Subject Area 10: State-of-Art in China – Research Progresss (Editor: Yong-Guan Zhu)

Research Article

Heavy Metals in Bark of *Pinus massoniana* (Lamb.) as an Indicator of Atmospheric Deposition Near a Smeltery at Qujiang, China

Yuan Wen Kuang[1,2], Guo Yi Zhou[1], Da Zhi Wen[1]* and Shi Zhong Liu[1]

[1] South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, China
[2] Graduate School of Chinese Academy of Sciences, Beijing 100039, China

---

* **Corresponding author** (dzwen@scbg.ac.cn)

DOI: http://dx.doi.org/10.1065/espr2006.09.344

Please cite this paper as: Kuang YW, Zhou GY, Wen DZ, Liu SZ (2007): Heavy Metals in Bark of *Pinus massoniana* (Lamb.) as an Indicator of Atmospheric Deposition Near a Smeltery at Qujiang, China. Env Sci Pollut Res 14 (4) 270–275

Abstract

Goal, Scope and Background. Rapid urbanization and the expansion of industrial activities in the past several decades have led to large increases in emissions of pollutants in the Pearl River Delta of south China. Recent reports have suggested that industrial emission is a major factor contributing to the damages in current natural ecosystem in the Delta area. Tree barks have been used successfully to monitor the levels of atmospheric metal deposition in many areas, but rarely in China. This study aimed at determining whether atmospheric heavy metal deposition from a Pb-Zn smeltery at Qujiang, Guangdong province, could be accurately reflected both in the inner bark and the outer bark of Masson pine (*Pinus massoniana* L.). The impact of the emission from smeltery on the soils beneath the trees and the relationships of the concentrations between the soils and the barks were also analyzed.

Methods. Barks around the bole of *Pinus massoniana* from a pine forest near a Pb-Zn smeltery at Qujiang and a reference forest at Dinghushan natural reserve were sampled with a stainless knife at an average height of 1.5 m above the ground. Mosses and lichens on the surface barks were cleaned prior to sampling. The samples were carefully divided into the inner bark (living part) and the outer bark (dead part) in the laboratory, and dried and ground, respectively. After being dry-ashed, the powder of the barks was dissolved in HNO_3. The solutions were analyzed for iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni) and cobalt (Co) by inductively coupled plasmas emission spectrometry (ICP, PS-1000AT, USA) and Cadmium (Cd) and lead (Pb) by graphite furnace atomic absorption spectrometry (GFAAS, ZEENIT 60, Germany). Surface soils (0–10 cm) beneath the sample trees were also collected and analyzed for the selected metals.

Results and Discussion. Concentrations of the selected metals in soils at Qujiang were far above their environmental background values in the area, except for Fe and Mn, whilst at Dinghushan, they were far below their background values, except for Cd and Co. Levels of the metals, in particular Pb and Zn, in the soils beneath the sample trees at Qujiang were higher than those at Dinghushan with statistical significance. The result suggested that the pine forest soils at Qujiang had a great input of heavy metals from wet and dry atmospheric deposition, with the Pb-Zn smeltery most probably being the source. Levels of Cu, Fe, Mn, Zn, Ni and Pb at Qujiang, both in the inner and the outer bark, were statistically higher than those at Dinghushan. Higher concentrations of Pb, Fe, Zn and Cu may come from the stem-flow of elements leached from the canopy, soil splash on the 1.5 m height and sorption of metals in the mosses and lichens growing on the bark, which were direct or indirect results from the atmospheric deposition. Levels of heavy metals in the outer barks were associated well with the metal concentrations in the soil, reflecting the close relationships between the metal atmospheric deposition and their accumulation in the outer bark of Masson pine. The significant ($p<0.01$) correlations of Fe-Cu, Fe-Cr, Fe-Pb, Fe-Ni, Pb-Ni, and Pb-Zn in the outer barks at Qujiang again suggested a common source for the metals. The correlation only occurred between Pb and Ni, Cd and Co in the outer barks at Dinghushan, which suggested that those metals must possibly have other uncommon sources.

Conclusions. Atmospheric deposition of the selected metals was great at Qujiang, based on the levels in the bark of *Pinus massoniana* and on the concentrations in the soils beneath the trees compared with that at Dinghushan. Bark of *Pinus massoniana*, especially the outer bark, was an indicator of metal loading at least at the time of sampling.

Recommendations and Perspectives. The results from this study and the techniques employed constituted a new contribution to the development of biogeochemical methods for environmental monitoring particularly in areas with high frequency of pollution in China. The method would be of value for follow up studies aimed at the assessment of industrial pollution in other areas similar with the Pearl River Delta.

Keywords: Atmospheric deposition; bark as bioindicator; China, inner bark; metal deposition; outer bark; Pearl River Delta; *Pinus massoniana*; smeltery

Introduction

The rapid population growth, urban sprawl, and industrial activities expansion have affected the chemical substances in our environment more and more. Many heavy metals emitted mostly from anthropogenic sources have now exceeded or equaled their natural emissions (Biney et al. 1994) and have been posing a serious threat to the ecosystems. Monitoring of the heavy metals has been of importance and new methods of observing the deposition have been constantly sought (Böhm et al. 1998, Kakulu 2003, Pacheco et al. 2003). Biological monitoring of airborne contaminants has made a great progress since the early observations of environmentally induced stress on plants and its applications have grown to an extent hardly envisaged just a few decades ago (Pacheco et al. 2002). Higher plants have emerged in recent years as a valuable tool enabling identification of environmental pollution (Markert et al. 1996, Carreras and Pignata 2002, Klumpp et al. 2002, Outola et al. 2003, Schröder et al. 2003, Adamoa et al. 2004, Jackson et al. 2004, Trimbacher and Weiss 2004). Increased metal concentrations and decreased pH values have been reported in the tree barks from the high industrial activity, increased urbanization, increased acidic precipitation and high traffic loads (Kuik and Wolterbeek 1994, Tüerkan et al. 1995, Santamaría and Martía 1997, Böhm et al. 1998, Pacheco et al. 2001, El-Hasan et al. 2002, Pacheco et al. 2002, Pacheco et al. 2003, Bellis et al. 2001, Harju et al. 2002). A problem exists with the interpretation of chemical data from the tree barks. Within the barks there are two parts outside the woody body according to Satake et al. (1996). One is the living part, the inner bark, and the other is the dead part, the outer bark. Although the tree bark is known to absorb and accumulate airborne contaminants due to their hard, rough and thick structure (Santamaría and Martía 1997), the relative importance of an accumulation of pollutant directly from the atmosphere compared with uptake from the soil in the different parts may be neglected. Due to the small part of the inner bark, most previous studies, except that from Satake et al. (1996), have sampled the whole bark for analysis, and thereby ignored the difference of the elements between the two parts. In the present study, inductively coupled plasma was used to analyze some heavy metals from the inner and outer bark of Masson pine (*Pinus massoniana* L.) growing in the vicinity of iron refinery, as compared with trees from a natural reserve largely unaffected by refinery emission. Masson pine is a ubiquitously distributed species of tree across subtropical China, which makes it a good candidate for comprehensive surveys of airborne contaminants. The purpose was to determine whether atmospheric emission from the refinery could be accurately reflected in the inner and outer bark. The potential impact of the soil on the metal levels in the bark was also detected. Due to the lack of similar investigations in both areas, we hope that the results from this study would provide preliminary information on the impact of these heavy elements on the ecosystem and may form baseline data for future impact assessments concerning heavy metal pollution.

1 Material and Methods

1.1 Site descriptions and sampling

Two woodlands situated in Qujiang and Dinghushan, southern China, respectively, were chosen for the study. The first is located about 5 km from a Pb-Zn smelting plant operational since 1960s in Qujiang (27°37'N, 113°30'E). The smelting plant is one of the main smelters in the Pearl River Delta of south China. Its industrial product capability has reached 240,000 tons Pb and Zn per year nowadays. The reference site is situated in Dinghushan (23°10'N, 112°32'E), a national reserve in China and a member of the Chinese Ecosystem Research Network. Trees from Qujiang, with a stem mean diameter at breast height (DBH) of 24.96 ± 3.0 cm, were 40–45-years-old and 10.3–11.8 m in height. The density of the Masson pine is about 1,500 per 10,000 m^2 with some shrub species (e.g. *Cinnamomum camphora*, *Callicarpa macrophylla*, *Gardenia jasminoides*, *Camellia oleifera*) and herbaceous species (e.g. *Dicranopteris dichotoma* and *Imperata cylindrical*). Trees from Dinghushan, with a mean DBH of 27.95 ± 2.01 cm, were 48–50-years-old and 11.0–12.8 m in height. The density of Masson pine here is smaller (1,200 per 10,000 m^2) than that of Qujiang, with shrub species (e.g. *Schima superba*, *Evodia lepta*, *Rhodomyrtus tomentosa*, *Clerodendron fortunatum*, *Melastoma candidum*) and the dominant herbaceous species *Dicranopteris pedata*.

Sampling was conducted in November 2002, during the dry season of the year, since rainfall could bring about a considerable reduction in the level of metal in the plants (Ho and Tal 1979). Ten Masson pine trees were selected for bark sampling from a pine forest. Sample trees were at least 100 m away from each other and 200 m away from the roads. The bark was carefully removed from the boles of the trees with a stainless steel knife at an average height of 1.5 m above the ground. To reduce the influence of the relative source position, a whole bark ring was completely sampled around the bole. The rings were 10 cm in height. The bark was cleaned with a synthetic hard brush to remove mosses and lichens prior to being collected. Bark samples were kept in a paper envelope and then placed in a polythene bag before transporting to the laboratory. Surface soil samples (0–10 cm) were collected around the selected trees using a stainless-steel trowel and transferred to the laboratory in paper bags.

1.2 Sample preparation

In the laboratory, the barks were divided into inner and outer barks, with a stainless steel knife used according to Satake et al. (1996) (**Fig. 1**). All samples were not washed and were dried in the oven at a temperature of 105°C for about 5 h to a constant weight. The dried samples were then ground. To avoid contamination, the mill was thoroughly cleaned and dried after each grinding.

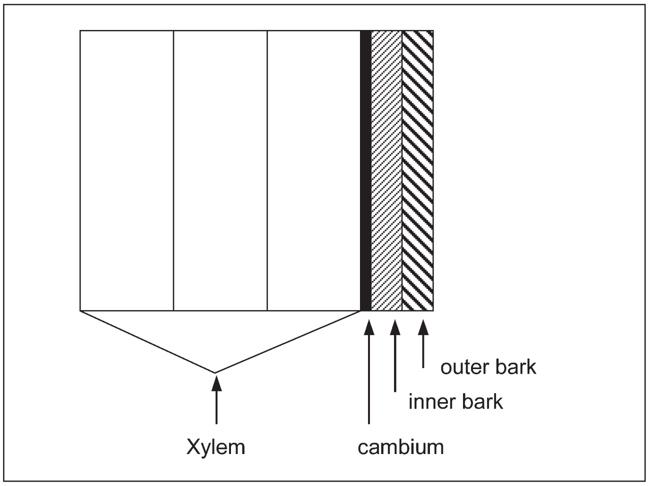

Fig. 1: Diagram of the tree bark

1.3 Chemical analyses

About 5 g of the powder of the barks was accurately weighed and transferred into a properly cleaned crucible, and pre-ashed on a hot plate until all the fumes disappeared. Then it was transferred into the muffle furnace and dry-ashed at 450–500°C for 8 h. After cooling, the ash was dissolved in HNO_3 solution and filtered through acid-washed (10% HNO_3) filter paper and diluted to 50 mL with distilled-deionized water (dd-H_2O). Solutions were analyzed for iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni) and cobalt (Co) by Inductively Coupled Plasma emission spectrometry (ICP, PS-1000AT, U.S.A) and Cadmium (Cd) and lead (Pb) by graphite furnace atomic absorption spectrometry (GFAAS, ZEENIT 60, Germany).

Soils were air-dried, ground and then were analyzed for total (5 mL HNO_3 and 5 mL HF) heavy metal concentrations using standard analytical procedures (Dong 1996) by ICP.

Precision and accuracy were determined through repeated analysis of National Standard Reference material of poplar leaves (GBW 07604) and agricultural soil (GBW E 070045). Recoveries from plant standards ranged between 90–96%, while heavy metal recoveries from soil standards were between 80–95%.

2 Results and Discussion

2.1 Soil metal concentrations

The difference in total concentrations of deposited metals between sites was indicative of a difference in total concentration in the soils, and could reflect the different sources within the surface soil horizon (Alloway 1990). Concentrations of the selected metals were presented in **Table 1**. The metal concentrations, except for Fe and Mn at Qujiang, were far above their background values, which was 35 200 μg·g^{-1} and 218.03 μg·g^{-1}, respectively (Li and Zheng 1989). Surface soil (0–10 cm) beneath the trees at Qujiang had significantly higher concentrations of the selected heavy metals, in particular Pb, compared with surface soil at Dinghushan. Lead contents from Qujiang ranged from 145.55 to 428.16 μg·g^{-1}. The average values were above 20 and 6 times those recorded at Dinghushan for Pb and Zn, respectively. Significantly higher total Cu, Zn, Cd, Pb, Ni and Co in the soil at Qujiang than those at Dinghushan indicated that the input of those metals from dry and wet atmospheric deposition may be great. The forest at Qujiang had received substantial atmospheric metal deposition, most probably originating from the Pb-Zn smelting plant.

The total concentrations of the heavy metals except for Cd and Co in the soils beneath the trees at Dinghushan were far below their environmental background values in the area (Li and Zheng 1989), indicating that this area received minimal atmospheric heavy metals inputs. However, high concentrations of Cd (10 times the background value) and Co (double the background value) in the surface soil horizon beneath the trees at Dinghushan may result from some unknown sources and should be concerned.

2.2 Metal concentrations in barks

Copper, Fe, Mn, Zn, Ni and Pb in the outer barks at Qujiang were statistically ($P<0.001$, $n=10$) higher than those at

Table 1: Total heavy metal concentrations (μg·g^{-1}) in surface soil (0–10 cm) beneath the sampled trees at Qujiang and Dinghushan. Numbers in the bracket after the averages are the standard deviations of the concentrations

Tree	Cu	Zn	Fe	Mn	Cd	Cr	Pb	Ni	Co
Dinghushan									
1	9.20	42.44	15,856.44	33.56	1.39	4.49	11.84	8.57	5.52
2	6.67	41.84	18,961.46	35.38	1.44	5.03	12.04	9.59	9.72
3	12.74	30.43	17,084.16	50.9	0.59	4.39	19.66	11.41	11.96
4	5.55	24.75	16,463.27	16.13	0.48	5.21	13.79	5.92	10.27
5	15.27	56.88	19,402.71	33.90	0.86	4.53	20.46	13.59	13.27
6	12.31	61.04	19,107.30	53.25	0.15	2.55	19.41	13.42	4.23
7	6.97	33.75	20,241.90	39.79	0.25	4.17	6.89	7.65	8.88
8	7.23	43.73	17,845.02	26.10	0.46	3.94	9.35	9.01	13.29
9	12.29	37.88	14,054.23	10.07	0.74	3.75	5.19	5.46	5.73
10	16.23	22.24	15,447.18	18.23	0.88	2.92	5.85	11.71	9.43
Average	10.44(3.68)	39.50(13.43)	17,446.37(1671.4)	31.73(12.40)	0.72(0.52)	4.10(0.87)	12.45(5.11)	9.63(2.91)	9.23(3.26)
Qujiang									
1	38.90	355.15	35,470.25	194.82	8.4	6.45	428.16	35.68	34.63
2	41.46	341.96	27,340.57	188.96	9.45	3.28	208.55	44.70	28.68
3	37.33	190.15	33,375.09	194.50	5.20	6.65	236.24	35.95	34.37
4	36.74	186.09	33,161.49	190.58	4.94	5.50	339.02	25.57	27.62
5	38.48	357.94	27,991.94	201.31	8.96	6.36	275.68	36.15	29.74
6	38.42	321.26	31,149.67	198.11	9.14	4.59	276.44	36.12	33.19
7	41.61	178.86	29,365.22	123.22	1.62	6.74	267.24	25.48	22.66
8	37.75	152.85	26,298.22	119.96	3.70	9.01	166.42	32.66	20.00
9	35.07	193.31	29,370.67	185.98	1.56	6.39	242.62	32.22	24.27
10	44.66	138.62	37,348.06	89.51	2.08	7.92	145.55	40.51	31.99
Average	39.04(2.80)	241.62(90.26)	31,087.12(3,657.8)	168.70(41.07)	5.51(3.25)	6.29(1.60)	258.59(81.95)	34.50(5.95)	28.72(5.08)

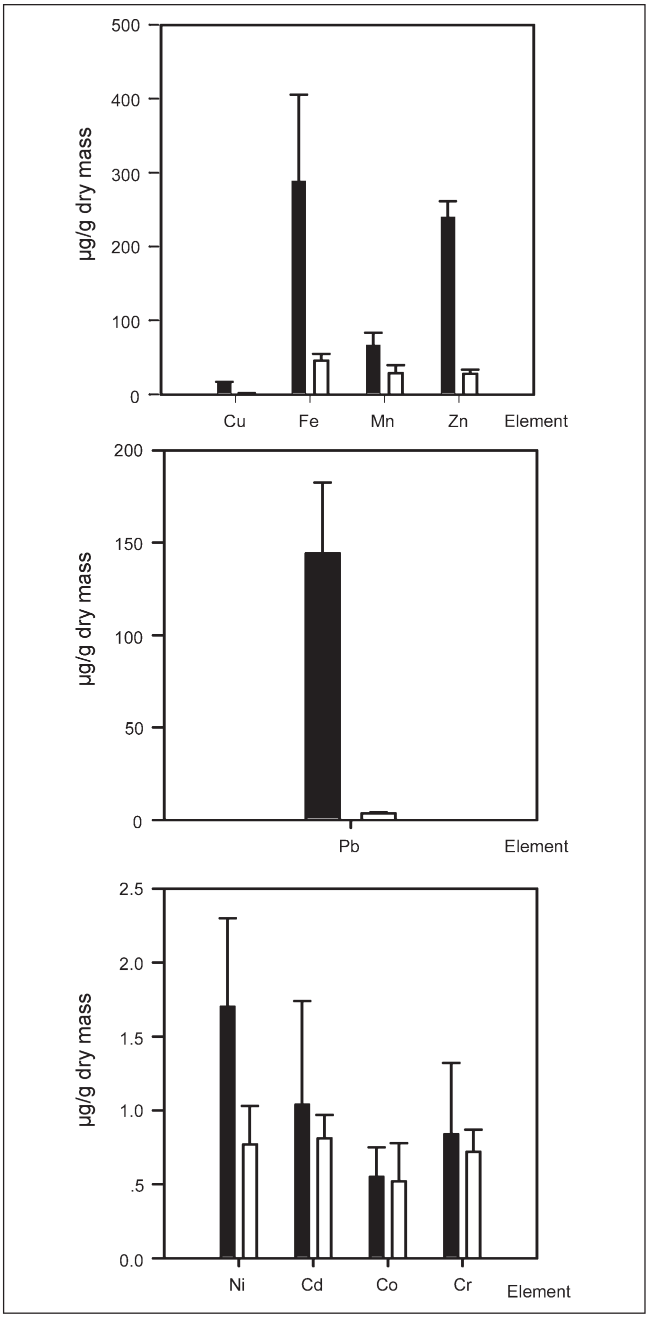

Fig. 2: Concentrations of the metals in the outer bark of Qujiang (■) and Dinghushan (□)

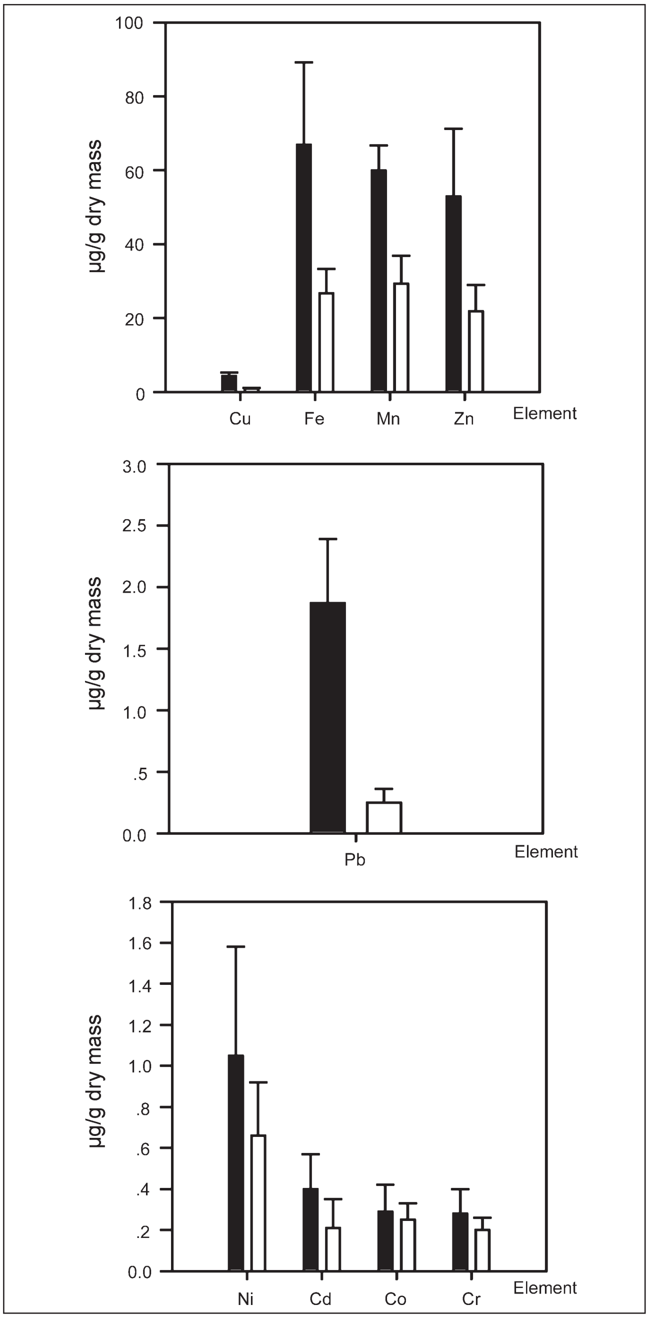

Fig. 3: Concentrations of the metals in the inner bark of Qujiang (■) and Dinghushan (□)

Dinghushan detected by Student's t-test (**Fig. 2**), which is similar to the patterns of the elements in the soil concentrations. Lead level in the outer barks at Qujiang was about 40 times that at Dinghushan, followed by Cu (10 times), Zn (8 times), Fe (6 times), Mn and Ni (2 times each). The concentrations of Cd, Co and Cr in the outer barks at Qujiang were not significantly different from those at Dinghushan. Iron and zinc were the highest heavy metals in the outer barks at Qujiang, and lead, the highest trace element.

The mean concentrations of Cu (4.29 ± 0.71), Fe (66.86 ± 10.93), Mn (59.94 ±7.15), Zn (52.96 ± 7.27), Pb (1.87 ± 0.24) and Ni (1.05 ± 0.19) in the inner barks at Qujiang were significantly ($p<0.05$, $n=10$) higher than those of Cu (0.95 ± 0.19), Fe (26.75 ± 1.53), Mn (29.26 ± 7.63), Zn (21.88 ± 7.07), Pb (0.25 ± 0.11) and Ni (0.66 ± 0.26) at Dinghushan (**Fig. 3**). Patterns of those metals in the inner barks were similar to those in the outer barks. Zinc and Fe in addition to Mn in the inner barks, both at Qujiang and Dinghushan, were statistically higher than Cu, whilst Pb and Pb-Ni were the highest trace metals in the inner barks.

Element concentrations in the barks reflect concentrations both within the phloem and absorbed to bark predominately via wet and dry atmospheric deposition (Lepp 1975). Much higher metal concentrations, even in the inner barks than their background values in the same species (Li and Zheng 1989), and much higher levels in the outer barks at Qujiang than those at Dinghushan might come from stem-flow of elements leached from the canopy, soil splash and sorption of metals in the mosses and lichens growing on the bark, which were originated directly or indirectly from the atmospheric deposition. Research has confirmed that industrial activity tends to increase the concentration of metallic contaminants of barks (Odukoya et al. 2000). Our results suggested that the emission from the Pb-Zn smelting plant was a most probable major source of Cu, Zn, Cd, Cr, Pb and Ni in the atmosphere at Qujiang. This confirmed that the presence of high levels of these metals in the outer bark and soils were indicative of atmospheric deposition at Qujiang.

The comparisons of the metal levels in the inner barks at the two sites also suggested that the availability of metals may be substantially greater at Qujiang, though plant-available metal concentrations was difficult to define (Lobersli and Steinnes 1987) because metals in the inner barks may originate from xylem or soluble metal ions transported from the bark surface or possibly from leaves. If the atmospheric deposition was similar at the two sites, levels of the metal in the inner barks at Qujiang and Dinghushan should not appear significantly different, since the topography and micro-climatology of both sites are similar. This evidence demonstrated that strong atmospheric deposition of metals at Qujiang impact the levels of the metal in the inner bark directly or indirectly.

In order to identify the possible source of the metal contaminations further, we detected the relationships among the metals. Significant correlations ($p<0.01$) between Fe-Cu, Fe-Cr, Fe-Pb, Fe-Ni, Pb-Ni, Pb-Zn in the outer barks at Qujiang were found, which suggested a common source for the metals. Significant correlations among those metals in the outer barks at Dinghushan only occurred between Pb and Ni, Cd and Co, which suggested that those metals must possibly have other uncommon sources.

2.3 Relationship between the total metals in soils and barks

Heavy metal concentrations of the barks were generally found to correlate very well with the heavy metal deposition (Huhn et al. 1995, Schulz et al. 1999). We found high total metal concentrations in the soil beneath the tree sample were generally followed by high metal levels in the outer bark at both sites (see Table 1 and Fig. 2). This again reflected the close relationships between the metal atmospheric deposition and their accumulation in the outer bark of Masson pine. The levels in sequence were almost similar in the outer bark as those in the soil for Fe, Zn, Pb, Mn, Cu and Ni at Qujiang, and for Fe, Mn, Zn, Pb and Cu at Dinghushan. Statistically significant ($P<0.01$) correlations between the soil concentrations and the outer bark levels for Fe, Mn, Zn, Pb and Cu ($r^2 \geq 0.80$) were found, which suggests that levels of heavy metals in the outer bark were associated with the total concentrations in the soil beneath the trees. However, relationships between the metal levels in the inner bark and the total metals in the soil were not similar at the two sites (see Table 1 and Fig. 3).

3 Conclusions

The concentrations of metals in both the outer and inner barks of Masson pine from Qujiang were statistically higher than those from Dinghushan, suggesting bark concentrations can be expected to be an indicator of metal loading at the time of sampling. The study demonstrated the suitability of the Masson pine barks, especially the outer barks, as an indicator of local atmospheric deposition. The comparison of the metal levels in the inner and outer barks from Qujiang with that from Dinghushan also suggested that the Masson pine barks can be used to evaluate atmospheric deposition of certain metals. The potential of the barks of Masson pine for indicating typical elements from industrial sources seems promising as well.

Acknowledgement. The authors wish to acknowledge the jointly financial support of the National Foundation of Sciences of China (Grant No. 30570349) and the CAS Orientation Project (Grant No. KSCX2-SW-120 and KACX2-SW-133) to the study.

References

Adamoa P, Arienzo M, Pugliese M, Roca V, Violante P (2004): Accumulation history of radionuclides in the lichen *Stereocaulon vesuvianum* from Mt. Vesuvius (south Italy). Environ Pollut 127, 455–461

Alloway BJ (ed) (1990): Heavy metals in soils. Blackwell, London, UK

Bellis D, Ma R, Bramall N, McLeod CW, Chapman N, Statake K (2001): Airborne uranium contamination-as revealed through elemental and isotopic analysis of tree bark. Environ Pollut 114, 383–387

Biney C, Amuzu AT, Calamari D, Kaba N, Mbome IL, Naeve H, Ochumba PBO, Osibanjo O, Radegonde V, Saad MAH (1994): Review of heavy metals. In: Calamari D, Naeve H (eds), Review of pollution in the African aquatic environment. Rome, Fao, pp 33–60

Böhm P, Wolterbeek H, Verburg T, MusÍlek L (1998): The use of tree barks for environmental pollution monitoring in the Czech Republic. Environ Pollut 102, 243–250

Carreras HA, Pignata ML (2002): Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. Environ Pollut 117, 77–87

Dong M (1996): Survey, Observation and Analysis of Terrestrial Biocommunities. Standards Press of China, Beijing, 154–159

El-Hasan T, AI-Omari H, Jiries A, AI-Nasir F (2002): Cypress tree (*Cupressus semervirens* L.) barks as an indicator for heavy metal pollution in the atmosphere of Amman City, Jordan. Environ Int 28, 513–519

Harju L, Saarela KE, Rajander J, Lill JO, Lindroos A, Heselius SJ (2002): Environmental monitoring of trace elements in barks of Scots pine by thick-target PIXE. Nucl Instrum Meth B 189, 163–167

Ho VB, Tal KM (1979): Effects of rain on lead levels in roadside vegetation in Hong Kong. B Environ Contam Tox 23, 658–660

Huhn G, Schulz H, Stark HJ, Tolle R, Schurmann G (1995): Evaluation of regional heavy metal deposition by multivariate analysis of element contents in pine tree barks. Water Air Soil Poll 84, 367–383

Jackson BP, Winger PV, Lasier PJ (2004): Atmospheric lead deposition to Okefenokee Swamp, Georgia, USA. Environ Pollut 130, 445–451

Kakulu SE (2003): Trace metal concentration in roadside surface soil and tree barks: A measurement of local atmospheric pollution in Abuja, Nigeria. Environ Monit Assess 89, 233–242

Klumpp A, Ansel W, Klumpp G, Belluzzo N, Calatayud V, Chaplin N, Garrec JP, Gutsche HJ, Hayes M, Hentze HW, Kambezidis H, Laurent O (2002): Eurobionet: A pan-European biomonitoring network for urban air quality assessment. Environ Sci Pollut Res 3, 199–203

Kuik P, Wolterbeek HTh (1994): Factor analysis of trace element data from tree-bark samples in the Netherlands. Environ Monit Assess 32, 207–226

Lepp NW (1975): The potential of tree-ring analysis for monitoring heavy metal pollution patterns. Environ Pollut 9, 49–61

Li J, Zheng CJ (1989): Handbook of data for environmental background values. Chinese Environmental Science Press, Beijing, pp 200–216

Lobersli EM, Steinnes E (1987): Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water Air Soil Pollut 37, 25–39

Markert B, Herpin U, Siewers U, Berkekamp J, Lieth, H (1996): German heavy metal survey by means of mosses. Sci Total Environ 182, 159–168

Odukoya OO, Arowole TA, Bamgbose O (2000): Pb, Zn, and Cu levels in tree barks as indicator of atmospheric pollution. Environ Int 26, 11–16

Outola I, Pehrman R, Jaakkola T (2003): Effect of industrial pollution on the distribution of ^{137}Cs in soil and the soil-to-plant transfer in a pine forest in SW Finland. Sci Total Environ 303, 221–230

Pacheco AM, Barros LIC, Freitas MC, Reis MA, Hipólito C, Oliveira OR (2002): An evaluation of olive-tree barks for the biological monitoring of airborne trace-elements at ground level. Environ Pollut 120, 79–86

Pacheco AM, Freitas MC, Barros LIC, Figueira R (2001): Investigating tree barks as an air-pollution biomonitor by means of neuron activation analysis. J Radioanal Nucl Ch 249, 327–331

Pacheco AMG, Freitas MC, Reis MA (2003): Trace-element measurements in atmospheric biomonitors – A look at the relative performance of INAA and PIXE on olive-tree barks. Nucl Instrum Meth A 505, 425–429

Santamaría JM, Martía A (1997): Tree barks as a bioindicator of air pollution in Navarra, Spain. Water Air Soil Poll 98, 381–387

Satake K, Tanaka A, Kimura K (1996): Accumulation of lead in tree bark pockets as pollution time capsules. Sci Total Environ 181, 25–30

Schröder P, Fischer C, Debus R, Wenzel A (2003): Reaction of detoxification mechanisms in suspension cultured spruce cells (*Picea abies* L. KARST) to heavy metals in pure mixture and in soil eluates. Env Sci Pollut Res 4, 225–234

Schulz H, Popp P, Huhn G, Stärk HJ, Schüürmann G (1999): Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks: Temporal and spatial variations. Sci Total Environ 232, 49–55

Trimbacher C, Weiss P (2004): Norway spruce: A novel method using surface characteristics and heavy metal concentrations of needles for a large-scale monitoring survey in Austria. Water Air Soil Pollut 152, 363–386

Tüerkan I, Henden E, Celik Ü, Kivilcim S (1995): Comparison of moss and bark samples as biomonitors of heavy metals in a highly industrialized area in Izmir, Turkey. Sci Total Environ 166, 61–67

Received: October 9th, 2005
Accepted: September 22nd, 2006
OnlineFirst: September 23rd, 2006

Env Sci Pollut Res 4 (10) 225–234 (2003)

Reaction of Detoxification Mechanisms in Suspension Cultured Spruce Cells (*Picea abies* L. KARST.) to Heavy Metals in Pure Mixture and in Soil Eluates

Peter Schröder[1]*, Claudia Fischer[1], Reinhard Debus[2] and Andrea Wenzel[3]

[1] Institute for Soil Ecology, GSF-National Research Center for Environment and Health, Neuherberg
[2] Technical University of Wiesbaden, Germany
[3] Fraunhofer Institute for Ecotoxicology and Environmental Chemistry, Schmallenberg, Germany

***Corresponding author** (peter.schroeder@gsf.de)

DOI: http://dx.doi.org/10.1065/espr2002.10.138

Abstract

Intention, Goal and Background. The widespread and unconcerned use of chemicals in the past has led to an accumulation of pollutants in our environment. Numerous sites are polluted with a mixture of organic chemicals and heavy metals. The future use of these sites and the safe consumption of groundwater from these areas depends on our ability to assess risk by determining the bioavailability of trace levels of pollutants in the respective soil solutions. Soil eluates containing heavy metals in mixture as well as pure heavy metals in aqueous solution were added to a spruce cell culture to set up such a test system.

Objective. The present study aims at evaluating the response of cultured spruce cells to heavy metals in aqueous solution, and at characterizing these basic cellular responses as potential biomarkers.

Methods. In order to characterize cell reactions toward heavy metals, spruce cell cultures were incubated with $CdSO_4$ (50 to 500 µM), Na_2HAsO_4 (1.5 to 80 µM) or $PbCl_2$ (10 to 150 µM). Alternatively, the cells were incubated with a standard heavy metal mixture containing 80µM Na_2HAsO_4, 150 µM $CdSO_4$ and 150 µM $PbCl_2$ in medium and with aqueous original soil eluates. Measurement of oxidative stress, antioxidants and basic detoxification enzymes involved in plant defence reactions were performed with the treated cells.

Results and Discussion. After 5 hrs of incubation, the onset of a strong oxidative burst was observed. H_2O_2 concentrations exceeded 40 µM in the culture media after 20 hrs. Concomitantly, glutathione levels showed drastic changes indicating the influence of the metals and/or the H_2O_2 on antioxidative systems. Following cadmium treatment, GSH and GSSG were elevated by 50 and 200% above controls. Whereas arsenic doubled GSSG levels, treatment with lead did not cause significant changes. However, a mixture of the metals decreased both metabolites by 50%. The effect of the metals was concentration-dependent and disappeared at high concentrations. Furthermore, strong induction of glutathione S-transferase (GST) subunits was observed and, although no novel subunit was expressed, the rise of a new GST isoform occurred. The most potent inducer of plant defence reactions is cadmium, followed by arsenate and lead in descending order of effectiveness. Counter ions seem to play an important role, e.g. lead chloride influenced the investigated parameters much more than lead acetate.

Conclusions. The investigated metals activate gene expression through signal transduction pathways previously not associated with these metals, which points to new end points for resistance and toxicity testing. Especially a monitoring of GST subunit behaviour together with quantifying the oxidative burst seem to be promising for a biomonitoring concept. The close regulation of plant answers observed may facilitate the setup of an integrated biotest for heavy metal pollution that could be based on enzymological as well as proteome data.

Recommendations and Outlook. Heavy metals cause stress to plant cells and elicit a whole range of answers, although specific for individual metal species. The differences observed in plant answers are suitable to distinguish between metals bioavailable in soil eluates and water samples, however only at concentrations in the µM range. It will be necessary to evaluate the effects on the RNA and transcript level. We recommend that similar plant metabolic end points and enzyme reactions be screened for their suitability as biotest systems.

Keywords: Arsenic; biotest; cadmium; glutathione; glutathione S-transferase; H_2O_2; heavy metals; lead; oxidative burst; spruce