near a busy highway. By contrast, the noticeable effect of water-washing was found in the needles of Aleppo pine (Al-Alawi and Mandiwana, 2007). It could be concluded that the washing effect differed with plant species and contaminants relating to the degree of penetration. As proposed by Al-Alawi and Mandiwana (2007), plant species that retain constant metal concentrations in washed and unwashed needles could prove a more suitable biomonitor of metal pollution, as embodied in Masson pine.

As discussed above, the pollutant concentrations of unwashed needles expressed the concentrations of the needles plus any particles deposited and retained on the needle surface. Since the latter portion particularly for PAHs was susceptible to wind and rainfall which were quite variable, we chose the washed needles for comparisons among different sites and different years.

3.3 Spatial variations of pollutants

Concentrations of heavy metals and PAHs presented in the needles of Masson pine demonstrated conspicuous contamination in the industrial areas of Guangdong and supported the ability of Masson pine needles to act as biomonitor of both inorganic heavy metals and organic PAHs in the environment. The heavy metal concentrations in the needles analyzed in this study were not lower

but even higher than those in the needles collected from Guangzhou and NKS in 2006 (Sun et al., 2009), suggesting the heavy metal emissions have not been controlled effectively. Needle Pb, Zn and Cd concentrations touched or exceeded their risky levels (10.0, 40.0 and 0.3 mg/kg for Pb, Zn and Cd, respectively) (Sun et al., 2009) at industrial sites, which should be taken seriously. The PAH load in this study was a bit higher than that at an urban site of Guangzhou in 2006 (Tian et al., 2008) but within the range between 19 and 3091 ng/g in rural/remote areas of the UK (Tremolada et al., 1996), and between 400 and 8325 ng/g in urban/industrial areas in the Czech Republic (Holoubek et al., 2000; Hwang et al., 2003). However, the difference between the industrial and rural areas in this study was much smaller than in the Czech Republic study, where total PAH concentrations in needles from industrial areas were about 7 times higher than that from rural areas (Holoubek et al., 2000). This might be related to the long distance diffusion of PAHs from the urban and industrial pollution sources over the whole region, resulting in a concerning regional contamination. Another possible explanation was that lowered physiological activities including decreased leaf stomatal conductance of trees under air pollution might inhibit the entering of PAHs into the needles. This adaptation to air pollution stresses by adjusting stomatal operation has been reported in several species (Wen et al. 2003) although no report for Masson pine.

The PAH distributions in pine needles could reflect a mixed transport and deposition mechanism (Lehndorff and Schwark, 2004). The higher proportion of LMW PAHs especially Nap in the needles at the remote site NKS was because of their vapor phase and their resulting wider-range transport (Yang et al., 1991). At the industrial sites, the 4-ring PAHs were the most representative and Phe, Fluo and Pyr were conspicuously enriched. This was in good agreement with other research (De Nicola et al., 2008; Orecchio, 2007). The relatively lower concentrations of Ace, DiH, Ant, BkF, BaP, Ind, BghiP, and DiB found in pine needles of all the sites could be ascribed to their physical and chemical properties. Ace, DiH and Ant were susceptible to photolysis (Wang et al., 2005), whereas BaP, Ind, BghiP and DiB mainly existed as particles, had low lipid solubility (Connell and Hawker, 1988), and were thus little adsorbed by needles (Simonich and Hites, 1994).

Recent studies have shown that PAH compositions of emissions are inherently variable with respect to combustion sources, and concentration ratios of various PAHs can be used for source discrimination (Hwang et al., 2003; Lehndorff and Schwark, 2004; Yunker et al., 2002). In the present study, we selected the Fluo/(Fluo+Pyr) and $\Sigma\text{COMB}/\Sigma\text{PAHs}$ ratios to estimate PAH sources. The former ratio covered the gaseous as well as the particulate-transported PAH and the value kept unmodified during transfer into different matrices (Yunker et al., 2002), while the latter was often used to indicate typical combustion origin PAHs. The Fluo/(Fluo+Pyr) ratio below 0.40 involved petroleum, and ratios above 0.50 indicated grass and wood or coal combustion, whereas ratios between 0.40 and 0.50 were more characteristic of fossil fuel combustion (Yunker

et al., 2002; Lehndorff and Schwark, 2004).

As shown in Fig. 4, the study sites could be categorized into three groups. Needles sampled from the remote site NKS were generally characterized as low $\Sigma\text{COMB}/\Sigma\text{PAHs}$ ratios but had enhanced Fluo/(Fluo+Pyr) ratios above 0.50, indicating low combustion activities and grass and wood or coal combustion involved (Orecchio, 2007; Yunker et al., 2002). The higher $\Sigma\text{COMB}/\Sigma\text{PAHs}$ ratio at SGS suggested more extensive combustion activities than at the other sites, since coke manufacturing, sintering, iron making, casting, mold poring and cooling, and steel making in the steel and iron industries could release PAHs (Yang et al., 2002). Moreover, Fluo/(Fluo+Pyr) ratios at SGS lay in a wide range between 0.30 and 0.66 with a mean of 0.49, probably inferring mixed sources but with dominant contributions of coal combustions related to the processes in the coal-fueled steel and iron industries. The low ratios of Fluo/(Fluo+Pyr) below 0.40 in the needles at DSK revealed the petroleum-dominated contribution from the nearby petrochemical industries, supported by the relatively high fraction of 2-ring PAH since fresh fuels resulted in an enrichment of LMW PAHs (Budzinski et al., 1997; Hwang et al., 2003). At XQS, the Fluo/(Fluo+Pyr) ratios in needles ranged from 0.30 to 0.44, which seemed to point to mixed sources such as petroleum and fossil fuel combustion (Lehndorff and Schwark, 2004; Yunker et al., 2002). These results were in good agreement with the real types of energy consumption of the studied sites and the results of other research on biomonitoring (Hwang et al., 2003; Orecchio, 2007).

4 Conclusions

Water-washing removed a large amount of needle surface deposit and consequently most of the particle-bound PAHs, especially for young C needles, but much less affected the elemental pollutants which might migrate into cuticular waxes after deposition on the needle surface. Both the unwashed and washed needles exhibited higher pollutant concentrations in the C+1 age class; the washed needles in particular more clearly highlighted the accumulation capacity of most PAHs over exposure time. Although differing adsorption and uptake strategies between heavy metals and PAHs might exist, significantly higher concentrations of both pollutants in Masson pine needles were recorded at the industrial sites, indicating conspicuous contamination due to industrial emissions there. PAHs with four rings dominated the industrial sites while those with two and three rings dominated the remote site. The distribution patterns of PAHs around the industrial and the remote sites accorded with the real types of energy consumption in the studied sites, indicating the potential of Masson pine needles for pinpointing local pollution sources.

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