Bull. Environ. Contam. Toxicol. (2003) 71:617–624

DOI: 10.1007/s00128-003-8931-9

Bulletin of Environmental Contamination and Toxicology

Copper, Manganese, Lead, and Zinc Concentrations on *Eucalyptus* sp. Leaves in a Small Coastal Town

C. Mispagel, N. Turoczy, F. Stagnitti

School of Ecology and Environment, Deakin University, Warrnambool, Vic 3280, Australia

Received: 5 March 2003/Accepted: 13 June 2003

Large amounts of organic substances and heavy metals are emitted into the atmosphere daily as a consequence of human activity. Exhaust fumes from internal combustion engines are the main source of environmental lead in urban areas in industrialized countries (Clarke et al 1996; Wróbel et al 2000; Halliwell et al 2000; Bilos et al 2001; Lau & Luk 2001; von Storch et al 2002). The evidence regarding the effects of lead fall-out is extensive, with both the environment and human health displaying serious toxicological effects (Pönkä, 1998; Francek, 1997). As a consequence of the progressive introduction of cars with catalytic converters, and the phase-out of leaded fuels, lead levels in urban areas of the world are decreasing. For example, investigations have reported atmospheric lead levels in the cities of Budapest and Oporto as decreasing by a factor of 4-9 and 50%, respectively (Salma et al., 2000; Vasconcelos & Tavares, 1998). Measurements in Germany in the 1980s and 1990s showed that atmospheric lead concentrations were halved about every 4 to 5 years (von Storch et al 2002). The same reduction in plants was also observed. Similar decreases have been observed for the USA and Australia (Pönkä, 1998; Mira et al., 1996). In the 1970s, Germany was the first European country to regulate lead additives in gasoline. The Arhus Treaty signed in 1998 by nearly all European countries stipulates the exclusive use of unleaded gasoline by the year 2005 (von Storch et al 2002).

Due to the potential toxicological effects of lead to the human population (eg. hematotoxicity, Alzheimer's disease, etc), blood lead levels have been monitored throughout the transition from leaded to unleaded fuels. Comparisons have shown a strong positive correlation between blood lead levels in adults and children and gasoline lead levels (Thomas et al 1999; Pönkä 1998) and the decrease in human blood lead levels are largely attributed, directly or indirectly, to the decrease in vehicular lead emissions following the introduction of unleaded gasoline. Von Storch et al (2002) demonstrate a very strong correlation in the decease in human blood lead levels in Germany with the decrease in atmospheric lead levels (eg. see Figure 4 in von Storch et al (2002)).

With the continuing decrease in gasoline lead content, the importance of lead as an indicator to vehicular emissions may diminish in value as levels decrease below current detectable limits, or if levels remain constant. Therefore, the use of different trace metals as indicators of traffic-induced emissions are required. Both barium and zinc, rather than lead, have been suggested as effective tracers of automotive traffic emissions based on a study using leaves (*Quercus ilex*) as a biomonitor (Monaci et al 2000). Metals such as zinc and copper in surface soils have been the focus of a number of investigations because of their long residence

---

Correspondence to: F. Stagnitti

time and their potential toxicity. Of particular interest are the trace metals copper, manganese and zinc due to their association with motor vehicle components. Tyre wear is an important source of both barium and zinc, with the latter also associated with fluid leakage, whereas manganese and copper are released with particles from vehicle brakes (Ball et al., 1991). In Australia, lead levels in fuels are expected to decrease to a maximum limit of 0.005g/L from the 1st January 2002 (Fuel Quality Standards Act, 2000). In agreement with international data, it is proposed that the proportion of vehicular emitted lead that will contribute to environmental levels in Australian urban areas will decrease as a result. It is therefore apparent that investigations measuring current urban lead levels should also include additional trace metals in their study.

This paper is the second part of a long-term study to investigate the effects of legislative changes in vehicle emissions on atmospheric pollution. The primary aim of the study is to evaluate the evolution of lead levels on eucalyptus leaves over the past decade, with reference to an earlier study by Halliwell et al (2000). We hypothesize that the concentration of lead on leaves is lower than that reported by Halliwell et al (2000). If so, it is postulated that the greatest contributor to the observed decline is probably the decreased use of lead in gasoline. In addition, copper, zinc and manganese levels on leaves will also be reported. Although lead levels have been previously monitored in the study area, the additional metals have not. Future investigations can utilize the presented data to continue monitoring the distribution of all measured trace metals in the study area.

MATERIALS AND METHODS

The study was conducted in Warrnambool, a small rural coastal town with a population of approximately 30,000 people. Warrnambool is located approximately 265 km southwest of Melbourne, Australia. The primary industry for the area is tourism and consequently the town has a large influx of tourists traveling by car and other road transport, especially in the summer months. The assumption is that vehicle emissions are the major source of atmospheric heavy metal contamination. The selected survey area coincides with a previous study conducted by Halliwell et al (2000), and encompasses 30 sites within a 10 km radius from the centre of the township. All sites were randomly selected based on the availability of trees within 20m from the roadside. Sampling was conducted on *Eucalyptus* sp. in accordance with the criteria described in detail by Halliwell et al (2000). In brief, *Eucalyptus* species are native to Australia, readily available in the area and movement of lead into the leaf from the surface is known to be relatively small. In addition to the survey conducted by Halliwell et al (2000), we also determined the concentration range of Cu, Zn, Pb and Mn in soil beneath eucalyptus trees previously sampled by Halliwell.

The method for the determination of lead on leaves described by Halliwell et al (2000) was adopted with modifications outlined below. For the current investigation, the accumulation period was reduced from 6 days (Halliwell et al 2000) to 5 days with the removal of between 15 and 30 leaves at each site. The accumulation time was reduced due to persistent precipitation during the study period. Leaves were sampled on the 24th September, 2001. The leaves were collected in clean polyethylene bags (~ 30 x 50 cm). To eliminate the soaking period in 0.1M Nitric Acid, ten polyethylene sample bags were tested for Cu, Mn, Pb and Zn contamination prior to their use. Results indicated no potential interference from the sample bags. Gloves were worn during field sampling to minimize skin contact, with sample bags sealed in the field by tying a knot. All samples were analyzed for Cu, Zn, Pb and Mn within 48 hours of collection. A syringe was used to administer 40mL of 0.1M nitric acid (ASIK) into the polyethylene bag and to the leaf surface. Whilst wearing gloves, the hole was clamped with thumb and forefinger and the bag was shaken vigorously to ensure complete washing. The washings were then centrifuged at 16 000 rpm for 5 minutes, and the supernatant was quantitatively transferred to 50.0ml volumetric flasks and made up to the mark with deionised water.

Two sites were chosen for the soil testing; an outer and centre site coinciding with low and high traffic flow respectively. Two soil samples were collected from each site. The soil samples were collected using polyvinyl chloride (PVC) corers to a maximum depth of ~10 cm. A new corer was used for each site, with three corers having a diameter of 2 inches and one of 1 inch. Each corer was cut in half and taped prior to use to enable the removal of soil cores without disruption to the profile. Core samples were collected on the 24th of August 2001. Within an hour after collection, all four cores were frozen whilst in the PVC corers until profile dissection two days later. Profile dissection entailed the removal of tape and one half of each corer leaving the frozen soil profiles in the bottom half of each corer. The separation of 1 cm depth increments entailed profile measurement with a standard metric rule and dissection with a small plastic serrated knife. Each depth increment was transferred to sealed plastic specimen containers and stored until analysis (maximum of 4 weeks).

Soil samples were digested using a pre-weighed (wet weight) homogenized portion of 1cm depth increments. Approximately 0.5 g of each sample was accurately weighed into 200mL Erlenmeyer flasks. The average wet weight of digested samples was 0.517g. Anti-bumping granules were added prior to the addition of 4mL of concentrated nitric acid (ASIK) to each flask. After the reaction subsided, an additional 4 ml of nitric acid was added followed by 50 mL of deionised water. The solutions were boiled for 1 hour, topping with deionised water when necessary. After cooling, digests were centrifuged at 16 000 rpm for 5 minutes. The supernatant was quantitatively transferred to 100 mL volumetric flasks and made up to the mark with deionised water. Dry weight of the soil at each depth was determined by drying a pre-weighed (wet wt) portion of each depth increment at 105^{o}C for 24 hours. Both the digest sample and drying sample were wet weighed at the same time to eliminate any error from ambient water loss over time. The dried sample was cooled in a dessicator for 20 min and re-weighed. The relative difference between wet weight and dry weight constituted the mass of water in the digested sample of the same 1 cm depth increment. This enabled the accurate determination of the mass of metal per gram of dry soil.

Working standard solutions for each metal were prepared by serial dilution of Spectrosol grade standard solutions for atomic absorption spectrophotometry from Ajax Chemicals. . Both the core samples and leaf washings were analyzed for Pb, Cu, Mn and Zn using a Hitachi 6000 Polarized Zeeman Atomic Adsorption Spectrophotometer with flame atomization using the standard conditions for these metals for the instrument. All glassware, plastics, and corers were soaked in 10% HNO_3 for a minimum of 24 hours prior to use. Inclusion of sample duplicates, spikes and blanks into the run-stream assessed the accuracy and precision of the results for both leaf and soil analyses. Duplicate readings were taken for all blanks, samples and standards, and the averages are reported. Spike recovery was between 90 and 100 percent. After washing, leaves were photocopied onto A3 paper, for determination of surface area by the cut and weigh method. The average area of sampled leaves was approximately 1051 cm^2. The concentration of metal on leaves is then expressed as ng/cm^2.

RESULTS AND DISCUSSION

Lead concentrations on leaves were below the method detection limit (estimated at 1 ng/cm^2) for a majority of the sites.. Lead was detected at only 6 sites out of 30 and ranged in concentrations from 1 ng/cm^2 to 8 ng/cm^2. No distinct patterns were evident from this data. Both zinc and copper accumulation on leaves ranged from below the instrumental detection limits to 40 ng/cm^2 and 6 ng/cm^2, respectively. For all thirty sites sampled, manganese accumulation on leaves was detected between the range of 2 ng/cm^2 to 43 ng/cm^2. Higher levels of zinc, manganese, and copper were recorded in central Warrnambool, with elevated levels of zinc extending along the Princes Highway (the major route into the city, see Figure 1 of Halliwell et al 2000). Manganese concentrations show a slightly different pattern in comparison to zinc and copper, with three areas of high concentration. The concentration of manganese on leaves was . 20ng/cm^2 for the town centre in addition to the northeast and northwest of the centre. With minimal samples removed from these outer areas, it must be noted that these elevated levels correspond to one sample site and therefore may not be representative of the entire area.

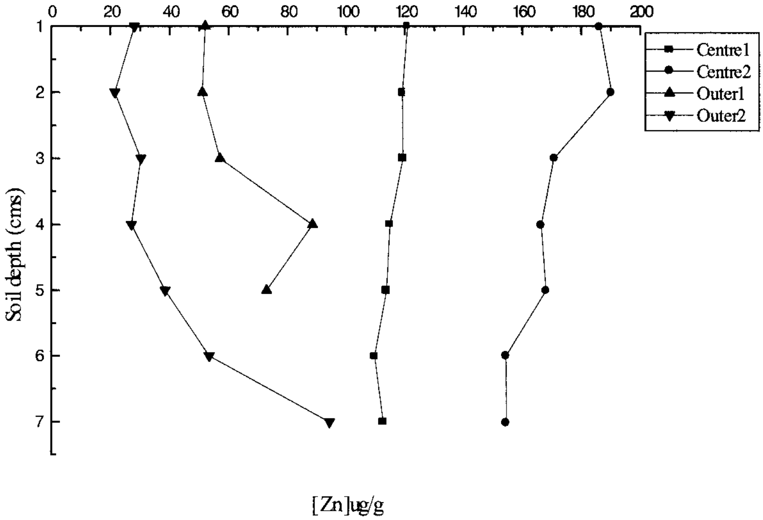

Figure 1. Depth profile of zinc in 4 soil cores at two sites in Warrnambool.

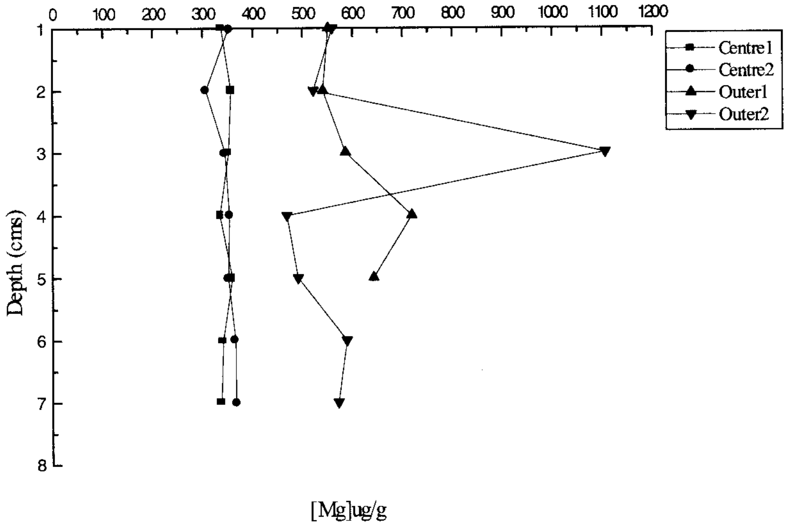

Figure 2. Depth profile of manganese in 4 soil cores at two sites in Warrnambool.

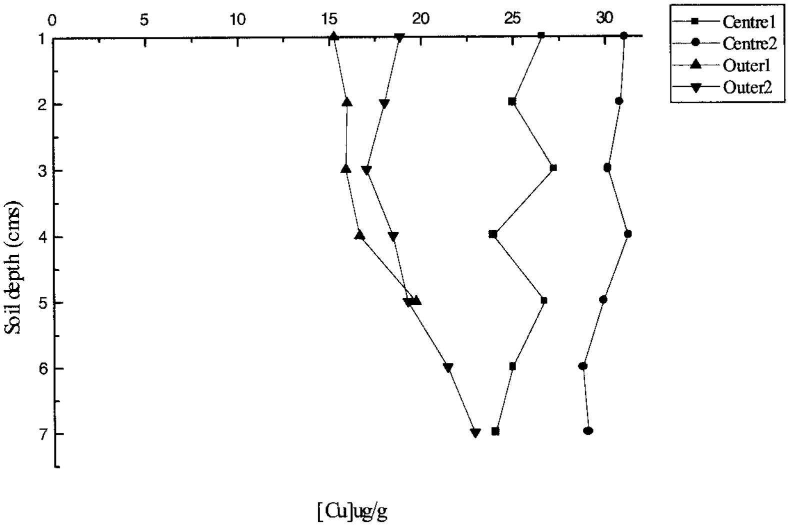

Figure 3. Depth profile of copper in 4 soil cores at two sites in Warrnambool.

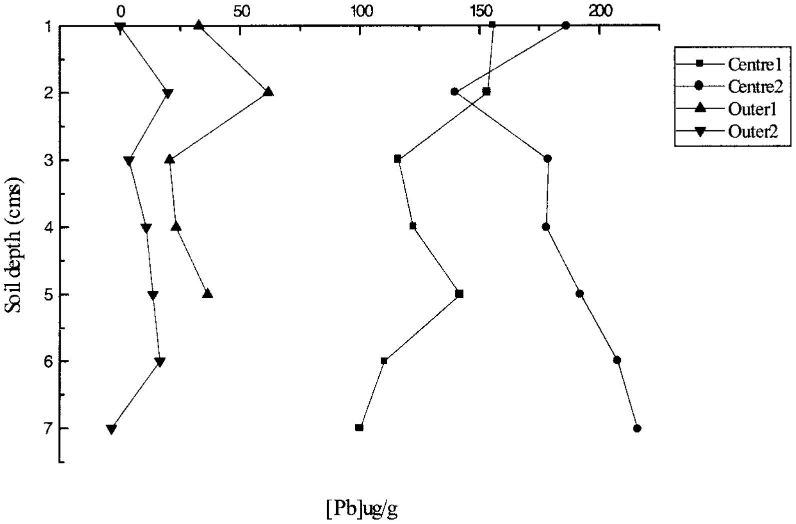

Figure 4. Depth profile of lead in 4 soil cores at two sites in Warrnambool.

The soil depth profiles of the concentration of zinc, manganese, copper and lead at both centre and outer locations are presented in Figures 1 to 4 respectively. The concentration of zinc, copper and lead in soils irrespective of depth, were consistently lower at the outer sites (1 & 2) in comparison to the central site (1 & 2). In contrast, manganese showed the reverse pattern, with lower concentrations in soil removed from the central sites. The outer sites are located on low traffic flow routes. The central cores show the least relative variation within each core, with a minimal change in Cu, Zn and Mn concentration with depth. However, there was an increase of 76 μg/g in the concentration of Pb in the Centre 2 profile. Cores removed from the outskirts of the township (Outer 1 & 2) show an increase in the variability of metal concentration across all depths. The concentration of Zn and Cu in the outer soil profile 2 shows an increase of approximately 43 μg/g and 4.03 μg/g with depth, respectively.

In contrast to the earlier study by Halliwell et al (2000), the current lead accumulations on *Eucalyptus* sp. leaves were lower, and generally below the method detection limit. Assuming that lead fall-out is largely generated from vehicular emissions, this observed reduction is a consequence of the progressive introduction of unleaded gasoline and is consistent with extensive international investigations of lead levels in urban areas after the phase-out of leaded gasoline (eg. Salma et al 2000; von Storch et al 2002). Perhaps surprisingly, the highest lead concentrations were not found in areas of known high traffic flow, such as sample sites located within the central commercial area. Samples from which lead was detected displayed no distinct spatial pattern in comparison to Halliwell et al (2000) data, although all but two sites were located either in the town centre, or near major roads. This results incidate that atmospherically deposited lead has decreased substantially over 5 years in Warrnambool.

Unlike lead accumulation, levels of copper, zinc and manganese on leaves were higher in central Warrnambool in comparison to low traffic flow areas, and indicating the possibility that these trace metals are associated with traffic-induced emissions. On a global basis, anthropogenic inputs of zinc and copper predominate over natural sources accounting for 66 and 56 % of the total emissions, respectively (Nriagu 1989). Areas of high human activity were generally associated with "hot spots" of both copper and zinc, although levels were quite diffuse for the former. Previous trace metal analysis of urban areas has shown that elevated levels of copper, zinc and manganese, which are associated with general vehicle deterioration can be used as tracers of traffic-induced emissions (Ball et al 1991). Manganese levels were high (i.e.. 20 ng/cm2) in three distinct areas, one of which coincides with high traffic flow. The additional areas of high manganese concentration are associated with residential and semi-rural areas. Traffic flow in these areas is lower than central sites and therefore traffic-induced emissions would not be expected to contribute significantly to these elevated levels. Previous investigations have shown increases in manganese soil content as a result of the introduction of an organic manganese compound as an anti knock additive to gasoline (Lytle et al 1995; Veysseyre et al 1998). MMT (methyl-cyclopentadienyl mangenese tricarbonyl) is currently used in the United States, Canada, Europe, Asia, as well as Central and South America but has yet to be sold to the Australian market. Considering MMT is not an additional anthropogenic source in Warrnambool, the observed results could be a result of crustal dust disturbance in areas of high activity rather that traffic emissions directly (Bilos et al 2001). A problem with this hypothesis is the low concentration of soil manganese in central sites in comparison to outer sites. If crustal dust disturbance effects atmospheric particulates, and manganese is higher on the outskirts of town, then we would expect manganese levels on leaves at these sites to also be high. This, however, was not apparent.

All trace metals, with the exception of manganese, indicated that traffic-induced emissions might affect trace metal concentration in soil. Lead levels in soil removed from central Warrnambool were between 1 to 2 orders of magnitude higher in concentration than in outer soil cores, indicating the extended influence traffic emissions can have on soil. The traditional trace metal paradigm, especially for lead, is relative immobility with an exponential decrease with depth (Sutherland 2000). This was not observed for any core samples, or for any trace metals in central or outer Warrnambool, although sample depth was limited to 8 cm. If we assume that the lead deposited on leaves is washed into the soil, and that there are on average approximately 100,000 leaves per tree (area of each leaf about 200 cm^2), then the mass of lead deposited under the tree would be about 100 g/yr. Assuming that the lead is deposited over an area of 2.5 m^2, and that this is bound within the first 10 cm of soil, and that the density of the soil is 2 g/cm^3, the concentration of accumulated lead in soil would be about 0.2 mg/g/yr. This outcome is not unexpected considering lead can migrate to significant soil depths (Sutherland 2000). This figure is very small and does not pose a significant risk to soil biota.

In conclusion, this study investigated the levels of copper, manganese, zinc and lead on a common native tree (*Eucalyptus* sp.) for a small coastal town. Compared with a previous study, lead levels have significantly been reduced, most likely as a consequence of the introduction of unleaded gasoline. High levels of copper and zinc were associated with high traffic flow areas. The concentration of manganese on leaves in the town centre were higher than outer areas, although this level was not limited to areas of high traffic flow. Metal concentrations in soil indicated that lead, copper and zinc were higher in the town centre with manganese showing the reverse pattern.

REFERENCES

Ball D, Hamilton R, and Harrison R (1991) The influence of highway-related pollutants on environmental quality. In Highway Pollution Hamilton R and Harrison R (eds) 1-47. Elsevier Science New York

Bilos C, Colombo JC, et al (2001) Sources distribution and variability of airbourne trace metals in La Plata city area. Argentina Environ Pollut 111: 149-158

Clarke AG, Chen J–M, et al (1996) Vehicular particulate emissions and roadside air pollution Sci Tot Environ 189/190: 417-422

Francek MA (1996) Soil lead levels in orchards and roadsides of mission peninsula Michigan Wat Air Soil Pollut 94: 373-384

Halliwell D, Turoczy N and Stagnitti F (2000). Lead concentrations in *Eucalyptus* sp in a small coastal town Bull Environ Contam Toxicol 65: 583-590

Lau OW and Luk SF (2001) Leaves of Bauhinia blakeana as indicators of atmospheric pollution in Hong Kong Atm Env 35: 3113-3120

Lytle CM Smith BN and McKinnon CZ (1995) Manganese accumulation along Utah roadways – a possible indication of motor vehicle exhaust pollution Sci Tot Environ 162: 105-109

Monaci F, Moni F, et al (2000) Biomonitoring of airbourne metals in urban environments: new tracers of vehicular emission in place of lead Environ Pollut 107: 321-327

Mira M and Bawden-Smith J (1996) Blood lead concentrations of preschool children in central and southern Sydney Med J Australia 164: 399-402

Nriagu JO (1989) A global assessment of natural sources of atmospheric trace metals Nature 338: 47-49

Pönkä A (1998) Lead in the ambient air and blood of children in Helsinki Sci Tot Environ 219: 1-5

Salma I, Maenhaut W, et al (2000) Impact of phase out of leaded gasoline on the air quality Budapest Microchem J 67: 127-133

Sutherland RA (2000) Depth variation in copper lead and zinc concentrations and mass enrichment ratios in soils of an urban watershed J Environ Qual 29: 1414-1422

Thomas VM, Socolow R H et al (1999) Effects of reducing lead in gasoline: An analysis of the international experience Environ Sci Technol 33: 3942-3948

Vasconcelos MTSD and Tavares HMF (1998) Atmospheric metal pollution (Cr Cu Mn Ni Pb and Zn) in Oporto city derived from results for low-volume aerosol samplers and for the moss *Sphanum auriculatum* bioindicator Sci Tot Environ 212: 11-20

Veysseyre A, van de Velde K et al (1998) Searching for manganese pollution from MMT anti-knock gasoline additives in snow from central Greenland Sci Tot Environ 221: 149-158

Von Storch H, Hagner M et al (2002). Reassessing past European gasoline lead policies Amer Geophys Union EOS 83(36): 393-399

Wróbel A, Rokita E and Maenhaut W (2000) Transport of traffic-related aerosols in urban areas Sci Tot Environ 257: 199-211