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## **Lichens as a biomonitoring tool for detecting heavy metal air pollution associated with industrial activities in Collie, south-western Australia**

Meenu Chaturika Vitarana  
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**Lichens as a biomonitoring tool for detecting heavy metal air pollution  
associated with industrial activities in Collie, south-western Australia**

Meenu Chaturika Vitarana

MSc.

This thesis is presented in fulfilment of the requirements

for the degree of

**Doctor of Philosophy**

Faculty of Health, Engineering and Science

School of Natural Sciences

Edith Cowan University

August, 2013



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## Abstract

During the last few decades, various techniques for using lichens as biomonitors have been developed for monitoring air pollution and forest ecosystem health. Lichens have been used effectively to determine the dispersion of heavy metals emitted by industrial point-sources; however the approach has not been commonly used in Australia. This thesis aimed to determine the effectiveness of using a lichen biomonitoring approach to measure the heavy metal pollutants emitted from coal-fired power stations and related industries in Collie, south-western Australia, an area with concern over poor air quality. Three different approaches to lichen biomonitoring were investigated. The first explored lichen community composition patterns in thirty-six study sites across an identified pollution gradient in the jarrah forest ecosystems of Collie. The second measured *in situ* *Usnea inermis* lichens for heavy metals, across wet and dry seasons in Collie. The third approach used lichen transplant bags of *Usnea inermis* to determine seasonal heavy metal accumulation patterns. Because the gaseous pollutants NO<sub>2</sub> and SO<sub>2</sub> are known to have a significant effect on lichen vitality and distribution, they were monitored by means of a direct measurement approach using Radiello<sup>®</sup> passive air samplers, to determine any confounding effects.

A total of twenty lichen taxa were recorded in the lichen community study, with an average species diversity of ten per site. The lichens *Usnea inermis* and *Cladonia rigida* occurred at all thirty-six study sites. High lichen diversity and abundance values were recorded from control sites, and crustose and squamulose species were more abundant across all study sites. The grouping of lichen communities into pollution-tolerant classes, based on information from other studies, showed that the jarrah forests surrounding the industries in Collie were dominated by pollution-tolerant lichen species, while sensitive species were infrequent and rare.

Spatial distribution maps of lichen diversity indices showed areas with low diversity values downwind from the coal mines and coal-fired power stations and near an alumina refinery, indicating a possible influence from these point-sources on lichen community composition. Pollution effects on lichen communities were observed with little influence from forest management practices, demonstrating the effectiveness of this method for monitoring air pollution influences in managed jarrah forests. The study also identified *Usnea inermis* as a suitable species for both the *in situ* and transplant

lichen biomonitoring experiments to explore heavy metal pollution in the area, because of its widespread distribution across the pollution gradient.

Low concentrations of NO<sub>2</sub> and SO<sub>2</sub> were recorded by Radiello<sup>®</sup> passive samplers, suggesting that these pollutants had very little confounding influence on lichen community composition and heavy metal accumulation patterns. However, seasonal differences in the dispersion of gaseous pollutants were observed, particularly in the summer season. The areas influenced by gaseous pollutants were also found to be those with low lichen diversity, suggesting that although low in concentration, the gaseous pollutants were having a demonstrable influence on the lichens in the jarrah forests in Collie.

Mean concentrations for the metals As, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Zn were low for *in situ Usnea inermis* lichens; however elevated concentrations of some metals were recorded at certain locations. Arsenic concentrations in spring were high from sites close to the coal mines and Mn was elevated in both seasons from sites near an alumina refinery. Higher metal concentrations were recorded in the higher rainfall autumn season compared with spring for most metals. The fallout patterns of heavy metals were explained by a power curve showing exponential decreases in concentrations, with very low concentrations found beyond the 8 - 10 km distance range from the closest pollution source. Spatial dispersion maps showed interpolated concentrations consistent with that expected if point-sources were responsible for the generation of high atmospheric heavy metal concentrations.

Transplanted *Usnea inermis* lichens did not show elevated metal concentrations, however seasonal variations were observed, with the highest concentrations recorded in the wet winter season. Metal uptake in both the *in situ* and transplanted lichen studies was favoured by low temperature and high rainfall, suggesting that metal uptake was promoted during periods of wet deposition. This highlights the importance of season of sampling if lichen biomonitoring studies are to be deployed in WA. The wetter and cooler winter season with more consistent rainfall patterns is recommended as optimal for conducting lichen biomonitoring studies in Collie. The transplants exposed over a 48 week period recorded the highest concentrations for most metals, however they also showed a loss of metal accumulation ability at the high exposure sites. Exposure periods of 24 – 32 weeks (6 – 8 months) are recommended for more reliable results when using lichen transplants. The transplant study also identified that the control sites were



affected by industrial emissions, suggesting that reference sites should be located at distances greater than those used in this study.

The results from all three biomonitoring approaches identified pollution dispersion patterns associated with industrial point-sources, and also identified a pollution influence at the control sites, an area previously considered to be unaffected by industrial pollution. Findings from this study support the idea that a lichen biomonitoring approach can be used as an effective tool for monitoring heavy metal air pollution in Western Australia and if used correctly it could replace the more expensive active sampling techniques. The study also provided essential baseline information for future studies on the effect of industrial pollution on lichen communities in WA.

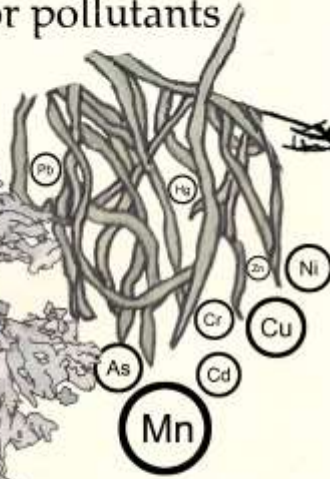


# Lichen Biomonitoring

Community Composition



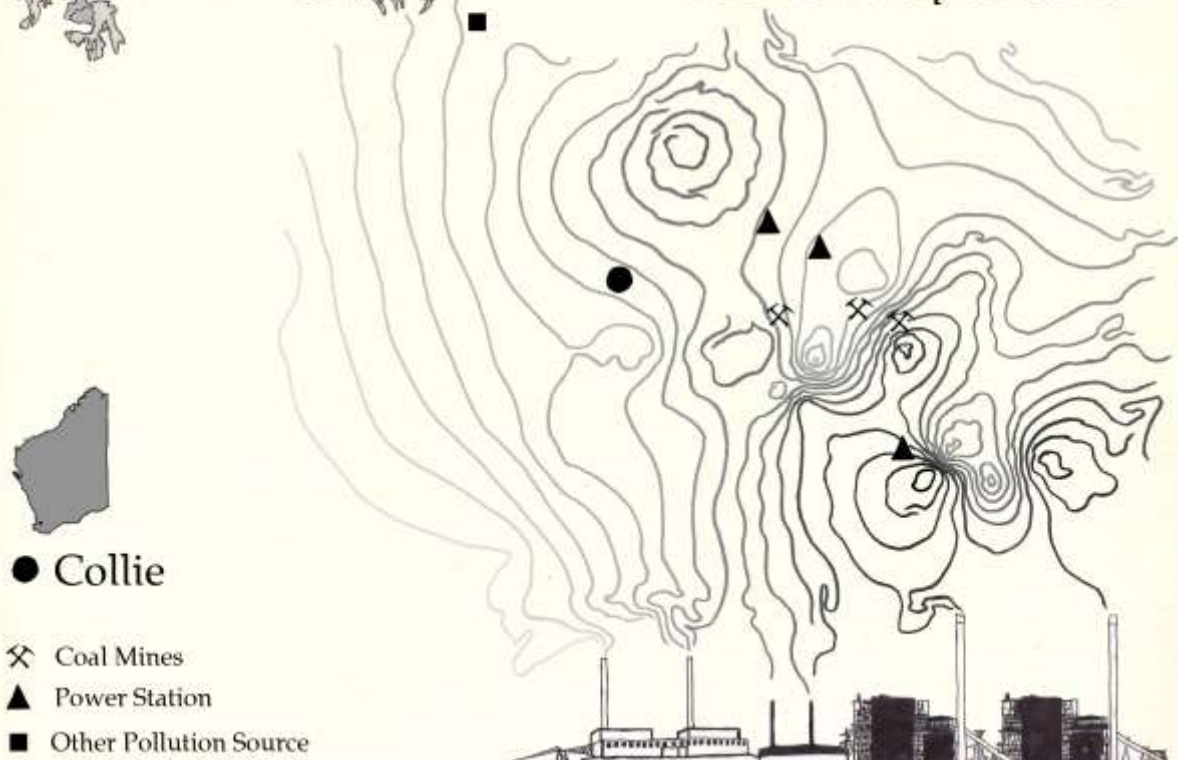
In situ analysis for pollutants



Lichen transplant analysis



Pollution Map of Collie



*Design created by Hannah Tyler for the Edith Cowan University Visualising Research Initiative 2012*



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## **Chapter 1 - General Introduction**

### **1.1 Air quality assessment**

Air pollution is a significant global issue with elevated concentrations of pollutants reported from many parts of the globe (Gaga et al., 2012; Josipovic, Annegarn, Kneen, Pienaar, & Piketh, 2010; Plaia & Ruggieri, 2011). This has led to increased interest in monitoring atmospheric pollutants, with many countries establishing nationwide monitoring programmes (Josipovic et al., 2010; Plaia & Ruggieri, 2011). Particular attention has been given to measuring and monitoring ambient levels of NO<sub>2</sub>, SO<sub>2</sub>, CO, O<sub>3</sub>, PM<sub>10</sub> and PM<sub>2.5</sub>, considered as criteria pollutants because of the high concentrations emitted from many sources (Gaga et al., 2012; Plaia & Ruggieri, 2011). There has been less attention given to measuring ambient levels of heavy metals in national monitoring programmes, although in recent years increased emissions by industrial activities and concern over bio-accumulation in food chains has focused attention on heavy metal monitoring (Babula et al., 2008; Bañuelos & Ajwa, 1999; Nelson, Shah, Strezov, Halliburton, & Carras, 2010).

Heavy metal emission by coal power generation is of particular concern, because of the elevated concentrations of heavy metal emissions reported, the persistent nature of heavy metals and their toxicity to biological systems (Bhargava, Carmona, Bhargava, & Srivastava, 2012; Fernandezturriel, Decarvalho, Cabanas, Querol, & Lopezsoyer, 1994). In Australia, coal power is the main source of energy generation, producing more than 54% of Australia's electricity (Australian Coal Association, 2012; Diniz Da Costa, Prasad, & Pagan, 2004). Coal use is viewed as a 'dirty' industry, with many efforts undertaken to reduce toxic emissions (Diniz Da Costa et al., 2004). Despite these efforts, the emissions from coal-fired power stations still remain a community concern, particularly in areas such as Collie in south-western Australia, which has intense coal mining and coal power generation activities (Nelson et al., 2010; Figure 1.1).

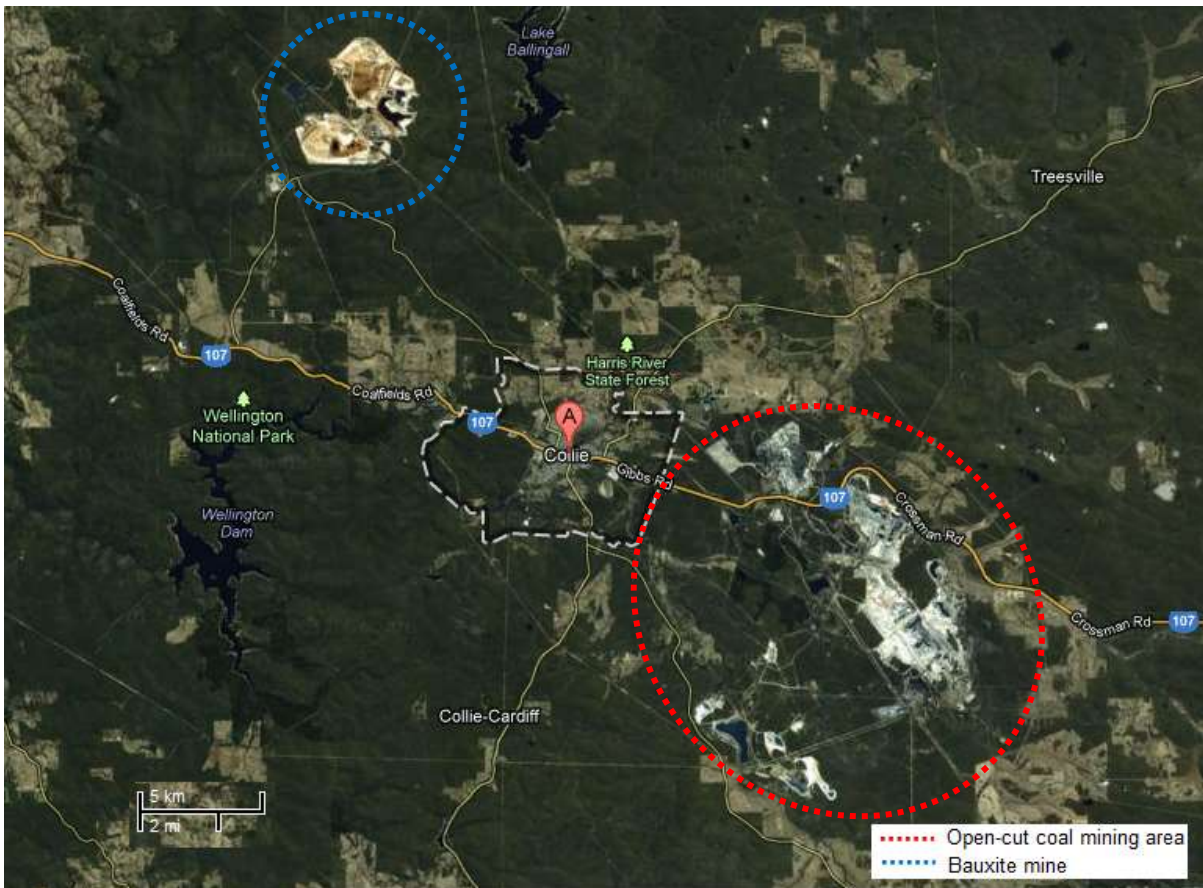
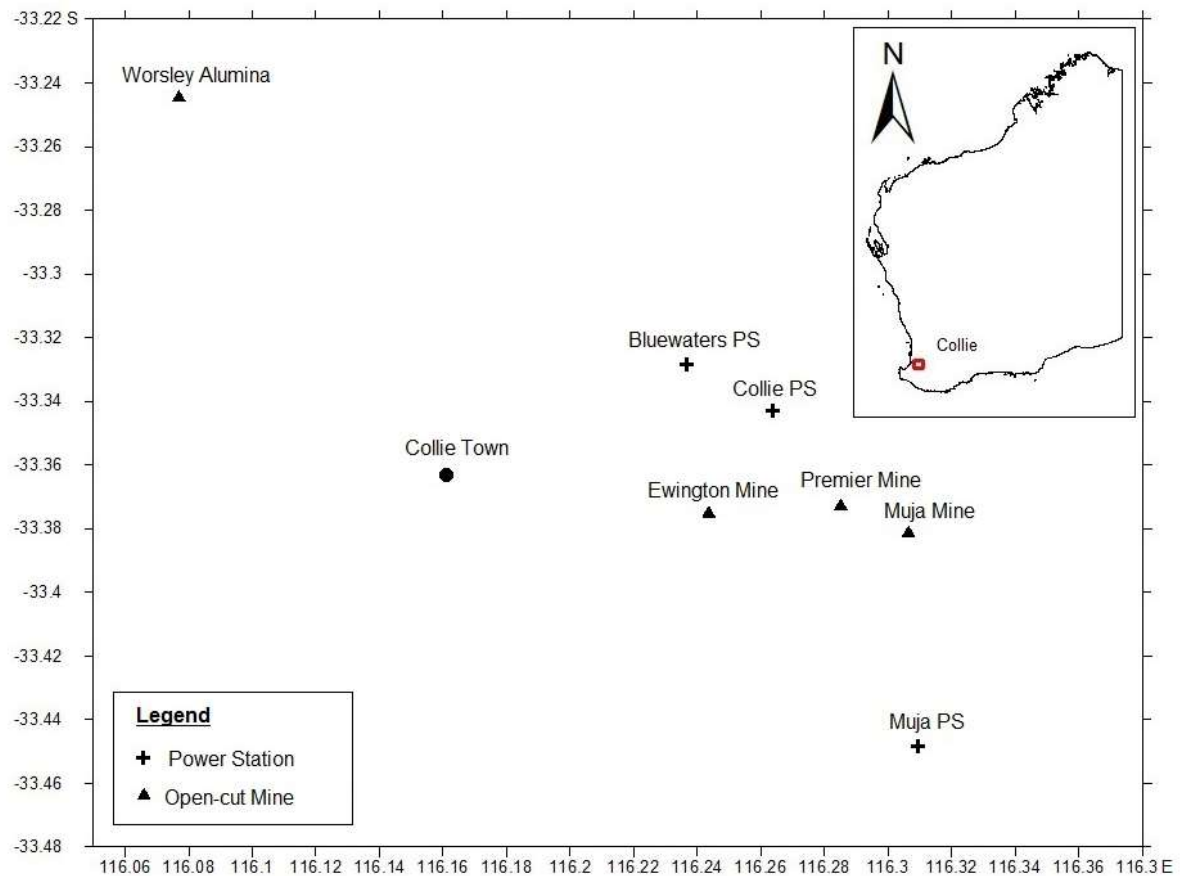


Figure 1.1: Satellite map highlighting the area covering open-cut coal mines and bauxite mining in Collie (Google Maps, 2012)

### 1.2 Air-shed of the study area

Collie, a region in south-western Australia rich in coal reserves, has received significant attention in recent years due to expansions to existing coal-fired power stations and other proposed coal-related industrial developments in the region (EPA, 2003, 2005a). Collie houses a number of coal-fired power stations, with a significant land area (over 50 km<sup>2</sup>) consisting of open-cut coal mine operations (Figures 1.1 and 1.2). Coal mining in Collie dates back to the late 1890s and the Collie coal basin is believed to have reserves of 2.4 billion tonnes of coal (MRG Metals, 2011). Three coal mines or ‘open-pits’ are currently in operation (Figure 1.2), with the Premier Coal company undertaking the majority of the operations, having produced approximately 90 million tonnes of coal from five underground mines and six open-cut operations over the period from 1950 to 2010 (Premier Coal, 2011). Bauxite mining is also undertaken north-west of Collie, in Worsley (Figures 1.1 and 1.2), which has an on-site alumina refinery. This refinery recently underwent an expansion which increased its capacity from 3.5 to 4.6 million tonnes per year of alumina production (BHP Billiton, 2011). Coal mines in Collie supply three coal-fired power stations in the area and also provide for an onsite

coal-fired power station at the Worsley refinery (EPA, 2005b). The coal-fired power stations that are currently in operation in Collie have a combined generation capacity of 1570 MW. Electricity generation is the main contributor of pollutants to the Collie airshed, emitting large quantities of CO<sub>2</sub>, SO<sub>2</sub>, NO<sub>2</sub>, particulate matter and heavy metals to the atmosphere (Figure 1.3). Future developments such as a proposed urea plant and expansions to two coal-fired power stations will add more pollutants to the Collie airshed (GHD, 2009).



**Figure 1.2: Main point-source emitters in Collie**

Despite community concern over the poor air quality in the Collie township because of the industrial activities, no studies have been undertaken to measure the industrial emissions or their impacts on a continuous basis (GHD, 2009). Current emission data (Figure 1.3) have been derived through estimating the emissions of all the coal-fired power stations in the area (National Pollutant Inventory, 2010). On site monitoring carried out in and around Collie is limited, not continuous, and lacks spatial components showing the dispersion of pollutants in the area (Table 1.1). Attention has been focused mainly on the effect of industrial pollutants in the Collie town (GHD, 2009), with little focus on the impacts on the surrounding jarrah forest ecosystem. Assessment of pollutant concentrations across the Collie air-shed has been limited to dispersion modelling, with few direct measurements to validate the patterns obtained from those dispersion models.

Predictive modelling maps show elevated particulate matter emissions from the industries being dispersed south-east of the Collie town, with maps for both coarse and fine particulate matter (PM<sub>10</sub> and PM<sub>2.5</sub>) emissions showing a substantial area with concentrations exceeding the National Environment Protection Measure (NEPM) air quality standards for particulates (GHD, 2009). The exceedance of particulate matter standards in Collie is of concern, especially because heavy metals are usually dispersed in the particulate matter emissions (US EPA, 2004). The environmental concerns associated with the release of heavy metals during the coal-burning processes (Nelson et al., 2010) and their dispersion in the particulate matter plume, strongly suggests that the Collie air-shed may have elevated concentrations of heavy metals (GHD, 2009). Heavy metals are toxic and persistent in nature (Bhargava et al., 2012) and are known to have ecological impacts leading to forest degradation and ecosystem stress, particularly in areas close to industrial point-sources (Budianu, Robu, & Macoveanu, 2010), highlighting the need to monitor heavy metal emissions in the jarrah forests ecosystems in Collie.

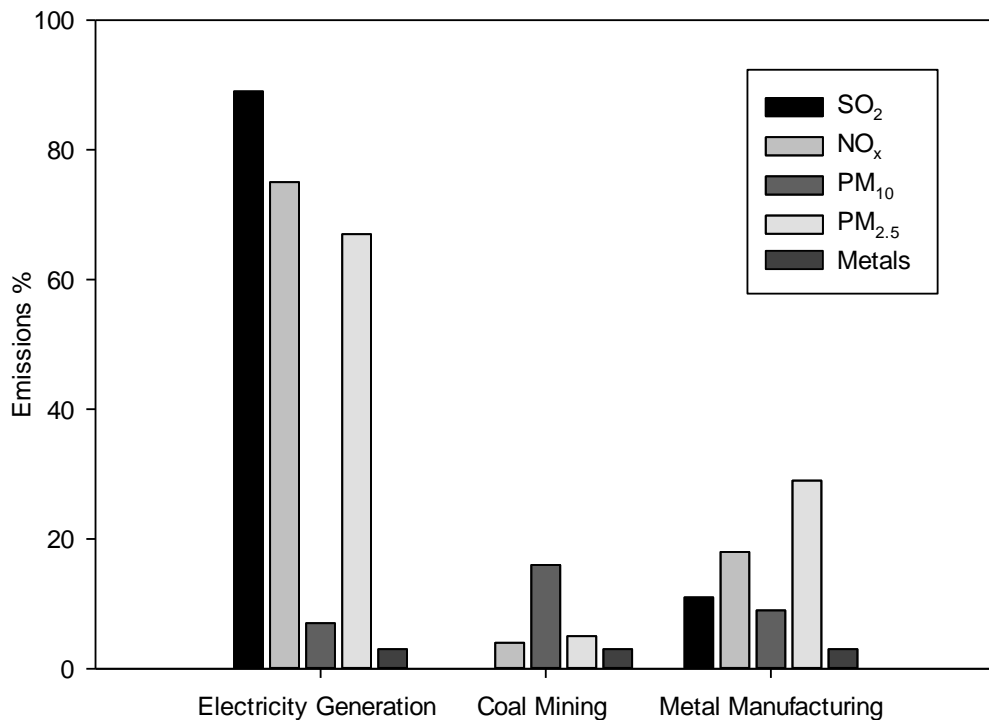


Figure 1.3: Estimated emissions of criteria pollutants in Collie based on pollution source for the years 2010/2011 (National Pollution Inventory, 2010)

Table 1.1: List of ambient air quality monitoring projects carried out in Collie from 1995 to 2013 (Sinclair Knight Merz, 2005)

Pollutant	Monitoring Location	Duration Measured	Measured by
SO <sub>2</sub>	Shotts	Mar 1995 – Nov 2003	Verve Energy Pty Ltd
	Collie town	Mar 1995 - present	Verve Energy Pty Ltd
	Cardiff	Mar 1995 – Nov 2003	Verve Energy Pty Ltd
	Jacksons's Farm	Dec 1996 – Nov 1998	Verve Energy Pty Ltd
	Bluewater's Farm	Nov 1998 – Nov 2003	Verve Energy Pty Ltd
SO <sub>2</sub> & NO <sub>2</sub>	Worsley (13 km from refinery site)	present	Worsley alumina
PM <sub>10</sub>	Collie town	1998 - present	Verve Energy Pty Ltd
	Bluewaters	2000 - 2003	Verve Energy Pty Ltd
PM <sub>2.5</sub>	Collie town	Feb 2003 - present	Verve Energy Pty Ltd

### 1.3 Biomonitoring using lichens

Biomonitoring is the use of biological material to quantitatively monitor pollution, by utilizing the capacity of organisms to accumulate pollutants over time (Markert, 2007; Markert et al., 1999; Markert et al., 2011). It is an alternative approach to the direct monitoring of pollutants using air monitoring equipment. It is also a cost-effective practice, making the study of heavy metal air pollution across time and space possible (Batzias & Siontorou, 2006). Although many organisms accumulate pollutants, only certain species have the physical, ecological and physiological features that make them practical biomonitors. The efficiency of a biomonitor depends on the capability of an organism to reflect the presence and amount of pollutants in the atmosphere (Garty, 2001). Mosses and lichens are the most popular biomonitors in studies of heavy metal air pollution because concentrations in their tissues are highly correlated with deposition rates (Conti & Cecchetti, 2001; Bari, Rosso, Minciardi, Troiani, & Piervittori, 2001).

The unique anatomy, physiology and ecology of lichens make them ideal biomonitors (Conti & Cecchetti, 2001; Hawksworth & Rose, 1976), since the absence of true roots restricts their nutrient uptake to atmospheric sources (Nash III, 1996). Lichens have also developed mechanisms for efficient extraction and accumulation of elements from low nutrient atmospheres, in comparison with nutrient-rich soil pools utilized by higher plants (Sweat, 2010). Since they lack a protective cuticle and associated stomata (Nash III, 1996), lichens have the ability to absorb atmospheric nutrients and other substances over the whole thallus surface (Hawksworth & Rose, 1976). The poikilohydric nature of lichens also enables them to passively change their water status with the surrounding environment and absorb nutrients directly through their thallus surface (Nash III, 1996; Sweat, 2010). Their slow-growing nature allows them to maintain a consistent morphology with time, resulting in minimal effects on net deposition rates (Nash III, 1996). Moreover, unlike deciduous higher plants, lichens have all tissue parts remaining intact throughout their lifespan, which ensures that accumulation patterns are consistent. Slow growth and long life-spans enable lichens to accumulate environmental pollutants within lichen thalli over time, with concentrations often exceeding their physiological requirements (Garty, 2001; Sweat, 2010). Lichens are also desiccation-tolerant organisms and can revive even after many months, making them extremophiles that can live in ecosystems such as the polar, alpine and desert regions (Kranner, Beckett, Hochman, & Nash, 2008). The locally abundant and broad geographic distribution of



many lichen species also facilitates the recording of the distribution patterns of pollutants from point-sources across multiple spatial scales.

With several advantages over other organisms for use in biomonitoring, lichen biomonitoring generally utilizes one of three approaches. These include (1) the measurement of community changes in relation to pollutants (species presence/absence, community composition and/or richness) (2) measurement of pollutants in the tissue of *in situ* lichen species, and (3) measurement of pollutants in the tissue of transplanted lichen species. These three methods (Figure 1.4) have been used either alone or in combination to gather quantitative data on environmental pollutants and their dispersion in the biosphere (Bajpai, Upretia, Nayakaa, & Kumarib, 2010; Conti & Cecchetti, 2001).

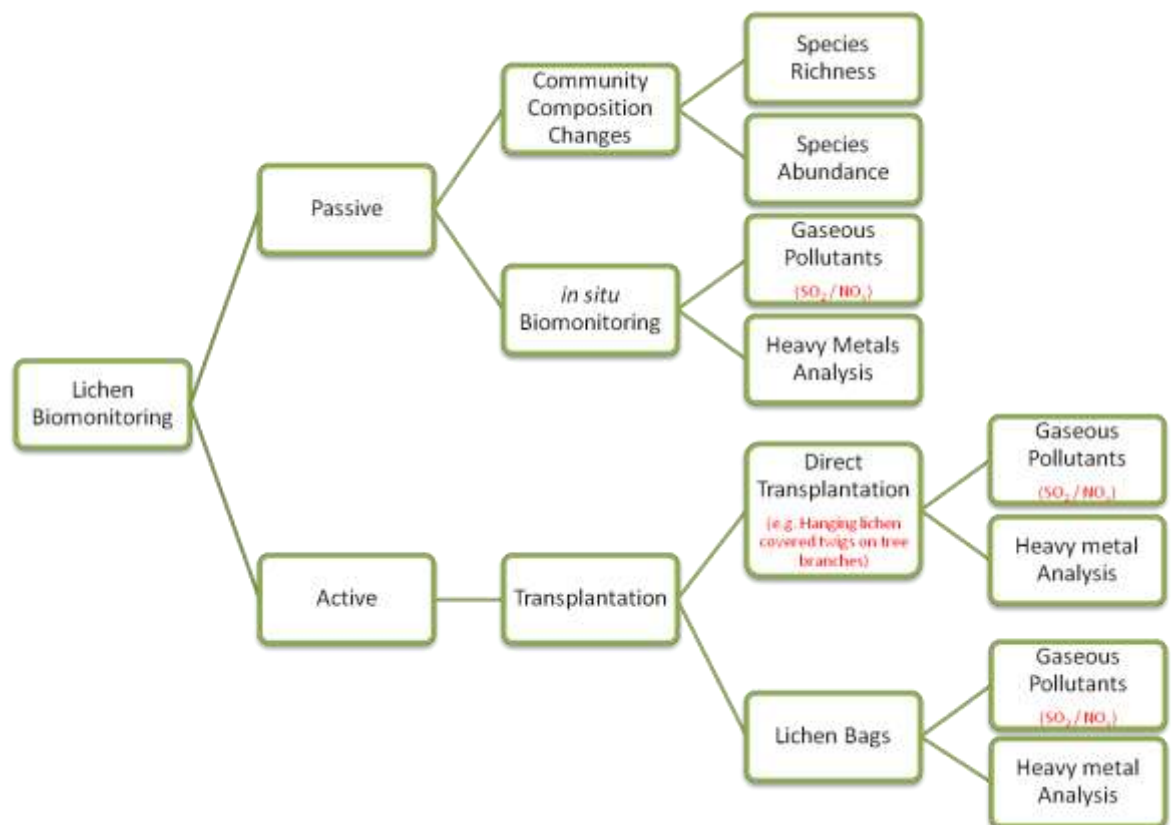


Figure 1.4: Conceptual diagram developed from literature to explain the theoretical basis of the lichen biomonitoring approaches used in the current study

The passive techniques (community composition changes and *in situ* biomonitoring; Figure 1.4) provide information on behavioural changes in monitor organisms and details of pollutants trapped within their tissue (Sloof & Wolterbeek, 1991).

Behavioural changes that have been monitored include species composition, diversity, abundance or richness, ecological performance and morphological changes (Sloof & Wolterbeek, 1991). Air pollutants can cause ecological disturbances giving rise to areas with lower diversity and abundance of lichen species, with decreases in sensitive species and increases in tolerant species often being observed (Markert et al., 1999). Such community compositional changes in lichens have been highly correlated with environmental pollution loads and researchers have developed a standardized index for mapping air quality using lichen compositional changes, referred to as the 'Index of Atmospheric Purity' or IAP (Conti & Cecchetti, 2001). This is a technique commonly used in Europe, with regional scale monitoring regimes that have provided useful information on pollution loads based on the IAP (Conti & Cecchetti, 2001). To date, no such approaches have been used in Australian studies.

*In situ* biomonitoring (Figure 1.4) helps to quantify specific pollutants in the atmosphere and identify their impact on ecosystems over periods of time, and between one location and another (Markert et al., 1999). The ability to obtain time-integrated measures over the longer term is a key advantage of biomonitoring (Batzias & Siontorou, 2009; Smodis & Parr, 1999), where lichens have been extremely useful in this effort. The strong connection of biomonitors with the local environment makes them representative of local conditions, enabling the identification of underlying biological effects over the longer term, especially when the effects are delayed and only visible much later than the actual pollution event (Batzias & Siontorou, 2009). Conventional monitoring instruments are not capable of identifying such interactive effects, especially those induced by multiple stressors (Batzias & Siontorou, 2009). Furthermore, the minimal costs associated with biomonitoring makes intensive sampling feasible in comparison with direct monitoring (Batzias & Siontorou, 2009), which requires expensive equipment (Seaward, 1995). This makes it possible to carry out extensive surveys over large geographic areas, enabling the mapping of the dispersion of pollutants from point-sources (Conti & Cecchetti, 2001; Garty, 2001).

However, passive biomonitoring is possible only in environments where indicator species are present, while the active monitoring approaches (transplantation; Figure 1.4) help overcome this obstacle in urban areas, where such species may not occur (Yildiz, Aksoy, Tug, Islek, & Demirezen, 2008). In active lichen biomonitoring, either lichen covered twigs removed from unpolluted environments are directly transplanted, or

lichen thalli are removed from their substrate and packed in nylon mesh bags and transplanted, by hanging on trees and other suitable substrates at desired study sites (Culicov & Yurukova, 2006; Yildiz et al., 2008). The lichen transplant approach has the advantage of allowing well-defined exposure times (Ceburnis & Valiulis, 1999). However, long exposure periods can sometimes be problematic when using the lichen bag method, where physiological changes may occur in the transplanted lichens, giving erroneous results (Anicic et al., 2009; Mikhailova, 2002). Therefore the transplantation approach is recommended for determining short-term accumulation patterns (Mikhailova & Sharunova, 2008).

#### 1.4 Pollutants monitored with lichens

Lichens have been used to monitor air pollutants such as SO<sub>2</sub> (Batziias & Siontorou, 2009; Hawksworth & Rose, 1976), nitrogen (Stevens et al., 2012), polycyclic aromatic hydrocarbons (Augusto, Máguas, Matos, Pereira, & Branquinho, 2010), other organic pollutants (Shukla & Upreti, 2009), organochlorides (Muir et al., 1993), radionuclides (Kahraman et al., 2009; Popovic et al., 2008), oxidants (Valencia-Islas, Zambrano, & Rojas, 2007), ozone (Nali et al., 2007) and heavy metals (Garty, 2001; Wolterbeek, 2002). Early lichen investigations focused mostly on gaseous pollutants, with the identification of SO<sub>2</sub> pollution as the cause of the ‘city’ effect with lichen-scarce zones reported in the nineteenth century in highly industrialized areas (Nash III, 1996). However, in the past two decades, lichen biomonitoring efforts have shifted focus towards measuring heavy metal pollution (Garty, 2001), particularly due to the high correlation rates observed between atmospheric deposition of metals and their concentrations within lichen thalli (Conti, Pino, Botre, Bocca, & Alimonti, 2009). Lichens are particularly useful for heavy metal pollution monitoring in the vicinity of mines (Cayir, Coskun, & Coskun, 2007; Pawlik-Skowronska, Wojciak, & Skowronski, 2008), power stations (Bajpai et al., 2010; Garty, Tomer, Levin, & Lehr, 2003) and similar point-source emitters (Freitas, 1995; Gur & Yaprak, 2011). Many studies have shown elevated concentrations of metals in lichens close to point-sources (Balabanova, Stafilov, Sajn, & Baeeva, 2012; Cayir et al., 2007; Nash III & Sommerfeld, 1981) and have reported significant correlations between lichen elemental levels and distance to point-sources (Wolterbeek, 2002).

One of the main issues related to lichen biomonitoring for heavy metal pollution is the confounding effect of gaseous pollutants on lichen distribution (van Dobben,

Wolterbeek, Wamelink, & Ter Braak, 2001). In lichen biomonitoring, NO<sub>2</sub> and SO<sub>2</sub> are considered to be the main pollutants that affect species composition and heavy metal accumulation patterns (Purvis et al., 2003; van Dobben et al., 2001). It is difficult to separate the effect of different pollutants on lichens, due to the synergistic interactions occurring in nature (Batzias & Siontorou, 2006). One approach to separating the effect of gaseous pollutants in lichen heavy metal biomonitoring studies is to determine the level of influence by measuring the atmospheric concentrations of these gaseous pollutants using direct sampling techniques (van Dobben & ter Braak, 1999). Many direct measurements of gaseous pollutants are concentrated near pollution sources, towns or cities, and are very rarely located in clean areas, such as forest ecosystems (van Dobben & ter Braak, 1999). However long-range dispersion of pollutants can give rise to elevated pollutant concentrations even in remote forest environments (Steinnes & Friedland, 2006). Field measurements of gaseous pollutants are useful to identify such unknown and unexpected sources of emissions (Batzias & Siontorou, 2009) and to determine their effect on the heavy metal accumulation patterns observed in lichen biomonitoring studies.

### 1.5 Ecology of the study area

The Collie region is dominated by the native eucalypt species commonly known as 'jarrah', a dominant vegetation type that covers over 1.8 million hectares in southwestern Australia (Cranfield, Robinson, Williams, & Tunsell, 2011; McCaw, Robinson, & Williams, 2011), of which a significant proportion occurs in the Shire of Collie. An estimated 79% of the Collie river catchment is state-managed jarrah forest (Lund, McCullough, & Kumar, 2012), and the biodiversity of the jarrah forest is unique due to the distinctive evolutionary processes and the antiquity of the region (Watson, 2006). These forests are rich in endemic vascular flora and hold significant silvicultural importance (McCaw et al., 2011; Watson, 2006). Lichen populations of the native jarrah forests have recently been investigated because of their importance in forest biodiversity and health (Cranfield et al., 2011). A total of 615 species of lichens have been recorded in Western Australia, with 280 species reported in jarrah forests, where they were found to predominantly colonize the coarse woody debris such as fallen tree trunks and stumps (Cranfield, 2004; Cranfield et al., 2011).

This study focused on biomonitoring using lichens which is a technique not commonly applied in Australia, where power generation from coal is a common and widespread

industry. The potential of lichen biomonitoring has previously been suggested as a possibility for pollution studies but was never developed (EPA, 2005a). This study of lichens in Collie aimed at determining the most practical, cost-efficient and effective biomonitoring approach for investigating the dispersion of heavy metal pollutants and the identification of heavily polluted areas.

### 1.6 Objectives of Thesis

The overall aim of this project was to undertake a lichen biomonitoring study to assess its applicability for monitoring the dispersion of pollutants in the Collie air-shed. The study was undertaken in four stages, using three different lichen biomonitoring approaches (Figure 1.4).

The specific aims of each stage are summarised below:

#### Stage 1 – Lichen community composition (Chapter 2)

The first stage investigated the distribution of lichen species and their community composition along a pollution gradient from the industries in Collie. It was hypothesized that the distribution of lichen communities would change (in species composition and abundance) along the dispersion gradients of industrial pollutants and that these would override natural site characteristics and forest management operations that influence lichen communities.

The following specific questions were addressed under this aim:

- (1) Do industrial pollutants have a significant effect on the species richness and abundance of lichens along the potential pollution gradient;
- (2) Which site characteristics play a key role in the distribution of lichen richness and abundance in the jarrah forests surrounding Collie;
- (3) Do forest management practices have an overriding effect on the species richness and the abundance of lichen species in the Collie area; and
- (4) Which lichen species would be most suitable for determining heavy metal concentrations in the Collie air-shed using *in situ* and transplant biomonitoring approaches.

#### Stage 2 – Passive sampling for gaseous pollutants (Chapter 3)

In order to develop an effective biomonitoring tool using lichens to understand the potential for a heavy metal pollution effect from coal-related industries in the Collie air-

shed, it was important to determine the effect of gaseous pollutants (SO<sub>2</sub> and NO<sub>2</sub>) that may confound the lichen community composition patterns and heavy metal accumulation patterns. Therefore, a study was carried out to measure the concentrations of SO<sub>2</sub> and NO<sub>2</sub> in the Collie air-shed using Radiello<sup>®</sup> passive air samplers.

The specific objectives of this study were:

- (1) To determine the concentrations of NO<sub>2</sub> and SO<sub>2</sub> in different parts of the Collie air-shed in order to understand the potential effect of these pollutants on native lichen communities in the jarrah forests surrounding Collie; and
- (2) To establish the seasonal differences in NO<sub>2</sub> and SO<sub>2</sub> concentrations to understand the influence of meteorological conditions on the dispersion of these pollutants in the Collie air-shed and their possible effects on lichen heavy metal accumulation.

#### Stage 3 – *In situ* lichen biomonitoring for heavy metals (Chapter 4)

This stage explored the use of the lichen *Usnea inermis* as a biomonitor to measure the concentrations of the heavy metals As, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Zn along a potential pollution gradient from industrial emissions in the Collie coal basin. The study aimed to:

- (1) Identify spatial and temporal patterns of metal concentrations in the lichen *Usnea inermis* along a potential pollution gradient;
- (2) Explore the differential concentrations of heavy metals associated with seasonal conditions, particularly the wet and dry seasons in the Collie region; and
- (3) Map the dispersion of heavy metals in the Collie air-shed, in order to identify the impact zones and key industrial pollution sources contributing heavy metals to the Collie air-shed.

#### Stage 4 – Lichen transplantation approach (Chapter 5)

This stage of the research used a ‘lichen bag’ transplantation approach to investigate short-term heavy metal accumulation patterns in lichens in the Collie region, in order to better understand the dispersion of heavy metal pollutants from the industries in the area.

The specific aims of this investigation were:

- (1) To use a lichen transplant approach to determine short-term metal accumulation patterns in the lichen *Usnea inermis*; and

(2) To elucidate seasonal variations in heavy metal accumulation by the *Usnea inermis* lichen transplants.

#### General discussion (Chapter 6)

Chapter 6 integrates the overall findings of the study, where the three stages are synthesized in a general discussion to address the main aims of the study.

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## **Chapter 2 - Distribution of lichen communities along a potential pollution gradient in a coal mining region of south-western Australia**

### **2.1 Introduction**

Lichens are dependent almost exclusively on atmospheric sources of nutrients, and have developed mechanisms for the efficient uptake of water and essential nutrients from their surrounding environment (Nash III, 1996). Despite their slow growth, lichens respond to changing concentrations of air pollutants and concentrations of these pollutants in the atmosphere are often highly correlated with those in lichen thalli, making them reliable biomonitors (Bari, Rosso, Minciardi, Troiani, & Piervittori, 2001; Sloof & Wolterbeek, 1991). During the last few decades, various techniques for using lichens as biomonitors have been developed (Svoboda, Peksa, & Vesela, 2010). These include lichen incidence and abundance, the species richness of lichen communities and elemental concentrations in lichen tissue, (or a combination of techniques) for monitoring air pollution and forest health (Manning, 2005; Miller & Watmough, 2009; Styers, Chappelka, Marzen, & Somers, 2010).

The number, abundance and composition of lichen species occurring on a defined section of a tree trunk has been used to assess the level of environmental stress imposed by various atmospheric pollutants (Giordani, 2006; Svoboda, 2007; Svoboda, et al., 2010). Many studies have investigated lichen species diversity changes along gradients of exposure to SO<sub>2</sub>, NO<sub>2</sub> and heavy-metals (Conti & Cecchetti, 2001) and a higher lichen species diversity and abundance have been reported in areas with low pollution (Bennett & Wetmore, 2010; Branquinho, Catarino, Brown, Pereira, & Soares, 1999; Fernandez-Salgui, Terron, Barreno, & Nimis, 2007; Kaffer et al., 2011). A positive correlations between lichen diversity and distance from pollution sources have also been reported (Bajpai, Upretia, Nayakaa, & Kumarib, 2010; Elsinger, Burrell, DeBruyn, Tanasichuk, & Timoney, 2007). Studies have observed significant increases in species richness in areas away from the direction of dominant winds from point-sources, highlighting the importance of meteorological conditions in pollution dispersion and lichen community composition (Bajpai, et al., 2010; Cameron, Neily, & Richardson, 2007; Fernandez-Salgui, et al., 2007). Today, long-term air pollution monitoring programs using lichens are common worldwide, particularly in the USA and Europe (Cameron, et al., 2007).

The species composition and abundance of lichens in a community also respond to environmental variables such as forest age, forest fragment size and stand density, particularly in non-urban forest ecosystems (Giordani, 2006; Svoboda, et al., 2010). Forest habitat quality directly affects lichen communities, where, for example, increased light intensity associated with reduced canopy cover can lead to changes in the lichen flora (Aragon, Martinez, Izquierdo, Belinchon, & Escudero, 2010; Nash III, 1996). A number of studies have reported a positive relationship between forest age and lichen species diversity in different forest habitats around the world, indicating the importance of old-growth forests for maintaining a higher species diversity (Ellis & Coppins, 2010; McMullin et al., 2010; Moning, et al., 2009; Nascimbene, Marini, & Nimis, 2010). Modern sustainable forest management practices are also considered to affect lichen diversity, especially by the conversion of old-growth forests into young, managed stands (Aragon et al., 2010; Moning et al., 2009). These variables can sometimes confound the lichen community composition patterns observed in biomonitoring studies (Svoboda, et al., 2010) and it is therefore important to identify and separate the influence of environmental and forest management variables, when carrying out lichen biomonitoring studies. Climate and topography can also affect lichen community composition; however this effect becomes problematic in biomonitoring studies only across large geographic scales (Will-Wolf, Geiser, Neitlich, & Reis, 2006).

Lichen diversity and abundance have been used as a tool to map the dispersion of pollutants associated with coal-fired power stations, with many studies observing reductions in lichen diversity and abundance closer to the associated emission sources (Bajpai et al., 2010; Fernandez-Salgui et al., 2007; Nimis, Castello, & Perotti, 1990). However, this cost-effective approach has not been applied in Australia, where coal power generation is an important industry, with much community concern expressed over poor air quality arising from coal-fired power stations (Diniz da Costa & Pagan, 2006; EPA, 2005). Collie is a significant coal mining area in south-western Australia, housing a number of coal-fired power stations and the region is a significant contributor of electricity for Western Australia. Collie produces 37% of the mineral and energy wealth of the South West region, primarily through the production of coal (New Collie Coal Inc., n.d.). These industries emit a variety of pollutants including CO<sub>2</sub>, NO<sub>x</sub>, SO<sub>2</sub>, and heavy-metals (Nelson, Shah, Strezov, Halliburton, & Carras, 2010). Other industrial activities in the area include the refining of alumina and open-cut coal mining (Varma et al., 2009). The air-shed of Collie is reported to have possible elevated concentrations of

As, Cd, Cr, Hg, Ni and Pb (GHD, 2009) emitted by these industries. These heavy-metals are usually present in particulate matter, mainly associated with the PM<sub>2.5</sub> (EPA, 2004; Office of Legislative Drafting, 2003) and are often not measured separately. Despite the rapid industrial developments in the region, no studies have investigated the health of the jarrah forest ecosystems surrounding Collie, particularly due to the effect of production of pollutants by the power generating plants. The prospect of future additions to existing coal power generation facilities draws attention to the need for monitoring the dispersion of pollutants in the Collie air-shed.

This study investigated the distribution of lichen communities along a pollution gradient from the industries in Collie, to identify areas impacted by pollution. It was hypothesized that the distribution of lichen communities would change in species composition and abundance along the dispersion gradients of industrial pollutants and that these effects would override natural site characteristics and forest management operations that influence lichen communities. The following specific questions were addressed in this study: (1) Do industrial pollutants have a significant effect on the species richness and the abundance of lichens along the potential pollution gradient? (2) Which site characteristics play a key role in the distribution of lichen richness and abundance in the jarrah forests surrounding Collie? (3) Do forest management practices have a significant influence on the species richness and abundance of lichen species in the Collie area? (4) Which lichen species would be most suitable for determining heavy metal concentrations in the Collie air-shed using in situ and transplant biomonitoring approaches.

## **2.2 Methodology**

Lichen community composition patterns were explored along a potential pollution gradient in the Shire of Collie, where fallen logs and old stumps were sampled for lichen diversity and abundance to identify the effect of industrial emissions on lichens and to map the dispersion of lichen diversity and abundance across the Collie air-shed. The study was conducted in August 2010, where thirty six study sites were sampled, which included nine control sites.

### 2.2.1 Study area

The study was carried out within the Shire of Collie in south-western Australia. The Shire covers an area of 1,685 km<sup>2</sup>, of which 80% is State Forest (Collie Shire Council, 2009). The Collie basin is a bilobate northwesterly valley in the Darling Plateau, with gently undulating uplands and shallow sandy valley floors. Soil type varies from pale to deep brown sands, with granite outcrops along the southern edge (GHD, 2009).

The climate in Collie is Mediterranean, with mean annual maximum and minimum temperatures of 22.5 °C and 8.4 °C respectively. January and February are the hottest months, while July is the coldest (Bureau of Meteorology, 2011). Average annual rainfall is 940 mm, with June having the highest average rainfall. Prevailing winds are strongest from the north-west throughout the year, while in autumn, winds are not very strong. Wind speeds average between 4.4 and 9.6 kmh<sup>-1</sup> in the morning and between 9.2 – 12.6 kmh<sup>-1</sup> in the afternoon, annually (GHD, 2009).

### 2.2.2 Pollution sources in Collie

Industries in the Collie area include power generation, coal mining, farming (grazing and dairy) and forestry. Significant economic contributors and point-source emitters of atmospheric gases and particulates are the coal-fired power stations (Muja, Collie and Bluewaters phases I & II), an alumina refinery with its own on-site coal and gas-fired energy generators and three open-cut coal mines (Figure 2.1) (GHD, 2009). New developments include stages III and IV of the Bluewaters coal-fired power station, scheduled for completion in 2013 and 2015 (Griffin Energy Group, 2007). Stages A and B of the Muja coal-fired power station were decommissioned in 2007, but were recommissioned in 2012 (Verve Energy, 2006). A new urea plant has also been proposed (GHD, 2009). These expansions will add to the existing air quality issues of the Collie air-shed (EPA, 2005). The coal-fired power stations were the highest emitters



of air pollutants to the Collie air-shed in the year of the current study, followed by the Worsley alumina refinery and the coal mines (Table 2.1). However, The National Pollution Inventory recorded paved and unpaved roads as the most significant contributor of heavy metals in 2011, followed by dust emissions, with the power stations and coal mines also contributing equally to heavy metal emissions in Collie (Table 2.2).

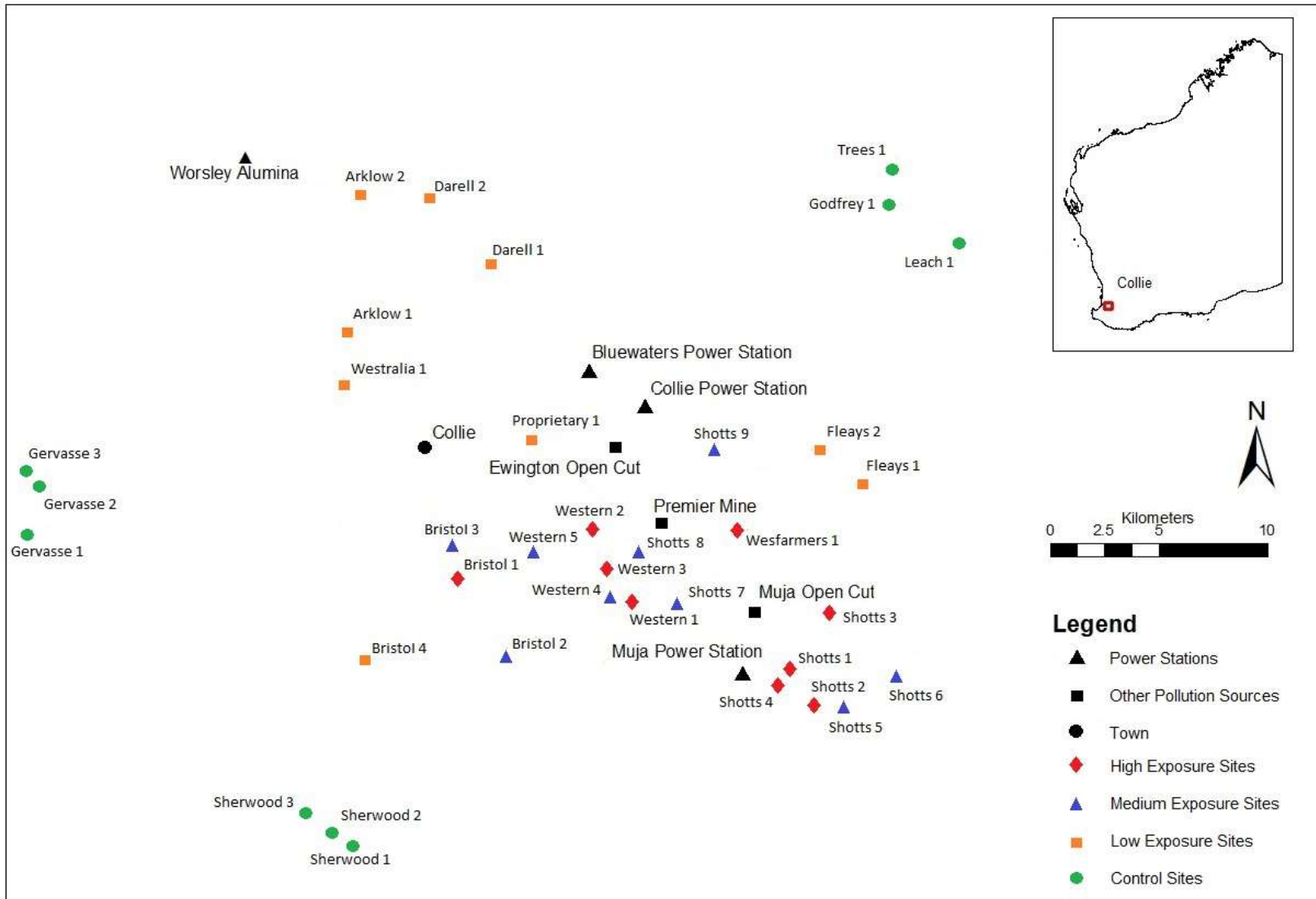


Figure 2.1: Map of Collie showing the distribution of sites for the lichen community study and pollution sources in the area

Table 2.1: Estimated emissions by each facility in the Collie region for the years 2010/2011 (National Pollution Inventory, 2010)

Facility	SO <sub>2</sub>	NO <sub>x</sub>	PM <sub>10</sub>	PM <sub>2.5</sub>	Metals Air (kg)								
	Air (kg)	Air (kg)	Air (kg)	Air (kg)	As	Cd	Cr	Cu	Pb	Mn	Hg	Ni	Zn
Worsley Refinery	7,100,000	4,200,000	1,400,000	180,000	28	9	736	220	340	410	370	210	540
Bluewaters I & II	11,000,000	6,400,000	800	620	5.3	51	121	42	0.3	120	0.1	290	600
Collie Power Station	23,000,000	5,200,000	410,000	190,000	40	28	100	130	150	260	40	140	220
Muja Power Station	25,000,000	6,300,000	710,000	230,000	44	44	147	130	180	360	67	260	43
Ewington Mine	5,000	640,000	1,400,000	13,000	8.8	0.3	100	85	310	1,100	0.9	37	540
Muja Mine	0.016	590	35	11	2.8	-	-	-	-	110	-	-	55
Premier Coal Mines	970	290,000	1,000,000	19,000	7	0.2	62	48	36	620	0.5	28	280

Table 2.2: List of important pollutants and their top emission sources in the Collie region estimated for the years 2010/2011 (National Pollution Inventory, 2010)

Pollutant	Emissions Based on Source - Air (kg)						Total Emissions - Air (kg)
	Electricity Generation	Coal Mining	Metal Manufacturing	Paved / Unpaved Roads	Windblown Dust	Other	
SO <sub>2</sub>	59,000,000	6,000	7,100,000	-	-	4,200 (Motor Vehicles) 9,200 (Fuel combustion)	66,000,000
NO <sub>x</sub>	18,000,000	930,000	4,200,000	-	-	440,000 (Biogenics) 220,000 (Motor Vehicles)	24,000,000
PM <sub>10</sub>	1,100,000	2,400,000	1,400,000	9,000,000	630,000	-	15,000,000
PM <sub>2.5</sub>	420,000	33,000	180,000	-	-	-	630,000
Metals (As, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Zn)	3619	3494	2863	92,170	4,648	31.65*	107,036

\*Sources include wildfires, fuel combustion and gaseous fuel burning

### 2.2.3 Ecology of the study area

This study was carried out within State-managed jarrah forests which are dominated by the Western Australian endemic *Eucalyptus marginata* Sm. Species. *Corymbia calophylla* K.D. Hill & L.A.S. Johnson (Marri) co-occurs with jarrah in this ecosystem, which is classified as a ‘dry sclerophyll forest’ on the Darling Plateau. Environmental variables such as topography, soil nutrients and fire regimes contribute to the species diversity within the jarrah forest and the height of dominant trees decreases down the rainfall gradient (Watson, 2006).

### 2.2.4 Selection of study sites/sampling design

The selection of sites was based on Particulate (PM<sub>2.5</sub>) dispersion maps generated by models of the pollution sources and emissions to the Collie air-shed (GHD, 2009). Predicted maximum 1 hour SO<sub>2</sub> concentration scenarios were also consulted to identify similarities in the emission distribution patterns, in order to determine possible confounding effects from major gaseous pollutants. It is essential to consider effects of gaseous pollutants, as they affect lichen vitality, and subsequently, their distribution (Riga-Karandinos & Karandinos, 1998). The dispersion modelling outcomes used for site selection considered effects from both existing and future industrial emission sources in the Collie region. These included Muja power station, Collie power station, Worsley alumina refinery and its expansions, Bluewaters power station, and open-cut coal mines in the area (Figure 2.1). Source emission rates used for the modelling were based on previous cumulative air-dispersion modelling assessments (CSIRO, 2004; Environ Australia, 2009; Sinclair Knight Merz, 2005). Three major zones were identified as high, medium and low pollution, based on air-dispersion modelling for PM<sub>2.5</sub>. The high exposure zone was considered as sites found at > 40 µg m<sup>-3</sup> PM<sub>2.5</sub> contours, medium zone for sites falling between 40 – 20 µg m<sup>-3</sup> PM<sub>2.5</sub> contours and low exposure zone for sites at < 20 µg m<sup>-3</sup> PM<sub>2.5</sub> contours. These were the zones that could be categorized from the available dispersion models and the drawbacks of using narrow ranges of PM<sub>2.5</sub> concentrations for the categories were noted at study onset. Suitable sites for these zones were randomly selected across the modelled area by observing satellite imagery obtained using Google maps and the COG-derived products from the Department of Environment and Conservation (DEC, 2010). Field visits were carried out to select sites with similar jarrah forest habitats and with easy access by road, across the different pollution categories. Nine sites per pollution category were selected for sampling (n=27), with a further nine control sites selected approximately 25 km away

from the major pollution sources (Figure 2.1). Jarrah forest sites were selected, where possible, so as to have similar post-logging ages and ecological characteristics (Appendix 2.3).

#### 2.2.5 Survey technique

At each site, ten fallen logs or old tree stumps within a radius of 500 m<sup>2</sup> were randomly selected for lichen sampling. Randomization was ensured by selecting ten random compass directions from the midpoint of the 500 m<sup>2</sup> plot and then choosing a fallen log or tree stump in each direction. Fallen logs/old tree stumps were preferred as substrates for this study because they are favoured sites for lichen establishment in the jarrah forest. Live trees were not sampled in this survey, since jarrah trees shed their bark regularly and lose their lichen cover, which makes surveying of such substrates problematic. A cumulative area of 20 x 20 cm was sampled on each log/stump by using either a 10 x 10 cm grid or/and a 20 x 5 cm grid, based on the substrate conditions. Each grid was placed in areas with a smooth surface, avoiding damaged parts of the trunks and parts with knots. The choice of grid (either 10 x 10 cm or 20 x 5 cm) was based on the ability to place the grid easily on each area of the substrate being surveyed. The first 10 cm of the trunk above the soil was avoided in grid selection, because of possible problems with soil splash which would introduce terrestrial lichens to the survey.

Data on the presence/absence of each lichen species and their abundance was recorded. Abundance was recorded as the percentage cover that each species occupied within each grid. A majority of the lichen species were identified only to the genus level during the survey, since species level identification requires expert skills in lichen identification such as chemical spot tests, thin layer chromatography or liquid chromatography (Cameron, et al., 2007); the use of these methods was not possible in the field. The use of a suite of recognisable 'indicator' species has been explored to overcome the challenge of lichen identification for monitoring studies and has been proven to be a reliable method for lichen biomonitoring (Cameron, et al., 2007; Giordani, 2006; Li, Liu, Wang, Ma, & Song, 2011) and hence this approach was applied in this study.

### 2.2.6 Environmental variables

Forest management variables for the study sites were recorded by obtaining all available site characteristics on the jarrah forest blocks (within which study sites were placed) from the Department of Environment and Conservation. Details considered for the respective forest blocks were age since last fire, logging history and forest patch area (Appendix 2.3). Forest-stand density, canopy cover, understory thickness and leaf litter density was estimated by collecting data from a 40 X 40 m quadrat at each study site. Stand density was obtained by counting all trees within the quadrat and canopy cover was estimated as a percentage by visual observation (Appendix 2.3).

### 2.2.7 Calculating biodiversity indices

Patterns of biodiversity were quantified by calculating Species Richness, Shannon-Weiner Index, Simpson's Index and Species Evenness (Magurran, 1988), using the equations given below:

Species Richness = the number of species (s) ----- Equation 2.1

Shannon-Wiener Index =  $(-1) * (\sum(P_i * \ln(P_i)))$  ----- Equation 2.2

Where  $P_i$  (relative abundance or proportion of species i) =  $n_i/N$ ;  $n_i$  = number of individuals in species i;  $N$  = total number of individuals in all species;  $\ln$  = natural log

Simpson's Index =  $1/\sum(P_i^2)$  ----- Equation 2.3

Where  $P_i$  (relative abundance or proportion of species i) =  $n_i/N$

Species Evenness =  $H/\ln(R)$  ----- Equation 2.4

Where  $H$  = Shannon-Wiener Index;  $R$  = species richness;  $\ln$  = natural log

### 2.2.8 Index of Atmospheric Purity (IAP)

Another biomonitoring method, introduced in 1970, is the calculation of an IAP or "Index of Atmospheric Purity", based on the number of species present, their coverage and frequency, and their specific tolerance to pollutants (LeBlanc & De Sloover, 1970). This method has been used widely and the formula that considers only the frequency (F) of the lichen species is considered to give a good correlation with pollution data (Conti & Cecchetti, 2001). The IAP value was calculated for all thirty-six sites in this study, using the frequency method formula.

$$IAP = \sum F_i \text{----- Equation 2.5}$$

Where  $F_i$  is the frequency of every  $i$ th species in ten substrates for each site

### 2.2.9 Data analysis

Descriptive statistics and graphical displays were generated using IBM SPSS Statistics 19 (IBM Corp., 2010) software. Correlation coefficients for identifying possible patterns between site characteristics, forest management parameters and distance to pollution sources were also calculated in SPSS. Correlations between parameters were assessed using Spearman's correlation coefficient for non-parametric data. Community composition patterns of lichen species were analysed by multivariate statistical techniques using PRIMER v6 (Plymouth Marine Laboratory, UK). A Principal Component Analysis (PCA) using percentage cover data was performed to describe differences in lichen species composition among the studied region (Clarke & Gorley, 2006). Dispersion maps for diversity indices were generated using the Surfer 8.0 software (Golden Software Inc., 2010) a grid-based contouring and surface modelling package for Windows. The gridding method used was kriging, which interpolates regularly-spaced grid points from irregularly-spaced data points and generates contour maps (Golden Software Inc., 2010).

## 2.3 Results

### 2.3.1 Lichen species composition and diversity indices

A total of twenty lichen taxa were recorded in the thirty six study sites (Table 2.3). Two taxa, *Usnea inermis* and *Cladonia rigida* occurred in all thirty-six study sites, while the foliose lichen *Hypogymnia subphysodes* and two squamulose lichens *Hypocenomyce scalaris* and *Thysanothecium scutellatum* were recorded in an average of 98% of the study sites (Table 2.3). Four species, *Cladia schizopora*, *Pannaparmelia wilsoni*, *Ramboldia* sp. and *Cladonia cervicornis* var. *Vetricillata* were present in an average of 68% of the study sites (Table 2.3). The remaining eleven taxa were recorded in an average of 24% of the study sites (Table 2.3). Of the lichens recorded, 3 species belonged to the fruticose growth form, with the Westralia 1 site (Figure 2.1) having the highest cover (43.5%) for the group (Table 2.3). Another five species belonged to the foliose group and the highest cover (57.9%) for the group was recorded from the Fleays 1 site (Table 2.3; Figure 2.1). Six species were recorded for the squamulose and crustose growth forms each, with the highest cover of these growth forms found at Shotts 4 and Darrell 1 sites respectively (Table 2.3; Figure 2.1).

*Cladonia rigida* was dominant at most sites in which it was found, with sixteen sites having the highest percentage cover of this species. *Usnea inermis* was the most abundant species at twelve sites, while *Thysanothecium scutellatum* was the most abundant species at six sites, and *Hypogymnia subphysodes* had the highest percentage cover at only two sites (Table 2.3). Although *Cladonia rigida* dominated in abundance, the highest percentage cover from the whole study was for *Usnea inermis*, with two sites having over 40% cover, while among the sixteen sites where *Cladonia rigida* was recorded, no sites had cover over 40% (Appendix 2.1).



Table 2.3: Lichen species recorded from the 36 study sites in the Collie area, including their frequency data and percentage cover based on growth forms

<b>Species Name</b>	<b>Growth Form</b>	<b>No. of Sites Present</b>	<b>% Sites Present</b>	<b>No. Sites with Highest Cover</b>
<i>Usnea inermis</i>	Fruticose	36	100%	12
<i>Cladonia rigida</i>	Squamulose	36	100%	16
<i>Thysanothecium scutellatum</i>	Squamulose	35	97%	6
<i>Hypogymnia subphysodes</i>	Foliose	34	94%	2
<i>Hypocenomyce scalaris</i>	Squamulose	34	94%	-
<i>Cladia schizopora</i>	Fruticose	27	75%	-
<i>Pannaparmelia wilsonii</i>	Foliose	24	67 %	-
<i>Ramboldia</i> sp.	Crustose	24	67%	-
<i>Cladonia cervicornis</i> var. <i>Vetricillata</i>	Squamulose	22	61%	-
<i>Punctelia pseudocoralloidea</i>	Foliose	13	36%	-
<i>Hypocenomyce australis</i>	Squamulose	13	36%	-
<i>Parmelina conlabrosa</i>	Foliose	13	36%	-
<i>Caloplaca elixii</i>	Crustose	10	28%	-
<i>Thysanothecium hookeri</i>	Squamulose	10	28%	-
<i>Xanthoparmelia elixii</i>	Foliose	9	25%	-
<i>Cladia aggregata</i>	Fruticose	7	19%	-
<i>Ochrolechia</i> sp.	Crustose	7	19%	-
<i>Candelariella xanthostigmoides</i>	Crustose	6	17%	-
<i>Tephromela alectoronica</i>	Crustose	5	14%	-
<i>Calicium</i> sp.	Crustose	3	8%	-

<b>Growth Form</b>	<b>Highest Cover</b>	<b>Site Recorded</b>	<b>Lowest Cover</b>	<b>Site Recorded</b>
Fruticose	43.5%	Westralia 1	0.8%	Gervasse 1
Foliose	40.1%	Fleays 1	2.3%	Arklow 2
Squamulose	57.9%	Shotts 4	0.4%	Arklow 2
Crustose	12.6%	Darrell 1	0%	7 Sites

The average species richness across all sites was ten, with a minimum richness of six, recorded from Arklow 2 and Gervasse 1 sites (Table 2.4; Figure 2.1). The Shannon-Wiener index which determines the number of species and their relative dominance averaged at 1.49 in this study (Table 2.4), indicating a community with low diversity. The Simpson's index which quantified the diversity of the lichen community by taking into account the number of species and their abundance, also indicated a community with low diversity (Table 2.4). The species evenness which measures how similar the abundance of different species are in a community, reported an average value of 0.64, indicating a community with similar proportions of all species (Table 2.4). The diversity

indices for the thirty six sites recorded the Arklow 2 site to have the lowest values for the Shannon-Wiener index, Simpson's index, species richness, species evenness and IAP values (Table 2.4). This suggested a potential influence from the Worsley alumina refinery, since the Arklow 2 site was located directly downwind from the refinery (Figure 2.1). The Sherwood 1 site recorded the highest values for the Shannon-Wiener index, Simpson's index and species evenness (Table 2.4) and was located approximately 20 km away from the industries in Collie (Figure 2.1).

Table 2.4: Lichen species richness, abundance, diversity indices and IAP values for 36 sites in the Collie area

<b>Biodiversity Index</b>	<b>Mean</b>	<b>Std. Deviation</b>	<b>Min. value</b>	<b>Site recorded from</b>	<b>Max. value</b>	<b>Site recorded from</b>
Shannon-Wiener Index	1.49	0.31	0.38	Arklow 2	1.88	Sherwood 1
Simpson's Index	3.53	0.98	1.19	Arklow 2	4.93	Sherwood 1
Species Richness	10.22	1.93	6.00	2 sites*	13.00	5 sites**
Species Evenness	0.64	0.11	0.21	Arklow 2	0.79	Sherwood 1
IAP	40.72	7.21	21.00	Arklow 2	58.00	Gervasse 3
Percentage Cover	51.11	9.64	30.00	Western 3	65.00	Shotts 3

\* Arklow 2 and Gervasse 1; \*\*Bristol 1, Wesfarmers 1, Shotts 6, Western 4 and Trees 1

### 2.3.2 Effect of industrial pollutants on lichen species composition

A Spearman's correlation analysis was performed to determine if distance to closest pollution source had an influence on lichen diversity. A significant but weak correlation was observed between the Simpsons' index and the distance to the closest power station (Figure 2.2), suggesting that lichen abundance increases as one moves away from the closest pollution source, but the weak correlation coefficient cannot be used to draw any strong conclusions about this relationship.

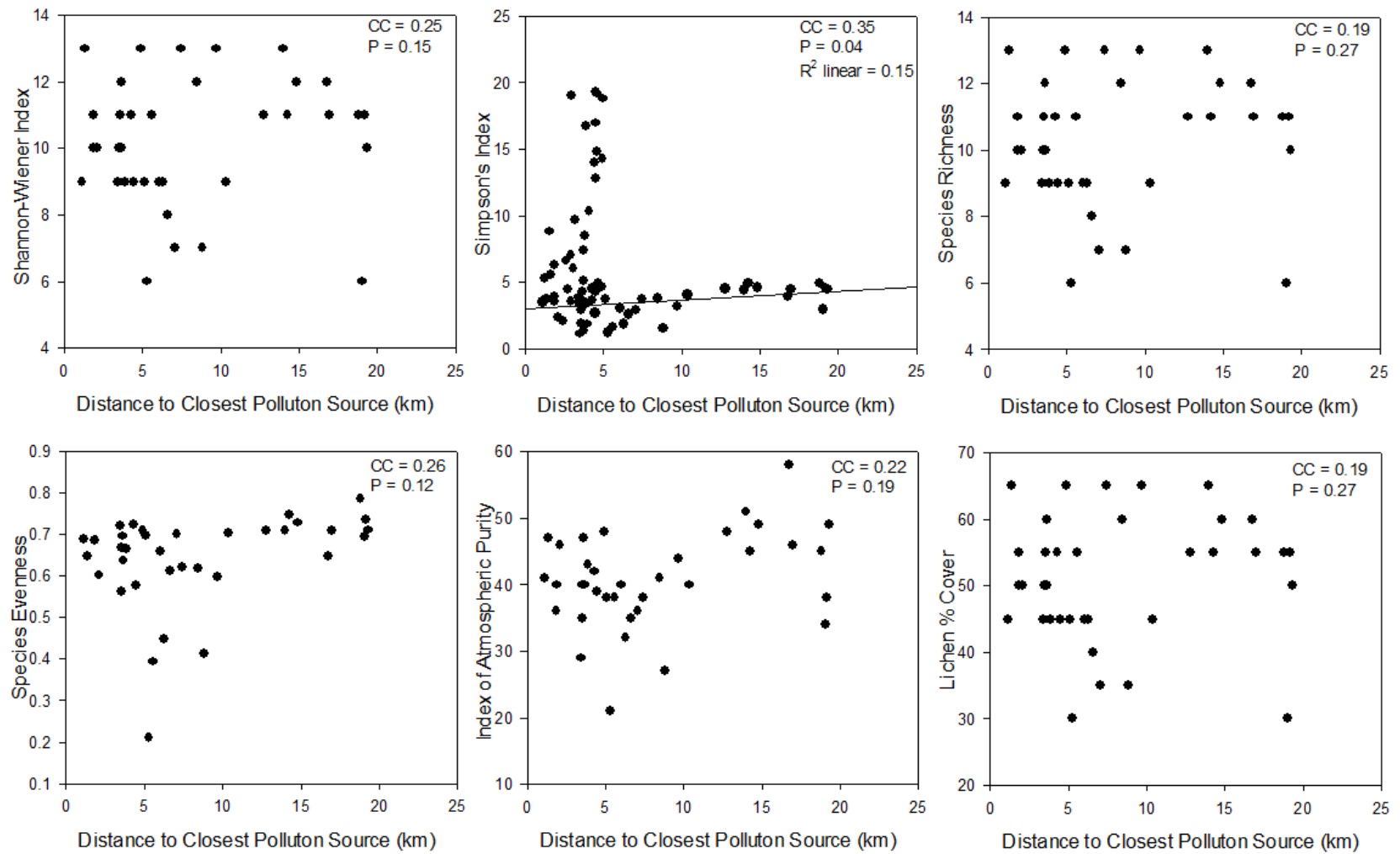


Figure 2.2: Relationship between diversity indices and distance to closest pollution source (n=36; CC = Spearman's correlation coefficient,  $P < 0.05$ )

### 2.3.3 Spatial dispersion of lichen communities

Study sites were clustered based on their similarity in lichen species composition by PCA analysis, where the first two principal components explained 73.2% of the variation for the dataset, which was a robust estimation of the overall projection of the dataset (Table 2.5). *Thysanothecium scutellatum* explained PC 1 in the negative direction with an eigen-value of -0.809 (Table 2.5), while *Usnea inermis* explained the variation along PC 2 with an eigen-value of 0.758 in the positive direction (Table 2.5), suggesting a strong influence from these two lichens in determining lichen species composition for jarrah forests in Collie. The pattern illustrated four distinct groups (Figure 2.3), with sites within each group having a similarity of 55% or more (Figure 2.3). A majority of the sites group together (20 or 56% of all sites) under group one, which was made up of mostly high and medium exposure sites, while ten sites (28%) made up a second group having most of the control sites (Figure 2.3). The next two groups were small clusters of four and two sites each, with all four sites in the third group located south-east from the Worsley alumina refinery (Figure 2.3). The grouping patterns were further explained by individual lichen species, with *Thysanothecium scutellatum* separating the group three sites in one direction, while *Usnea inermis* was defining groups one and two. *Hypogymnia subphysodes* also helped separate group two. *Usnea inermis* and *Hypogymnia subphysodes* appear to have a dominant influence at the control sites. However, *Usnea inermis* is also driving the exposed site group (group 1), suggesting a tolerance to industrial pollution in the lichen. *Cladonia rigida* illustrates the grouping of Gervasse 1 and Fleays 1 sites (group 4), but did not show any strong patterns between the other groups and was the most evenly distributed lichen for this study. The other lichen species did not show any distinct patterns with the PCA groups and played no significant role in explaining the spatial patterns of distribution.

Of the twenty one sites clustering in the largest group (Figure 2.3), thirteen have Muja power-station as their closest pollution source, with eleven of these sites located less than 7km from the power station. Therefore, this PCA group had separated the sites with the highest pollution influence, particularly because the Muja power station is the most prominent emission source for the area (Table 2.1). Seven sites in PCA group two are located more than 15 km away from all power stations and were classified as control sites *a priori*. Leach 1, which is also a control site, however, clustered in group one and not with the majority of the control sites in group two. This site is classified as wandoo woodland (dominant tree species is *Eucalyptus wandoo*), while all other sites are

located within jarraah forest ecosystems (DEC Forest Management Branch, Personal Communication, June 2011), possibly explaining why the site did not cluster in group two with the other control sites in the PCA. It may have ecological characteristics very different from the other control sites. The final control site (Gervasse 1) clustered separately with Fleays 1 (group 4), which can be explained by the lack of *Thysanothecium scutellatum* in these two sites (Appendix 2.1). The Gervasse 1 site recorded the lowest species richness for this study with only six lichen species recorded (Table 2.4), and therefore appears to be a 'disturbed' site compared with the other control sites. The PCA results provided a means of reclassifying the sites based on lichen composition, and patterns were shown that were different from those that might be expected from the pollution gradients identified from the modelling results used for *a priori* site selection. This difference in site classification will be explored further in a proceeding section of the current chapter.

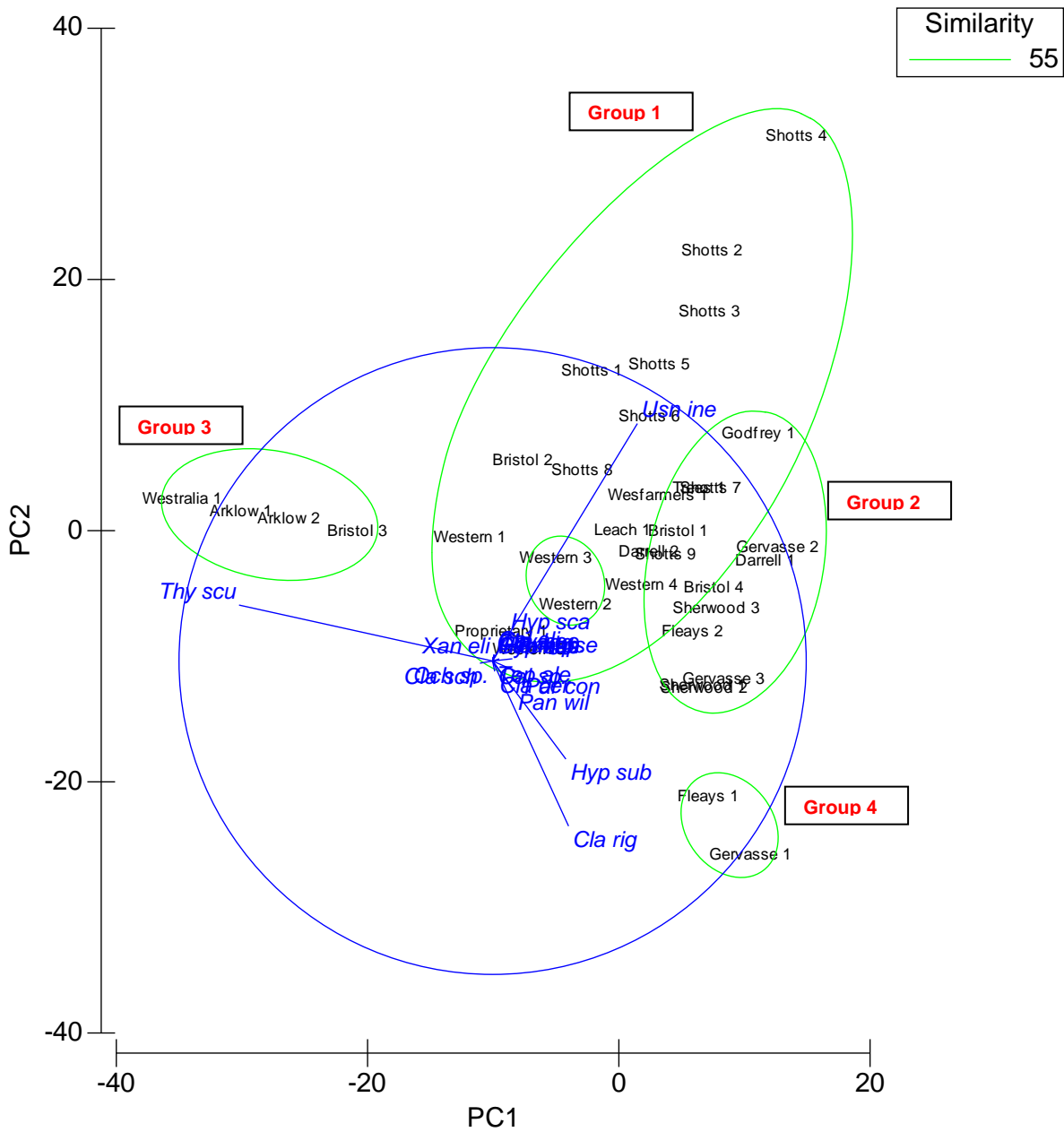


Figure 2.3: Ordination plot of principal component analysis (PCA) for lichen community composition percentage cover data with cluster overlays

Table 2.5: Eigenvalues and Eigenvector scores for the principal component analysis (PCA) for lichen community composition percentage cover data

**Eigenvalues**

PC	Eigenvalues	% Variation	Cum. % Variation
1	142	39.3	39.3
2	122	33.8	73.2
3	37	10.2	83.4
4	26.3	7.3	90.6
5	13.9	3.8	94.5

**Eigenvectors**

(Coefficients in the linear combinations of variables making up PC's)

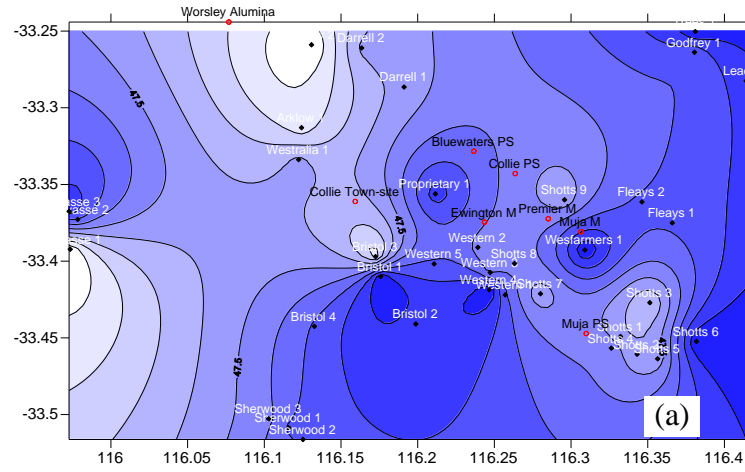
Variable	PC1	PC2	PC3	PC4	PC5
<i>Thy scu</i>	-0.809	0.179	0.277	0.446	0.181
<i>Usn ine</i>	0.462	0.758	0.307	0.315	0.059
<i>Hyp sub</i>	0.234	-0.313	-0.408	0.795	-0.008
<i>Cla rig</i>	0.242	-0.527	0.788	0.133	0.090
<i>Cla cer</i>	0.007	-0.033	0.011	0.114	-0.185
<i>Pan wil</i>	0.065	-0.091	0.008	0.061	0.032
<i>Cla sch</i>	-0.041	-0.007	0.036	0.009	-0.007
<i>Hyp aus</i>	0.003	0.001	0.001	-0.006	0.016
<i>Hyp sca</i>	0.044	0.082	0.074	0.047	-0.111
<i>Pun pse</i>	0.063	0.007	-0.177	0.071	0.564
<i>Ram sp.</i>	0.021	0.007	-0.015	0.036	0.021
<i>Cla agg</i>	0.001	0.013	0.015	-0.012	0.011
<i>Och sp.</i>	-0.003	-0.002	0.011	0.003	0.003
<i>Can xan</i>	0.001	0.003	-0.003	-0.011	-0.012
<i>Cal sp.</i>	0.003	-0.008	0.006	-0.011	0.010
<i>Cal eli</i>	0.004	0.022	-0.012	-0.017	-0.035
<i>Par con</i>	0.087	-0.038	-0.042	-0.158	0.767
<i>Xan eli</i>	-0.002	0.001	0.020	0.024	-0.004
<i>Tep ale</i>	0.002	-0.001	-0.001	0.005	-0.003
<i>Thy hoo</i>	0.007	0.020	0.013	0.010	-0.017

2.3.4 Spatial dispersion of diversity indices

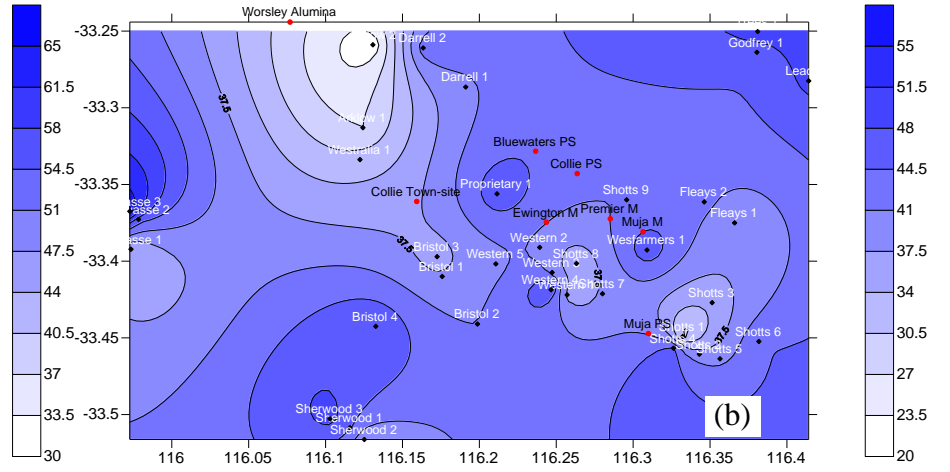
Patterns observed in the contour maps for the six biodiversity indices calculated from the lichen community study data were related to the major point-source emitters in the Collie region. The maps clearly show contours dispersing in the south-eastern direction, where diversity indices increase in value along this axis, highlighting the effect of dominant north-westerly winds characteristic to the area (Bureau of Meteorology, 2011) in the dispersion of pollutants from the point-source emitters. Low contour peaks were observed for all indices around the Muja power station and the Worsley alumina refinery, indicating low diversity in these areas. The low contour pattern near the Worsley refinery was very distinct for all six indices, while the low peak near the Muja power station is prominent only for the lichen percentage cover data, the IAP values and

for the Simpsons' index (Figure 2.4). A third low contour peak is observed near Gervasse 1 site in the lichen total percentage cover (Figure 2.4a) and species richness maps (Figure 2.4e), which confirm the separation of Gervasse 1 site in the PCA ordination from the other control sites (Figure 2.3). When the abundance of lichens in relation to growth form was estimated, the Gervasse 1 site had the lowest abundance of fruticose lichens and had no crustose species, while all other control sites had a more equal distribution of lichen species across all four growth forms (Appendix 2.3). The Gervasse 1 site also had the lowest species richness (six species) among the control sites (Table 2.4). Another low contour peak was visible south-east from the Collie town for the lichen percentage cover and species richness maps, showing a pollution influence from the Collie town at the sites closest to the town (Figures 2.4a and 2.4e). The similarity between the lichen percentage cover and species richness spatial maps suggests that lichen abundance and diversity is inter-related, with areas having low abundance always showing low lichen species richness. The spatial variation in the diversity indices correspond to the patterns observed in the PCA and both results highlight the disturbance in lichen diversity and abundance, with the spatial maps showing a possible influence from the major pollution emitters in Collie, particularly the Muja power station and the Worsley alumina refinery. The spatial maps also suggest that the pollution plumes from the Worsley alumina refinery could be having an influence as far as the Wellington National Park, resulting in the disturbance of lichen communities in the areas that were selected as control sites in this study.

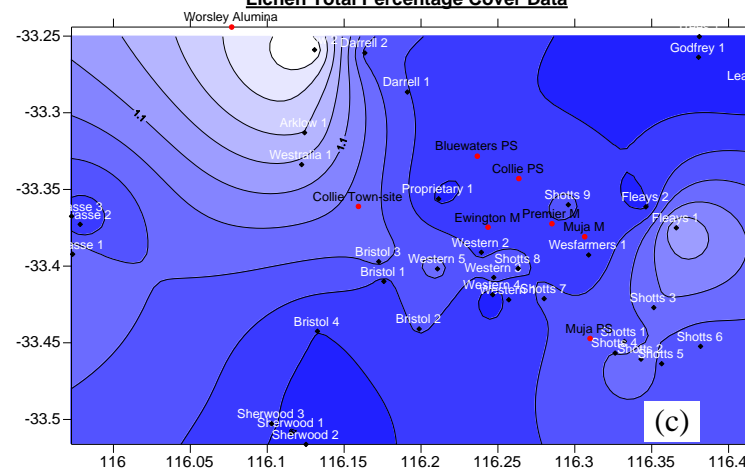




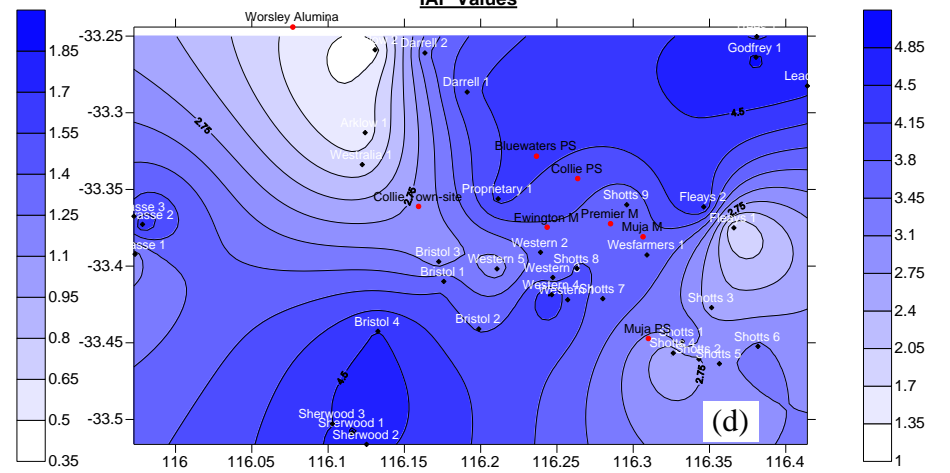
**Lichen Total Percentage Cover Data**



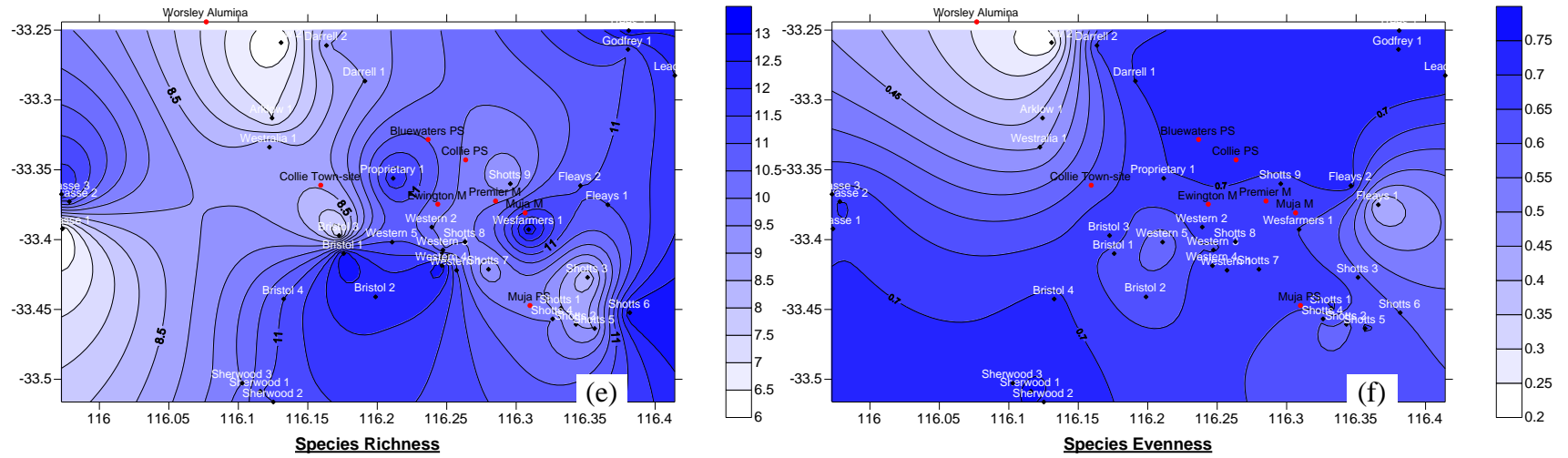
**IAP Values**



**Shannon-Wiener Index**



**Simpsons Index**



**Figure 2.4: Spatial variation patterns of lichen biodiversity indices in the surveyed area**

### 2.3.5 A priori site classification and lichen diversity

When the lichen community study results were analysed based on the exposure categories under which the original site selection was based, all indices except for species evenness were highest at the control sites (Table 2.6). This pattern was expected, as these sites were unlikely to be under the influence of pollutants from the industries in Collie. However, the medium and high exposure sites had higher values than the low exposure sites for all biodiversity indices (Table 2.6), which was not expected. This suggested either that pollution had little effect on lichen biodiversity in the region or that the pollution gradients were not accurately predicted in the air-dispersion models. The data were re-analysed based on the PCA results, and relationships between the initial exposure categories (*a priori*) and the grouping of sites in the PCA were determined (Figure 2.5). The PCA group 1 was dominated by high and medium exposure sites (85%), distinguishing the group as a ‘highly exposed’ group, while group 2 was dominated by control sites (70%), making it an ‘unexposed’ group. Group 3 was a ‘moderately exposed’ group, having only medium and low exposure sites, while group 4 consisted of only two sites having relatively unexposed sites (Figure 2.5). A chi-square test revealed statistically significant differences between the *a priori* site groups (expected) versus the PCA groups (observed), indicating that the *a priori* categorization of sites as ‘high’, ‘medium’ and ‘low’ based on predictive modelling gave a different pattern to that generated by the lichen community data.

Table 2.6: Biodiversity indices for lichen community study data based on predicted exposure type

<b>Biodiversity Index</b>	<b>Exposure Type</b>	<b>Mean</b>	<b>Median</b>	<b>Variance</b>	<b>Std. Deviation</b>
Species Richness (R)	C	4.37	4	2.7	1.64
	H	3.37	3	1.57	1.25
	M	3.51	3	1.8	1.34
	L	3.73	3	2.11	1.45
Shannon-Wiener Index (H)	C	1	1.07	0.17	0.41
	H	0.93	0.96	0.1	0.32
	M	0.93	0.85	0.12	0.35
	L	0.93	0.97	0.13	0.36
Simpson's Index (D)	C	2.45	2.21	1.12	1.06
	H	2.26	2.22	2.14	0.59
	M	2.24	2.18	2.09	0.69
	L	2.26	2.22	0.75	0.87
Species Evenness (E)	C	0.72	0.74	0.05	0.23
	H	0.84	0.84	0.1	0.31
	M	0.8	0.81	0.06	0.24
	L	0.76	0.77	0.07	0.26
IAP	C	46.11	46.00	49.61	7.04
	H	38.89	39.00	30.11	5.49
	M	40.33	40.00	15.75	3.97
	L	37.56	40.00	83.78	9.15
Percentage Cover	C	60.28	60.00	109.17	10.45
	H	49.52	45.50	375.88	19.39
	M	51.48	49.63	118.10	10.87
	L	49.63	50.50	100.41	10.02

C=control sites; H=high exposure; M=medium exposure; L=low exposure

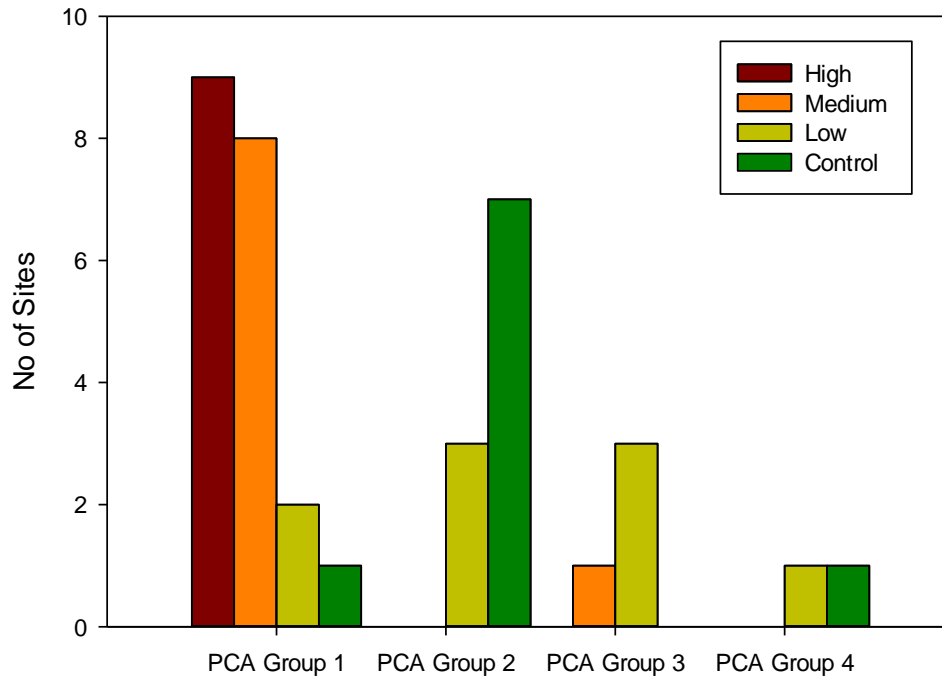


Figure 2.5: Distribution of study sites among the PCA groups based on exposure categories; Chi square test for expected (*a priori* groups) versus observed (PCA groups) statistically significant at  $p < 0.05$  (Group 1  $X^2 = 305.0$ , Group 2  $X^2 = 76.8$ , Group 3  $X^2 = 201.0$ , Group 4  $X^2 = 44.0$ )

### 2.3.6 Effect of site characteristics and forest management variables on lichen species composition

A Spearman's correlation analysis was performed to determine whether the patterns of lichen distribution and abundance observed for this study could be influenced by other confounding factors such as differences in ecological characteristics between sites (forest fragment size, tree density, canopy cover and log density) or forest management characteristics, such as fire age and harvesting history (Moning, et al., 2009). During the site selection process for the current study, areas with similar forest management data were considered, therefore the variation in the forest management variables were kept to a minimum, explaining the weak correlations observed (Table 2.7). However, the results of the correlation analysis showed that log density had an influence on the lichen community composition data, since lichen density increased significantly ( $P < 0.05$ ) with log density (Table 2.7). In the jarrah forests, fallen logs were preferred substrates for lichen establishment, which explained this relationship. Other variables did not show

any statistically significant associations with lichen community composition data (Table 2.7).

Table 2.7: Pearson correlation analysis between ecological and forest management variables and lichen community study data (n=36)

	IAP Values	Percentage Lichen Cover	Shannon-Wiener Index	Simpson's Index	Species Richness	Species Evenness
% Canopy Cover	0.111	0.101	0.174	0.287	-0.045	0.258
Tree Density	-0.109	-0.053	-0.249	-0.127	-0.281	-0.087
Log Density	0.012	<b>0.435*</b>	0.017	0.086	0.086	0.012
Years since last Fire	-0.036	0.252	0.038	0.066	-0.174	0.169
Decades Since Last Harvesting	0.078	0.163	-0.051	-0.045	-0.162	0.053
Forest Block Size	0.192	0.083	0.101	0.062	0.129	0.137

\* Spearman's Correlation is significant at P<0.05 level (2-tailed)

## 2.4 Discussion

Results from this study show that lichen communities in native jarrah forest in Collie might be affected by emissions from coal-fired power stations and coal mining activities, since a higher lichen diversity and abundance was recorded in control sites which were not under the influence of emissions from these industrial pollution sources. The spatial dispersion maps for diversity indices showed low values closer to the coal-fired power stations. There were also clear dispersion patterns of increases in diversity indices when moving away from the emission sources, suggesting that the lower lichen diversity and abundances observed was an influence from the power station emissions. Local site characteristics and forest management variables did not appear to have an important role in the lichen community composition patterns that were observed.

### 2.4.1 Lichen species composition and pollution sensitivity classes

In this study, *Usnea inermis* and *Cladonia rigida* were found to be lichens with a higher tolerance to atmospheric pollution, due to their ubiquitous nature, with both species found in all study sites (Table 2.3). Ryan (1990) classified *Usnea* spp. and *Cladonia* spp. as sensitive to intermediate genera for SO<sub>2</sub> pollution, but the survey referred to different species from the species reported in the current study. *Usnea* spp. is reported to be much more tolerant of acid rain and associated pollution compared with other species in England (Richardson & Cameron, 2004), whereas Cameron *et al.* (2007) classified it as an intermediate pollution-tolerant genus for Nova Scotia.

Other lichens with ubiquitous distribution in this study were *Thysanothecium scutellatum*, *Hypogymnia subphysodes* and *Hypocenomyce scalaris* (present in 97% – 94.5% of study sites) and the latter two species are reported as intermediate to tolerant species to oxidant and SO<sub>2</sub> pollution respectively (Ryan, 1990). Hawksworth and Rose (1970) reported SO<sub>2</sub> tolerance of up to 70 µg m<sup>-3</sup> in *H. subphysodes*. Unusual substrates associated with anthropogenic pollution have been reported for *Thysanothecium scutellatum* (Lepp, 2011), but no studies have investigated its tolerance to pollution, possibly due to its restricted distribution in the Australasian region (Australian Biological Resources Study, 2011). The widespread occurrence of the pollution-tolerant taxa *Hypogymnia subphysodes* (Pawlik-Skowronska, Wojciak, & Skowronski, 2008) and *Hypocenomyce scalaris* (Ryan, 1990), suggested that the Collie air-shed is experiencing a pollution effect from the surrounding industries. *Cladia schizopora*, *Pannaparmelia wilsonii*, *Ramboldia* sp. and *Cladonia cervicornis* var. *Vetricillata* were

moderately common taxa in this study (75 – 66% distribution), also indicating some tolerance to pollution in these species. A survey in Tasmania recorded *Cladia schizopora* as confined to decaying or charred logs of Eucalypt forests, similar to the ecosystem and substrates for the current study (Kantvilas & Jarman, 1988). Cranfield *et al.* (2011) reported a more frequent occurrence of *Pannaparmelia wilsonii* in unharvested forests than silviculturally-treated forests in south-western Australia. This study also recorded a higher abundance of *P.wilsonii* in the sites thought to have low pollution exposure, which had slightly longer periods between harvesting than other sites (Appendix 2.3). *Ramboldia* spp. are recorded frequently from urban areas (Hill and Davies, 2008), indicating a tolerance to urban pollution in these species, while *Cladonia cervicornis* var. *Vetricillata* had been associated with nitrogen deposition in Great Britain (Stevens *et al.*, 2012). These comparisons suggest that the more common lichen species in the Collie area are those that show better tolerance to air pollution. The establishment of these pollution-tolerant lichens suggests that the Collie air-shed is possibly experiencing a pollution influence from the point-sources in the area (Elsinger *et al.*, 2007).

Of the remaining eleven taxa, four species (*Parmelina conlabrosa*, *Xanthoparmelia elixii*, *Ochrolechia* sp. and *Calicium* sp.) are described as pollution-sensitive (Ryan, 1990) and were low in abundance and distribution in the current study (Table 2.3). Of these species, *Ochrolechia* spp. are reported as rare or absent in polluted sites, but common in sites away from pollution sources (Elsinger *et al.*, 2007). No reports of pollution tolerance in the Australian species *Hypocenomyce australis* have been recorded. However, a survey in Tasmania (Kantvilas & Jarman, 1988) found *H. australis* to be confined to burnt wood of *Eucalyptus*, *Baeckea* and other Myrtaceae. The current study recorded a wider distribution for *H. scalaris* compared with *H. australis* (Table 2.3), suggesting the latter to be more pollution-sensitive. *Punctelia* sp. also appeared to be pollution-sensitive in the current study. This classification is confirmed by a study in Spain that observed *Punctelia* sp. to reappear in areas with a decline in SO<sub>2</sub> pollution (Crespo, Divakar, Arguello, Gasca, & Hawksworth, 2004). *Caloplaca* taxa were infrequent and rare in the surveyed area of the current study (similar trends reported in polluted European oak forests), and are often categorized as being pollution-sensitive (Svoboda *et al.*, 2011). However, they have been recorded accumulating high concentrations of heavy metals, which indicates tolerance of certain



pollutants (Garty, Galun and Hochberg, 1986). The absence of *Candelariella xanthostigmoides* from a majority of the study sites (84%) suggested it to be a pollution-sensitive species. However, in other studies, *Candelariella xanthostigmoides* was classified as an intermediate pollution-tolerant species (Ryan, 1990), while another species of *Candelariella* (*Candelariella Müll. Arg.*) was reported as tolerant to metal pollution in India (Majumdar et al., 2009).

A survey across all the jarrah forests of south-west Australia (ForestCheck) found a higher frequency of occurrence for *Tephromela alectoronica* in unharvested forests (Cranfield et al., 2011), similar to the results of the current study. However, the ForestCheck survey records *Cladia aggregata* from 77% of the grids sampled (Cranfield, Robinson, Williams, & Tunsell, 2011), but the current study observed a less frequent distribution for this species. This was probably because the ForestCheck survey explored a number of different substrate types including soil lichens, and *Cladia aggregata* more frequently occupy soil-associated substrates. The current study, however, only examined epiphytic lichens. Although low in density, *Thysanothecium hookeri* is more prevalent in exposed sites than control sites in the current survey (Appendix 2.1); however there is insufficient literature to determine its pollution tolerance capacity. *Tephromela alectoronica*, *Cladia aggregata* and *Thysanothecium hookeri* was found at 25% of the sites, and are broadly classified as rare and sensitive taxa (Ryan, 1990). When the pollution tolerance classes in the lichen species identified in this study were classified based on the study findings, and by comparison with the information available in the international literature, an interesting trend was observed, which indicated that the more abundant species showed a higher tolerance to air pollution, while the rare species were those sensitive to air pollution (Table 2.8). The lower abundance of sensitive species in the current study suggests that the lichen communities of the Collie region are possibly being affected by pollution sources present in the area.

Table 2.8: Pollution tolerance classes identified for the lichen species recorded in Collie

Species Name	Growth Form	No. Sites Present	% Cover Exposed Sites	% Cover Control Sites	Abundance Class	Pollution Class (This study)	Pollution Class (Other studies)	Reference
<i>Usnea inermis</i>	Fruticose	36	14.7	12.8	Dominant	T	I / I-S	USFS <sup>1</sup> / Ryan 1990
<i>Cladonia rigida</i>	Squamulose	36	14.0	17.8	Dominant	T	S / I-S	Ryan 1990
<i>Thysanothecium scutellatum</i>	Squamulose	35	11.7	2.7	Dominant	I-T	-	-
<i>Hypogymnia subphysodes</i>	Foliose	34	2.0	12.1	Dominant	I-T	I-T	USFS
<i>Hypocenomyce scalaris</i>	Squamulose	34	2.1	1.1	Dominant	I-T	I-T	Ryan 1990
<i>Cladia schizopora</i>	Fruticose	27	1.1	1.0	Moderate	I	-	-
<i>Pannaparmelia wilsonii</i>	Foliose	24	1.2	3.2	Moderate	I	T	USFS
<i>Ramboldia</i> sp.	Crustose	24	0.8	1.3	Moderate	I	-	-
<i>Cladonia cervicornis</i>	Squamulose	22	1.7	3.0	Moderate	I	T	USFS
<i>Punctelia pseudocoralloidea</i>	Foliose	13	0.8	5.9	Infrequent	S	-	-
<i>Hypocenomyce australis</i>	Squamulose	13	0.3	0.3	Infrequent	S	-	-
<i>Parmelina conlabrosa</i>	Foliose	13	4.0	6.7	Infrequent	S	S	Ryan 1990
<i>Caloplaca elixii</i>	Crustose	10	1.0	0.1	Infrequent	S	-	-
<i>Thysanothecium hookeri</i>	Squamulose	10	0.6	0.1	Infrequent	S	-	-
<i>Xanthoparmelia elixii</i>	Foliose	9	0.5	1.5	Infrequent	S	I / S	Ryan 1990
<i>Cladia aggregata</i>	Fruticose	7	1.7	0.7	Infrequent	S	-	-
<i>Ochrolechia</i> sp.	Crustose	7	0.4	0.2	Infrequent	S	S	Ryan 1990
<i>Candelariella xanthostigmoides</i>	Crustose	6	0.4	0.5	Rare	S	I / S	Ryan 1990
<i>Tephromela alectoronica</i>	Crustose	5	0.1	0.4	Rare	S	-	-
<i>Calicium</i> sp.	Crustose	3	0.6	1.0	Rare	S	S	Ryan 1990

Pollution Sensitivity: S = sensitive, I = intermediate, T = tolerant; <sup>1</sup>USFS – Unites States Forest Service national lichens & air quality database and clearinghouse

In terms of lichen growth forms, the higher species richness in crustose and squamulose species in comparison with other growth forms also suggested a polluted air-shed, since crustose lichens are considered to be more pollution-tolerant than the other growth forms (Backor & Loppi, 2009; Wetmore, 1981a). Although fruticose groups are considered as pollution-sensitive (Wetmore, 1981b), the presence of *Usnea* sp. in the current study at all sites and especially in close proximity to the coal-fired power stations further suggested a pollution tolerance capacity in this species. Good establishment of species like *Usnea* have also been attributed to an open forest structure (Storaunet, Rolstad, Toeneiet, & Rolstad, 2008), such as that found in the jarrah forest, where canopy cover never exceeded 40% for all exposed sites and 70% for control sites (Appendix 2.3). Cameron *et al.* (2007) suggested that *Usnea* sp. is more pollution-tolerant than other fruticose taxa, which also appears to be the case in the current study. The ubiquitous nature of the *Usnea inermis* species, the thallus structure which makes sample collection and analysis an easier task and the potential pollution tolerance of the species indicated by the current survey, identified *Usnea inermis* as the most suitable lichen to be used for measuring heavy metal air pollution associated with the industrial emissions in Collie.

#### 2.4.2 Distance to pollution source

Although no significant ( $P < 0.05$ ) correlations were observed with lichen community data and distance to the closest pollution source, the spatial dispersion maps showed a decrease in diversity in areas downwind from prominent pollution sources (Figure 2.4). Lower frequencies and diversity of lichen species in areas under the direct influence of coal-fired power stations have been reported in the USA (Spencer, 2001, McClenahan, Davis, & Hutnik, 2007) and in Northern Spain (Fernandez-Salgui *et al.*, 2007), with findings similar to the current study. A study in India (Bajpai, *et al.*, 2010), looking at the influence of prolonged exposure to pollutants from a coal-based thermal power station, reported an increase in lichen diversity with increasing distance from the pollution source, with the highest diversity observed at distances over 15 km from the power station, while a very low lichen diversity was observed at a 5 km distance. Elsinger *et al.* (2007) found that species richness in localities that were far from an industrial area was roughly twice the species richness observed closer to the pollution sources. Other studies in the USA (Bennett & Wetmore, 2010), Canada (Cameron, *et al.*, 2007) and Central Argentina (Estrabou, Filippini, Soria, Schelotto, & Rodriguez,

2011) found that lichen diversity in areas closer to industrial and/or urban disturbances was affected by air pollution, similar to the findings from the current study.

The non-significant and weak correlations between the lichen diversity indices and distance to source in the current study could be due to the presence of several point-sources in the area (Figure 2.1), which could be having a synergistic pollution effect on many of the sites in the area, and therefore did not show any clear patterns of lichen community decline with distance to the closest pollution source (Figure 2.2). In the presence of several pollution sources, the dispersion of pollution is often more complex and identifying the influence from a single source can be difficult (Elsinger et al., 2007).

#### 2.4.3 Spatial dispersion of diversity indices

The contour maps of lichen community characteristics generated in this study showed areas with higher industrial activity to have distinct zones of low diversity, while the sites which were not under the influence from the emissions sources had areas of higher diversity (Figure 2.4). This is particularly true for the low exposure area observed in proximity to the Worsley alumina refinery (Figure 2.4). The lowest values for all diversity indices were reported from the site closest to the Worsley alumina refinery (Arklow 2), downwind (south-east) from the refinery. This finding suggested a pollution effect from the Worsley alumina refinery on lichen communities in the jarrah forests surrounding the refinery. The refinery runs two coal-fired power stations to generate power for its operation and the emissions from these plants and other bauxite processing activities might be having an influence on the lichen communities in the area (GHD, 2009).

#### 2.4.4 *A priori* site classification and lichen diversity

There is little similarity between the '*a priori*' site categories based on dispersion models and the grouping of sites obtained from the PCA ordination analysis (Figure 2.5). In the grouping obtained by the PCA analysis for the exposed sites, a clear distinction could not be made between high, medium and low exposure categories (Figure 2.5). The classification criteria used for these exposure zones might be too narrow to observe any clear patterns in lichen community composition. Interestingly, most 'low' exposure sites did not group together in the PCA and were distributed among all four main groups (Figure 2.5). Some of these 'low' exposure sites showed a higher disturbance by pollution with lower values recorded for diversity indices (Table

2.6), which was not expected. The contour maps (Figure 2.4) for diversity indices also confirm this pattern, where many 'low exposure' sites unexpectedly had areas with low diversity, especially in proximity to the Worsley alumina refinery. This indicated that the use of predictive modelling data to select sites for the current study was problematic, as these maps showed patterns very different from those generated using lichen community composition data. Dispersion modelling maps are based on prognostic meteorological and air pollution concentrations (Hurley, 2008), utilizing mathematical formulations to quantify the atmospheric processes that disperse a pollutant emitted by a source (Grigoras, Cuculeanu, Ene, Mocioaca, & Deneanu, 2012). Such models are considered important tools in the management of the impact of industrial emissions on surrounding ecosystems (Zoras, Evagelopoulos, Pytharoulis, & Kallos, 2010) and are often the only tools used for environmental impact assessments for proposed industries. Whether these models are representative of actual air pollution levels remains undetermined, and field measurements are essential for the validation of dispersion models (Batziias & Sinotorou, 2006). Further investigations using other lichen biomonitoring techniques to quantify the pollutant levels in the Collie air-shed are required to improve the reliability of the dispersion models generated for the Collie area.

#### 2.4.5 Effect of site characteristics and forest management variables on lichen species composition

When considering natural ecological variables among sites, a weak correlation was observed between log/stump density and lichen abundance data (Table 2.7), suggesting that lichen density increases with log/stump density. Although the correlation was too weak to confirm this relationship, field observations showed fallen logs and old stumps to be preferred substrates for lichen establishment in jarrah forest areas surveyed in this study. Live trees did not have a rich lichen cover, since jarrah trees shed their bark on a regular basis and lose their lichen cover during this process (Griffin & Conran, 1994).

Other studies have shown that forest management practices play a key role in the distribution of lichen communities in forest ecosystems (Moning, et al., 2009). However, the current study did not identify any prominent patterns in lichen communities that were associated with forest management variables (Table 2.7). This is

possibly because the site selection process for the current study was aimed at selecting sites with similar forest management, fire and harvesting histories to minimize the variability introduced by these parameters, on the air pollution effects being studied (Pinho et al., 2004). Harvesting and forest fires are reported to significantly affect the distribution of lichens in forested areas with low pollution effect (Giordani, 2006), but forest areas in this study were under the cumulative influence of several coal-fired power stations and mining activities. Previous studies have shown that in the presence of multiple factors (climate, topography, management practices, etc), the negative effect of air pollution is more pronounced than the other variables on the lichen community composition patterns observed (Svoboda, et al., 2010). Therefore, the influence from industrial pollutants could also have an overriding effect on forest management variables of lichen communities in the Collie area.

## 2.5 Conclusions

The findings from this study demonstrated that lichen community composition was affected by emissions from the industrial sources in Collie. The abundance of pollution-tolerant crustose and squamulose species and the lower abundance of sensitive species are indicative of a polluted air-shed. The results from this study enabled the establishment of pollution classes for lichen species in the jarrah forests which can be used in future pollution studies in the area. The mapping of diversity indices identified areas impacted by the pollution plumes from the industries in Collie. The study also identified *Usnea inermis* as a suitable biomonitoring species for *in situ* and transplant experiments to explore heavy metal pollution in the area because of its abundance and widespread distribution. Although the popular practice for many established lichen biomonitoring programmes around the world of sampling live trees was not used in this study, the sampling of fallen logs and dead stumps was equally effective in identifying spatial patterns of pollution effects on the forest ecosystems surrounding the industries in Collie. Furthermore, the pollution effects observed had little influence by forest management practices in this study, suggesting that with well controlled site selection, this method in monitoring air pollution would be highly effective in the jarrah forests if the Collie area. The study's identification of pollution effects in areas that were not predicted from dispersion modelling emphasizes the need for field measurements to validate the predictive tools so that future management decisions in disturbed ecosystems such as the jarrah forests of the Collie area may be implemented.

## 2.6 References

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## **Chapter 3 - Effect of gaseous pollutants (NO<sub>2</sub> and SO<sub>2</sub>) on the distribution of lichen communities in Collie, south-western Australia**

### **3.1 Introduction**

A key factor associated with lichen biomonitoring is the effect of gaseous pollutants that affect community composition (Batzias & Siontorou, 2009). Gaseous pollutants, particularly NO<sub>2</sub> and SO<sub>2</sub>, have been identified as the main pollutants that alter lichen community composition in polluted environments, mostly in industrial areas, at both the species and community level (Conti & Cecchetti, 2001; Geiser & Neitlich, 2007; Purvis et al., 2003). Pollution-sensitive species are categorized on the basis of their capacity to tolerate NO<sub>2</sub> and SO<sub>2</sub>, and are grouped as acidophilic, nitrophobic and nitrophilous species. Major shifts in species diversity and abundance of these functional groups have been observed in polluted environments (Geiser & Neitlich, 2007; Hawksworth & Rose, 1970; Larsen et al., 2007; Pinho et al., 2012; Pinho et al., 2011), but little is known of lichen responses to NO<sub>2</sub> and SO<sub>2</sub> in Australian environments.

The toxic effects of NO<sub>2</sub> and SO<sub>2</sub> on lichens have been studied at the population, anatomical, morphological, physiological and biochemical levels (Meysurova, Khizhnyak, & Pakhomov, 2011). Increased NO<sub>2</sub> levels causes reductions in species richness, with time-and-dose dependent effects subject to differential sensitivity among functional groups (Munzi, Pisani, Paoli, & Loppi, 2010). Gaseous nitrogen emissions from traffic pollution (Davies, Bates, Bell, James, & Purvis, 2007; Geiser & Neitlich, 2007), domestic heating (Loppi & Corsini, 2003), agriculture (Aragon, Martinez, Izquierdo, Belinchon, & Escudero, 2010) and mining activities (Bargagli, Monaci, Borghini, Bravi, & Agnorelli, 2002) have reported to result in diversity losses in lichen communities.

Effects of SO<sub>2</sub> pollution on lichens have been detected as early as the nineteen-seventies (Hawksworth & Rose, 1970) and researchers since then have carried out assessments of air quality based on lichen sensitivity to SO<sub>2</sub> (Batzias & Siontorou, 2009; Nimis, Castello, & Perotti, 1990). These studies have included time-integrated estimates of bioavailable levels of SO<sub>2</sub> in the atmosphere (Batzias & Siontorou, 2009), as well as having developed quantitative scales of SO<sub>2</sub> tolerance in indicator species (Nash III, 1976). The decline in SO<sub>2</sub> pollution in recent times has resulted in recolonisation by sulfur-sensitive species in many parts of the world, indicating that the effect of SO<sub>2</sub> on lichen distribution is not as pronounced as it used to be (Loppi & Corsini, 2003).

Although the absorption of the gaseous pollutants NO<sub>2</sub> and SO<sub>2</sub> by lichens is considered a passive process, accumulation patterns are characteristic in different lichen species (Meysurova et al., 2011). Laboratory experiments have revealed that long-term exposure to low concentrations of SO<sub>2</sub> can be destructive to foliose species such as *Hypogymnia physodes* (Meysurova et al., 2011), whereas crustose species can tolerate high concentrations of both gaseous pollutants because the thallus is pollutant resistant (Massara, Bates, & Bell, 2009). Lichen responses to different gaseous pollutants can also vary. For example, rare species may show a greater response to SO<sub>2</sub> through their decline in areas with elevated SO<sub>2</sub>, whereas certain common species may increase in areas with higher nitrogen pollution (van Dobben & ter Braak, 1999). Other critical factors affecting accumulation of pollutants by lichens are the concentration and exposure time (Meysurova et al., 2011). Both short-term high-exposure episodes and long-term low-exposure episodes can be critical to lichen communities and may result in the alteration of community composition, although different species may respond in a very different manner (Meysurova et al., 2011). Pollution-tolerant lichen species have efficient detoxification mechanisms for the removal of gaseous pollutants taken up by the thalli. For instance, SO<sub>2</sub>, upon absorption, is oxidized within the thallus and subjected to reduction and neutralization by transformation of sulfite to sulfate, without causing any thallus damage (Meysurova et al., 2011). Meteorological parameters can alter the effect of gaseous emissions on lichen communities; for example, during cool, wet conditions, lichens are physiologically active and accumulate more pollutants through wet deposition (Monaci, Fantozzi, Figueroa, Parra, & Bargagli, 2012; Nash III & Gries, 1995). Forest canopies are also known to reduce the influence of gaseous pollutants on lichen communities on the forest floor through the interception of the gases by the forest canopy (Haffner et al., 2001).

In Australia, motor-vehicle emissions are the main cause of NO<sub>2</sub> pollution, with metal refining and coal-fired power generation as secondary contributors (Commonwealth of Australia, 2010). Ambient concentrations of SO<sub>2</sub> in Australian cities are generally low (Kocken & Adeeb, 2008). However, increased industrial activities in certain regions have reported elevated concentrations and therefore require ambient levels to be monitored and regulated (Commonwealth of Australia, 2010; Government of Western Australia, 2009). This is particularly important in areas associated with coal-fired power stations, because nearly 50% of SO<sub>2</sub> emissions in Australia are from electricity generation associated with coal and other sources (Commonwealth of Australia, 2010).

In 1998, the National Environment Protection Measure (NEPM) was established, where standards were set for six pollutants, including NO<sub>2</sub> and SO<sub>2</sub> (Commonwealth of Australia, 2010). The ambient NEPM standards established for one hour NO<sub>2</sub> and SO<sub>2</sub> are 225 µgm<sup>-3</sup> and 530 µgm<sup>-3</sup> respectively. In Collie, where the current study was carried out, significant exceedances of air standards have been reported for NO<sub>2</sub> and SO<sub>2</sub> based on predictive modelling (GHD, 2009). Currently, only one station carries out monitoring of air quality in Collie and it is situated at Roche Park in the Collie town centre (GHD, 2009; Kocken & Adeeb, 2008). The jarrah forests surrounding Collie may also be affected by the industrial emissions from the area, but no studies have explored pollution impacts in the surrounding forest ecosystems.

To understand the lichen distribution and community patterns, it was necessary to measure the concentrations of gaseous pollutants and their dispersion patterns in the Collie air-shed. For this purpose, Radiello<sup>®</sup> passive air samplers were used because of their compact nature, cost-effective operation and ease of analysis (Krol, Zabiegala, & Namiesnik, 2012; Namiesnik, Zabiegala, Kot-Wasik, Partyka, & Wasik, 2005). These samplers have a coaxial geometry allowing a larger diffusive surface, which enables higher sampling rates than traditional passive samplers (Krol et al., 2012; Radiello<sup>®</sup>, 2006). They perform well over longer exposure periods at low concentrations for both NO<sub>2</sub> and SO<sub>2</sub> (Swaans et al., 2007), with good results observed during field experiments (Swaans et al., 2007). Temperature, humidity and wind speed are the main parameters that can affect the performance accuracy of the Radiello<sup>®</sup> samplers (Gerboles, Detimmermann, Amantini, & De Saeger, 2000), with measurements tending to be underestimated at lower ranges for these parameters (Gerboles et al., 2000). However, within short-range fluctuations of temperature and humidity, the Radiello<sup>®</sup> design allows consistent sampling (Pennequin-Cardinal et al., 2005; Radiello<sup>®</sup>, 2006). These samplers have been used for many monitoring studies and have provided reliable data on pollution levels (Roukos, Locoge, Sacco, & Plaisance, 2011), especially at detailed spatial scales (Soares & Pereira, 2007).

The aim of the current study was to quantify the levels of NO<sub>2</sub> and SO<sub>2</sub> in the Collie air-shed to determine the dispersion of these pollutants emitted by the coal-fired power stations and other industries in Collie. This information on the gaseous pollutants would enable the assessment of potential confounding effects of these gases on community composition and heavy metal accumulation patterns of lichens in Collie. The study also investigated the seasonal variations of NO<sub>2</sub> and SO<sub>2</sub> concentrations in an effort to

understand the influence of meteorological conditions on the dispersion of these pollutants in the Collie airshed.



## **3.2 Methods**

Radiello<sup>®</sup> passive air samplers were used to measure atmospheric SO<sub>2</sub> and NO<sub>2</sub> concentrations, in order to ascertain the presence of gaseous pollutants and quantify their concentrations in the jarrah forests surrounding the industries in Collie. Spatial and temporal measurements were obtained in fifteen sites in spring 2011, summer 2012, autumn 2012 and winter 2012.

### 3.2.1 Site selection

Radiello<sup>®</sup> samplers were deployed at fifteen study sites, which included five high-exposure sites, five low-exposure sites and five control sites (Figure 3.1). These exposure categories were identified and categorized based on the heavy metal results obtained in Chapter 4. The Radiello<sup>®</sup> study sites were overlapping with the sites where lichen transplant bags were deployed (Chapter 5), where the rationale behind site selection is described (Chapter 5.3). For the last passive sampling campaign in the winter season (July 2012), five more intermediate-exposure sites were sampled (T16 – T20 in Figure 3.1), to obtain better spatial representation for mapping the dispersion of gaseous pollutants in the Collie airshed.

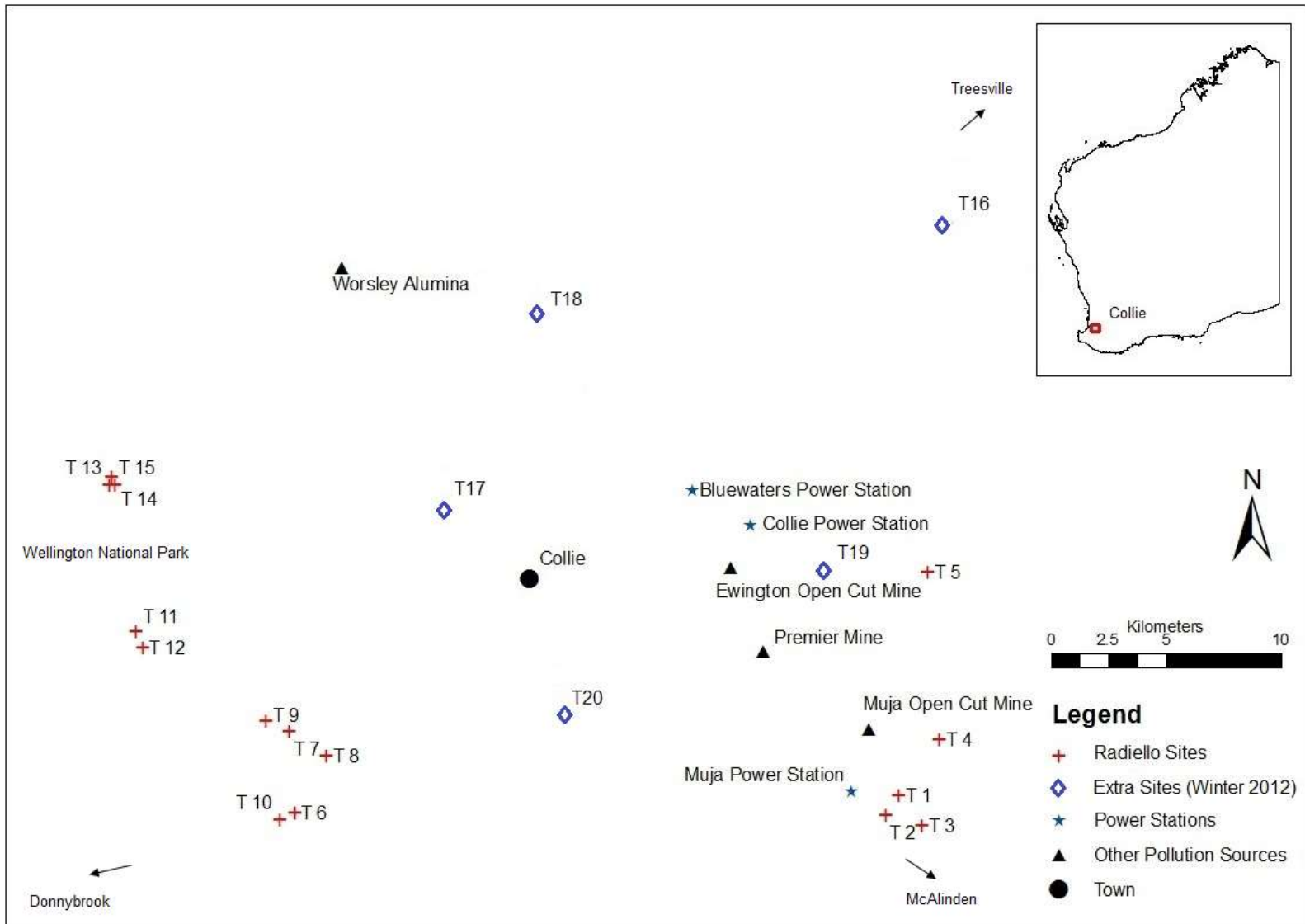


Figure 3.1: Study site locations in Collie where gaseous pollutants were measured using Radiello<sup>®</sup> passive samplers

### 3.2.2 Radiello<sup>®</sup> passive air samplers for NO<sub>2</sub> and SO<sub>2</sub>

At each study site, the triethanolamine (TEA) coated Radiello<sup>®</sup> cartridges (Sigma-Aldrich<sup>®</sup>, product code: RAD166) were removed from the sealed bag and inserted into the Radiello<sup>®</sup> blue diffusive body (Sigma-Aldrich<sup>®</sup>, product code: RAD1201), which was then sealed using a vertical adaptor (Sigma-Aldrich<sup>®</sup>, product code: RAD122), and mounted on a dome-shaped shelter. The shelters were made up of recycled unplasticised polyvinyl chloride (*uPVC*) pipes that provided protection against extreme weather, whilst allowing for ventilation (Figure 3.2). Passive samplers were hung on suitable trees, with one sampler at each study site, and exposed over a one-week period, during each season, in order to obtain seasonal weekly averages of gaseous pollutant concentrations.

At the end of the sampling week, the Radiello<sup>®</sup> samplers were removed from the shelter by unscrewing the vertical adaptor, and the exposed cartridges were placed in plastic tubes, sealed and stored in a dark container for transportation to the laboratory in Perth. The time of starting and ending of exposure was recorded to the minute for each sampler. Exposed cartridges were stored in a dark container in the refrigerator at 4 °C and analysed within two weeks of exposure. Two unexposed cartridges were transported to the field and returned to the laboratory without exposure and were used as field blanks for determining any background concentrations introduced during transportation and sampling.

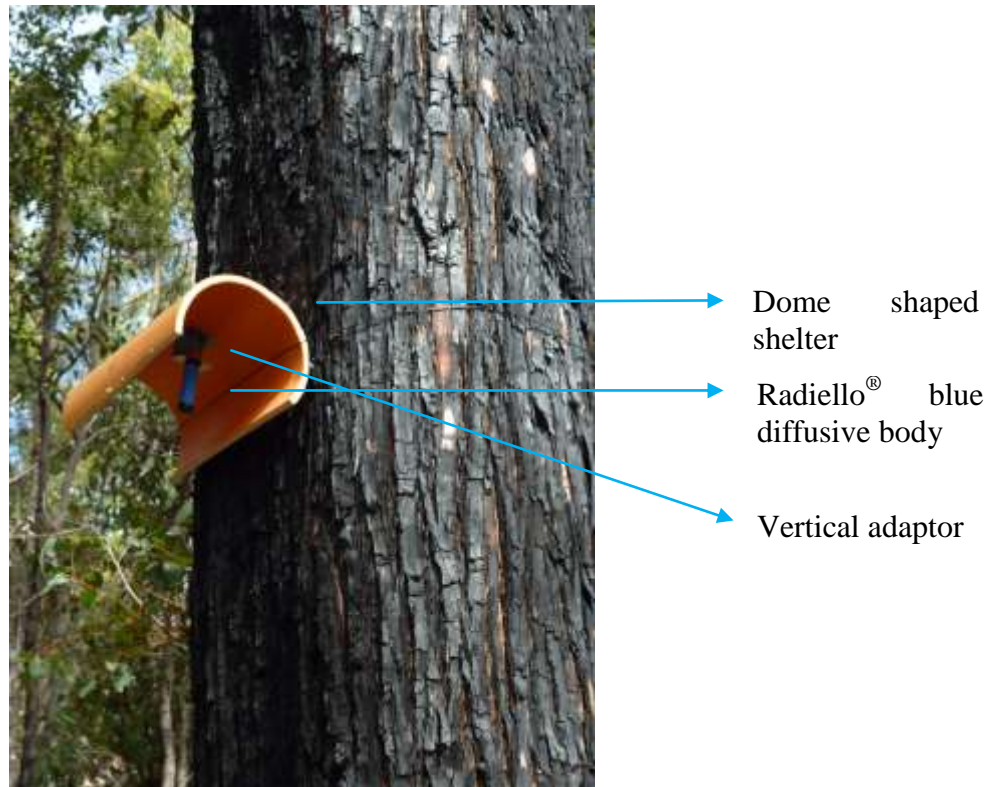


Figure 3.2: Radiello<sup>®</sup> passive air sampler mounted on a tree at a study site in Collie

### 3.2.3 Sampling rate of Radiello<sup>®</sup> samplers

The diffusive sampler is a closed, cylindrical box, with a diffusive surface that facilitates the absorption of gaseous molecules on to an adsorbing surface coated with triethanolamine (TEA). NO<sub>2</sub> and SO<sub>2</sub> are chemi-adsorbed onto TEA as nitrite and sulfite or sulfate ions.

The concentration of NO<sub>2</sub> follows the equation below and is given in ppb / μgL<sup>-1</sup> (Radiello<sup>®</sup>, 2006):

$$C_{NO_2} = \frac{m_{NO_2}}{Q_k \cdot t}$$

Equation 3.1

Where m<sub>NO<sub>2</sub></sub> is nitrite mass in nano grams (ng) found on the cartridge, t is exposure time in minutes and Q<sub>K</sub> is the sampling rate value at the temperature K in kelvin. The

sampling rate value  $Q$  at 298 K (25 °C) and 1013 hPa is  $0.141 \pm 0.007 \text{ ng}\cdot\text{ppb}^{-1}\cdot\text{min}^{-1}$ . Sampling rate of  $\text{NO}_2$  varies based on ambient temperature (in kelvin) following the equation:

$$Q_k = Q_{298} \cdot \left( \frac{T}{298} \right)$$

Equation 3.2

Where  $Q_k$  is the sampling rate at the temperature  $T$ , ranging from 263 to 313 K (from -10 to 40 °C) and  $Q_{298}$  is the reference value at 298 K.

The concentration of  $\text{SO}_2$  follows the equation below and is given in  $\text{ppb} / \mu\text{gL}^{-1}$  (Radiello<sup>®</sup>, 2006):

$$C_{\text{SO}_2} = \frac{m_{\text{SO}_4}}{0.466 \cdot t}$$

Equation 3.3

Where  $m_{\text{SO}_4}$  is the overall sulfate ( $\text{SO}_4^{-2}$ ) mass in nano grams (ng) found in the cartridge and  $t$  is exposure time in minutes. The mass of sulfite ( $\text{SO}_3^{-2}$ ) adsorbed onto the cartridge is converted to sulfate by multiplying its mass by 1.2. Sampling rate for  $\text{SO}_2$  does not vary with temperature between 263 and 313 K (10 to 40 °C), therefore  $Q$  remains constant and has been quantified as  $0.466 \pm 0.022 \text{ ng}\cdot\text{ppb}^{-1}\cdot\text{min}^{-1}$  at 298 K (25 °C) and 1013 hPa (Radiello<sup>®</sup>, 2006).

Sampling rate is constant for both gases with humidity in the range 15 - 90% and with wind speed between 0.1 and  $10 \text{ m}\cdot\text{s}^{-1}$  and is linear from 10,000 to 5,000,000  $\text{ppb}\cdot\text{min}$  (Radiello<sup>®</sup>, 2006). A seven-day exposure limit is usually recommended (Radiello<sup>®</sup>, 2006).

#### 3.2.4 Sample preparation for analysis

Exposed cartridges stored in plastic tubes were removed from the refrigerator (4 °C) prior to analysis and allowed to warm to room temperature. To each tube, 5 mL of Milli-Q water was added and stirred vigorously by a vortexer for 1 minute at a medium speed. Two unexposed cartridges (field blanks) were also prepared in this manner to obtain the background concentrations or blank values for the quantification of final

ambient air concentrations. For NO<sub>2</sub> analysis, 1 mL from each cartridge vial was transferred to 10 mL plastic test tubes and diluted to a final volume of 4 mL (4x dilution). For SO<sub>2</sub> analysis, 2 mL of original sample was diluted to a final volume of 10 mL (5x dilution).

### 3.2.5 Analysis of exposed Radiello<sup>®</sup> samplers for NO<sub>2</sub>

NO<sub>2</sub> concentrations adsorbed by the Radiello<sup>®</sup> samplers were quantified using the Lachat Quickchem<sup>®</sup> 8500 Series 2 flow injection analysis system, following the 30-107-04-1-A method (Lachat Instruments, 2009). In the Lachat analyser, the sample is run through a copperised cadmium column, where the nitrate (NO<sub>3</sub><sup>-</sup>) in solution is reduced to nitrite (NO<sub>2</sub><sup>-</sup>) and the subsequent nitrite is measured colorimetrically. The nitrite in solution reacts with sulphanilamide under acidic conditions and forms the diazonium ion, which is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to form a pink dye, which has an absorption at 540 nm. Nitrate concentration is determined by subtracting the nitrite values, which can be calculated from the nitrite + nitrate values (Lachat Instruments, 2009). The method was calibrated using a nitrite standard (NaNO<sub>2</sub>) prepared in deionised water. Standard solutions prepared for calibration were from 5 – 50 µgL<sup>-1</sup> and the calibration curve is shown in Figure 3.3. Duplicate analysis was carried out for calibration standards and the relative percent differences were calculated for quality assurance purposes (Appendix 3.1). The final concentration in µgL<sup>-1</sup> was calculated according to the Equation 3.1, where the value for Q was adjusted for the average temperature for the exposure period, based on Equation 3.2 (Radiello<sup>®</sup>, 2006). Temperature values for the adjustments were obtained from the Collie weather station (Bureau of Meteorology, 2011/2012).

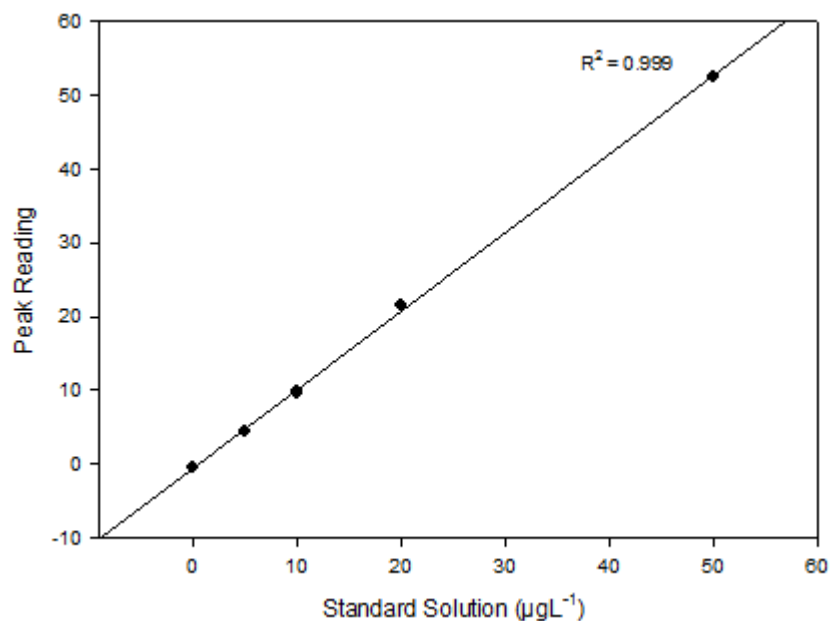


Figure 3.3: Calibration Graph for NO<sub>2</sub> measured using NaNO<sub>2</sub>

### 3.2.6 Analysis of exposed Radiello<sup>®</sup> samplers for SO<sub>2</sub>

Sulfite and sulfate concentrations in exposed Radiello<sup>®</sup> samplers were analysed by ion chromatography (Metrohm 850 Professional Ion Chromatography System). The column was 6.1006.530 Metrosep A SUPP 5-250 with a MSM (Metrohm Suppressor Module) suppressor, having a flow of 0.6 mL/min and the injection volume was 20 µL. For the stabilization of sulfite, 2.0% acetone and 0.5% isopropanol (IPA) was added to all exposed samples, unexposed blank samples and calibration standards. Calibration standards were prepared from a Na<sub>2</sub>SO<sub>3</sub> stock solution for sulfite ions and using a CertiPUR<sup>®</sup> sulfate standard solution (Merck & Co. Inc.). Standards were prepared in the range of 0.2 – 2 mgL<sup>-1</sup>. The calibration curve achieved from the standard solutions is displayed in Figure 3.4. Relative percent differences were obtained by duplicate analysis for calibration standards (Appendix 3.2). Although the SO<sub>2</sub> adsorbed onto the Radiello<sup>®</sup> cartridge is converted into sulfite and sulfate ions with variable ratios, the sum of the two ion equivalents is linear with exposure to SO<sub>2</sub> (Radiello<sup>®</sup>, 2006). Therefore, sulfite was converted to sulfate by multiplying its mass by 1.2 (Radiello<sup>®</sup>, 2006). The final concentration for SO<sub>2</sub> in µgL<sup>-1</sup> was calculated by applying Equation 3.3, with the value for Q considered at 298 K (25 °C) and 1013 hPa (Radiello<sup>®</sup>, 2006). No temperature adjustments were necessary for SO<sub>2</sub>, as sampling rate does not vary with temperature between 263 and 313 K (from -10 to 40 °C).

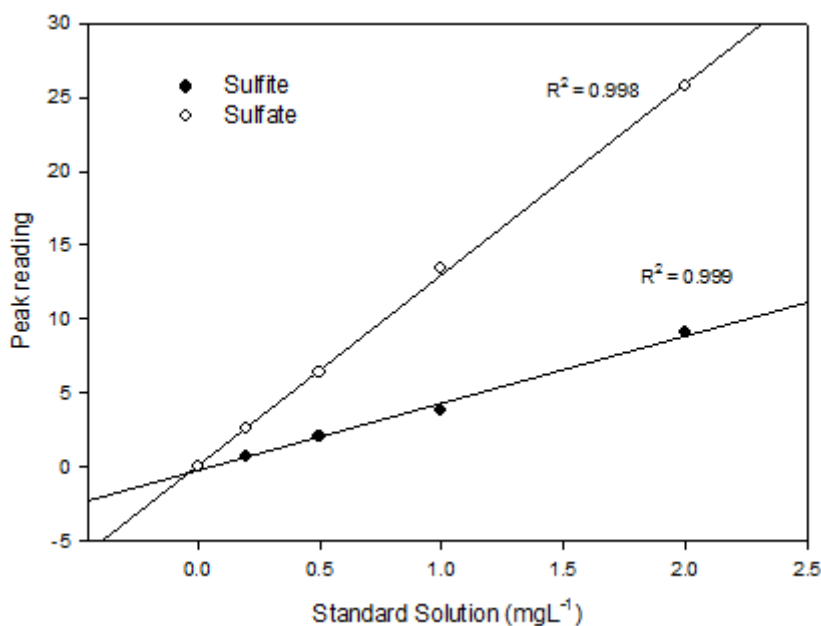


Figure 3.4: Calibration Graph for SO<sub>2</sub> measured using Na<sub>2</sub>SO<sub>3</sub> for sulfite and using CertiPUR<sup>®</sup> sulfate standard for sulfate

### 3.2.7 Blank values

During each sampling season, two unexposed Radiello<sup>®</sup> samplers were carried to the field with the other samplers that were exposed, left unexposed and brought back to the laboratory. These were used as field blanks to account for the contamination caused during transportation. The unexposed Radiello<sup>®</sup> cartridges (field blanks) were also analysed for NO<sub>2</sub> and SO<sub>2</sub> following the methods outlined above and their final concentrations were measured using the Equation 3.1 for NO<sub>2</sub> and Equation 3.3 for SO<sub>2</sub>. The average concentrations of these controls were subtracted from the concentrations obtained for each exposed sampler to determine the final concentration at each sampling location. This made it possible to eliminate background concentrations introduced during transportation and chemical analysis.

### 3.2.8 Calculation of final concentrations

The average concentration obtained after a seven-day exposure period during each season was considered as the ambient seasonal average for the season (Table 3.1), in order to obtain some understanding about the seasonal fluctuations in gaseous emissions from the industries in the area and determine their possible effects on lichen distribution. The final concentrations were converted from µgL<sup>-1</sup> to µgm<sup>-3</sup> for better comparison with air-quality standards and values reported in the international literature.



### 3.2.9 Data analysis

All data analyses were performed in IBM SPSS Statistics 19.0 software. Graphs were created using SigmaPlot 12.0 version. Data were tested for normality using the Shapiro–Wilk test (Shapiro & Wilk, 1965). The distributions were normal, with equal variances (Levine’s test results) for most seasonal data-sets and therefore parametric tests were performed. A repeated measures ANOVA was performed to determine the seasonal differences in gaseous concentrations at the 15 study sites. SO<sub>2</sub> to NO<sub>2</sub> ratio was calculated based on mean data for all seasons, which was used as a source identification tool (Fiedler et al., 2009; Nirel & Dayan, 2001). The influence of point-source emitters on the gaseous concentrations in the Collie atmosphere were determined by scatter plot analysis. A power regression curve with a negative exponent value was used to explain the relationship between gaseous concentrations and the distance to the closest pollution source. Dispersion maps for gaseous pollutants for each season were generated using the Surfer 8.0 contouring and surface modelling package for Windows (Golden Software Inc., 2010). The gridding method used was kriging, which interpolates regularly spaced grid points from irregularly spaced data points. These maps showed the dispersion of the two pollutants in the Collie air-shed. Meteorological data for the exposure periods across the four seasons were obtained from the weather station at Collie (Bureau of Meteorology, 2011/2012).

### 3.3 Results

#### 3.3.1 NO<sub>2</sub> and SO<sub>2</sub> concentrations in Collie

The average concentrations recorded for NO<sub>2</sub> and SO<sub>2</sub> in this study were 1.1 µgm<sup>-3</sup> and 0.6 µgm<sup>-3</sup> respectively, with the NO<sub>2</sub> concentrations approximately two times higher than the SO<sub>2</sub> concentrations. These average values were very low when compared with Australian National Ambient Air Quality Standards (NEMP) and international air quality standards (WHO) for NO<sub>2</sub> and SO<sub>2</sub> (Table 3.2). The highest concentrations recorded for both gases in this study were also much lower than the national and international air quality standards (Table 3.2). The mean SO<sub>2</sub> concentrations, however, were comparable to the monitoring carried out in Collie by the Department of Environment and Conservation (DEC) in 2007 using Radiello<sup>®</sup> passive samplers (Kocken & Adeeb, 2008).

Table 3.1: Average NO<sub>2</sub> and SO<sub>2</sub> concentrations (µgm<sup>-3</sup>) for each sampling season; lower case letters indicate statistically significant differences (P<0.05) between seasons based on repeated measures ANOVA Bonferroni *post hoc* test

Season	Exposure Period	NO <sub>2</sub> (µgm <sup>-3</sup> )		SO <sub>2</sub> (µgm <sup>-3</sup> )	
		Mean (Min, Max)	SD	Mean (Min, Max)	SD
Spring	5 - 11 Nov 2011	1.34 <sup>a</sup> (0.06, 3.57)	1.35	0.42 <sup>c</sup> (0.02, 1.56)	0.59
Summer	4 - 11 Feb 2012	2.52 <sup>c</sup> (1.54, 3.67)	0.57	1.46 <sup>a</sup> (0.03, 3.53)	1.11
Autumn	29 Apr – 6 May 2012	0.38 <sup>ab</sup> (0.01, 1.25)	0.32	0.06 <sup>ab</sup> (0.01, 0.1)	0.03
Winter	5 - 12 Aug 2012	0.29 <sup>b</sup> (0.08, 0.84)	0.18	0.64 <sup>b</sup> (0.21, 1.32)	0.29

Table 3.2: Comparison of mean and maximum NO<sub>2</sub> and SO<sub>2</sub> concentrations for the whole year with annual national and international air quality standards (given as a percentage of the standard)

Standard	Year Est.	NO <sub>2</sub>			SO <sub>2</sub>		
		1 Yr St. (µgm <sup>-3</sup> )	% of Mean	% of Max.	1 Yr St. (µgm <sup>-3</sup> )	% of Mean	% of Max.
NEPM	1998	56	2.02%	6.55%	53	1.26%	8.38%
WHO	2005	40	2.83%	9.18%	30	2.23%	14.8%

NEPM - Australian National Ambient Air Quality Standards; WHO - WHO guideline values for health; St. – Air quality standard

### 3.3.2 Seasonal variations in gaseous concentrations

Despite the relatively low concentrations, the values for both gases showed significant seasonal variations, with the highest concentrations recorded in summer for both gases and lowest concentrations recorded in winter and autumn for NO<sub>2</sub> and SO<sub>2</sub> respectively (Table 3.1). There were statistically significant differences between spring, summer and winter concentrations for both gases (repeated measures ANOVA Bonferroni *post hoc* test significant at P<0.05), indicating seasonal differences in the dispersion of gaseous pollutants (Table 3.1). Autumn NO<sub>2</sub> concentrations showed statistically significant differences with summer NO<sub>2</sub> concentrations, while autumn SO<sub>2</sub> concentrations showed statistically significant differences with spring SO<sub>2</sub> concentrations (Table 3.1). These results indicated that seasonal differences were more distinct between spring, summer and winter seasons, while autumn recorded similar patterns with at least two other seasons.

### 3.3.3 Meteorological data

The seasonal differences in the mean gaseous concentrations and their dispersion patterns were explained by seasonal meteorological conditions, particularly by the rainfall patterns and wind roses for the study periods (Figures 3.5 and 3.6). The highest rainfall was recorded during the autumn exposure period; while spring and winter recorded much lower rainfall and summer recorded no rainfall (Figure 3.5). The Radiello samplers recorded the highest concentrations in the dry summer exposure period, while the lowest values were recorded in autumn with the highest rainfall,

indicating an inverse relationship between rainfall and gaseous concentrations for this study.

Wind patterns showed dominant wind directions from the north-west for the spring, autumn and winter exposure periods, while summer winds were from the east-south-east direction (Figure 3.6). Summer also recorded the highest mean wind speed (Figure 3.6), which could also explain the higher gaseous concentrations in the summer sampling period.

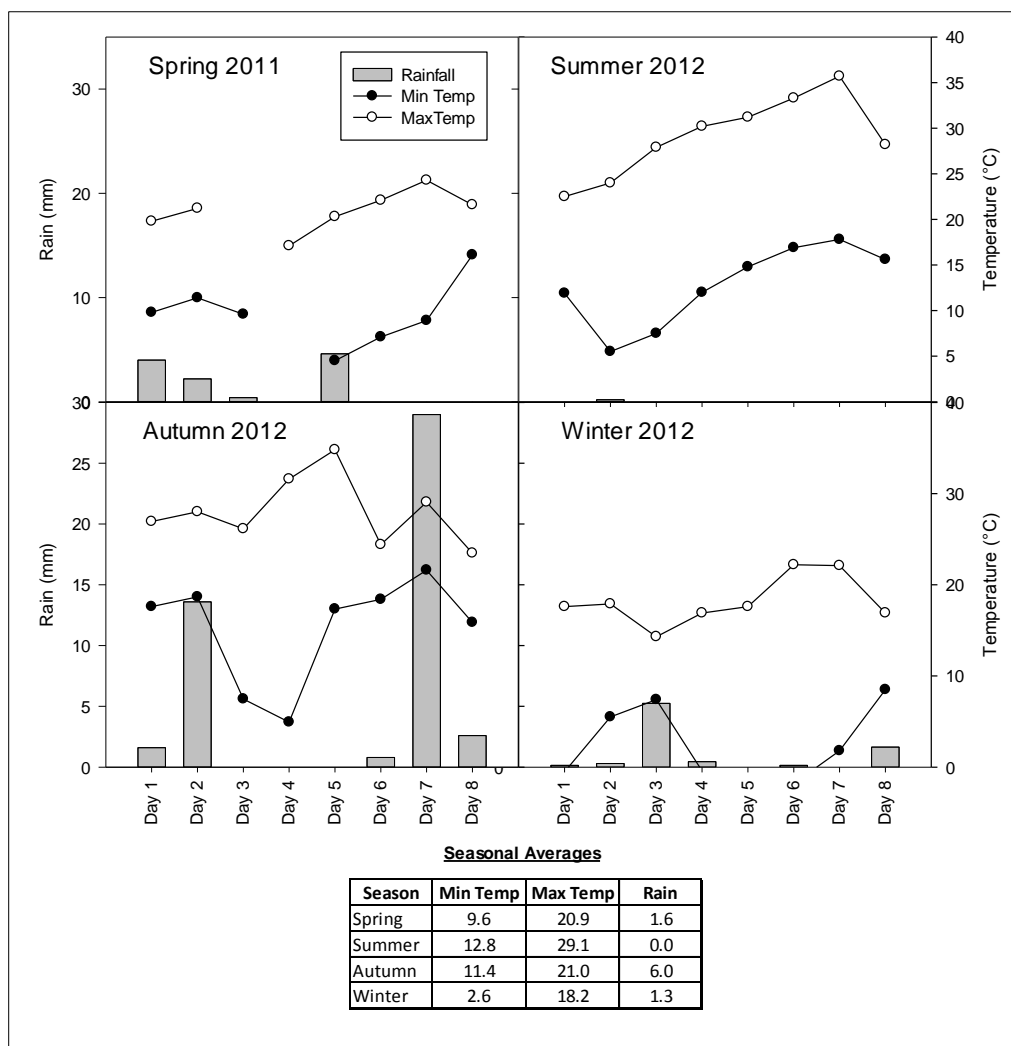
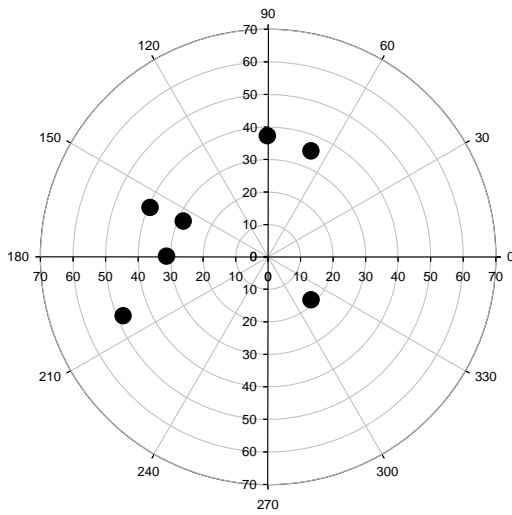


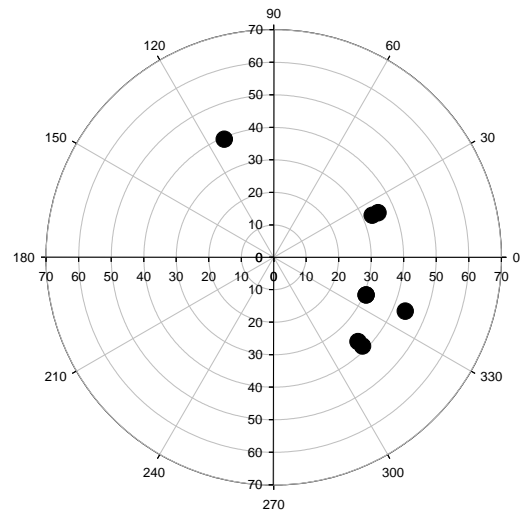
Figure 3.5: Daily temperature and rainfall data in Collie for Radiello® exposure periods (7 days) during each sampling season (Bureau of Meteorology, 2011/2012)

**Spring 2011**  
(5th - 12th November 2011)



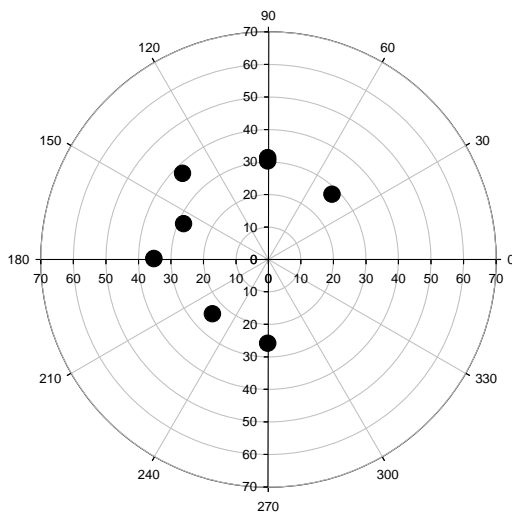
Mean wind speed = 33.9 kmh<sup>-1</sup>

**Summer 2012**  
4th - 11th February 2012



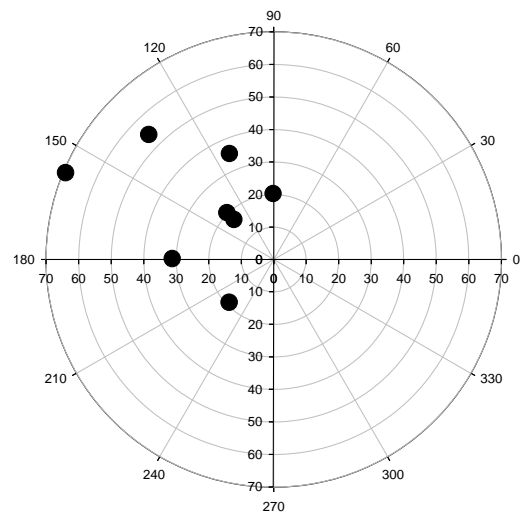
Mean wind speed = 36.1 kmh<sup>-1</sup>

**Autumn 2012**  
29th April - 6th May 2012



Mean wind speed = 29.9 kmh<sup>-1</sup>

**Winter 2012**  
5th - 12th August 2012



Mean wind speed = 33.1 kmh<sup>-1</sup>

Figure 3.6: Wind patterns in Collie for the Radiello<sup>®</sup> exposure periods (7 days) during each season showing the fastest daily wind speed (Bureau of Meteorology, 2011/2012)

### 3.3.4 SO<sub>2</sub>/NO<sub>2</sub> ratio

The ratio of SO<sub>2</sub> to NO<sub>2</sub> was compared to determine whether the source of pollution could be determined. The theory applied was that the sulfur content in fuel used for electricity generation and that used for transportation is significantly different, where electricity production would exhibit lower SO<sub>2</sub>/NO<sub>2</sub> ratios than fuel oil burning associated with traffic, and this characteristic difference can be used as a signature for source identification (Fiedler et al., 2009; Nirel & Dayan, 2001). The SO<sub>2</sub>/NO<sub>2</sub> ratio for this study was 0.6, which is within the range reported for power stations by Nirel and Dayan (2001) and Fiedler *et al.* (2009) (Table 3.3). This indicated that the gaseous pollutants picked up by the Radiello<sup>®</sup> passive samplers in this study, although low in concentration, were associated with the emissions from the coal-fired power stations in Collie.

Table 3.3: SO<sub>2</sub>/NO<sub>2/x</sub> emission ratios based on source and country

Emission Source	SO <sub>2</sub> /NO <sub>2/x</sub> Ratio		
	Nirel & Dayan, 2000	Fiedler <i>et al.</i> , 2009*	Duncan <i>et al.</i> , 1996
Traffic	0.12 - 0.48	<0.1	0.05
Power stations	0.43 - 1.0	1 - 3	2.67 - 4.56
Industry	0.74 - 1.9 (Low Wind)	1 - 5	-
	2.7 - 4.3 (High Wind)		

Country	SO <sub>2</sub> /NO <sub>2/x</sub> Ratio	Reference
Australia*	0.6	Fiedler <i>et al.</i> , 2009
USA*	0.7	Fiedler <i>et al.</i> , 2009
Africa*	0.1	Fiedler <i>et al.</i> , 2009
European countries	1	Duncan <i>et al.</i> , 1996
Some central and eastern European countries	>3	Duncan <i>et al.</i> , 1996
North America	>1	Duncan <i>et al.</i> , 1996

\*Gives the mol/mol ratio

### 3.3.5 Influence of distance to the closest emission source on gaseous pollutant concentrations

The gaseous concentrations and distance to the closest emission source showed exponential decreases in gaseous concentrations for the spring and winter seasons for both gases, where a power regression function with a negative exponent best described the relationship (Figures 3.7 and 3.8). The coal mines were not included in the analysis because they were not considered as major emitters of gaseous pollutants. The autumn scatter plots did not show any clear influence from the closest point-source, where very low concentrations were recorded for both pollutants. However, the summer scatter plots showed higher concentrations when moving away from the source, and for SO<sub>2</sub>, the power regression curve showed a positive exponent, indicating an increase in SO<sub>2</sub> concentration with increasing distance from the closest pollution source. The south-easterly wind patterns observed in the summer season, which is very different from the wind patterns observed during the other seasons (Figure 3.6) could explain the increasing concentrations with distance to source. The sites at which these elevated concentrations were found were located in the Wellington National Park, an area considered previously to have no influence from emission sources in Collie.

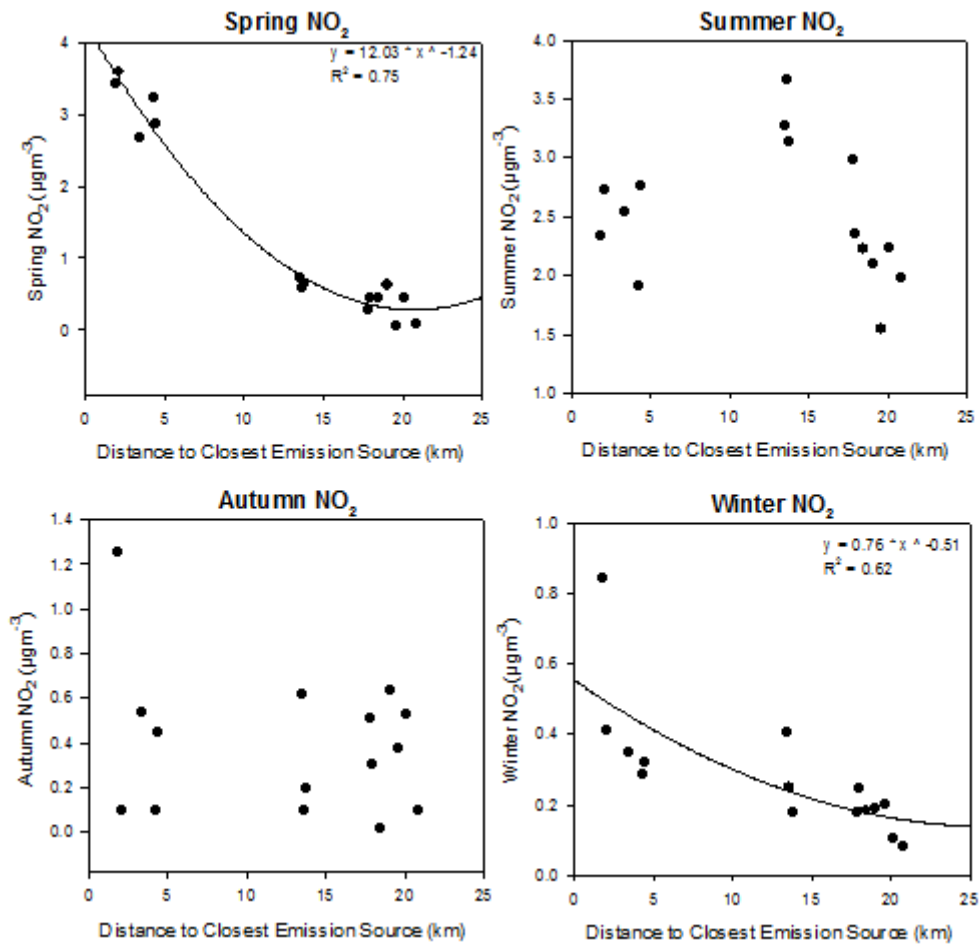


Figure 3.7: Relationship between distance to closest emission source (not including coal mines) and NO<sub>2</sub> concentrations, showing statistically significant ( $P < 0.05$ ) power regression curves



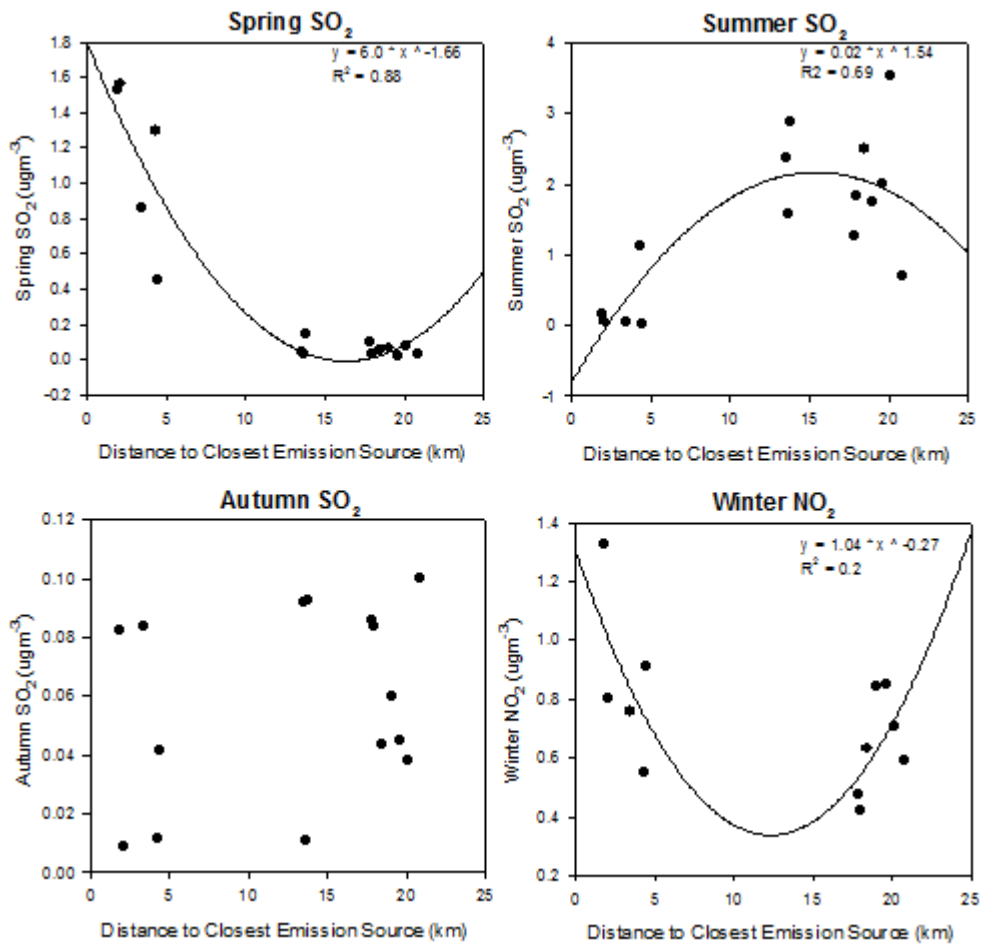


Figure 3.8: Relationship between distance to closest emission source (not including coal mines) and SO<sub>2</sub> concentrations, showing statistically significant ( $P < 0.05$ ) power regression curves

### 3.3.6 Spatial dispersion maps for gaseous pollutants

Pollution dispersion maps generated using Surfer 8.0 software to explore spatial representation of the gaseous pollutants showed high concentrations around power stations in all four seasons, except for SO<sub>2</sub> in summer (Figures 3.9 & 3.10). These maps showed the clear dispersion of gaseous pollutants corresponding to the prominent wind direction, where for spring, autumn and winter, the prominent north-westerly winds were dispersing the gaseous pollutants in the south-east direction (Figures 3.9 & 3.10 a, c, d); however this pattern is not prominent in the autumn SO<sub>2</sub> map because of the much lower concentrations recorded in autumn (Figure 3.10c). The summer dispersion maps showed a very different pattern to other seasons, because the dominant east-south-easterly winds in summer were dispersing pollutants west-north-west of the Collie town, showing high exposure contours in the Wellington National Park area. A high concentration area north-east from the coal-fired power stations was observed in the winter SO<sub>2</sub> spatial map (Figure 3.10d). This SO<sub>2</sub> peak could be a result of the emissions from a prescribed burn that was carried out east of the Collie town during this period (DEC, 2012).

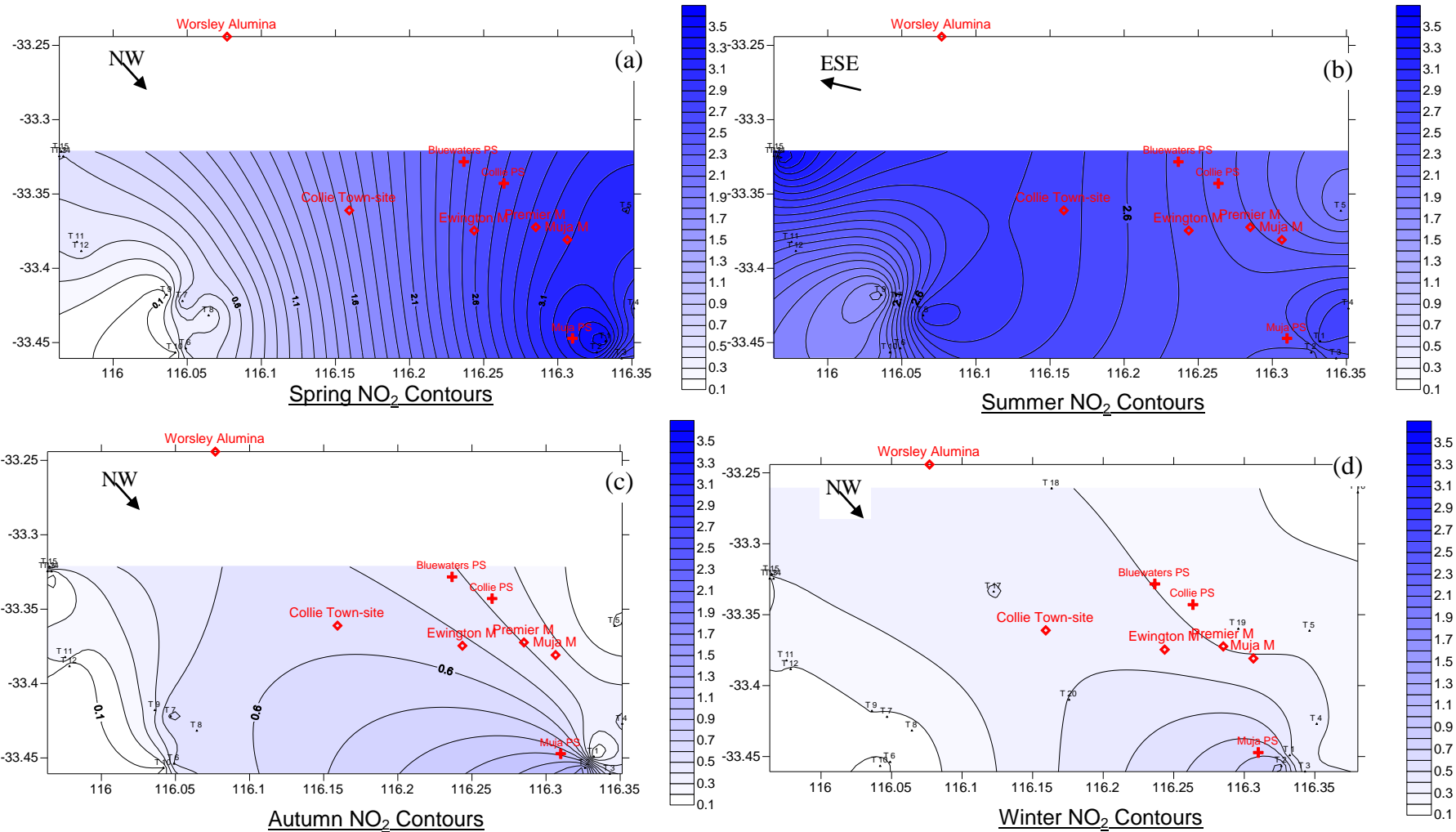


Figure 3.9: Contour maps displaying spatial distribution of NO<sub>2</sub> concentrations for each sampling season (Concentrations in μgm<sup>-3</sup>)

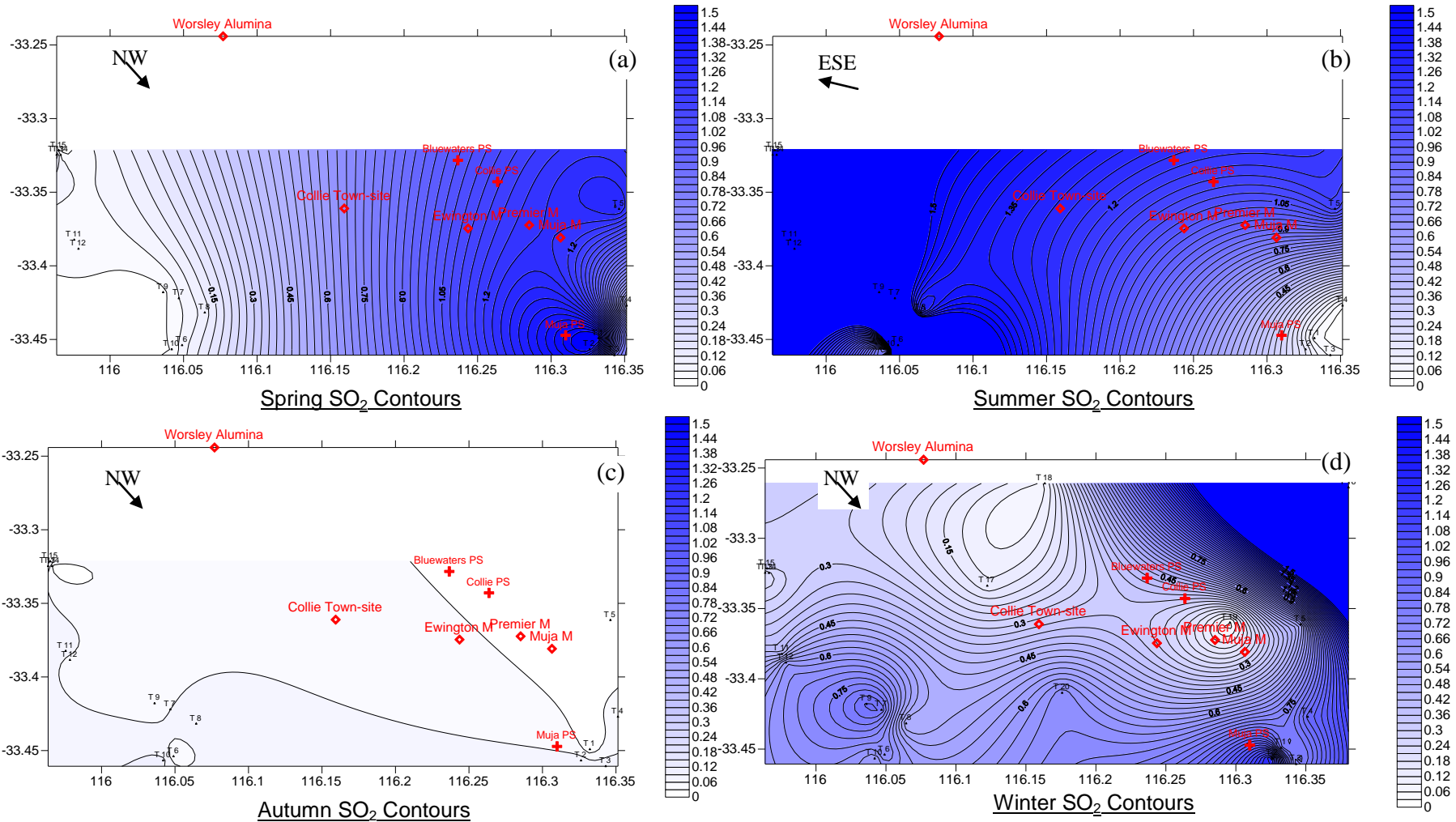


Figure 3.10: Contour maps displaying spatial distribution of SO<sub>2</sub> concentrations for each sampling season (Concentrations in μgm<sup>-3</sup>)

### 3.4 Discussion

#### 3.4.1 NO<sub>2</sub> and SO<sub>2</sub> concentrations in Collie

The results of the passive monitoring using Radiello<sup>®</sup> samplers in Collie showed very low concentrations of both gaseous pollutants, and the air standards established by the National Environment Protection Measures (NEPM) were not exceeded for both NO<sub>2</sub> and SO<sub>2</sub>. However, the air dispersion modelling conducted for the area by the Department of Environment and Conservation using the same pollution sources predicted significant exceedances of one hour NEPM standards for both NO<sub>2</sub> (130% of the standard) and SO<sub>2</sub> (450% of the standard), across the modelled area (GHD, 2009). This suggested that the emissions from the power stations might result in elevated levels of NO<sub>2</sub> and SO<sub>2</sub> in the Collie airshed at least in the short-term. The spatial dispersion maps showed higher concentration areas around the coal-fired power stations compared with other areas (Figure 3.9 and 3.10 a, c, d), which is similar to the predictions of the modelling outputs (GHD, 2009), indicating that the coal-fired power stations do affect gaseous pollutant concentrations in the Collie air-shed. Considering the higher concentrations predicted by the dispersion modelling and emissions reported by the National Pollution Inventory for Collie (National Pollution Inventory, 2010), it is thought that the gaseous pollutants might be rapidly dispersed away from the Collie river valley, thus explaining the lower concentrations observed in the current study. The relatively higher concentrations, observed in summer, further away from the emission sources, is indicative of long-range dispersion of pollutants, which is also seen in the low lichen diversity area observed near the Wellington National Park in Chapter 2 (Figure 2.4). It would appear therefore that gaseous pollutants are rapidly dispersed away from the point-sources in Collie, and do not accumulate in the immediate surroundings (Boubel, Fox, Turner, & Stern, 1994), possibly as a consequence of strong and consistent winds in the area.

Another explanation for the low concentrations of SO<sub>2</sub> in this study as well as the DEC study (Kocken & Adeeb, 2008) could be related to the low sulfur content in Collie coal (Kocken & Adeeb, 2008). The sulfur content of the fossil fuel is known to be a major factor determining SO<sub>2</sub> emissions to the atmosphere, with the sulfur content in coal ranging from 1 – 9 % (Chou, 1996), while NO<sub>2</sub> emissions are more dependent on combustion conditions than the type of fuel used (Benkovitz et al., 1996). The three power stations in Collie are fuelled by coal from the mines in the area, which in general have a sulfur percentage of 0.6% (Kocken & Adeeb, 2008). Furthermore, various flue

gas desulfurization control devices are used in Australian coal-fired power stations to remove SO<sub>2</sub> from flue gas, in order to reduce emissions to the atmosphere and similar mechanisms are used in two of the power stations in Collie (Benkovitz et al., 1996; Griffin Energy Pty Ltd, 2004, 2005).

Natural processes such as chemical transformation, dry deposition and absorption by vegetation can also remove gaseous pollutants from the atmosphere (Shukla, Misra, Sundar, & Naresh, 2008), resulting in lower concentrations being recorded by air sampling devices. Because the sampling sites for this study were located within native jarrah forests, the vegetation cover (40% and >) may have acted as a buffer for removing some of the gaseous emissions from the coal-fired power stations (Govindaraju, Ganeshkumar, Muthukumar, & Visvanathan, 2012). The sampling sites for the previous Radiello<sup>®</sup> study (Kocken & Adeeb, 2008) were located in the Collie town, close to the main road network and away from the surrounding forest ecosystems, with less buffering influence from vegetation. Therefore, it recorded some elevated concentrations (approximately two-fold higher values in certain months) compared to those of the current study.

#### 3.4.2 Seasonal variations in gaseous concentrations

The summer gaseous concentrations recorded in this study were the highest values recorded for both NO<sub>2</sub> and SO<sub>2</sub> and a similar trend was recorded for SO<sub>2</sub> in the DEC study which used active samplers (Kocken & Adeeb, 2008). Summer is the driest season in Collie, with little or no rainfall (Figure 3.5). These conditions appear to have contributed to higher gaseous concentrations in the Collie air-shed because the removal of gaseous pollutants from the atmosphere in the dry summer months is less efficient than in other seasons. Precipitation scavenging is a key mechanism by which pollutants are removed from the atmosphere and this requires rain events (Shukla, Sundar, Misra, & Naresh, 2008), which could explain the lower concentrations observed in the autumn season. Although autumn is generally a dry season in south-western Australia, the 2012 season experienced unusually high rainfall, immediately before and during the week the Radiello<sup>®</sup> samplers were deployed (Figure 3.5). Rain also prevents the dispersion of pollutants away from the point-sources, resulting in localised fallout close to the source, which is evident in the relationships with distance to source (Figures 3.7 and 3.8). The inverse relationship between rainfall and atmospheric levels of gaseous pollutants were

found in the DEC study (Kocken & Adeeb, 2008) which is consistent with the pattern observed in the current study.

Low temperatures in winter may have led to the accumulation of SO<sub>2</sub> in the atmosphere, since low temperatures restrict the movement of air masses (Atkinson, 2000), thus constraining the dispersion of pollutants away from the point-sources. The prescribed burns carried out east of the Collie town in July 2012, just before the winter sampling for gaseous pollutants commenced (DEC, 2012) could be another explanation for the high SO<sub>2</sub> concentrations. A fire effect was not seen in the NO<sub>2</sub> concentrations possibly because of the differential behaviour of the two gases in the atmosphere, where the dispersion rate of the two pollutants can often differ based on meteorological conditions (Duncan et al., 1996; Fiedler et al., 2009). The removal of NO<sub>2</sub> and SO<sub>2</sub> from wet and dry deposition and chemical reaction is also highly variable (Duncan et al., 1996).

#### 3.4.3 SO<sub>2</sub>/NO<sub>2</sub> ratio

Significant differences in SO<sub>2</sub>/NO<sub>2</sub> ratios are observed between mobile sources such as vehicular emissions and stationary sources such as industrial emissions (Duncan et al., 1996; Fiedler et al., 2009). SO<sub>2</sub> is primarily emitted by the burning of sulfur containing fossil fuels, and the sulfur levels in different types of fossil fuels also differ significantly, resulting in different concentration ratios during combustion (Nirel & Dayan, 2001). NO<sub>2</sub> on the other hand, is more abundant in vehicular emissions and therefore gives a lower SO<sub>2</sub>/NO<sub>2</sub> ratio when related to pollution associated with vehicular traffic (Nirel & Dayan, 2001). Burning of coal for electricity generation is considered to result in lower SO<sub>2</sub>/NO<sub>2</sub> ratios than low-temperature boilers burning fuel oil with high sulfur content (Nirel & Dayan, 2001). The SO<sub>2</sub>/NO<sub>2</sub> ratio of 0.6 in the current study indicated that the gaseous concentrations measured by the Radiello<sup>®</sup> samplers were more likely to be associated with the coal-fired power stations in Collie (Table 3.3), because this ratio would be much lower if associated with vehicular emissions and much higher if associated with other industrial sources (Table 3.3).

#### 3.4.4 Spatial dispersion of gaseous pollutants

The coal-fired power stations are reported as the key emitters of NO<sub>2</sub> and SO<sub>2</sub> in the Collie area (National Pollutant Inventory, 2010) and the spatial dispersion maps in the current study confirmed the influence of the power stations on gaseous emissions in Collie, where areas downwind from the power stations showed higher concentrations compared to other areas (Figures 3.9 and 3.10). The influence of wind patterns in the

dispersion of pollutants is clearly visible in the dispersion maps, where the prominent east-south-easterly winds blowing in from the direction of the power stations in summer appear to be dispersing pollutants as far as the control sites in the Wellington National Park area, showing different dispersion patterns compared with the other seasons (Figure 3.9 b & Figure 3.10 b). The dry summer conditions could also be facilitating the dispersion of pollutants further from the point-sources than in the other seasons. The positive power regression curve with distance to pollution source observed only for the summer SO<sub>2</sub> data (Figure 3.8) also confirm the dispersion of pollutants further away from the point-sources. Although low in concentration, the fallout of gaseous pollution plumes in the Wellington National Park area by summer winds is of concern, because the impact from industries in this area have not been identified before this study.

All other seasons showed prominent winds from the opposite north-westerly direction (Figure 3.6). The spatial contour maps and the wind patterns in the spring and winter seasons suggested that the fallout of the main gaseous plumes could be further south-east from the power stations, towards McAlinden (Figure 3.1). Many studies have reported similar wind-related dispersion patterns, with higher concentrations observed downwind from point-source emitters (Chang, Liu, Zhang, Ji, & Song, 2012; Gaga et al., 2012; Ge, Xu, Lin, Li, & Wang, 2012; Josipovic et al., 2010). The dispersion of pollutants by local winds and the fallout of pollutants away from pollution sources is a common phenomenon. This is important for pollution removal through dispersion, because stagnation can lead to severe pollution events (Boubel et al., 1994; Topcu, Incecik, & Unal, 2003). The latter condition can be detrimental to lichens and higher plants (Percy & Ferretti, 2004), with high toxicity resulting in elimination of indicator species (Hawksworth & Rose, 1976; Boubel et al., 1994).

#### 3.4.5 N& S sensitivity of lichen species in Collie

The gaseous pollutant patterns observed in this study were compared with the lichen community composition patterns recorded in Chapter 2, to determine the influence of gaseous pollutants on lichen communities in Collie. The sensitivity of lichens to nitrogen and SO<sub>2</sub> was used as a measure to classify lichen species recorded in the community study into functional groups, which enabled the determination of the effect of these pollutants in the distribution of lichen communities in Collie (Table 3.4). Most of the abundant species were classified as intermediately tolerant to SO<sub>2</sub>, while the rare



species were sensitive to SO<sub>2</sub> (Table 3.4), indicating that lichen communities in Collie are dominated by SO<sub>2</sub> tolerant species. This pattern suggested that although the NO<sub>2</sub> and SO<sub>2</sub> concentrations reported by passive sampling were low, there could be an influence from this pollutant on the native lichen vegetation. However, there appeared to be little effect from NO<sub>2</sub> on species distribution, since some of the rare species are thought to be eutrophic or tolerant of high concentrations of nitrogen (Table 3.4).

Table 3.4: Comparison of N and SO<sub>2</sub> tolerance in lichen species recorded in the lichen community study (Chapter 2) with international literature

Species Name	Growth Form	No. of Sites Present	N Requirement*	SO <sub>2</sub> tolerance**	Closest Species	References
<i>Usnea inermis</i>	Fruticose	36	O	I / I-S	<i>Usnea hirta</i> / <i>Usnea</i> spp.	USFS <sup>1</sup> / Ryan 1990
<i>Cladonia rigida</i>	Squamulose	36	E	S / I-S	<i>Cladonia fimbriata</i> / <i>Cladonia</i> spp.	Ryan 1990
<i>Thysanothecium scutellatum</i>	Squamulose	35	-	-	-	-
<i>Hypogymnia subphysodes</i>	Foliose	34	E	I-T	<i>Hypogymnia physodes</i>	USFS
<i>Hypocenomyce scalaris</i>	Squamulose	34	-	I-T	<i>Hypocenomyce scalaris</i>	Ryan 1990
<i>Cladia schizopora</i>	Fruticose	27	-	-	-	-
<i>Pannaparmelia wilsonii</i>	Foliose	24	M	T	<i>Parmelia hygrophila</i>	USFS
<i>Ramboldia</i> sp.	Crustose	24	-	-	-	-
<i>Cladonia cervicornis</i>	Squamulose	22	-	T	<i>Cladonia gracilis</i>	USFS
<i>Punctelia pseudocoralloidea</i>	Foliose	13	-	-	-	-
<i>Hypocenomyce australis</i>	Squamulose	13	-	-	-	-
<i>Parmelina conlabrosa</i>	Foliose	13	-	S	<i>Parmelina</i> sp.	Ryan 1990
<i>Caloplaca elixii</i>	Crustose	10	-	-	-	-
<i>Thysanothecium hookeri</i>	Squamulose	10	-	-	-	-
<i>Xanthoparmelia elixii</i>	Foliose	9	E	I / S	<i>Parmelia saxatilis</i> / <i>Xanthoparmelia</i> sp.	Ryan 1990
<i>Cladia aggregata</i>	Fruticose	7	-	-	-	-
<i>Ochrolechia</i> sp.	Crustose	7	-	S	<i>Ochrolechia</i> sp.	Ryan 1990
<i>Candelariella xanthostigmoides</i>	Crustose	6	E	I / S	<i>Candelariella concolor</i> / <i>Candelariella xanthostigmoides</i>	Ryan 1990
<i>Tephromela alectoronica</i>	Crustose	5	-	-	-	-
<i>Calicium</i> sp.	Crustose	3	-	S	<i>Calicium</i> sp.	Ryan 1990

\*N Requirement: O = oligotroph, M = mesotroph, E = eutroph; \*\*SO<sub>2</sub> Sensitivity: S = sensitive, I = intermediate, T = tolerant; <sup>1</sup>USFS – Unites States Forest Service national lichens & air quality database and clearinghouse

### **3.5 Conclusions**

Low concentrations were recorded for both NO<sub>2</sub> and SO<sub>2</sub> pollutants in the current study, and the spatial dispersion patterns of gaseous pollutants indicated that the main pollution plumes were possibly falling outside the Collie area. The low diversity peaks for lichen communities (Chapter 2) coincide with the gaseous pollutant fallout areas. The current study also identified the Wellington National Park as an area affected by the emissions from the industries in Collie, which had not been identified before. Classification of lichen species in Collie, based on pollution tolerance classes showed better establishment of SO<sub>2</sub> tolerant lichens in the area, indicating that even though the concentrations for gaseous pollutants were low, there appears to be some influence from these pollutants on lichen communities in the Collie air-shed.

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**Chapter 4 - In situ biomonitoring of heavy-metals with the lichen *Usnea inermis* in  
Collie, south-western Australia**

#### **4.1 Introduction**

Atmospheric pollution leading to degradation of forest ecosystems has been observed in many parts of the world and the continuous increase in industrial activities has focused attention on the topic (Markert et al., 1999; Styers, Chappelka, Marzen, & Somers, 2010). Air pollution can lead to structural and functional changes in plant communities, affecting various levels of ecosystem organization, from individual leaves to plant assemblages and entire ecosystems. Processes such as plant growth, productivity, root dynamics, and nutrient cycling can all be affected (Gregg, Jones, & Dawson, 2003; Lovett et al., 2000; Pouyat, McDonnell, & Pickett, 1995). Stressed ecosystems are also more susceptible to pests and diseases, leading to the poor health of forest ecosystems (Percy & Ferretti, 2004). Rapid industrial development has resulted in the release of persistent chemical substances into the environment in very large quantities, giving rise to additional impacts on plants (Percy & Ferretti, 2004). Included in these pollutants are heavy metals, substances of increasing concern because of their persistent nature and toxicity to biological systems (Bhargava, Carmona, Bhargava, & Srivastava, 2012). Although many metals are essential at low concentrations, for biological systems in carrying out various physiological processes, they become toxic at high concentrations (Babula et al., 2008).

The use of coal for the generation of energy is known to emit a variety of pollutants and heavy metals that are potentially toxic to biological systems (Fernandezturriel, Decarvalho, Cabanas, Querol, & Lopezsoyer, 1994). Metals emitted by coal power generation include As, Cd, Cr, Cu, Pb, Hg, Mn, Ni and Zn (Sabbioni, Goetz, & Bignoli, 1984; Shah, Strezov, & Nelson, 2010; Shah, Strezov, Prince, & Nelson, 2008), most of which are toxic to biological systems at high concentrations (Babula et al., 2008). Energy generation using coal releases more emissions to the atmosphere than other energy generating processes, such as natural gas, nuclear power and renewable energy sources (Diniz Da Costa, Prasad, & Pagan, 2004). However, the use of coal for energy generation continues because it is the most abundant and the cheapest fuel in the world, therefore providing energy security for the future (Diniz Da Costa et al., 2004).

Australia is a country rich in coal reserves, making it an inexpensive energy source to supply the fast-growing demand for energy within the country (Diniz Da Costa et al., 2004). Although new technologies are being developed to reduce the gaseous and particulate emissions, these continue to be a significant environmental issue (Nelson, Shah, Strezov, Halliburton, & Carras, 2010). Therefore, there is a critical need to monitor the pollutants emitted from coal-related industries in Australia, to evaluate the potential impact on ecosystems, particularly those impacts from the continuous release of persistent heavy metals into the atmosphere (Nelson et al., 2010).

The air-shed of Collie in south-western Australia has received attention in recent years because of the increase in industrial activities associated with coal power generation (EPA, 2005a). Coal mining and electricity generation is a significant contributor of atmospheric heavy metals in Collie (National Pollutant Inventory, 2010), with the presence of three open-cut coal mines and three coal-fired power stations in the area, and proposed expansions to two existing power stations. The air-shed of Collie is considered to have elevated concentrations of As, Cd, Cr, Hg, Ni and Pb, emitted from coal-burning processes (GHD, 2009). However, no studies have measured heavy metal concentrations across the Collie region, despite the literature reporting significant emissions from coal-burning processes (Shah, Strezov, & Nelson, 2010; Shah, Strezov, Prince, & Nelson, 2008). In 2010, electricity generation in Collie was estimated to have emitted 1,100,000 air kilograms of PM<sub>10</sub>, 420,000 air kilograms of PM<sub>2.5</sub> and 3619 air kilograms of heavy metals into the atmosphere (National Pollutant Inventory, 2010). Heavy metals are usually present in the particulate matter, mainly the PM<sub>2.5</sub> fraction (US EPA, 2004) and hence are not measured separately (Nelson et al., 2010). However, the toxic and persistent nature of heavy metals highlights the importance of measuring their concentrations separately, in order to better assess their impact on the surrounding environment (Babula et al., 2008; Jiao, Chen, Chang, & Page, 2012).

The standard approaches for assessing heavy metals in the environment are: 1) use modelling tools to estimate the dispersion of emissions based on the properties of coal, topographic and meteorological conditions; 2) calculate emission factors by considering a database of emissions for a large number of coal-fired power stations, or 3) the direct measurement of pollutants at specific locations (Nelson et al., 2010). The conventional technique used for direct measurement is 'precipitation analysis', which requires the deployment of large numbers of precipitation collectors (Harmens et al., 2004). This is

an expensive and time-consuming operation, which is one reason why the direct measurement of heavy metals is rarely undertaken (Nelson et al., 2010; Wolterbeek, 2002). Biomonitoring using a particular species of moss, lichen or higher plant is an alternative approach (Batzias & Siontorou, 2009). It is a cost-effective practice, making the study of heavy metal air pollution across time and space possible. Mosses and lichens are the most popular biomonitors in studies of heavy metal air pollution because concentrations in their tissues are highly correlated with deposition rates (Bari, Rosso, Minciardi, Troiani, & Piervittori, 2001; Conti & Cecchetti, 2001). Many lichen species are able to accumulate mineral elements, especially heavy metals in polluted environments, at levels exceeding their metabolic requirements without losing vitality, making them especially suitable for the biomonitoring role (Backor & Loppi, 2009; Garty, 2001).

Lichens sampled at varying distances around metal smelters, steelworks, power stations, mining sites and mixed industrial centers have revealed abrupt decreases in metal concentrations, often at a distance of a few kilometers (Garty, 2001). Research as far back as the 1970s developed mathematical models to predict the fallout of metals from emission sources in lichen biomonitoring studies (Campbell, 1976). A study in India found fallout of metals 4 - 5 km away from a busy city centre (Bajpai, Upreti, & Dwivedi, 2009), while a study in Canada found an exponential decrease in Pb concentrations in lichens at a distance of 50 km from an oil-processing site (Graney, Landis, & Krupa, 2012). The dispersion and fallout of metals depends on many characteristics such as topography, meteorological conditions, type of emission source, stack height and metal characteristics (Campbell, 1976). Investigations have also considered long distance transport of heavy metals from industrial areas and their effect on the remote areas to which these pollutants are transported (Agnan, Séjalon-Delmas, & Probst, 2013). Wind direction is considered a crucial factor in distance-dependent transportation of heavy metals (Garty, 2001).

Elevated heavy metal concentrations associated with point-sources have been reported in many lichen biomonitoring studies. A study in Canada in a gold-mining area with elevated As concentrations reported values as high as  $2300 \mu\text{g g}^{-1}$  dry weight in lichens growing near a mine tailing pond (Koch, Wang, Reimer, & Cullen, 2000). Another study by Skowronska *et al.* (2008) found high Zn and Pb in a number of lichen species collected close to mining operations in Poland, noting that epiphytic lichens (both

crustose and foliose) accumulated more metals than epigeic (soil based) lichens. Reports of Hg accumulation by lichens near metal-producing sites have noted very high concentrations for Hg, with mean values ranging between 0.1 and 2.8 mgg<sup>-1</sup> dry weight (Lackovicová, Martiny, Pisút, & Stresko, 1994; Horvat, Jeran, Spiric, Jacimovic, & Miklavcic, 2000). For a gold-mining area in Ghana, elevated Mn levels of 1097 µgg<sup>-1</sup> in lichens were reported (Boamponsem, Adam, Dampare, Nyarko, & Essumang, 2010). Manganese has also been associated with soil-borne pollution, especially when present in very high concentrations in lichen tissue (Hauck & Paul, 2005). The above studies indicate that lichens are capable of absorbing atmospheric metals at very high concentrations, making them ideal organisms for measuring heavy metal pollution.

*Usnea* species are useful biomonitors because of their fast growth, ease of collection and ubiquitous nature in areas of interest (Conti et al., 2012; Conti et al., 2009; Monaci, Fantozzi, Figueroa, Parra, & Bargagli, 2012). The *Usnea* genus has a worldwide distribution, with over 600 species identified (Monaci et al., 2012). It has a fruticose growth form and is a common epiphyte on living trees, but is also found on old stumps and logs (Monaci, Bargagli, & Gasparo, 1997). Various studies have explored the biomonitoring capacity of *Usnea* species to measure heavy metal pollution and have observed clear patterns with atmospheric pollution (Conti et al., 2012; Culicov & Yurukova, 2006; Jayasekera & Rossbach, 1996; Pino et al., 2007; Rossbach, Jayasekera, Kniewald, & Thang, 1999). As a biomonitor, *Usnea* has been particularly successful in Mediterranean environments (Conti et al., 2012; Conti et al., 2009), both as *in situ* monitors (Conti, et al., 2009) and when transplanted from control to impacted sites (Conti, et al., 2012). The genus is also believed to have an intermediate tolerance to pollution (Jansson, Palmqvist, & Esseen, 2009) and is abundant in the southern jarrah forests of Western Australia, where the current study was conducted (Cranfield, Robinson, Williams, & Tunsell, 2011).

The aim of the current study was to investigate the dispersion patterns of heavy metals in the Collie air-shed emitted from coal power generation and other industrial sources. The metals As, Cd, Cr, Cu, Pb, Mn, Hg, Ni and Zn were measured in *Usnea inermis*, along potential pollution gradients. The specific objectives of this study were to:

- 1) Identify spatial and temporal patterns of heavy metal concentrations in *Usnea inermis* along potential pollution gradients;

- 2) Explore the effect of season on heavy metal concentrations in *Usnea inermis* tissue;  
and,
- 3) Map the concentrations of heavy metals and their dispersion in the Collie air-shed,  
in order to identify zones of impact from known pollution sources in the area.

## **4.2 Methodology**

### 4.2.1 Study design

Sampling of lichens for heavy metals was carried out over two seasons, the first at the end of the wet season (spring) in November 2010 and the second at the end of the dry season (autumn) in May 2011. In Collie, heavy metal emissions have not been the focus of any previous studies and metals such as As, Cd, Cr, Cu, Pb, Hg, Ni and Zn should be present since these metals are commonly emitted during coal power generation (Sabbioni, Goetz, & Bignoli, 1984). The fruticose lichen, *Usnea inermis* Motyka was selected as the most suitable lichen species to be used as a biomonitor. This lichen species was chosen because of its availability at all study sites. It was also found during Stage 1 of this study (Chapter 2) that *Usnea inermis* is relatively pollution-tolerant, making it a suitable species for the biomonitoring of heavy metals, since it occurs across a range of sites with different levels of pollution.

### 4.2.2 Study sites

The same thirty-six sampling sites used for the lichen community study (Chapter 2) were used for this study (Figure 4.1) and details of the study-site selection criteria are given in Chapter 2.2. However, since the high, medium and low exposure categories did not show clear patterns of pollution effects on lichen communities, the sites were not grouped using an *a priori* categorization but were rather analysed using a gradient approach (n=36, Figure 4.1).

### 4.2.3 Sample collection

Lichen thalli of similar size were carefully removed by hand from old tree stumps or fallen logs, and placed into paper bags. Gloves were used at all times during lichen collection to avoid contamination. Three replicates were collected from each study site, with each replicate containing approximately 2 g of lichen material, collected from three different stumps/logs within a 500 m radius. Where enough material could not be collected from one stump/log, a pooled sample from several substrates close to each other was considered as one sample replicate. Samples were sealed and brought to the laboratory and stored in a dry place until analysis.

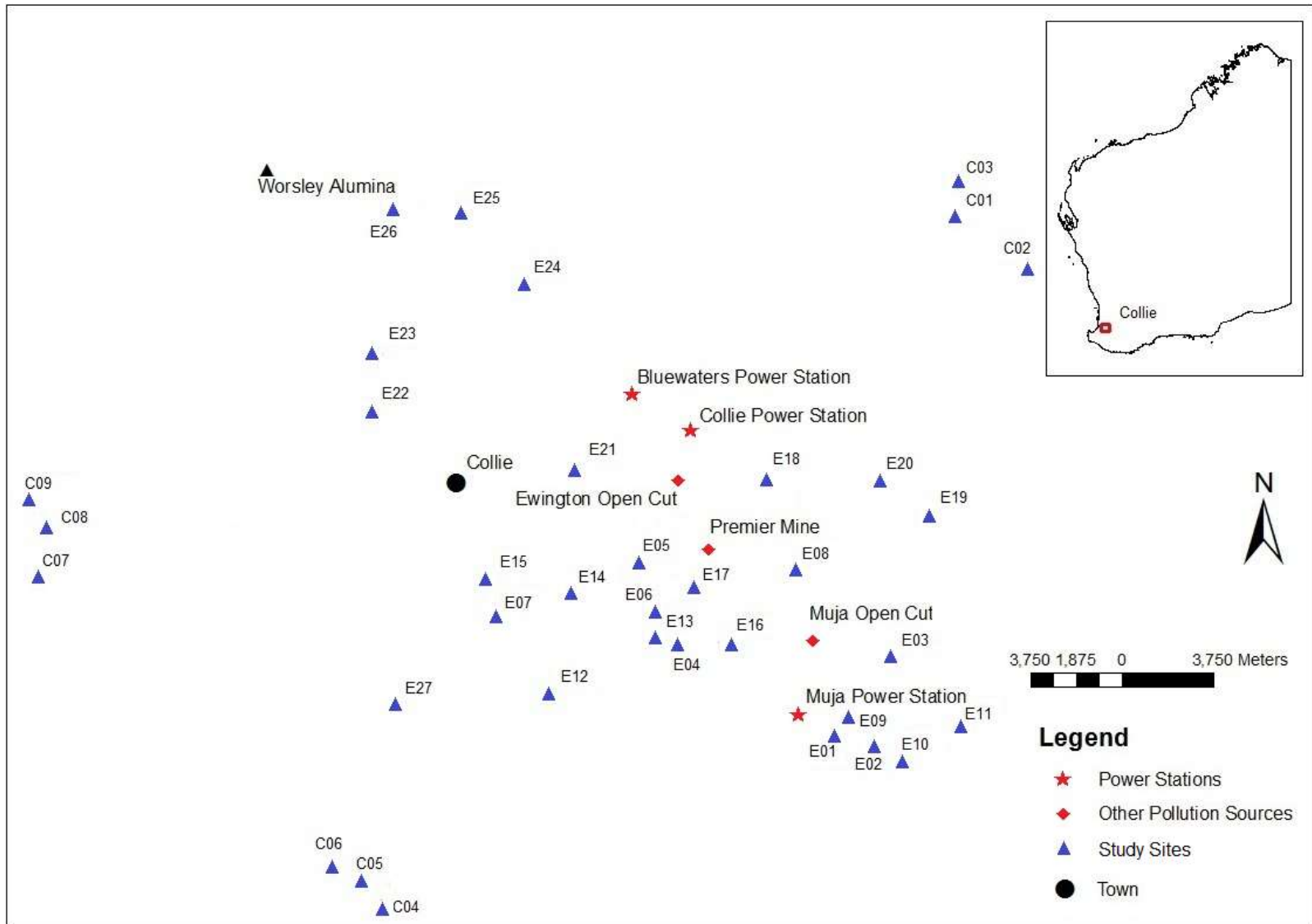


Figure 4.1: Locations of study sites for lichen *in situ* heavy metal biomonitoring in Collie

#### 4.2.4 Sample preparation

Before chemical analysis, all samples were cleaned of extraneous material by using nylon tweezers and brushing with a soft brush. Sample washing was not carried out to prevent the possibility of the leaching of heavy metals (Majumdar et al., 2009). After cleaning, the samples were oven-dried at 40 °C to a constant weight. Dried samples were ground to powder with liquid nitrogen until homogenous (Garty, Tomer, Levin, & Lehr, 2003), using a porcelain mortar and pestle. After grinding, the samples were redried overnight at 40 °C.

#### 4.2.5 Sample digestion

From each powdered sample, 0.500 g was weighed out to a beaker and digested with an oxo-acid mixture of 1:1 HNO<sub>3</sub> and 30% H<sub>2</sub>O<sub>2</sub> at a 3:1 ratio, inside a fume hood. All chemicals used for the digestion were of high purity and quality and were of trace-metal grade. The method was modified from the wet-ashing method described by Narin *et al.* (2004). A 6 mL volume of HNO<sub>3</sub> was added to each sample and heated to 130 °C for 3 hours. This was followed by the addition of 2 mL H<sub>2</sub>O<sub>2</sub>, with the samples heated to 130 °C for 1 hour. After digestion, the samples were filtered using Whatman No. 4 filter paper to remove any residues and the final volume was made up to 50 mL with high purity deionised Milli-Q water (Millipore Corporation). Blanks, spiked blanks and spiked samples were digested along with the study samples, to determine the accuracy and precision of the digestion and analytical methods. A 10 mL aliquot of each sample was sealed in sterile plastic tubes and sent for chemical analysis to the National Measurement Institute, Sydney.

#### 4.2.6 Chemical analysis and quality assurance

An Inductively Coupled Plasma Atomic Mass Spectroscopy (ICP-MS) technique was used to determine the concentrations of heavy metals. This technique has detection limits as low as 0.5 µg L<sup>-1</sup>, which ensured results with high sensitivity for the current study. All samples were analysed by ICP-MS (Perkin Elmer, Elan DRC II) at the National Measurement Institute in Sydney. Instrument specifications and operational conditions of the ICP-MS are given in Appendix 4.1. In order to confirm analytical accuracy, sample blanks, elemental spikes and sample spikes were included in each batch of analysis. The elemental spikes were prepared by spiking a known metal



concentration solution into three randomly selected lichen samples and three blank samples, prior to digestion. The metal concentrations were measured and percentage recovery was calculated to assess the accuracy of the analytical technique. The recoveries for elemental spikes were between 75 to 108% for most metals (except mercury and manganese), in both sampling seasons (Appendix 4.2).

Further quality assurance (QA) tests were carried out by the National Measurement Institute (NMI) and the QA reports for all sample batches analysed at NMI are averaged and presented in Table 4.1. NMI's acceptable recovery range was 75-120% and all analytes fell within this range in the quality assurance report. Duplicate analysis gave very good relative percent difference (RPD) values (Table 4.1), which show the precision of duplicates to be within the acceptable range (See Appendix 4.3 for detailed report).

Table 4.1: Instrument reporting limits and quality assurance data for heavy metals analysed in lichen samples using the ICP-MS technique (National Measurement Institute)

Analyte	Reporting Limit ( $\mu\text{gL}^{-1}$ )	Blank ( $\mu\text{gL}^{-1}$ )	RPD*	% Recoveries	
				Lab Control	Matrix Spike
Arsenic	0.5	<0.5	3.0	99.7	101.0
Cadmium	0.5	<0.5	1.0	94.3	95.0
Chromium	0.5	<0.5	2.3	92.7	102.0
Copper	0.5	<0.5	1.7	105.7	98.3
Lead	0.5	<0.5	2.7	94.0	100.7
Manganese	0.5	<0.5	1.0	97.7	100.3
Mercury	0.5	<0.5	9.3	93.0	92.0
Nickel	0.5	<0.5	1.3	97.3	97.7
Zinc	0.5	0.86	0.0	98.7	102.3

\*Relative percent difference for duplicate samples

#### 4.2.7 Data analysis

A large number of samples had values below the detection limit for Cd and Hg in both seasons (Table 4.2; Appendix 4.4 & 4.5). These non-detect values were replaced with half of the analytical detection limit for data analysis. All data analyses were performed in IBM SPSS Statistics 19.0 software, while graphical representations were generated using SigmaPlot 12.0. The heavy metal concentrations were tested for normality using

the Shapiro–Wilk test (Shapiro & Wilk, 1965). When the heavy metal concentrations were averaged for each site, only the Zn data-set for both seasons had a normal distribution, all other data-sets had positively skewed distributions. Square root, natural log and inverse transformations of the metals data did not make the data distribution normal, therefore no transformations were performed on the datasets, and geometric mean values were reported. Non-parametric tests were used to identify patterns and significant differences ( $P < 0.05$ ) in metal concentrations between seasons. Non-parametric techniques are generally considered to be less sensitive but more effective than parametric techniques in handling skewed data, since they make fewer assumptions and their applicability is much wider than the corresponding parametric methods (Pallant, 2010). The Friedman test was performed for comparison between the spring and autumn sampling seasons. The influence of distance to the closest pollution source on metal concentrations was visualized using scatter plots and the relationships plotted using non-linear power regression function with a negative exponent value, since this model best described the fallout of metals from the point-sources. The distance of metal fallout from the closest point-source for each metal was calculated as the distance where a 50% decrease in metal concentration was predicted from the power curve. Spatial maps for metals were produced in Surfer 8.0 (Golden Software Inc., 2010) a grid-based contouring and surface-modelling package for Windows. The gridding method used was *kriging*, which interpolates the spaced data points to generate contour maps showing the concentrations gradients of each metal (Golden Software Inc., 2010). Data were averaged from the concentrations of the three replicates analysed for each study site for generating the spatial maps.

#### 4.2.8 Meteorological parameters

The meteorological parameters for the area were obtained from the Bureau of Meteorology website for the weather station in Collie, which is situated close to the Collie town (Bureau of Meteorology, 2011). Daily weather data for temperature, rainfall, highest wind speed and direction, 9 am and 3 pm wind speed and direction and the number of calm days were obtained. These parameters were considered useful in explaining the dispersion of heavy metals across the region (Žibret & Šajin, 2008).

## 4.3 Results

### 4.3.1 Heavy metal concentrations in lichen tissue

The geometric mean concentrations of the nine heavy-metals measured in lichen tissue were generally low in this study (Table 4.2). Manganese and Zn had the highest concentrations, with mean values of  $19.8 \mu\text{gg}^{-1}$  and  $12.5 \mu\text{gg}^{-1}$  respectively (Table 4.2). Cadmium and Hg were the lowest in concentration, with many samples reporting concentrations below the analytical detection limit (Table 4.2; Appendix 4.4 & 4.5).

Although the geometric mean concentrations were low for the heavy metals reported in this study, elevated concentrations were recorded from several sites for selected metals, with maximum concentrations exceeding the values recorded for similar lichen biomonitoring studies from other parts of the world (Appendix 4.7). The metals As, Cr, Pb, and Mn were elevated at some study sites, suggesting a potential pollution signal at these locations. The spring As concentrations were elevated at eight sites, with concentrations between  $4.6 \mu\text{gg}^{-1}$  –  $11.0 \mu\text{gg}^{-1}$  (Appendix 4.4). Sites E04, E05, E12, E13 and E21 were located close to the Ewington open-cut coal mine and sites E11, E19 and E20 were near the Muja open-cut coal mine (Figure 4.1), indicating that the elevated As could be as a result of emissions from coal mines. Elevated Cr concentrations were recorded from sites E22 and E26 in the autumn season ( $2.8 \mu\text{gg}^{-1}$  and  $2.6 \mu\text{gg}^{-1}$ ), which were approximately four times higher than the average concentration (Appendix 4.5). These sites were located close to the Worsley alumina refinery (Figure 4.1). Elevated Pb ( $11.7 \mu\text{gg}^{-1}$ ) was recorded in the autumn season at site E06 located close to the Premier mine (Figure 4.1; Appendix 4.5). Elevated Mn concentrations were observed from several sites, particularly at sites E26 and E23 which were near the Worsley alumina refinery (Figure 4.1), with concentrations of  $62.2 \mu\text{gg}^{-1}$  and  $325.2 \mu\text{gg}^{-1}$  in spring and  $100.3 \mu\text{gg}^{-1}$  and  $123.0 \mu\text{gg}^{-1}$  in autumn respectively (Appendix 4.4 and 4.5). Sites E03, E08, E09 and E18 also had elevated Mn concentrations in autumn ( $44 \mu\text{gg}^{-1}$  –  $79 \mu\text{gg}^{-1}$ ), which were located close to the mines and at C03 ( $83.3 \mu\text{gg}^{-1}$ ) which was considered as an unpolluted site (Figure 4.1; Appendix 4.5).

Table 4.2: Geometric mean, range and standard deviation values for heavy-metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) in the lichen *Usnea inermis* for the two sampling seasons and the average values for both seasons; n=36; asterisks indicate statistically significant ( $P<0.05$ ) differences between seasons based on Friedman's test statistics

<b>Metal</b>	<b>Average for Both Seasons</b>	<b>SD</b>	<b>Spring Season</b>	<b>SD</b>	<b>Autumn Season</b>	<b>SD</b>
As*	0.24 (<DL - 11.02)	2.35	0.58 (0.08 - 11.02)	3.05	0.10 (<DL - 0.29)	0.12
Cd*	0.04 (<DL - 0.78)	0.10	0.03 (<DL - 0.08)	0.02	0.06 (<DL - 0.78)	0.09
Cr*	0.53 (0.16 - 2.59)	0.46	0.37 (0.16 - 1.29)	0.24	0.76 (0.38 - 2.59)	0.85
Cu*	1.21 (0.63 - 2.28)	0.35	1.09 (0.63 - 2.02)	0.37	1.33 (0.93 - 2.28)	1.36
Pb	0.73 (0.19 - 11.75)	1.36	0.71 (0.31 - 1.79)	0.32	0.75 (0.19 - 11.75)	1.13
Mn	19.97 (6.14 - 325.17)	41.44	16.8 (6.14 - 325.17)	52.35	23.74 (7.99 - 123.01)	31.28
Hg	0.06 (<DL - 0.36)	0.05	0.06 (<DL - 0.1)	0.02	0.05 (<DL - 0.36)	0.07
Ni*	0.36 (0.16 - 1.42)	0.18	0.31 (0.16 - 0.57)	0.11	0.41 (0.27 - 1.42)	0.45
Zn	12.5 (4.93 - 25.77)	4.36	12.16 (4.93 - 25.77)	4.99	12.85 (7.21 - 20.54)	13.34

<DL – values below detection limit;

#### 4.3.2 Seasonal variations in heavy metal concentrations

The heavy metal concentrations between the two sampling seasons showed some significant differences in metals concentrations, with the autumn season having higher geometric mean values than the spring season for all metals except As (Table 4.2). Statistically significant (Friedman's test statistics significant at  $P < 0.05$ ) seasonal differences were recorded for the metals As, Cd, Cr, Cu and Ni (Table 4.2). Mn, Zn and Cu were the highest ranking metals in terms of concentrations across both seasons, while the other metals showed differences in ranking based on season (Table 4.2). Arsenic was ranked as the fifth highest metal concentration in spring and as the seventh highest in autumn (Table 4.2), with elevated concentrations observed at some sites in the spring season (Appendix 4.4). Chromium was not elevated in spring and was the sixth highest ranking metal, while in autumn, Cr was the fourth highest metal concentration, with the maximum value two-fold higher than the spring maximum and having a higher geometric mean value than Pb. Autumn Cd concentrations ranked higher than Hg, with the maximum concentration eight-fold higher than the spring result (Table 4.2).

#### 4.3.3 Meteorological influence on heavy metal fallout patterns

Daily temperature, rainfall and wind data were obtained from the Bureau of Meteorology website for the two seasons, spring of 2010 and autumn of 2011, in which the lichen sampling for heavy metal analysis was carried out. This data assisted in explaining the different metal fallout patterns observed in the two seasons. Temperatures were generally high in autumn than in spring during the study period, and the temperature fluctuations were also high in autumn, particularly in March (Figure 4.2). In Collie, autumn is generally considered as a drier season than spring; however, in the current study, the autumn experienced higher rainfall than the spring season (Figure 4.2). The wet weather conditions combined with high temperature fluctuations may have facilitated better accumulation of heavy metals in lichens, explaining the elevated concentrations in autumn.

Long-term records show the spring season in Collie to have the strongest winds, with dominant north-westerly winds, while in autumn wind speeds are low and show no dominant direction (Bureau of Meteorology; 2011). However, during the two sampling seasons for the current study, the wind speed and direction were found to be similar across the two seasons, with no dominant direction recorded for the maximum wind speeds, although some dominance from the north-west was observed in spring for the 3

pm wind patterns (Figure 4.3). Autumn experienced more calm days than spring, particularly in the mornings (9 am wind patterns), indicating that winds were not consistent in autumn (Figure 4.3). The more consistent winds in spring could explain the lower concentrations when compared with autumn, where pollutants were possibly dispersing further away from the point-sources.

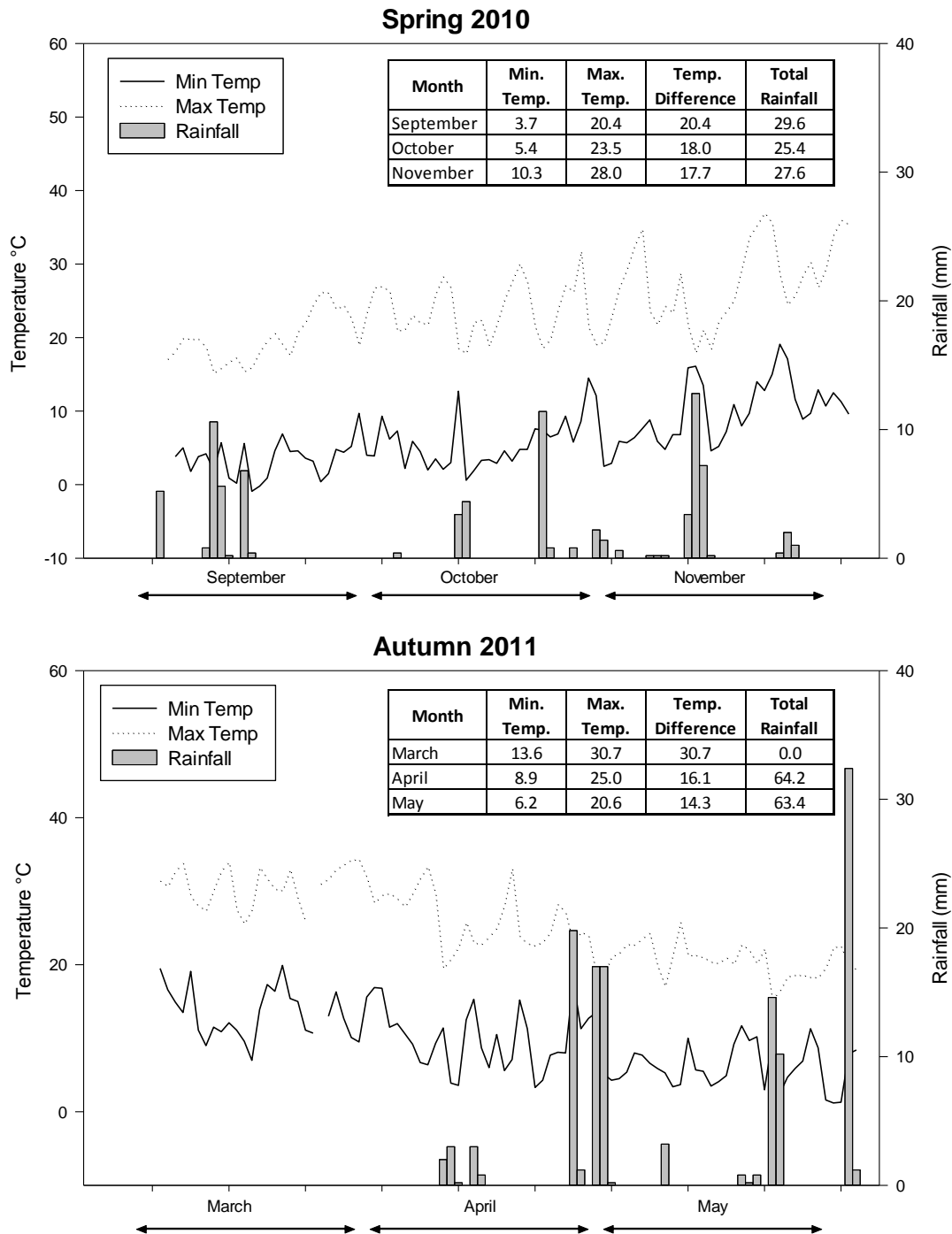


Figure 4.2: Daily minimum and maximum temperature and rainfall data for Collie leading up to the two sampling seasons for the lichen biomonitoring study (monthly averages are given in the table)

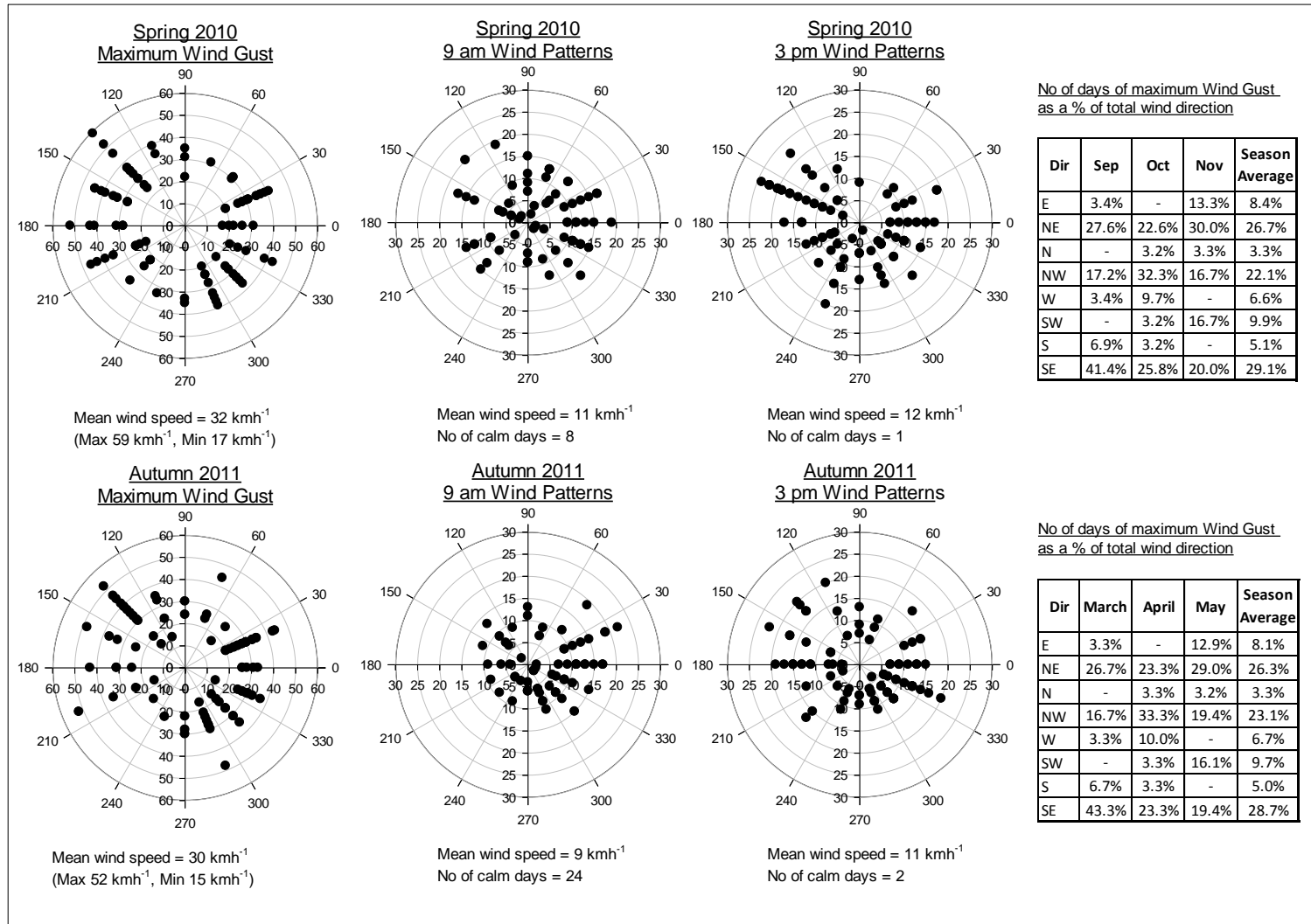


Figure 4.3: Wind data for Collie leading up to the two sampling seasons for the lichen biomonitoring study showing the maximum wind direction/speed and the wind direction/speed at 9 am and 3 pm

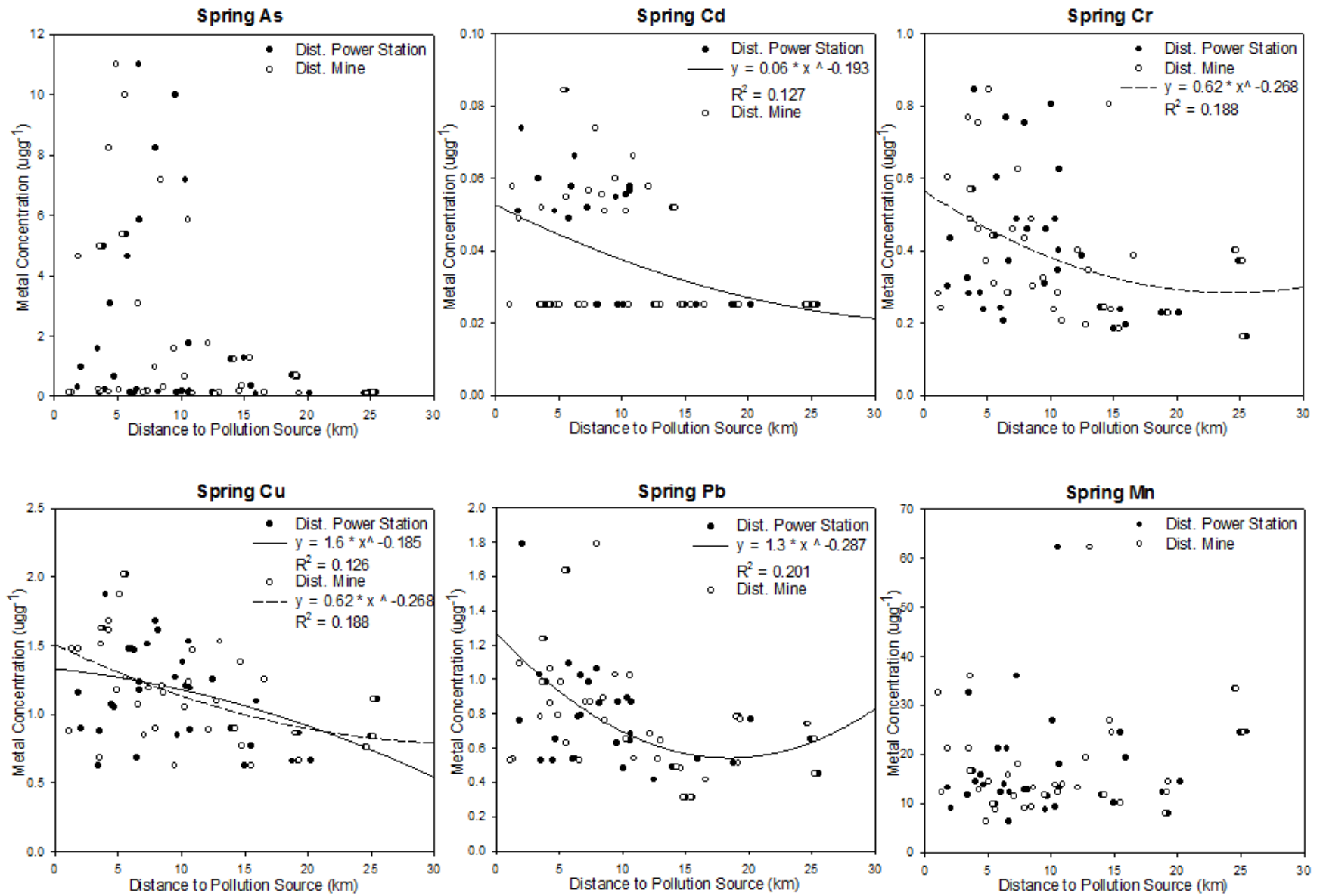
#### 4.3.4 Influence of distance to the closest point-source

The influence of distance to the closest point-source on lichen metal concentrations was assessed using scatter plots and regression power curves. These showed that Cd, Cr, Cu, Pb, Ni, Zn concentrations all decreased with increasing distance from source for either one or both seasons (Figures 4.4 and 4.5). Exponential decreases in spring metal concentrations associated with increasing distance were evident for Cd, Cu, Pb, Ni and Zn, (Figure 4.4), while only As and Ni showed a decrease in concentrations with increasing distance in the autumn season (Figure 4.5). The power curves very clearly indicate the influence of the industrial sources on the metal concentrations, and this influence is most prominent in the spring sampling season.

When the spring data were analysed based on distance to the closest mine or power station, Cu, Ni and Zn concentrations significantly ( $P < 0.05$ ) decreased when both sources were considered (Figure 4.4). Cadmium and Pb concentrations significantly ( $P < 0.05$ ) decreased with distance to the closest power station, while Cr concentrations significantly ( $P < 0.05$ ) decreased with distance to the closest mine (Figure 4.4). The spring Mn and Hg scatter plots suggest no effect from a point-source, showing no clear trend in concentrations with distance to pollution sources (Figure 4.4). Although the spring As concentrations did not show a statistically significant power curve, the high concentrations were all recorded from  $>10$  km from the closest pollution source (Figure 4.4). Despite higher concentrations recorded for all metals, autumn data showed only a few statistically significant power curves, for As and Ni (Figure 4.5).

The fallout of most metals was found to be relatively close to the point-source, with a 50% decrease in concentrations observed within 5 to 8 km for Cr, Pb, Ni, and Zn in the spring season (Figure 4.4), and for As in the autumn season (Figure 4.5). The spring Cu and autumn Ni concentrations were reduced by 50% approximately 12 km away from the point-source (Figures 4.4 and 4.5).





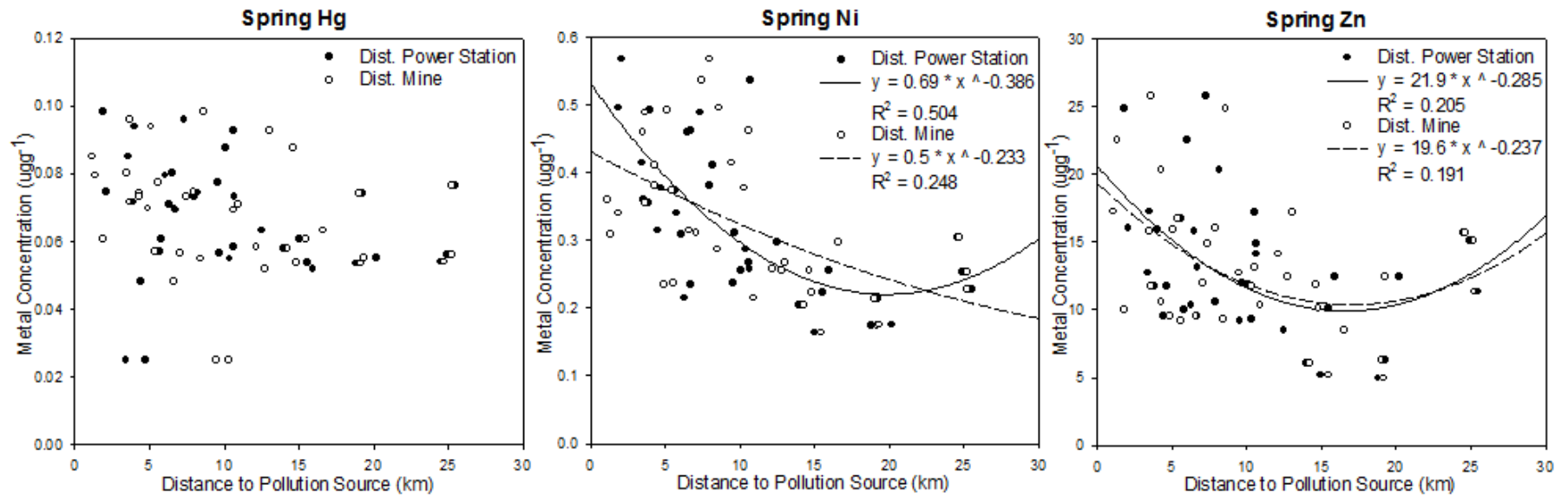
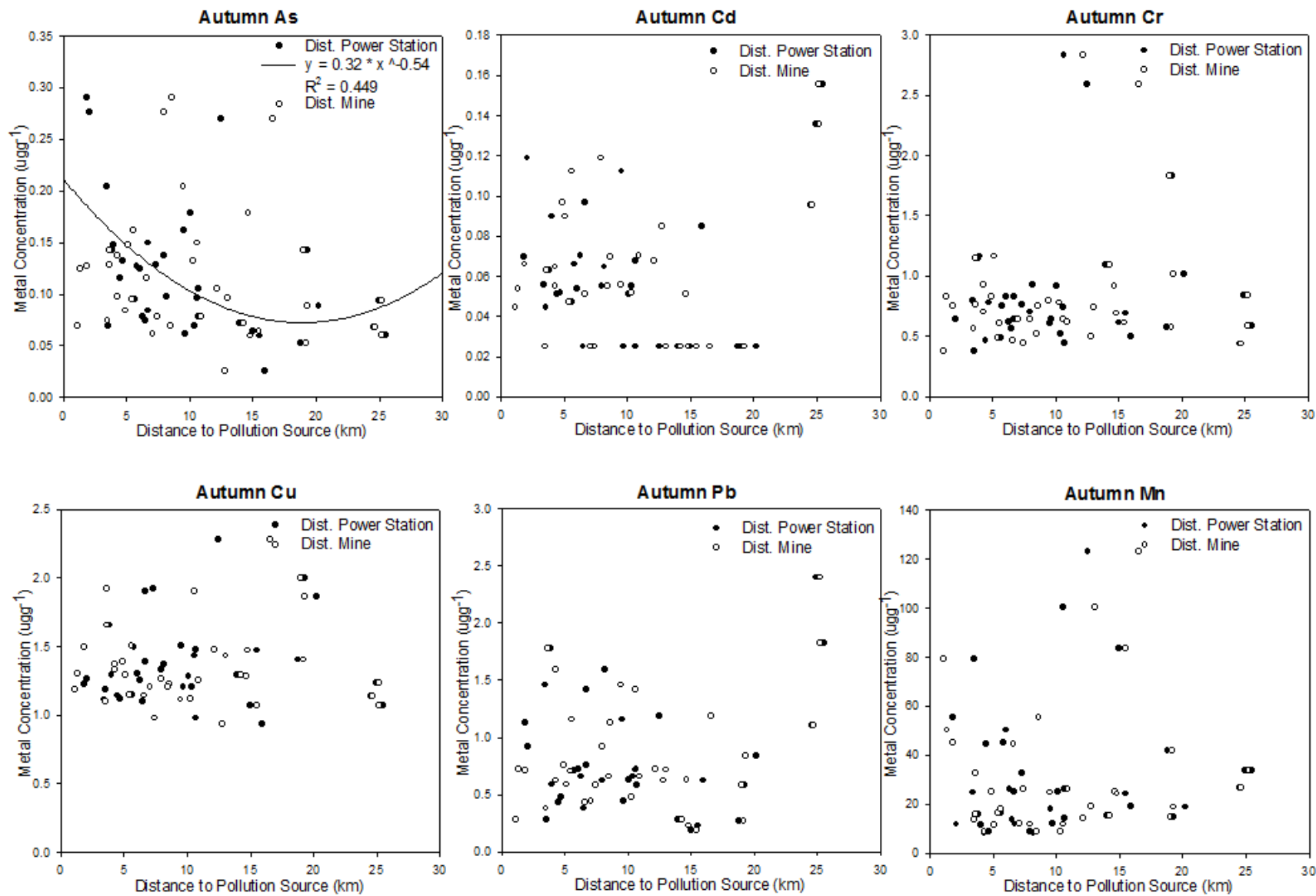


Figure 4.4: Relationship between distance (km) to closest power station (closed circles) and distance (km) to closest mine (open circles) and metal concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$ ) for the spring sampling season showing statistically significant power regression curves



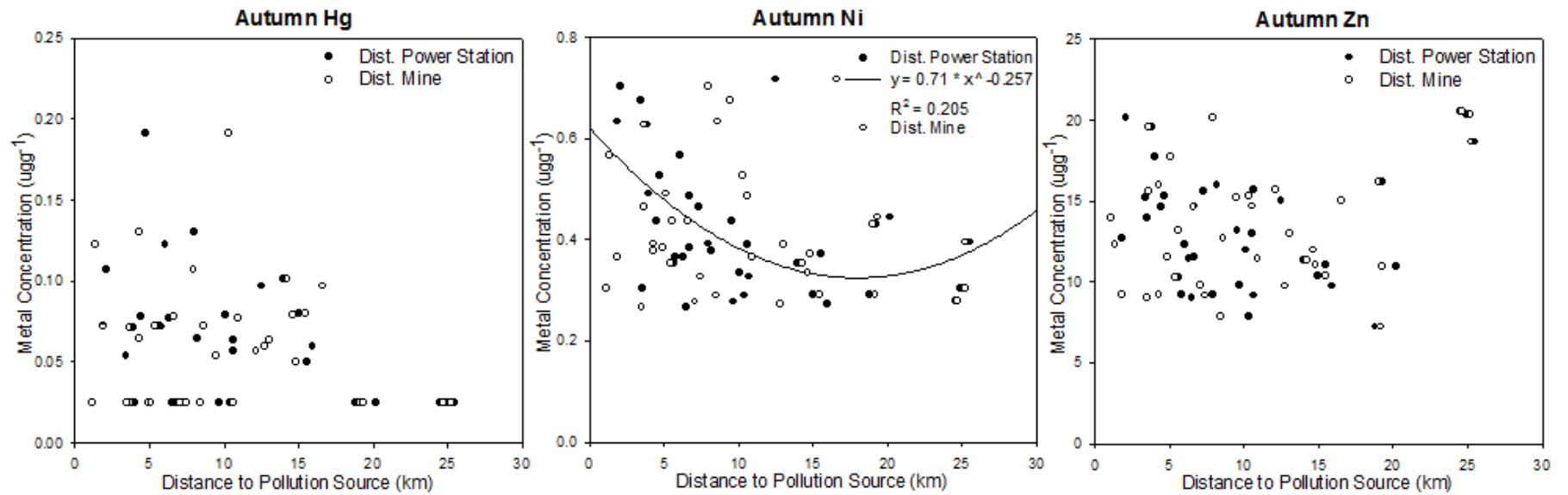
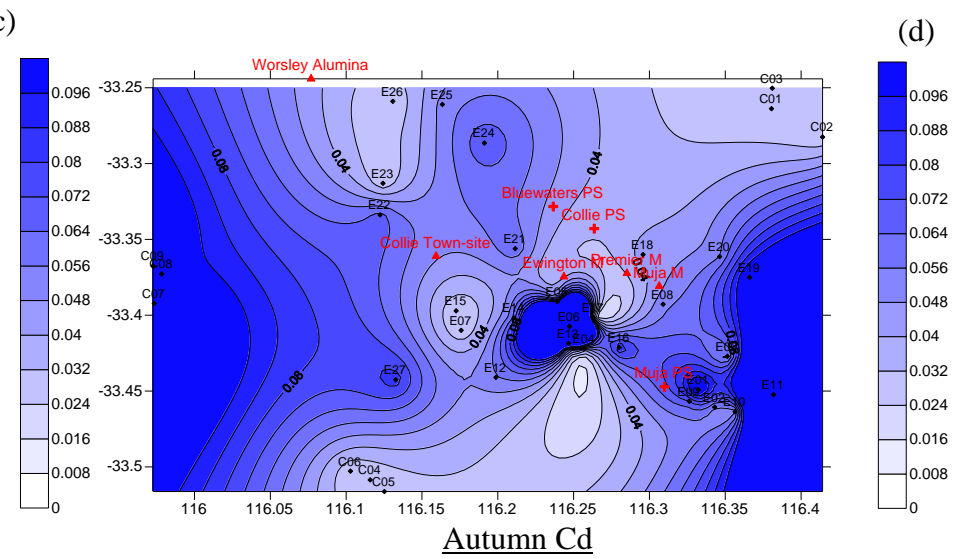
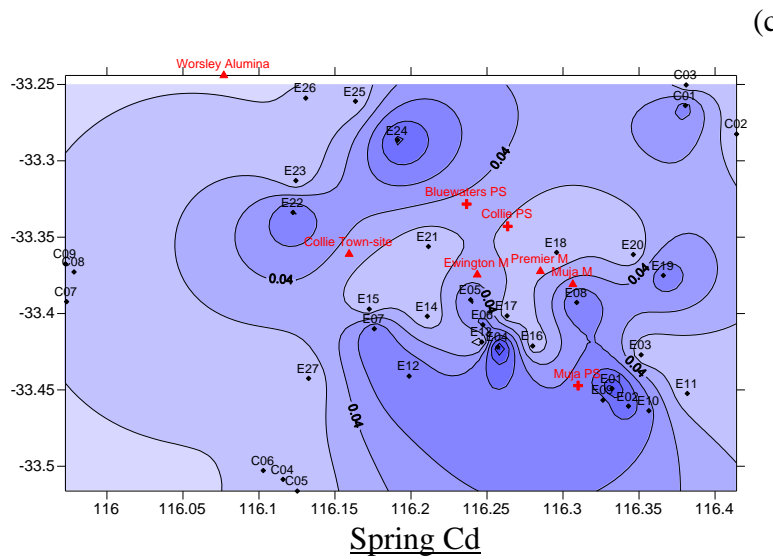
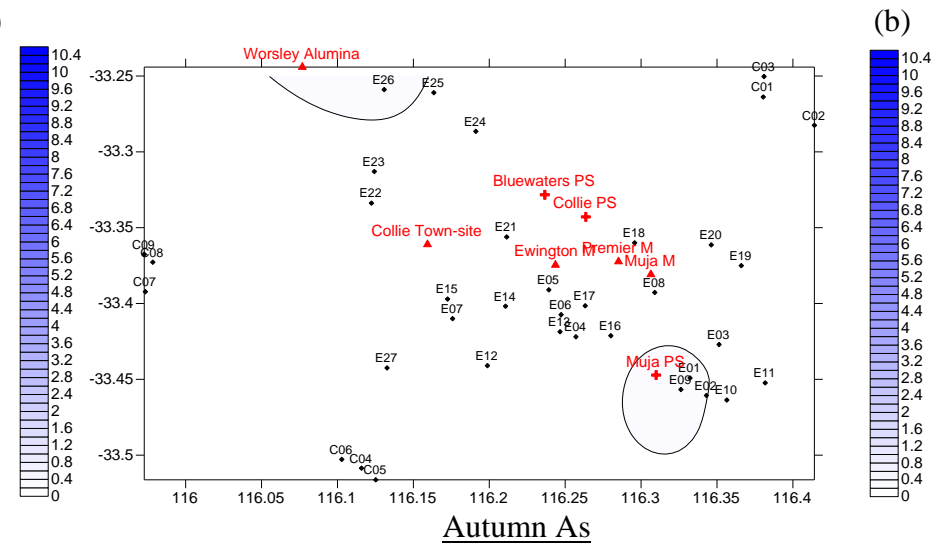
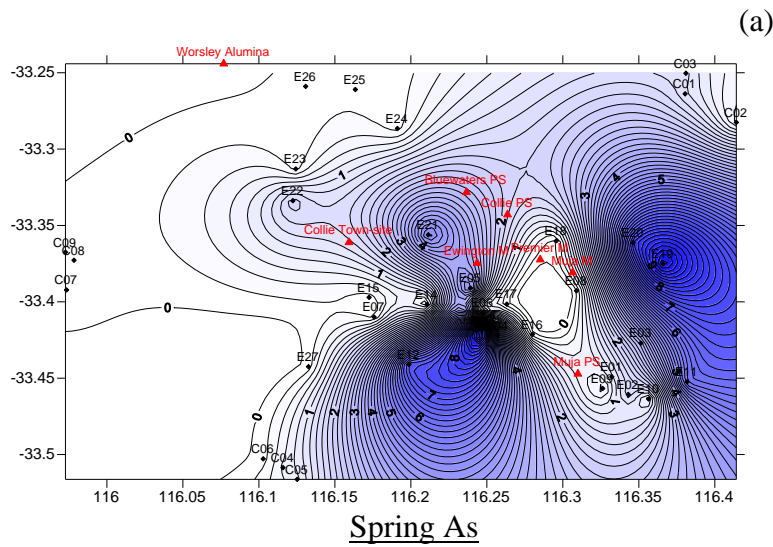


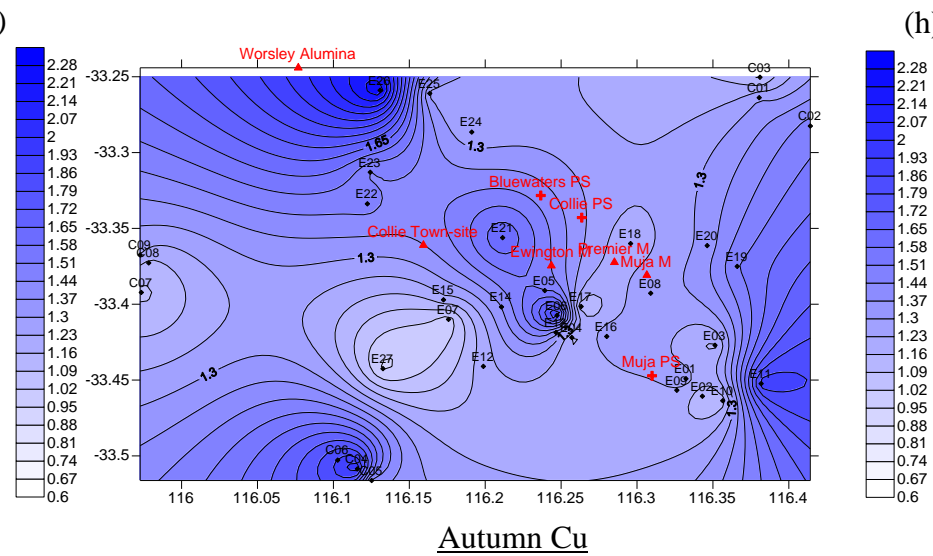
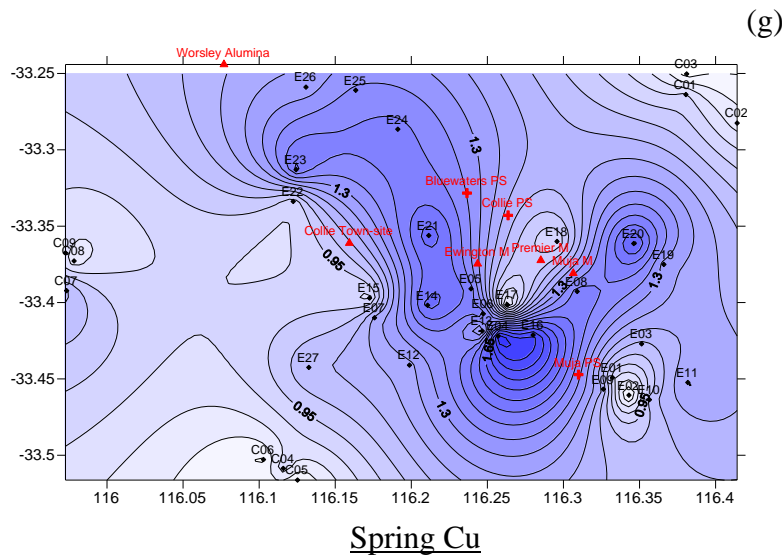
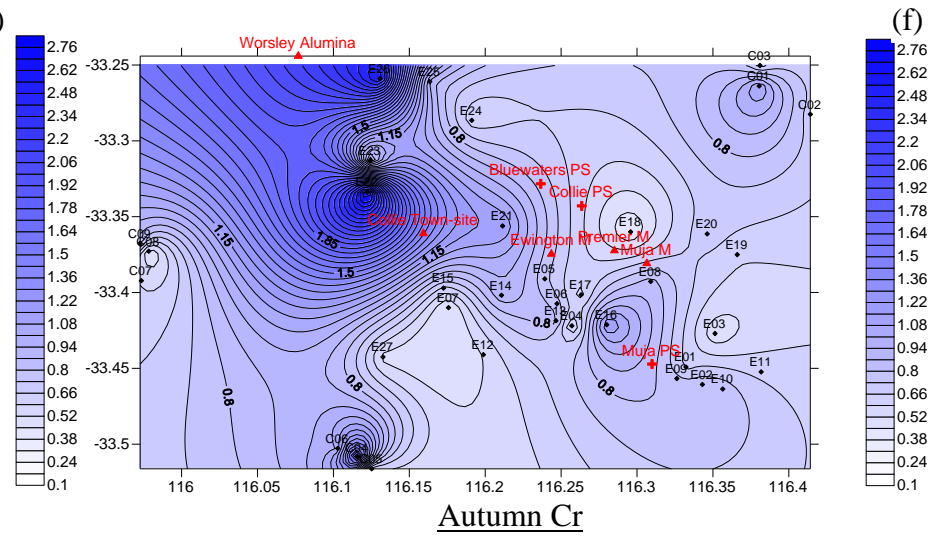
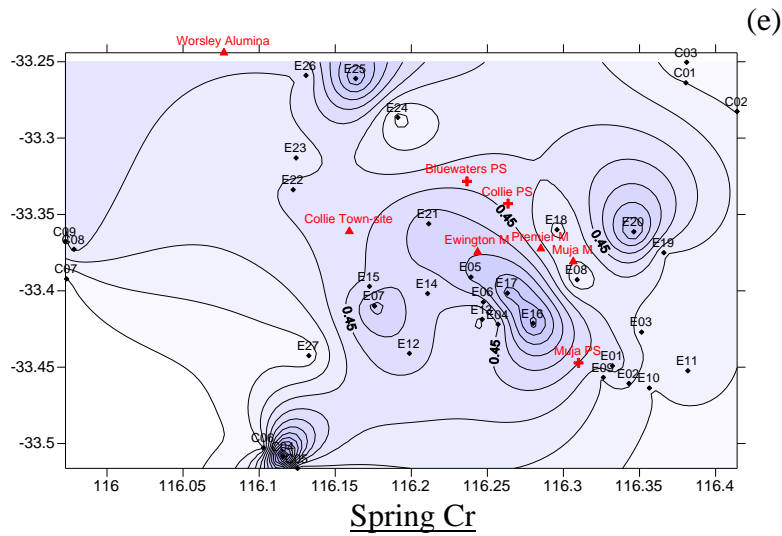
Figure 4.5: Relationship between distance (km) to closest power station (closed circles) and distance (km) to closest mine (open circles) and metal concentrations ( $\mu\text{g g}^{-1}$ ) for the autumn sampling season showing statistically significant power regression curves

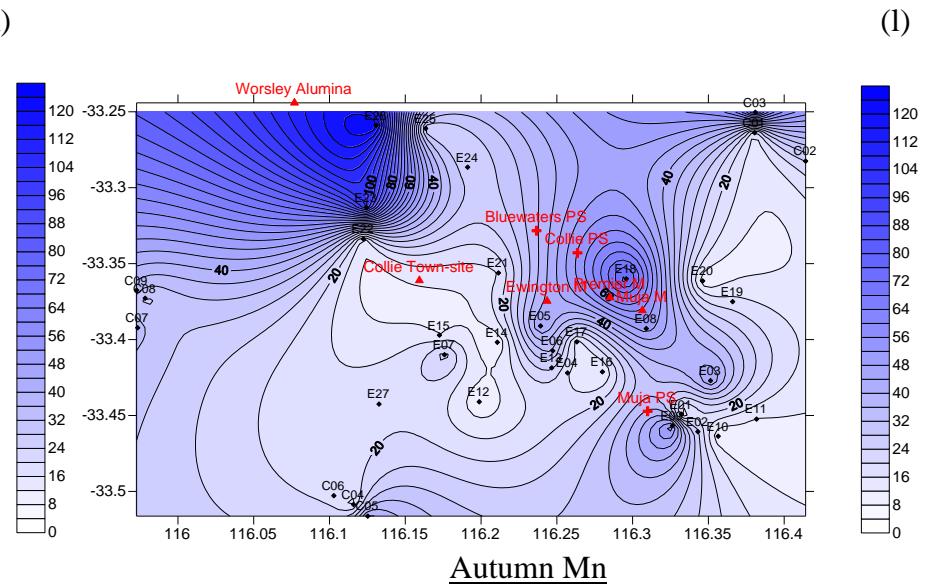
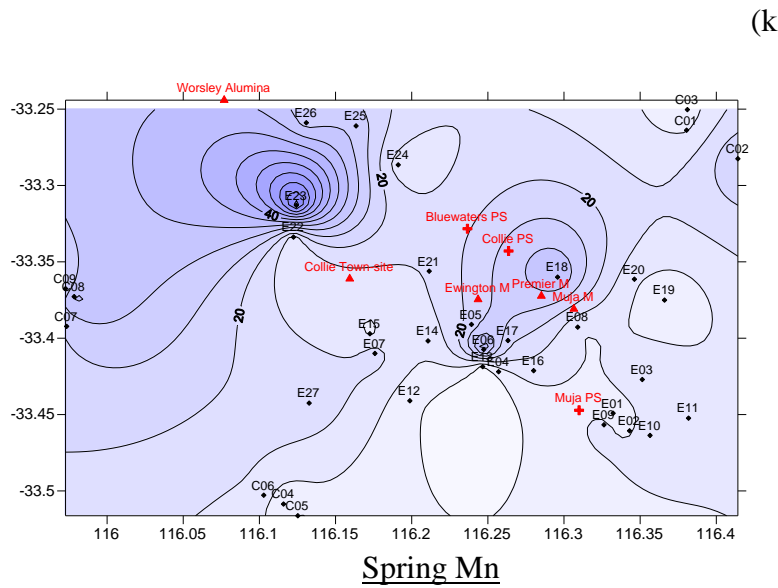
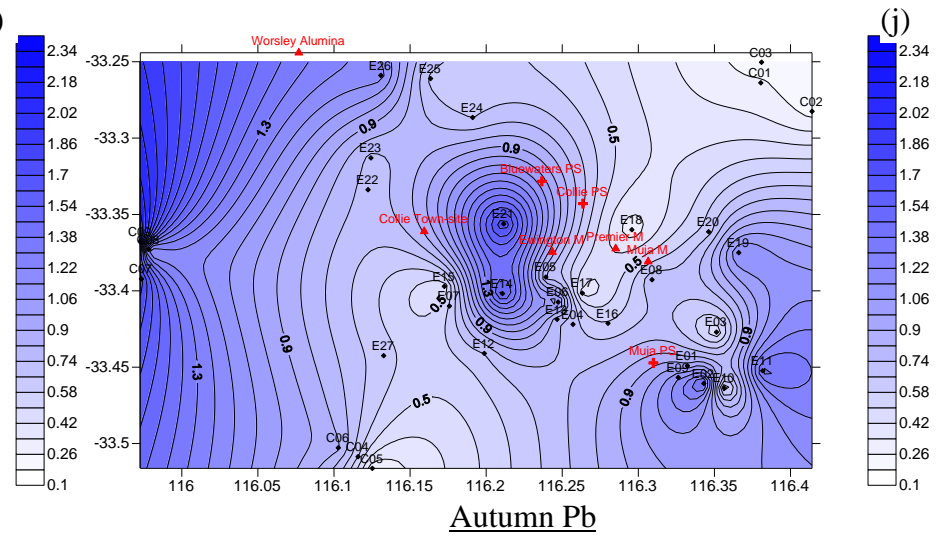
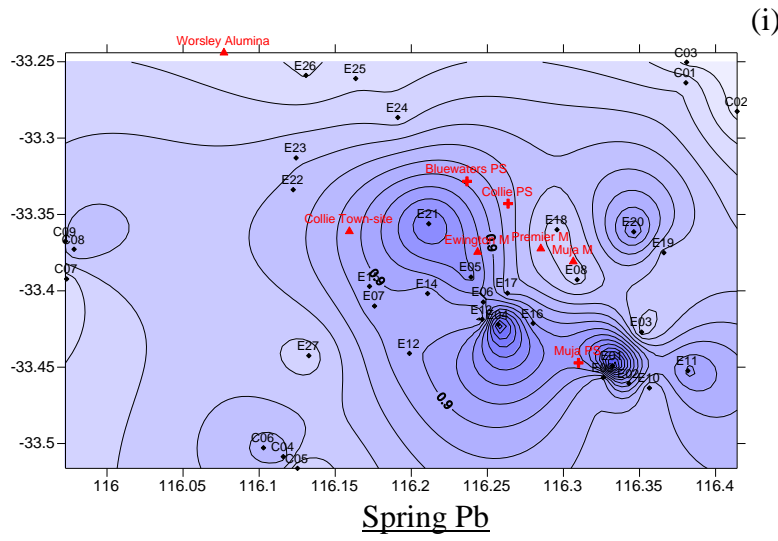
#### 4.3.5 Spatial trends in heavy metal dispersion

Spatial dispersion maps of metal concentrations showed a south-easterly dispersion of metals from the key point-source emitters (Figure 4.6). This pattern was prominent for Cr, Cu, Pb, Ni and Zn across both seasons (Figure 4.6). The spatial map for As in spring showed a strong influence from point-sources in the Collie area, with high exposure contours found south and east from the mines and power stations (Figure 4.6a). Autumn As concentrations were much lower than the spring concentrations (Appendix 4.4) and therefore did not show clear dispersion patterns in the spatial map (Figure 4.6b). The contour maps for Cd for both seasons were different from the dispersion patterns for the other metals (Figures 4.6c&d), probably because many sites recorded values below the analytical detection limit for Cd (Appendix 4.4). The autumn Cr map indicated that the elevated Cr concentrations may be influenced by the Worsley alumina refinery operations, or from another unidentified source (Figure 4.6f). The Cu spatial maps showed a low concentration area close to the Muja power station and below the coal mines, for both seasons (Figures 4.6g&h). The Cu and Pb maps both indicated an influence from the alumina refinery, with contour patterns south-east from the refinery showing elevated areas. The autumn Pb map also showed a prominent high exposure area south-west from the refinery.

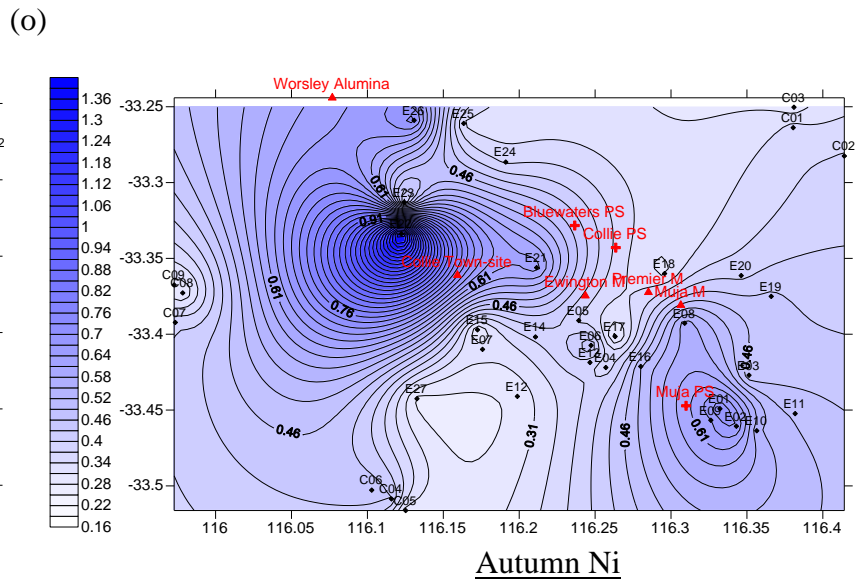
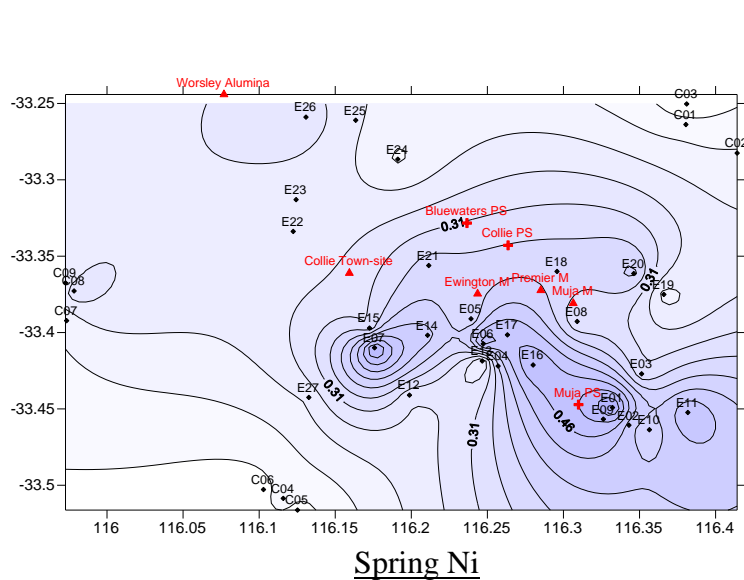
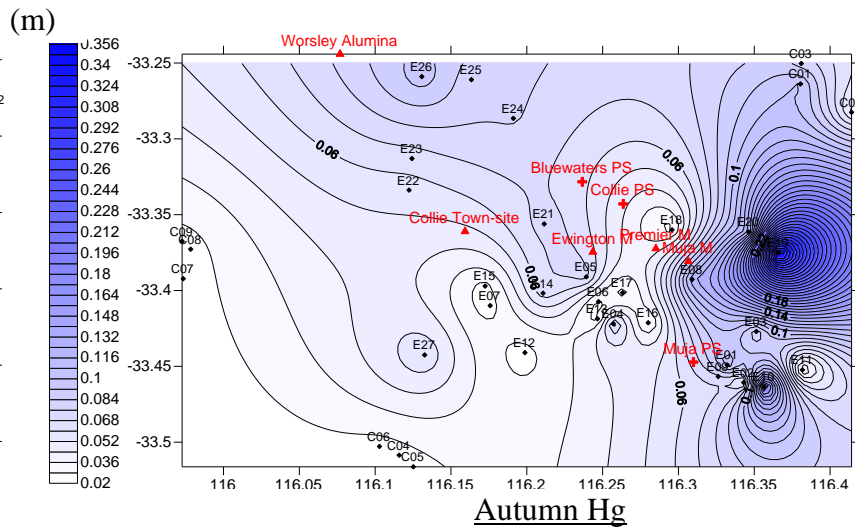
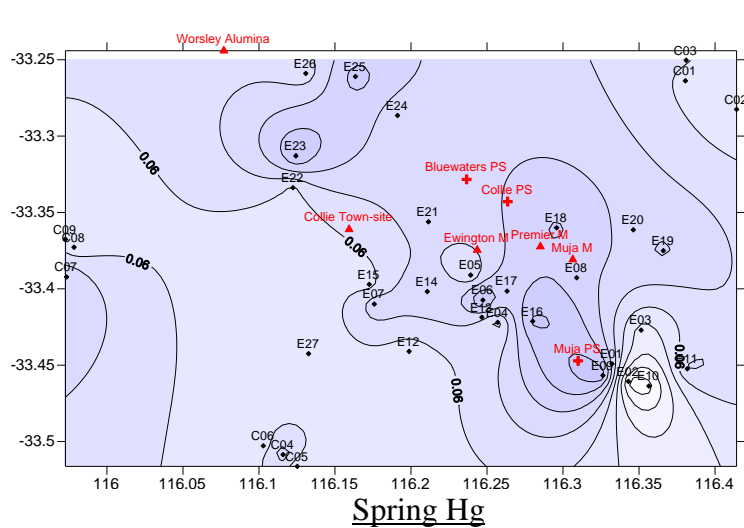
Mn in both seasons recorded high exposure areas in the south-east direction from the Worsley alumina refinery, suggesting that this metal might be associated with the refinery operations. The Hg maps showed elevated concentrations south-east and east from the coal mines and power stations in spring and autumn respectively (Figures 4.6m&n). The contour map for Ni in spring showed two prominent contour peaks, one south-east from the Collie town and one south-east from the coal mines (Figure 4.6o). The autumn Ni map, on the other hand, showed a high exposure contour south-west from the Worsley alumina refinery, very close to the Collie town (Figure 4.6p). The Zn contour maps for both seasons showed elevated metal contours south-east from the mines and power stations, as well as south-west from the Worsley refinery (Figures 4.6q&r), indicating them as sources of exposure.











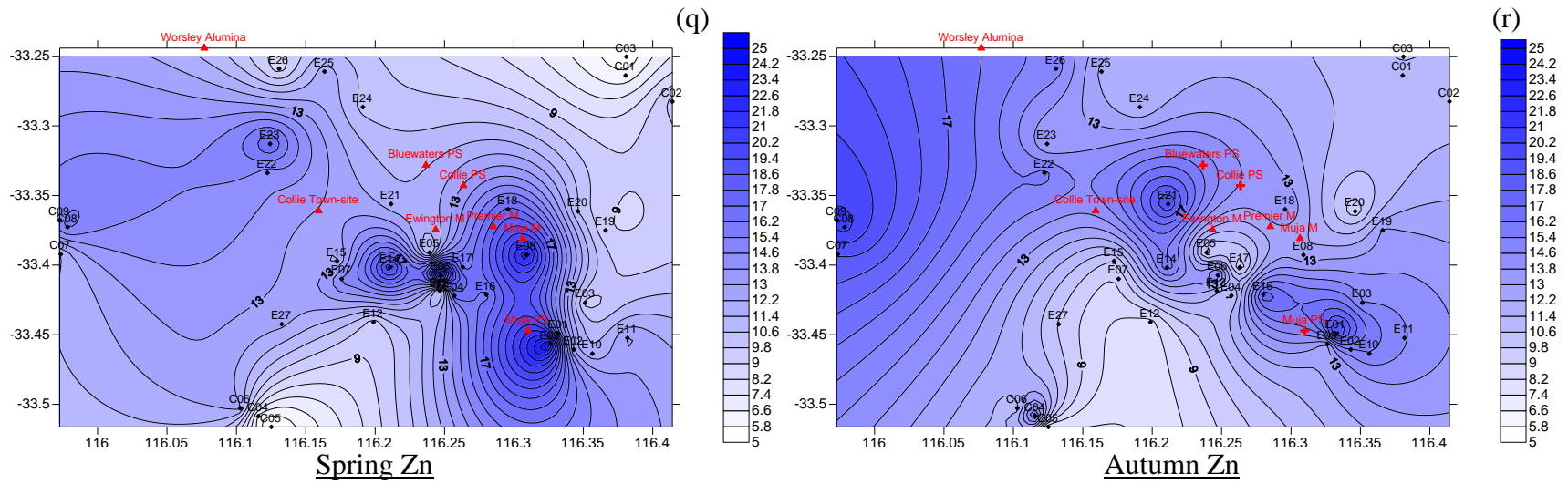


Figure 4.6: Spatial variation patterns of heavy metal concentrations in the Collie air-shed for the two sampling seasons, highlighting the influence of point-source emitters

## 4.4 Discussion

The results of this study showed that, while for most metals the concentrations found in lichen tissue were low, elevated concentrations were observed at locations possibly influenced by known industrial point-sources. The study also showed differences in heavy metal dispersion associated with the two sampling seasons, indicating seasonal variations in the dispersion of heavy metals in the Collie air-shed.

### 4.4.1 Heavy metal concentrations in lichen tissue

The most abundant metals recorded in this study were Mn and Zn, with mean concentrations falling within the concentration range generally found in plants (Hansch & Mendel, 2009). Both Mn and Zn are essential micronutrients for plants (Broadley, White, Hammond, Zelko, & Lux, 2007; Millaleo, Reyes-Diaz, Ivanov, Mora, & Alberdi, 2010) and are found in most biological materials. Manganese concentrations in the current study were similar to those reported near a coal-fired power station in Israel (Garty et al., 2003) but lower than concentrations reported in other international lichen biomonitoring studies in areas associated with industrial activities (Appendix 4.7). The Cu and Pb concentrations in the current study were also similar to the results obtained for other studies which had limited industrial activity (Conti et al., 2009; Riget, Asmund, & Aastrup, 2000), but were lower than the concentrations reported in lichens from areas polluted by coal-fired power stations in Israel and Turkey (Garty et al., 2003; Gur & Yaprak, 2011). Mean As and Ni concentrations were also found to be low compared with other biomonitoring studies (Appendix 4.7), with mean values approximately 50 times lower than those reported near a coal-fired power station in Turkey (Gur & Yaprak, 2011). Extremely low concentrations of Cd and Hg were found in *Usnea inermis* tissue in the current study, when compared with lichen biomonitoring studies from other Mediterranean environments (Bennett & Wright, 2004; Conti et al., 2009), and areas associated with coal power generation (Freitas, Reis, Alves, & Wolterbeek, 1999).

The low heavy metal concentrations found in this study, suggest that heavy metal emissions are low in Collie or that the metals are being transported out of the area as a consequence of local weather conditions. There could be several other reasons for the comparatively low concentrations of heavy metals in the current study. Collie coal is reported to have a high volatile content and low ash (Premier Coal, 2011) and these

unique features in Collie coal could account for the low release of heavy metal emissions to the surrounding atmosphere. The Bluewaters power station which produces 50% of the coal-fired energy in Collie uses precipitator and filter bag technology for the removal of flue dust which ensures very low emissions of heavy metals to the atmosphere (Griffin Energy Pty Ltd, 2004, 2005). This might also explain the low heavy metal concentrations observed for lichens in the current study, although the coal power production capacity was high compared with other locations that reported elevated heavy metals in lichens (Bajpai, Upretia, Nayakaa, & Kumarib, 2010; Fernandez-Salgui, Terron, Barreno, & Nimis, 2007; Freitas, 1994; Gur & Yaprak, 2011).

The open canopy structure of jarrah forests in Collie could also be a reason for the lower heavy metal concentrations observed in lichen tissue, where the majority of the metal fallout is trapped by the trees and then washed down the trunks. Forest canopies are known to be important sources of atmospheric wet and dry deposition (Levia et al., 2011) and are a source of deposition onto epiphytic lichens growing on live trees. Since this study sampled lichens on fallen logs and old tree stumps which were mostly in the open, the contribution of the canopy leachate for the heavy metals in lichen tissue would be much lower than other studies that used live trees as substrates for lichen collection.

However, some elevated metal concentrations were found at some locations for As, Cr, Pb and Mn (Table 4.2), with maximum values exceeding concentrations reported in other lichen biomonitoring studies (Appendix 4.7). Elevated concentrations of As in spring at selected study sites exceeded the values reported in other lichen biomonitoring studies by 3 - 20 fold (Allen-Gil et al., 2003; Aznar, Richer-Lafleche, & Cluis, 2008; Bargagli, Monaci, Borghini, Bravi, & Agnorelli, 2002; Freitas, 1994). Coal may contain As in elevated concentrations, where it can be mobilized in coal fly-ash (Pandey, Singh, Singh, Singh, & Yunus, 2011; Li, An, Zhou, Liu, & Waalkes, 2006; Yudovich & Ketris, 2005). Bituminous coal generally has average As content of  $9 \mu\text{gg}^{-1}$ , with  $50 \mu\text{gg}^{-1}$  in fly-ash (Yudovich & Ketris, 2005). Therefore, the sub-bituminous coal in Collie (GHD, 2009; Perdaman Industries, 2009) may also have elevated As content that can be mobilized in the Collie air-shed through the coal mining and power generation processes (EPA, 2003). However, the National Pollution Inventory (NPI) reported As amongst the lowest emitted metals in Collie (Appendix 4.8 & 4.9). It is apparent from

the NPI data that elevated As concentrations in the range reported in this study have not been accounted for in any monitoring or modelling carried out in the Collie area.

The sites with elevated Mn concentrations were found to be located close to the Worsley alumina refinery and these data were supported by the dispersion maps (Figure 4.6 k&l), with concentrations sixteen-fold higher near the refinery than the average concentrations for the two sampling seasons. Metal processing has been reported to emit dust associated with trace metals into the atmosphere (Birch & Scollen, 2003) and Mn is an important component of these local dust emissions. The Worsley alumina refinery also operates two on-site coal-fired power stations supplied by the coal mines in the area (EPA, 2005b). Collie coal is reported to contain Mn in moderate concentrations (Sappal, 1995) which can be mobilized in coal fly-ash in the oxide form (Ngu, Wu, & Zhang, 2007). However, it is most likely that the elevated Mn concentrations associated with the refinery could be a combination of Mn from both the power stations and the dust from the alumina processing dumps at Worsley. The methods used in this study were unable to determine whether the Mn found in lichen tissue was directly associated with emissions from the alumina refinery or is from dust associated with bauxite processing.

Some sites in the current study had Cr and Pb concentrations significantly higher than ambient concentrations reported for *Usnea* spp. in the United States (United States Forest Service, 2012). The spatial dispersion maps also showed high concentrations near the coal mines for Pb, suggesting the coal mines were a likely source of Pb in the lichens. Elevated Cr on the other hand was likely to be associated with the Worsley refinery, where the spatial maps for both seasons showed clear dispersion of metals downwind from the refinery (Figure 4.6e&f).

#### 4.4.2 Seasonal influence on heavy metal concentrations

The heavy metals for the two sampling seasons recorded similar concentration ranks for most metals (Table 4.2), indicating that the relative abundances of these heavy metals remained consistent across the seasons. However, spring showed lower concentrations than autumn for all metals, and these results might be a consequence of the atmospheric conditions, since wind, temperature and rainfall are known to play a vital role in the dispersion of pollutant plumes from industrial point-sources (Žibret & Šajin, 2008). During the current study, the wind speed and direction were found to be similar for both

sampling seasons, but autumn experienced more calm days than spring (Figure 4.3). Furthermore, the spring of 2010 had very low rainfall compared with the autumn of 2011 (Figure 4.2). The dry weather and more constant winds in spring could have dispersed most of the metal emissions from the coal mines and coal-fired power stations away from the Collie area, resulting in the lower concentrations recorded than those found in the autumn season (Steinnes & Friedland, 2006). The higher rainfall in autumn could also have facilitated higher wet deposition, where pollutants emitted by the point-sources would have been efficiently removed from the atmosphere and deposited onto lichen tissue (Shukla, Misra, Sundar, & Naresh, 2008), resulting in the higher mean concentrations recorded in autumn (Table 4.2).

The only exception to the higher heavy metal concentrations in autumn was As, where spring samples had higher mean concentrations (Table 4.2), with elevated concentrations reported at some sites which were not reflected in the autumn sampling. Laboratory studies looking at As absorption in lichens have demonstrated a dramatic reduction in As concentrations in lichen tissue within a few hours of uptake, because of excretion as arsenite back into the exposure medium (Mrak, Slejkovec, Jeran, Jacimovic, & Kastelec, 2008). A similar phenomenon may have occurred at the sites where elevated As was reported in this study, where the autumn rains may have facilitated the leaching of As from lichen tissue, resulting in lower concentrations in autumn.

#### 4.3.3 Influence of distance to the closest point-source

The fallout patterns of heavy metals showed exponential decreases in concentrations away from the sources, indicating that the heavy metal emissions from the coal-fired power stations and coal mines in Collie were settling out very close to the identified point-sources. Heavy metals were found to disperse to a maximum distance of about 10 - 12 km from the closest pollution source, with very low concentrations observed beyond this distance for most metals (Figures 4.4 and 4.5). Similar exponential decreases in metal concentrations, with distance to an oil-sand mine were observed in a lichen biomonitoring study in Canada (Graney et al., 2012). A study in Slovenia used a power-fraction model similar to the current study to explain the fallout of heavy metals from a zinc smelter (Žibret & Šajn, 2008). Fallout distances comparable to the current study have also been reported in other lichen biomonitoring studies, where higher heavy

metal concentrations closer to point-sources such as metal smelters, coal-fired power stations and mining sites have been reported (Aznar et al., 2008; Bajpai et al., 2010).

The lower concentrations observed in the spring season compared with autumn suggested that, although there is some fallout close to the source (Figure 4.5), most metal emissions may be dispersing further away from the Collie valley in spring, and depositing at far-off distances. The much lower concentrations closer to the source in spring could be due to the fallout from the larger particulate fraction ( $PM_{10}$ ), whereas the finer particulate fraction ( $PM_{2.5}$ ) in which most heavy metals are found would have been dispersed longer distances by the stronger wind conditions in spring (Graney et al., 2012). The main fallout of spring heavy metals further away from point-sources could not be assessed because the current study was carried out at sites only within a 30 km radius from the coal mines and coal-fired power stations in Collie. Long-range transport of pollutants has received global attention because it can potentially impact unsuspected areas and cause long-term detrimental effects (Steinnes & Friedland, 2006). Further investigation at greater distances from point-sources would be required to determine the long-range dispersion of heavy metals from the industries in Collie.

The main autumn fallout, on the other hand, was observed at 15 – 25 km distance range from the closest pollution source (Figure 4.5). Because of the higher concentrations recorded at this distance range compared with the immediate surroundings from the source, the power equation did not fit the scatter plots for most autumn metals in predicting their dispersion in the Collie air-shed (Figure 4.5). There was also the possibility of an unidentified source contributing to the higher concentrations observed at the sites further away from the closest point-source. The spatial dispersion maps for heavy metals in the autumn season identified the Worsley alumina refinery as a possible source contributing to the higher concentrations observed at these sites, where high concentration contours downwind from the refinery was observed for most metals (Figure 4.6). The Worsley alumina refinery underwent expansions in early 2011 (BHP Billiton, 2011), at the time when the sampling was carried out for the autumn season. The expansion operations could have increased the emissions from the refinery to the Collie air-shed, which could have been reflected in the autumn biomonitoring campaign.

#### 4.4.4 Spatial trends in heavy metal dispersion

The spatial dispersion maps for metals showed the areas possibly impacted by heavy metal concentrations in the Collie airshed (Figure 4.6). The maps clearly showed the influence of the coal-fired power stations and coal mines on areas downwind from these point-sources. A second area of possible impact is seen downwind from the Worsley alumina refinery, where the spatial-dispersion maps for some metals in spring (Cr, Cu, Hg, Mn and Zn) and most metals (except Cd and Hg) in autumn showed high concentrations closer to the refinery. This influence was observed as far as the Wellington National Park (locations for sites C06, C07 and C08) for some metals, which had been assumed to be a relatively pristine environment. The influence of the Worsley alumina refinery is seen in the results of this chapter as well as the lichen community composition study (Chapter 2) and the passive sampling of gaseous pollutants (Chapter 3). It is therefore important to investigate the impact on the Wellington National Park in future monitoring regimes for air pollutants in the Collie air-shed, as it is an area of rich biodiversity for the jarrah forest ecosystems of Western Australia.

#### 4.4.5 Limitations and future recommendations

In this study, the changes in heavy metal concentrations in lichens were assessed across two seasons, where distinct differences in metal dispersion were observed. However, there could be differences in the concentrations in the other seasons, because of the differences in wind direction, wind speed, temperature changes, rain patterns and other meteorological conditions associated with these seasons (Mikhailova & Sharunova, 2008). Measuring metal concentrations across all four seasons might have provided a more complete understanding of the influence of seasonal conditions on heavy metal dispersion in the Collie air-shed and their subsequent fallout. The current study also used only one lichen species, *Usnea inermis* to explore the dispersion of heavy metals in the Collie air-shed. Another lichen species such as *Hypogymnia subphysodes*, which was also found to be ubiquitous in the jarrah forests of Collie, could have been used as a second biomonitor to compare and confirm the heavy metal emissions and their dispersion across the Collie airshed. *Hypogymnia* spp. have been used extensively in lichen biomonitoring studies and might have provided better comparisons with the international literature for heavy metal concentrations (Balabanova, Stafilov, Sajn, & Baeeva, 2012; Egger, Schlee, & Turk, 1994; Lackovicová et al., 1994). Comparing



heavy metal concentrations obtained from two lichen species would have also improved our confidence in the effectiveness of using the lichen biomonitoring approach to determine the heavy metal dispersion patterns in the Collie air-shed.

#### **4.5 Conclusions**

This investigation of lichen biomonitoring in Western Australia confirms that lichens can be used as effective tools to map the dispersion of heavy metal pollution associated with industrial emissions. The mean heavy metal concentrations recorded were generally low, indicating limited contribution of heavy metals to the atmosphere from coal-burning in Collie. However, elevated concentrations from certain sites for As and Mn, in particular, indicated that the influence of mining and metal processing point-sources on heavy metal concentrations in the Collie air-shed cannot be ignored and require further investigation. This study has also shown distinct seasonal patterns of heavy metal dispersion in the Collie air-shed.

The heavy metal concentrations reported in this study provide useful baseline data for the monitoring of heavy metal emissions from coal-related industries in Western Australia, where the lichen *Usnea inermis* and possibly other species can be used as an effective tool for continuous monitoring of heavy metals in polluted air-sheds. The measurement of heavy metals in lichens at distances >25 km from the Collie area is needed if there is interest in the long-range dispersion of heavy metals from the industries in Collie.

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## **Chapter 5 - Seasonal variations in the rate of heavy metal accumulation in *Usnea inermis* lichen transplants in Collie, south-western Australia**

### **5.1 Introduction**

The elemental composition of biological systems is known to reflect the chemical composition of their nutrient sources (Fрати, Brunialti, & Loppi, 2005) and this relationship is strong in lichens, whose main nutrient source is the atmosphere (Bari, Rosso, Minciardi, Troiani, & Piervittori, 2001). Absorption levels in lichens are strongly correlated with atmospheric concentrations, making them effective biomonitors (Nash III, 1996). However, lichens may be absent in certain industrial and urban areas, thus making spatial *in situ* biomonitoring difficult (Yildiz, Aksoy, Tug, Islek, & Demirezen, 2008). Biomonitoring techniques have been developed in which lichen thalli are collected from areas with little or no air pollution and transplanted to polluted areas of interest for a fixed exposure period (Garty & Hagemeyer, 1988; Godinho, Freitas, & Wolterbeek, 2004). This approach enables the accumulation rates of pollutants across spatial and temporal scales, including seasonality, to be determined (Bergamaschi, Rizzio, Giaveri, Loppi, & Gallorini, 2007).

The lichen-bag technique is a popular lichen biomonitoring method, in which transplanted lichen thalli are loosely packed in non-metallic mesh bags that allow effective ventilation, which are suspended on trees or other suitable substrates at locations of interest (Goodman & Roberts, 1971, Culicov & Yurukova, 2006; Yildiz et al., 2008). This method allows for defined exposure periods, making it possible to measure seasonal variations in the accumulation rate of pollutants (Bergamaschi et al., 2007). It is important to collect lichen thalli of similar size, as accumulation rates can be age dependent (Armstrong, 1997; Garty, 2001). Exposure time can be a limiting factor with the lichen-bag technique, since prolonged exposure periods in non-native environments can lead to physiological stress, desiccation and leaching of elements (Anicic, Tasic, et al., 2009; Anicic, Tomasevic, et al., 2009; Mikhailova, 2002). Exposure periods of four weeks are ideal for transplant bags of moss, with ten weeks the maximum for determining positive accumulation rates (Anicic, Tasic, et al., 2009). However, since lichens are more desiccation-tolerant, longer exposure periods are possible (Pilegaard, 1979; Tretiach et al., 2007), and some studies have been conducted successfully for 9 - 12 months (Bari et al., 2001; Marques et al., 2004). This is

particularly advantageous for heavy metal monitoring, because direct measurements for prolonged periods using conventional techniques can be expensive (Aprile, Di Salvatore, Carratu, Mingo, & Carafa, 2010; Bari et al., 2001). However, some researchers prefer shorter exposure periods of 1 - 3 months for lichen transplants because of physiological degradation and structural changes (Bargagli & Mikhailova, 2002). Most lichens are known to show changes in elemental composition within short periods, possibly making short-term monitoring more accurate compared with longer-term studies using transplants (Garty, 2001; Mikhailova & Sharunova, 2008). Conversely, as removal time for metals in lichen tissue is considered to be 2 - 5 years (Walther et al., 1990), long-term monitoring can be undertaken, provided lichen vitality is sustained.

Metal uptake by lichens is usually a combination of active and passive processes (Guttova, Lackovicova, Pisut, & Pisut, 2011), including ion exchange and particulate trapping (Spagnuolo, Zampella, Giordano, & Adamo, 2011). Metals can occur either as dry particulates or in dissolved form (Frati et al., 2005), with several uptake mechanisms occurring simultaneously (Bergamaschi et al., 2007). The accumulation rate also depends on the element being captured (Garty, 2001). Soluble metals are absorbed faster (Bergamaschi et al., 2007), with many metals (Cd, Cr, Pb and Zn) absorbed within a few minutes into extracellular or intracellular spaces (Antonelli, Ercole, & Campanella, 1998).

The rate of accumulation also depends on meteorological parameters such as temperature, rainfall, wind patterns and precipitation (Bergamaschi et al., 2007). Rainfall enriched with elements is a key contributor of wet deposition and may determine seasonal differences under constant pollution loads (Knops, Nash III, Boucher, & Schlesinger, 1991). Wind direction can also affect accumulation rates, where lichen transplants oriented towards the dominant wind direction can accumulate more pollutants emitted by industrial point-sources (Freitas, Reis, Marques, & Wolterbeek, 2001). Temperature is not a major limiting factor for lichen transplants; however, extreme temperatures in dry conditions can desiccate the lichen transplants, resulting in lower metal accumulation rates (Cabrajic, Moen, & Palmqvist, 2010). Precipitation, on the other hand, is an important factor affecting lichen transplants (Branquinho et al., 2008), where low precipitation levels can reduce metal accumulation rates (Tretiach et al., 2007).

Physiological and structural features of the lichen species also influence heavy metal accumulation rates (Cercasov, Pantelica, Salagean, Caniglia, & Scarlat, 2002; Conti & Cecchetti, 2001; Garty, 2001; Mikhailova, 2002). Foliose and fruticose lichens generally have higher accumulation rates (Guttova et al., 2011), the latter usually having fine divisions that attract particulate matter enhance accumulation (Ribeiro et al., 2013). Many studies have shown species-dependent differences in accumulation in different lichen transplants (Aprile et al., 2010; Bergamaschi et al., 2007; Culicov & Yurukova, 2006; Godinho, Verburg, Freitas, & Wolterbeek, 2009; Piccotto, Bidussi, & Tretiach, 2011). Selecting suitable species should be based on criteria such as its availability in the study area, its sensitivity to pollution, stress tolerance and ease of collection (Conti & Cecchetti, 2001).

Successful transplant experiments rely on the correction of baseline concentrations of the pollutants entrapped within lichen thalli before exposure in new sites (Frati et al., 2005). However, this can be problematic, because of the high variability in background concentrations (Frati et al., 2005; Policnik, Batic, & Ribaric Lasnik, 2004). This may arise from substrate differences, soil interactions, involvement of non-pollution sources, and long-range transport of pollutants from great distances (Policnik et al., 2004). Variability within the microenvironment can also be erroneously interpreted as differential accumulation in lichens (Nash III & Gries, 1995). An interpretative scale based on the exposed-to-control ratio (EC ratio) has been established to determine the accumulation levels of biomonitors by accounting for the natural fluctuations in heavy metals observed in lichen tissue (Frati et al., 2005). The EC ratio allows comparison between different lichen species and different transplant studies (Bergamaschi et al., 2007).

This current research project used the lichen-bag approach to measure heavy metal pollution in the Collie region, in order to determine the dispersion patterns of pollutants from local industries. A fruticose lichen, specifically *Usnea inermis* was used for transplantation, as discussed above. The morphology of the thalli for removal enabled easy collection of intact thalli for transplantation. The ability to clean exogenous material from fruticose species (Frati et al., 2005) was advantageous as was the desiccation tolerance of these growth forms (Singh, Ranjan, Nayaka, Pathre, & Shirke, 2013). The lichen transplant process was used so that short-term seasonal accumulation

patterns could be determined in the Collie area, for the purpose of explaining the spatial and temporal heavy metal patterns identified in the previous chapter.

## **5.2 Methodology**

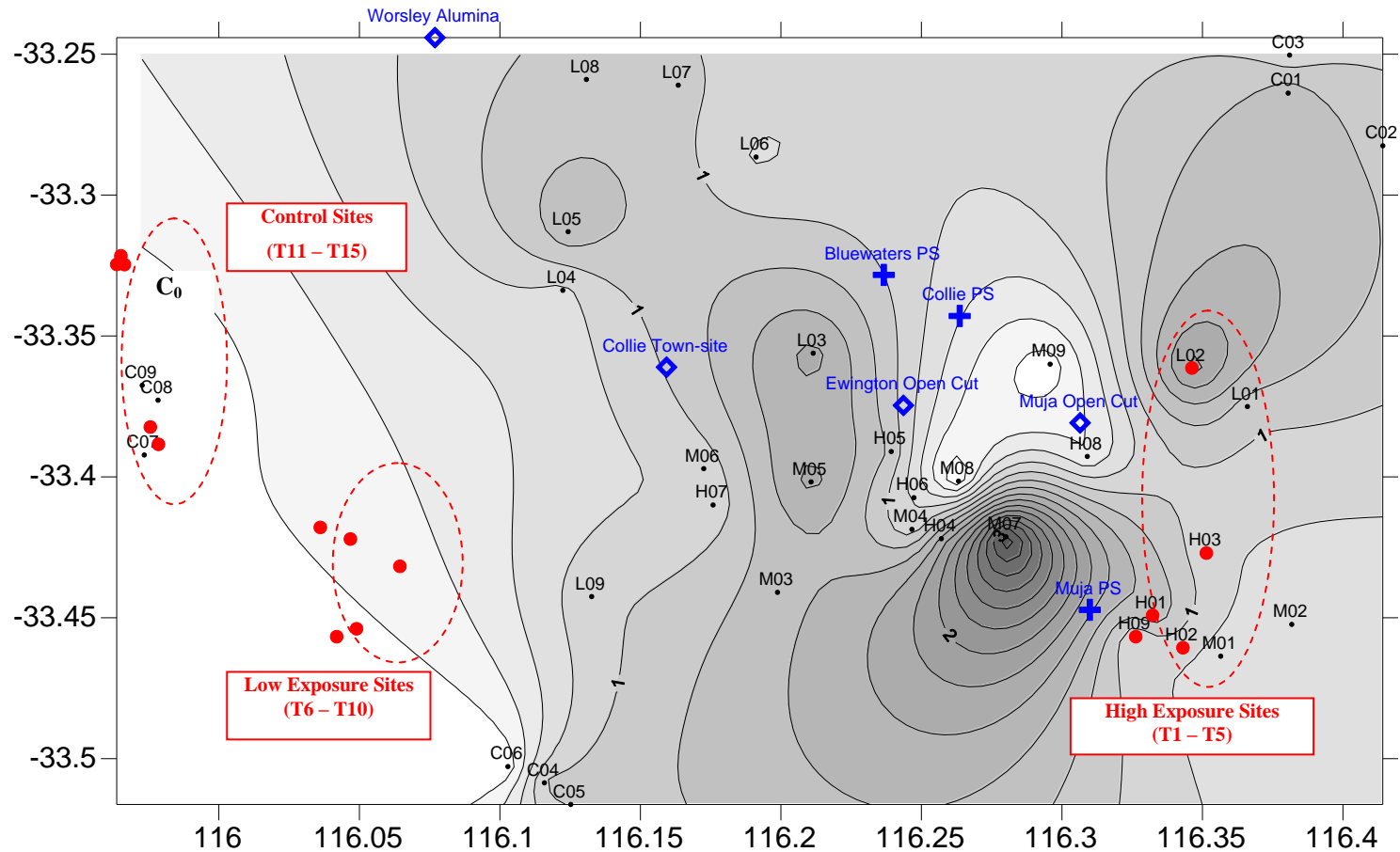
### 5.2.1 Study design

*Usnea inermis* lichen transplant bags were deployed at 15 study sites across the Collie air-shed, on four occasions over a one year period. After the 12 week exposure periods, transplants were harvested and analysed for the metals As, Cr, Cu, Pb, Mn, Ni and Zn using the inductive coupled plasma mass spectrophotometry (ICP-MS) technique, as previously described (Chapter 4.2). Lichen transplants were also exposed for 48 weeks to show the long-term accumulation of metals across the sampling seasons. At the start of each sampling season, *in situ* lichen samples collected from the control sites (where lichens were harvested for transplantation) were also analysed for heavy-metals, to determine the background metal concentrations in the lichen transplants.

### 5.2.2. Site selection

Fifteen sites were selected for the transplant study, with three categories of pollution exposure (control, low and high), based on the results of *in situ* biomonitoring in Chapter 4. Five study sites were located close to the coal-fired power stations and coal mines in the area (Figure 5.1), and were considered as ‘high exposure’ sites. These sites overlapped with study sites categorized as ‘high exposure’ sites in Chapters 2 and 4. Five ‘low’ exposure sites were selected in areas with low concentrations observed in the contour graphs for metal concentrations generated in Chapter 4 (Figure 4.6). Five ‘control’ sites were located in the same area where lichens were collected for transplantation (Figure 5.1).

Sites were selected to ensure maximum vertical representation of study sites in the Collie air-shed, covering a wide spatial range in the area. Figure 5.1 gives a typical heavy metal dispersion contour map, obtained in Chapter 4 (Cu concentrations in the spring season), that depicts the rationale behind site selection for the transplant experiment.



**Metal Distribution Map Example from Stage 2 (Spring Cu Data)**

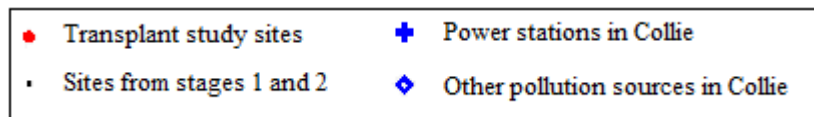


Figure 5.1: Site selection for transplant experiment based on *in situ* lichen biomonitoring data (C<sub>0</sub> – area of lichen collection for transplantation)

### 5.2.3 Collection of lichens for transplant bags

The first sampling season began in July 2011, when lichens were harvested from control sites (C<sub>0</sub>, Figure 5.1) for lichen-bag preparation. The location of the control site was considered to be far removed from the emissions from the industries in Collie, and was a ‘pristine’ environment located in the Wellington National Park. The lichen *Usnea inermis* was selected because of its wide distribution in the Collie area, and its identification as a pollution-tolerant lichen in Chapter 2. *Usnea inermis* thalli of similar size were collected by hand, using rubber gloves, then cleaned of extraneous material using a soft nylon brush. They were loosely packed in nylon mesh bags which are used for fruit packaging (60 x 8 cm; mesh size 0.3 cm), with three clusters per bag, each cluster having 5 - 6 lichen thalli separated with cable ties (Figure 5.2a). These ‘lichen-bags’ were hung on nylon ropes suspended 5 - 6 m above the ground between two trees, at each study site (Figure 5.2c). Two sets of nylon rope, each carrying three lichen-bags, were hung at two different locations at each study site, to ensure that at least one set of lichen-bags would survive any adverse weather events and vandalism.

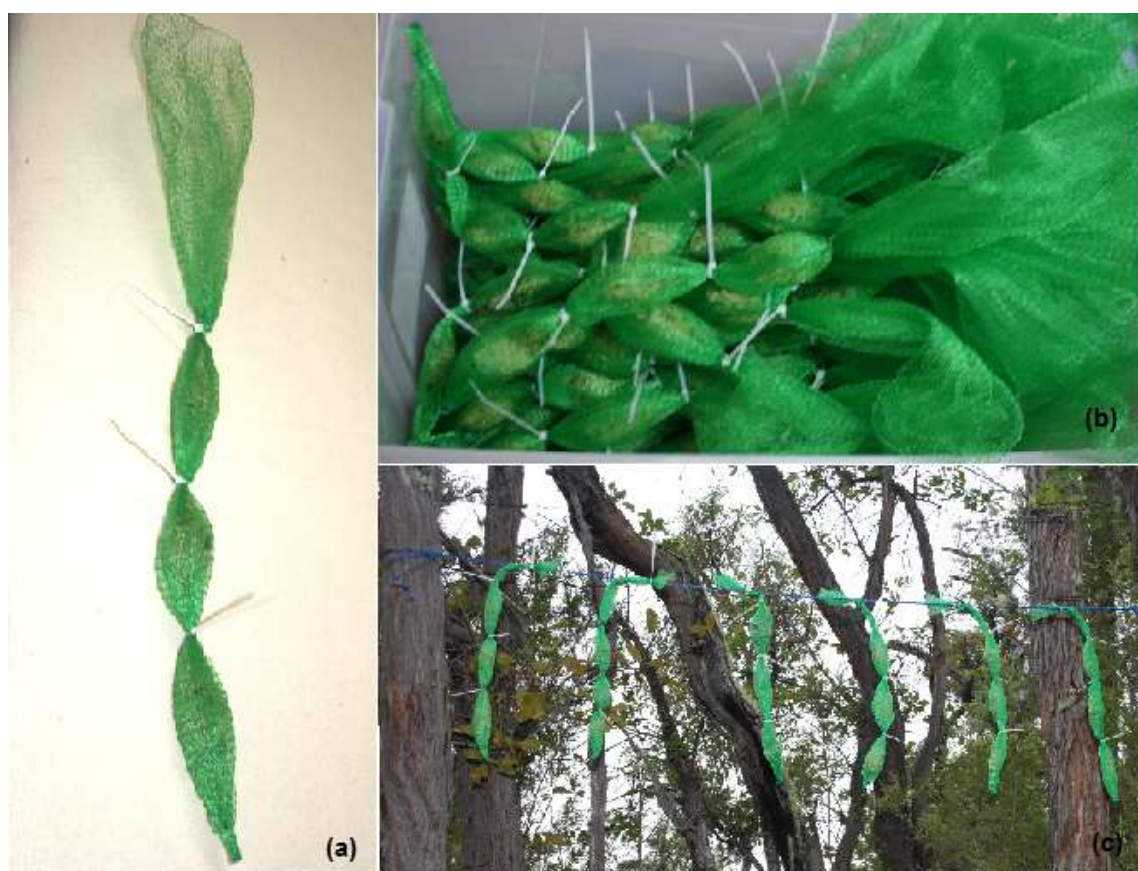


Figure 5.2: Lichen transplant bags – (a) A single bag with three replicate clusters; (b) Lichen-bags before exposure; (c) Lichen-bags suspended on nylon rope at a study site

#### 5.2.4 Sampling seasons

Four sampling seasons were used for the transplant experiment, with the first set of transplants deployed in July 2011 during the winter season, with the last samples collected in May 2012 (Table 5.1). Fresh lichen transplants (three bags) were hung during each season at all 15 study sites (including the control sites for determining the transplant effect), exposed for a period of 12 weeks (3 months), then collected at the end of each season, in order to determine seasonal variation in metal accumulation. A further three lichen-bags were exposed for 48 weeks (all year) at all 15 sites. This made it possible to determine the total accumulation load over the duration of the experiment and also allowed the comparison of long and short-term exposure results.

Table 5.1: Details of sampling dates and durations for the transplant experiment

<b>Sampling Season</b>	<b>Time Period</b>	<b>Duration</b>	<b>Season</b>
S1	4 <sup>th</sup> Week of Apr 2011 – 4 <sup>th</sup> Week of Jul 2011	12 weeks	Winter
S2	4 <sup>th</sup> Week of Jul 2011 – 1 <sup>st</sup> Week of Nov 2011	12 weeks	Spring
S3	1 <sup>st</sup> Week of Nov 2011 – 1 <sup>st</sup> Week of Feb 2012	12 weeks	Summer
S4	1 <sup>st</sup> Week of Feb 2012 – 1 <sup>st</sup> Week of May 2012	12 weeks	Autumn
S5	4 <sup>th</sup> Week of Apr 2011 – 1 <sup>st</sup> Week of May 2012	48 weeks	All Year

#### 5.2.5 Sample collection

At the end of each season, three lichen-bags were removed, sealed in paper bags and transported to the laboratory, where lichen samples were air-dried within the sealed paper bags and stored in a dry place until analysis.

#### 5.2.6 Background concentrations and calculating the transplant effect

At the start of each sampling season, six samples of lichen thalli (each approximately 2 g) were collected from the control sites separately, for the determination of background levels of metal concentrations at the onset of the transplant experiments. These measurements allow for the determination of accumulation rates and transplantation effect on *Usnea inermis* lichens by comparison with the final concentrations in lichen bags exposed at the control sites.

The concentration difference between the background concentrations and transplants exposed at the control sites was calculated to determine the ‘transplant effect’ on metal



accumulation. The net accumulation for each season was calculated by deducting this transplant effect from the final concentrations obtained after transplantation in the high and low exposure sites.

#### 5.2.7 Meteorological data

Meteorological data for the duration of the transplant study were obtained for the Collie weather station (Bureau of Meteorology, 2011/2012). Daily weather data were obtained for temperature, rainfall, wind speed and direction, since these parameters are known to affect metal accumulation in lichens (Mlakar et al., 2011).

#### 5.2.8 Sample preparation and analysis

Chemical analysis for heavy metals in lichen transplants from all sampling seasons was carried out at the end of the transplant experiment. One lichen-bag from each study site for each season was analysed. Acid-digested lichen samples were analysed for the metals As, Cr, Cu, Pb, Mn, Ni and Zn at the National Measurement Institute (NMI), using the inductively coupled plasma mass spectrometry (ICP-MS) technique. Steps in sample preparation, digestion and analysis were identical to the procedure followed in the second stage of this research (Chapter 4). Detailed sample preparation, digestion and analysis techniques are given in Chapter 4.3.

Since the metals Cd and Hg had a very high number of non-detects in the second stage of the study (Chapter 4), the transplant samples were not analysed for these two metals. Quality assurance procedures followed by the NMI included duplicate analysis and laboratory controls, and the results showed acceptable percent differences for duplicates, as well as good recovery for laboratory controls and matrix spikes (Appendix 5.1). Sample blanks, elemental spikes and sample spikes were also included for quality assurance and the recoveries were between 76% - 102% (Appendix 5.2).

#### 5.2.9 Data analysis

Data analysis was performed in IBM SPSS Statistics 19.0 software and graphs were created using SigmaPlot 12.0. According to the Shapiro–Wilk test statistics, the metal accumulation data for the five sampling seasons were normally distributed (Shapiro & Wilk, 1965). Levine’s test results showed equal variances (homogeneity of variance) for seasonal data. A one way ANOVA was performed for comparison between the different sampling seasons and a Tukey’s *post hoc* test was used to determine which seasons

were statistically significant from each other. A one way ANOVA was also performed to identify any differences between metal concentrations in background samples and metal accumulation at control sites in lichen transplants, to determine if the transplantation approach was introducing any variation to the metal concentrations recorded.

#### 5.2.10 Calculation of exposed-to-control ratio (EC ratio)

The accumulation of each metal was calculated using the exposed-to-control ratio (EC ratio) technique proposed by Frati *et al.* (2005). This was done by dividing the metal concentrations in transplanted lichens after exposure for each sampling season, by the background metal concentrations measured for lichen samples collected at the start of each transplant period. This ratio was compared against an interpretative scale developed by Frati *et al.* (2005), where five classes were used to determine the accumulation or loss of metals in transplanted lichen thalli. These five classes were developed based on the EC ratio, where a 25% difference for each class was considered adequate to include natural variation observed in metal accumulation in lichens (Frati *et al.*, 2005; Loppi, Giordani, Brunialti, Isocrono, & Piervittori, 2002). This scale describes the trends in metal accumulation in transplanted lichens and allows for inter-study comparisons (Godinho, Verburg *et al.*, 2009).

## 5.3 Results

The transplant study reported low concentrations for all heavy metals (Table 5.2), with values similar to those recorded for *in situ* lichens in Chapter 4 (Table 4.2). The mean metal concentrations for transplant lichens across the four seasons ranked in concentration from Mn>Zn>Pb/Cu>Ni/Cr>As (Table 5.2). No elevated concentrations were recorded for any metals; however, seasonal differences in metal accumulation were evident in lichen transplant bags.

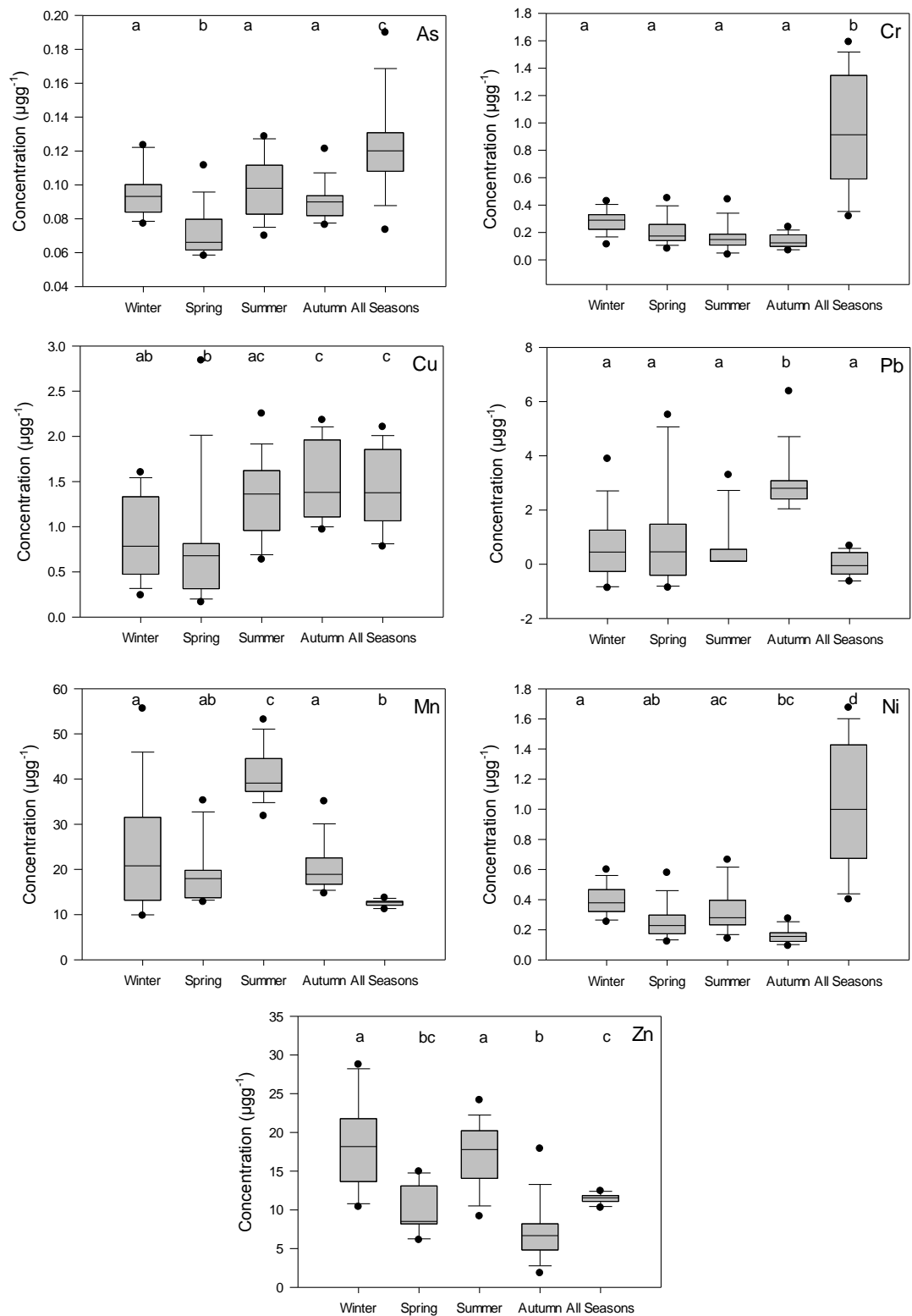
### 5.3.1 Seasonal differences in metal accumulation in lichen transplants

When the metal concentrations were corrected for the ‘transplantation effect’ as described in section 5.2.6, the net accumulation patterns showed seasonal variations for most metals (Figure 5.3). The highest mean concentrations were recorded in winter for almost all metals, while the lowest concentrations were recorded in autumn for most metals (Figure 5.3). Statistically significant seasonal accumulation patterns were observed based on one way ANOVA Tukey’s *post hoc* test statistics, where most metals showed some differences in accumulation at least across two seasons, with the exception of Cr (Figure 5.3). The transplants exposed for 48 weeks showed statistically significant differences from seasonal data for most metals (Figure 5.3).

Higher accumulation was recorded for transplants exposed for 48 weeks compared with the 12 week exposure periods, for all metals except Pb, Mn and Zn (Figure 5.3), with Pb recording negative accumulation. However, the 48 week transplants recorded higher accumulation in the low-exposure and control sites compared with the high-exposure sites, showing statistically significant differences between the three exposure categories (one way ANOVA Tukey’s *post hoc* test), for all metals except As (Figure 5.4).

Table 5.2: Geometric mean, minimum & maximum values (in parenthesis) and standard deviations for heavy metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) in the lichen *Usnea inermis* transplants for the four sampling seasons (12 week exposures) and for the transplants exposed for 48 weeks (n=15)

Metal	<u>Winter</u>		<u>Spring</u>		<u>Summer</u>		<u>Autumn</u>		<u>48 Weeks (1 year)</u>	
	Geometric Mean	SD	Geometric Mean	SD	Geometric Mean	SD	Geometric Mean	SD	Geometric Mean	SD
As	0.09 (0.07, 0.12)	0.01	0.08 (0.07, 0.12)	0.01	0.09 (0.06, 0.12)	0.02	0.08 (0.06, 0.11)	0.06	0.11 (0.07, 0.18)	0.03
Cr	0.35 (0.18, 0.50)	0.08	0.26 (0.15, 0.52)	0.10	0.23 (0.12, 0.53)	0.01	0.18 (0.13, 0.30)	0.13	0.92 (0.39, 1.66)	0.40
Cu	0.92 (0.38, 1.74)	0.44	0.65 (0.23, 2.90)	0.66	1.01 (0.42, 2.04)	0.43	0.93 (0.52, 1.73)	0.52	1.50 (0.92, 2.24)	0.41
Pb	1.76 (0.65, 5.40)	1.23	1.70 (0.45, 6.82)	1.89	0.11 (<DL, 3.20)	0.98	0.46 (<DL, 4.36)	0.03	1.45 (0.88, 2.19)	0.41
Mn	31.68 (20.09, 65.94)	12.97	21.25 (15.64, 38.08)	6.78	22.53 (13.81, 35.16)	5.61	23.74 (18.33, 38.70)	18.33	22.88 (21.54, 24.07)	0.71
Ni	0.32 (0.19, 0.54)	0.10	0.27 (0.16, 0.61)	0.11	0.29 (0.13, 0.65)	0.16	0.23 (0.17, 0.35)	0.17	0.87 (0.34, 1.61)	0.40
Zn	17.49 (10.29, 28.66)	5.57	15.48 (11.86, 20.69)	2.97	10.62 (3.63, 18.63)	4.14	10.06 (5.45, 21.52)	5.45	11.33 (10.18, 12.34)	0.60



**Figure 5.3: Box plots showing heavy metal accumulation in lichen transplants across the seasons after correcting for the transplant effect;  $n=10$ ; bullets indicate outlier values; ANOVA, As:  $F_{4,70} = 17.1$ ,  $P=0.00$ ; Cr:  $F_{4,70} = 43.9$ ,  $P=0.00$ ; Cu:  $F_{4,70} = 6.9$ ,  $P=0.00$ ; Pb:  $F_{4,70} = 13.4$ ,  $P=0.00$ ; Mn:  $F_{4,70} = 31.4$ ,  $P=0.00$ ; Ni:  $F_{4,70} = 40.8$ ,  $P=0.00$ ; Zn:  $F_{4,70} = 24.2$ ;  $P=0.00$ ; lowercase letters indicate Tukey's *post hoc* differences between seasons at  $P < 0.05$**

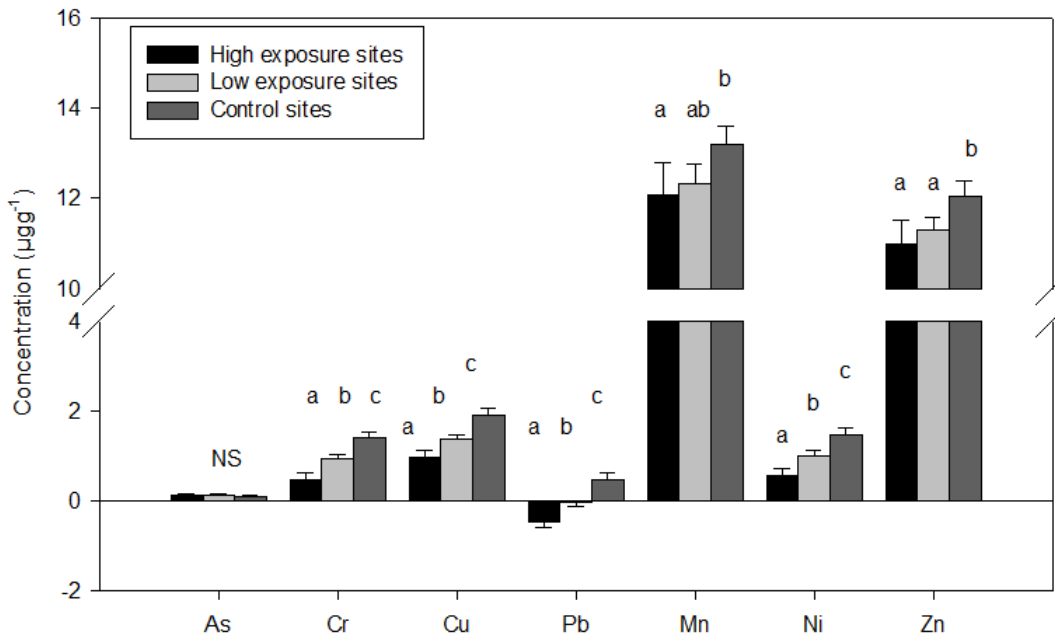


Figure 5.4: Metal accumulation patterns (after corrected for the transplant effect) in different site groups based on exposure category for sites for the 48 weeks exposure transplants;  $n=5$ ; error bars indicate the standard deviation; ANOVA ( $P<0.05$ ), As:  $F_{2,12} = 1.9$ ,  $P = 0.2$ ; Cr:  $F_{2,12} = 59.0$ ,  $P = 0.00$ ; Cu:  $F_{2,12} = 64.3$ ,  $P = 0.00$ ; Pb:  $F_{2,12} = 64.6$ ,  $P = 0.00$ ; Mn:  $F_{2,12} = 6.0$ ,  $P = 0.02$ ; Ni:  $F_{2,12} = 58.4$ ,  $P = 0.00$ ; Zn:  $F_{2,12} = 9.2$ ,  $P = 0.00$ ; lower case letters indicate Tukey's *post hoc* differences between exposure categories at  $P<0.05$

Temperature, rainfall and wind data for the entire study period were used to determine their influence on the metal accumulation observed in this study (Figures 5.5, 5.6 and 5.7). Collie has a Mediterranean-type climate with hot, dry summers and cool, wet winters (GHD, 2009). In the study period, rain was highest in winter months, while some rainfall was also experienced in spring (Figure 5.6). Autumn on the other hand, had very little rain and was the driest season in Collie, with only a few showers experienced towards the end of the season. Temperature differences were higher in both summer and autumn, with winter and spring showing lower temperature fluctuations (Figure 5.5). Wind patterns did not show any dominant wind directions across the seasons and the mean wind speed was also consistent across the seasons (Figure 5.7). However, winter and spring experienced higher maximum wind speeds compared with summer and autumn (Figure 5.7). The dry autumn period with the least rainfall recorded the lowest metal concentrations in lichen transplants (Table 5.2), while the highest concentrations for all metals were recorded in the winter season, where the highest rainfall was observed during the study. The transplants exposed in summer recorded

higher concentrations than autumn, possibly explained by the relatively higher rainfall when compared with autumn (Figure 5.6). Summer also had higher maximum wind speeds and fewer calm days than autumn, conditions which may have facilitated better dispersion of pollutants in summer (Figure 5.7).

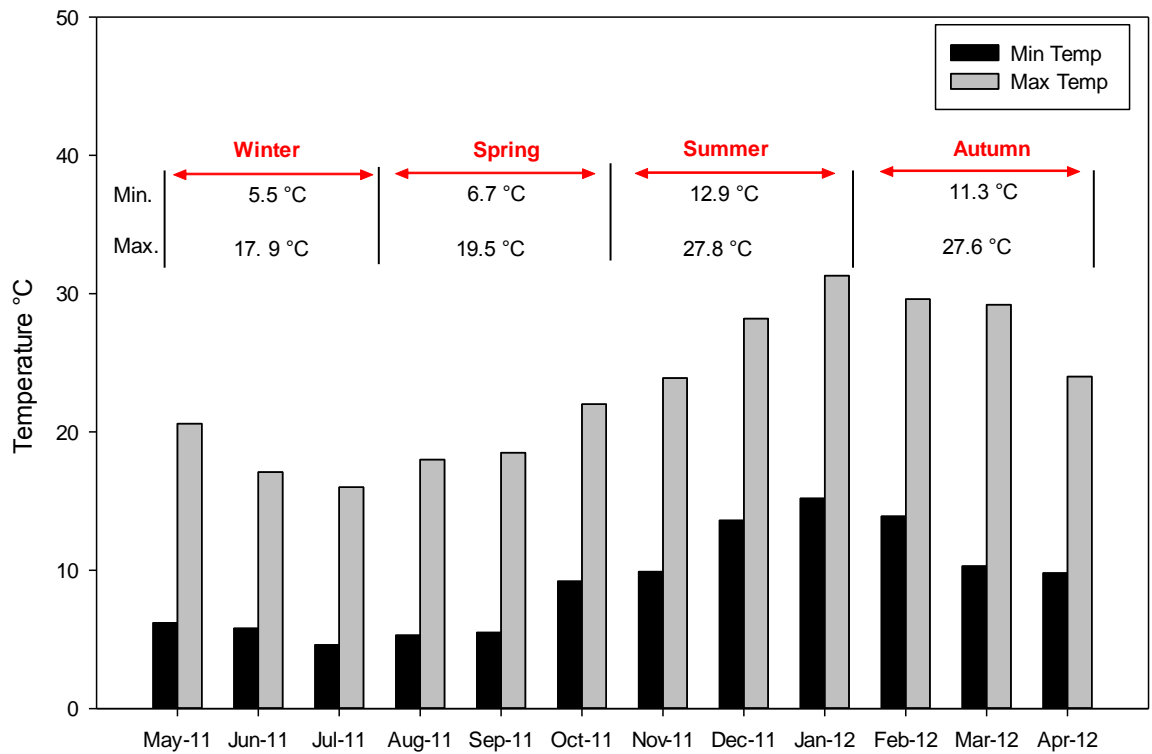


Figure 5.5: Monthly average air temperatures during the transplant study, highlighting the temperature differences over the four seasons - data from the Collie weather station (Bureau of Meteorology, 2011/2012)

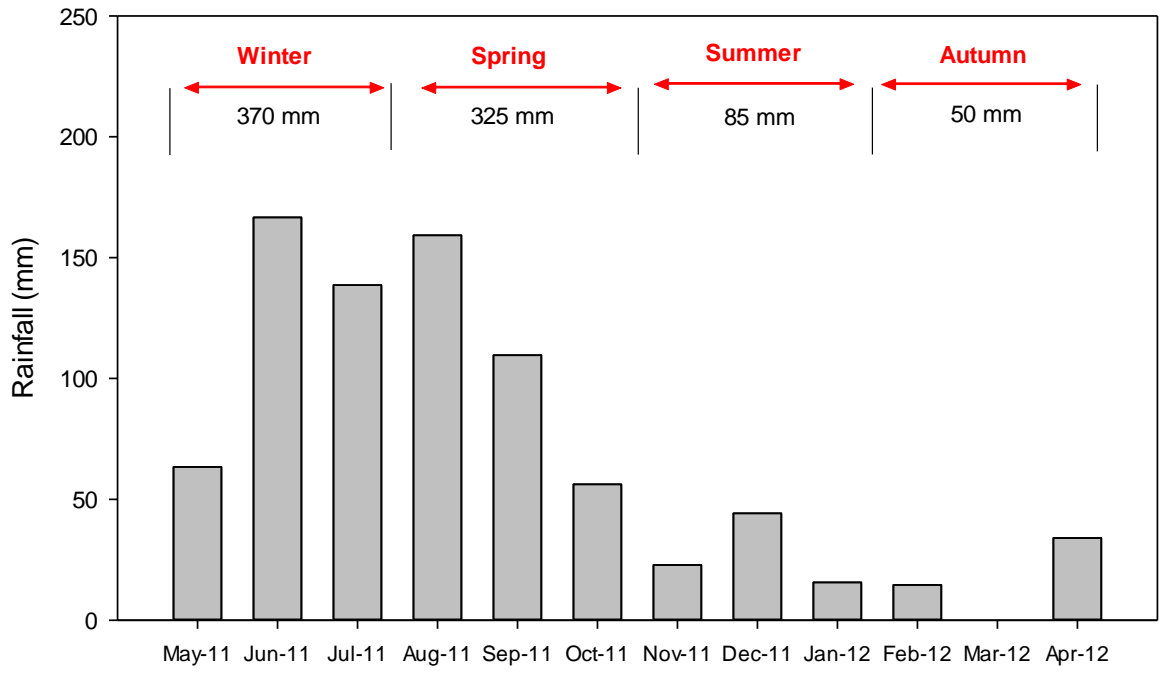


Figure 5.6: Monthly total rainfall data for the duration of the transplant study recorded at the Collie weather station (Bureau of Meteorology, 2011/2012)



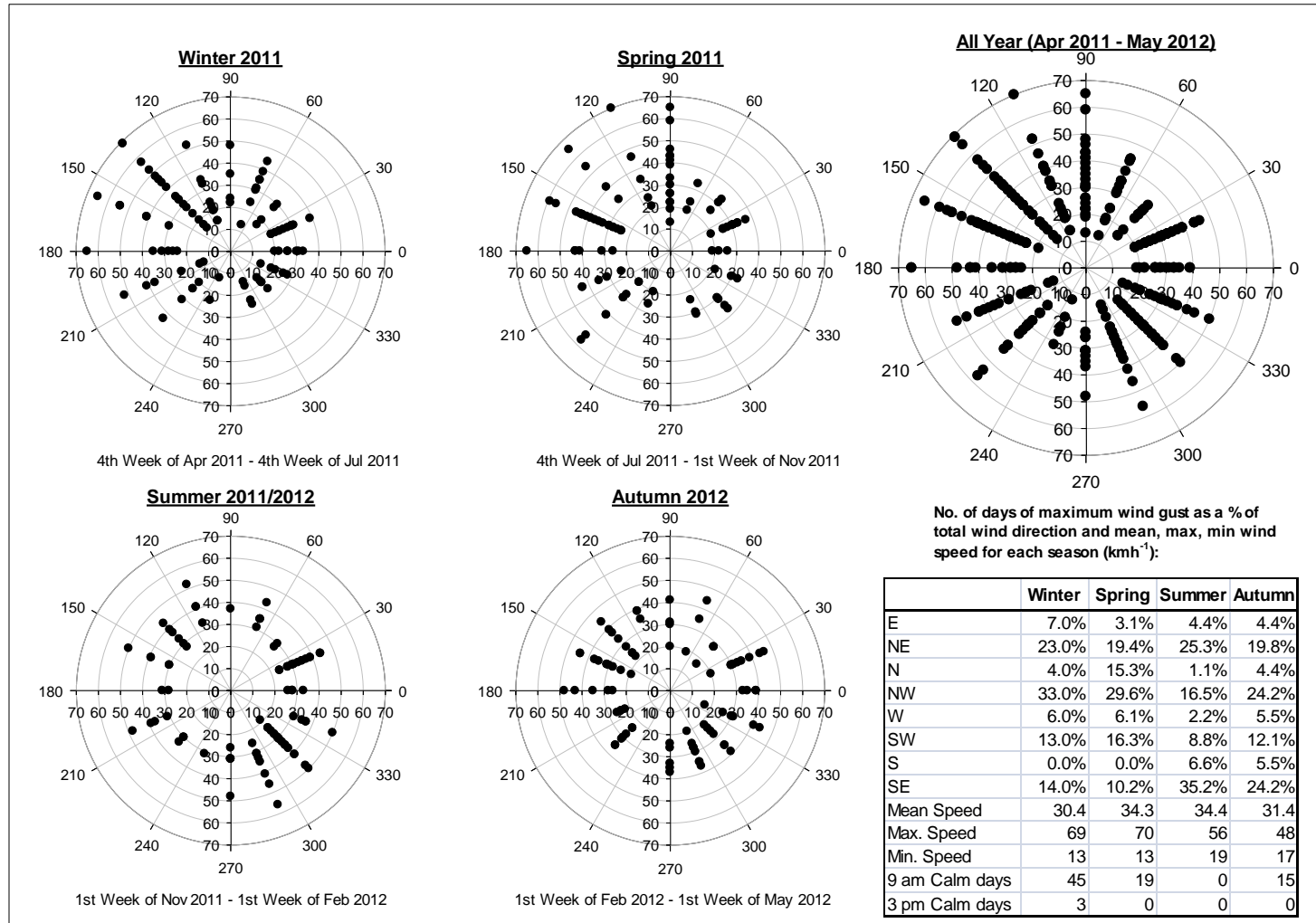


Figure 5.7: Wind patterns in Collie for the four sampling seasons of the transplant experiment showing maximum wind direction/speed; seasonal mean, maximum, minimum wind data, and number of calm days at 9 am and 3 pm are also tabulated (Bureau of Meteorology, 2011/2012)

### 5.3.2 Transplantation effect on metal accumulation

The heavy metal concentrations in the background *in situ* samples before exposing in transplant bags showed some elevated values, and were higher than the transplants exposed at the control sites, particularly in autumn for most metals (Figure 5.8). The higher background concentrations indicated that there was a pollution effect at the control sites and that the area was not ideal for collecting lichens for transplantation experiments.

### 5.3.3 Exposed-to-control ratios (EC ratios) for lichen transplants

The exposed-to-control ratios (EC ratio) for the 12 week and 48 week transplants were calculated (Frati et al., 2005), to identify trends in metal accumulation by transplanted lichens (Table 5.3). When considering the overall dataset, higher exposed-to-control ratios were recorded for Pb, with high accumulation observed in all seasons, except summer, from the high exposure and control sites (Table 5.3). However, this trend was very different for Pb when the accumulation patterns were corrected for the transplant effect, which showed negative accumulation (Figure 5.3 and 5.4), indicating that different approaches used to correct for the transplant effect can show different patterns in metal accumulation in transplant experiments.

Higher exposed-to-control ratios were recorded in the 48 week transplants for all metals except for Pb, Mn and Zn (Table 5.3). Inefficient uptake of As was evident compared with other metals across the four seasons (Table 5.3). Efficient uptake of Cr was revealed across the seasons, with summer and one-year transplants recording the highest EC ratios (Table 5.3). Seasonal variation was highest for Mn, where summer recorded leaching; autumn recorded inefficient uptake; while winter and spring recorded high accumulation (Table 5.3). None of the metal EC ratios recorded values in the 'severe loss' class and only a few metals showed values in the 'loss' class (Table 5.3).

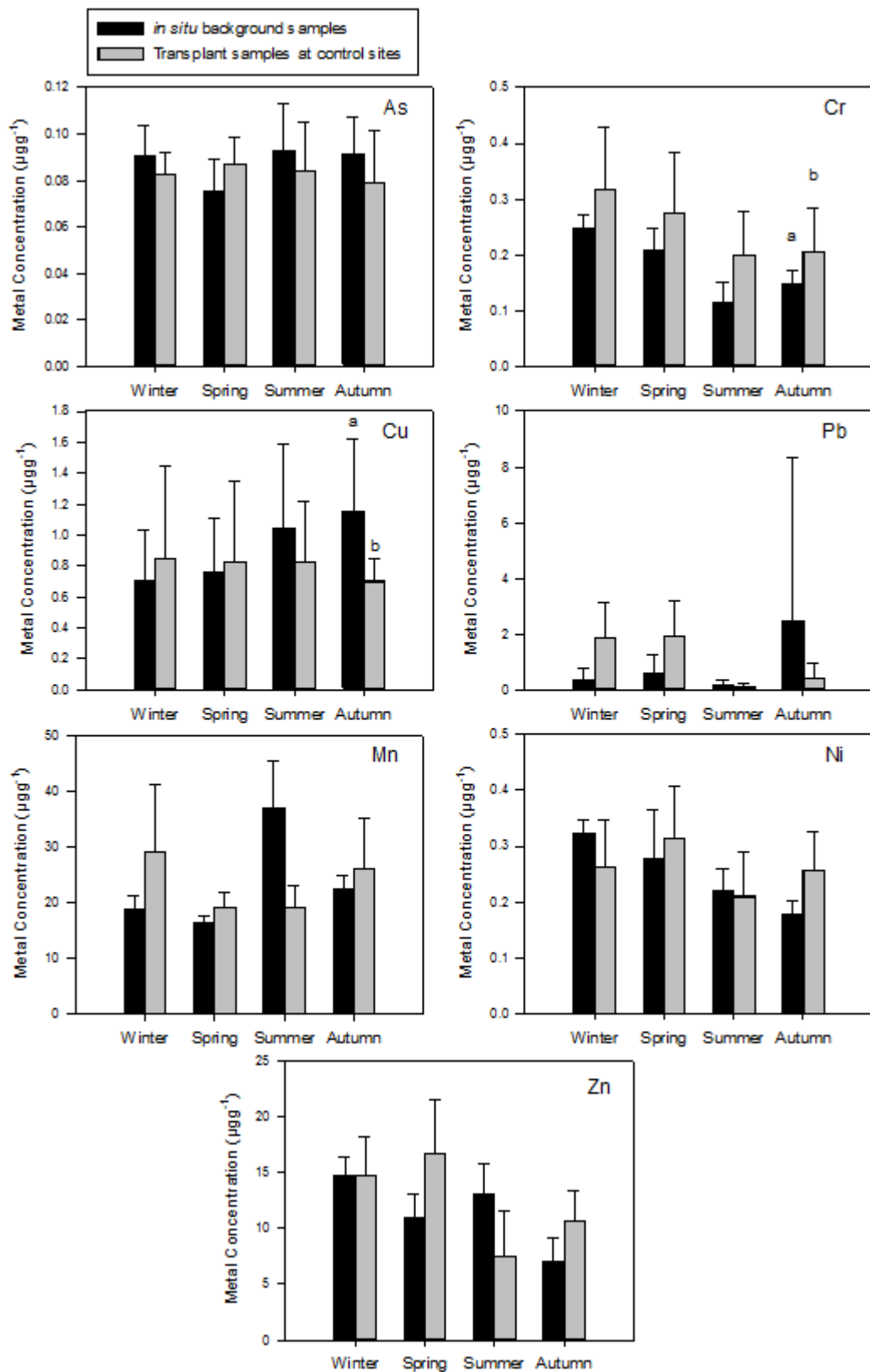


Figure 5.8: Bar graphs showing the variability in metal concentrations for transplants exposed at the control sites and the background concentrations for *in situ* lichens before exposure, the difference between which was used to calculate the ‘transplant effect’; n=5; lowercase letters indicate statistically significant differences (P<0.05) in concentrations between control and background samples for each metal based on independent sample t-test statistics

Table 5.3: EC ratios of transplanted lichens for each season averaged for each exposure category and their relative accumulation/loss (A/L) class (Scale for interpretation of EC ratios: 0–0.25 Severe Loss; 0.25–0.75 Loss; 0.75–1.25 Normal; 1.25–1.75 Accumulation; >1.75 Severe Accumulation)

Metal	Season	BC*	High Exposure		Low Exposure		Control Sites		Summary of A/L Classes
			EC Ratio	A/L Class	EC Ratio	A/L Class	EC Ratio	A/L Class	
As	Winter	0.09	0.95	N	1.09	N	0.94	N	3 N
	Spring	0.07	1.14	N	1.08	N	1.19	N	3 N
	Summer	0.09	1.23	N	0.94	N	0.98	N	3 N
	Autumn	0.09	0.91	N	0.87	N	0.91	N	3 N
	48 weeks	0.09	1.43	A	1.41	A	1.12	N	2 A, 1 N
Cr	Winter	0.24	1.57	A	1.51	A	1.30	A	3 A
	Spring	0.19	1.39	A	1.45	A	1.42	A	3 A
	Summer	0.11	3.00	SA	2.16	SA	1.88	SA	3 SA
	Autumn	0.14	1.13	N	1.51	A	1.46	A	2 A, 1 N
	48 weeks	0.24	2.28	SA	4.08	SA	6.03	SA	3 SA
Cu	Winter	0.63	1.85	SA	1.63	A	1.34	A	1 SA, 2 A
	Spring	0.66	1.82	SA	0.65	L	1.25	N	1 SA, 1 N, 1 L
	Summer	0.87	1.62	A	1.19	N	0.95	N	1 A, 2 N
	Autumn	1.06	1.01	N	1.18	N	0.66	L	2 N, 1 L
	48 weeks	0.63	1.75	A	2.41	SA	3.22	SA	2 SA, 1 A
Pb	Winter	0.20	8.28	SA	13.25	SA	9.38	SA	3 SA
	Spring	0.34	9.78	SA	4.55	SA	5.50	SA	3 SA
	Summer	0.09	0.66	L	17.07	SA	0.85	N	1 SA, 1 N, 1 L
	Autumn	0.17	10.52	SA	3.68	SA	2.62	SA	3 SA
	48 weeks	0.20	5.30	SA	7.42	SA	9.96	SA	3 SA
Mn	Winter	18.18	2.15	SA	1.80	SA	1.61	A	2 SA, 1 A
	Spring	16.01	1.50	A	1.43	SA	1.20	N	1 SA, 1 A, 1 N
	Summer	34.70	0.71	L	0.74	L	0.55	L	3 L
	Autumn	21.80	0.99	N	1.15	N	1.20	N	3 N
	48 weeks	18.18	1.23	N	1.25	N	1.30	A	1A, 2 N
Ni	Winter	0.32	1.20	N	1.15	N	0.82	N	3 N
	Spring	0.25	1.36	A	0.85	N	1.25	A	2 A, 1 N
	Summer	0.21	2.29	SA	1.19	N	0.98	N	1 SA, 2 N
	Autumn	0.17	1.17	N	1.47	A	1.47	A	2 A, 1 N
	48 weeks	0.32	1.61	A	2.98	SA	4.47	SA	2 SA, 1 A
Zn	Winter	14.38	1.40	A	1.40	A	1.02	N	2 A, 1 N
	Spring	10.43	1.51	A	1.42	A	1.60	A	3 A
	Summer	12.10	1.20	N	1.02	N	0.62	L	2 N, 1 L
	Autumn	6.59	1.38	A	1.82	SA	1.62	A	1 SA, 2 A
	48 weeks	14.38	0.76	N	0.78	N	0.83	N	3 N

\*BC – Background Concentrations

#### 5.3.4 Distance to the closest pollution source

There were no patterns observed between metal accumulation in lichen transplants and distance to closest pollution source for the four seasons. The transplants exposed over 48 weeks also did not show any statistical relationships with the distance to the closest pollution source when all three types of sites were included (Figure 5.9). However, the high exposure sites showed the lowest accumulation for all metals in the 48 week transplants, while control sites showed the highest accumulation (Figure 5.9), which was an unexpected pattern.

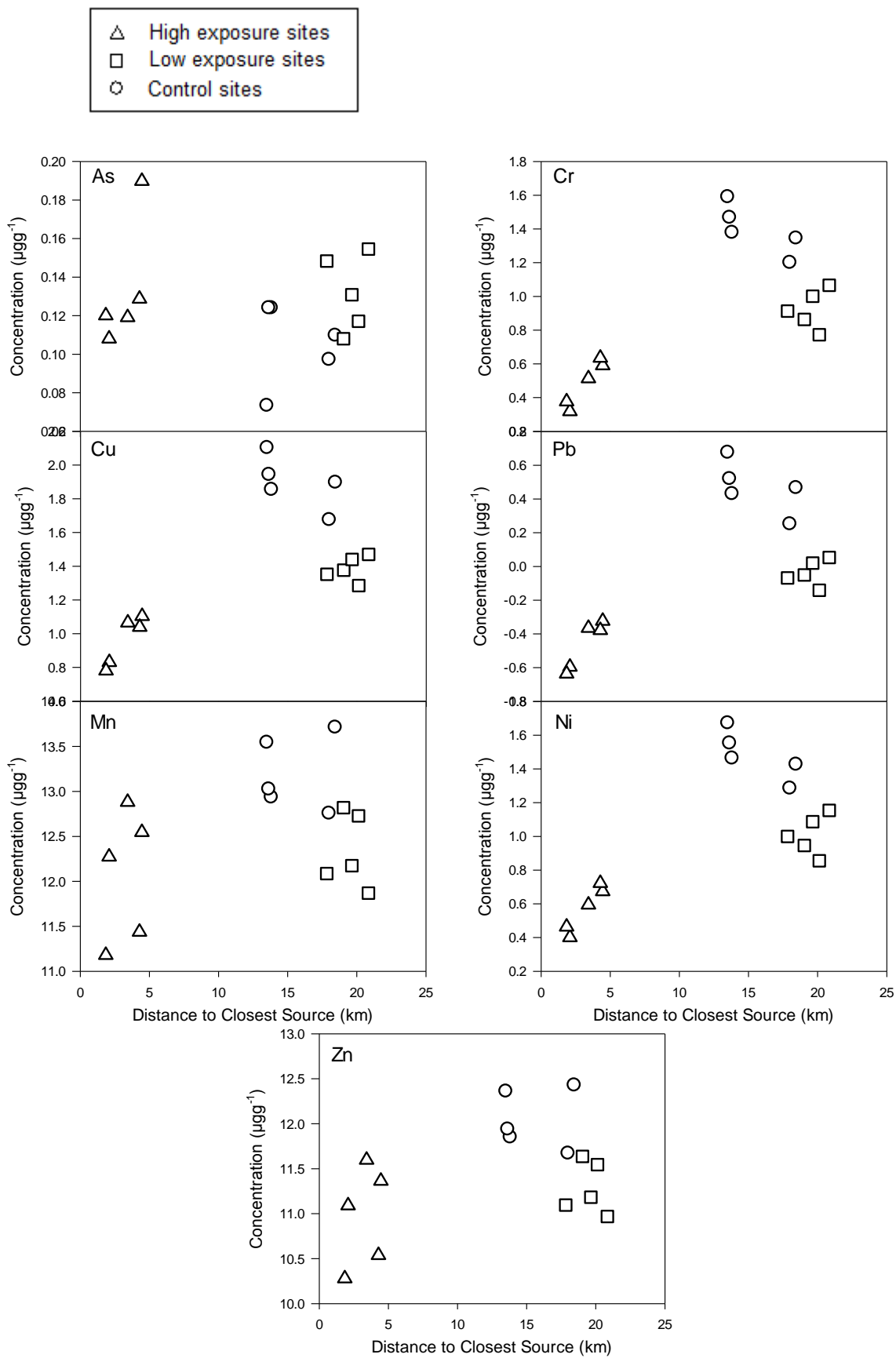


Figure 5.9: Scatter plots showing the relationship between transplants exposed for 48 weeks and the distance to the closest pollution source

## 5.4 Discussion

The results from the transplant experiment revealed very low rates of heavy metal accumulation by lichen transplant bags and did not show any elevated concentrations even at sites close to emission sources. The heavy metal concentrations found in this study were low when compared with other lichen transplant experiments using *Usnea* species in areas associated with industrial emissions (Bergamaschi et al., 2007; Conti et al., 2012; Culicov & Yurukova, 2006; Monaci, Fantozzi, Figueroa, Parra, & Bargagli, 2012) as well as remote, unpolluted environments (Conti et al., 2012; Monaci, Fantozzi, Figueroa, Parra, & Bargagli, 2012). This may indicate either, that for some reason *Usnea inermis* lichens did not accumulate the heavy metals emitted by the industries, or that the Collie air-shed is relatively unpolluted by heavy metal emissions.

### 5.4.1 Seasonal differences in metal accumulation in lichen transplants

Despite the low concentrations recorded, seasonal differences in heavy metal accumulation were observed in the lichen transplants. These differences were explained by meteorological conditions characteristic of the different seasons. The highest concentrations for all metals were recorded in the winter season (Table 5.2), where the highest rainfall was observed in Collie (Figure 5.6). Rain can wash metals from the atmosphere, facilitating the entry of larger amounts of heavy metals into lichen tissue via wet deposition (Knops, Nash III, Boucher, & Schlesinger, 1991). *Usnea* species favour hygrophilic environments and depend on atmospheric wet deposition for nutrient absorption, thus explaining why the wet winter months showed the highest accumulation of heavy metals in the transplants (Monaci et al., 2012). Since rains are more regular during winter, the lichens are also able to reach equilibrium in their metal uptake mechanisms during the prolonged wet season. The consistent cold and wet winter conditions are also considered to be ideal for metabolic activities of lichens, as indicated by increased growth and uptake of elements in lichen thalli (Nash III & Gries, 1995). Therefore, the higher metal concentrations in winter may be related to the increased metabolic activities in lichens facilitating the increased uptake of metals from the surrounding environment. The north-westerly winds prevalent in the winter months (Figure 5.7), which blow from the direction of the point-source emitters, may also have contributed to the higher accumulation of metals in the lichens, particularly at the high exposure sites.

The lowest metal concentrations in lichen transplants were recorded in autumn (Table 5.2), a pattern very different to that of the *in situ* lichen analysis in Chapter 4, where autumn recorded the highest concentrations for heavy metals (Table 4.2). However, the 2011 autumn, in which the *in situ* lichen analysis was conducted (Chapter 4), experienced higher rainfall compared with the 2010 spring *in situ* lichen sampling (Figure 4.2), which may have facilitated *in situ* lichens collected in autumn to absorb heavy metals by wet deposition, while the 2012 autumn months, in which the transplant bags were exposed, was the driest period with the lowest rainfall for the transplant experiment (Figure 5.6). These annual differences could explain the different seasonal patterns recorded for metal concentrations between the *in situ* lichen study (Chapter 4) and the lichen transplant experiment.

The low autumn concentrations in 2012 lichen transplants (Figure 5.3) were also associated with higher temperature fluctuations typical of the season (Figure 5.5). Large fluctuations in temperature and hotter daytime hours can increase the leaching of metals from lichen tissue (Backor & Loppi, 2009). Chlorolichens such as *Usnea* species are particularly sensitive to intense light exposure in the dry state (Gauslaa, Coxson, & Solhaug, 2012). Lichens are also known to release soluble intracellular material when hydrated after prolonged dry periods (Brown & Brown, 1991), which was the case for the autumn transplants that were exposed to a prolonged dry period followed by a few rain events at the end of the season (Figures 5.3 and 5.6).

Lichen physiological characteristics such as tissue age, growth rate and vitality, as well as the species type can also result in variable accumulation rates (Olowoyo, van Heerden, & Fischer, 2011), resulting in different patterns observed in different locations and seasons. This influence was minimized for the study by selecting *Usnea inermis* tissue of similar size (Conti, Tidono, Stripeikis, & Cecchetti, 2004), which corresponds to similar thallus age in lichens (Armstrong, 1997); however the different growth rate in different seasons may also have contributed some variability to the heavy metal concentrations observed.

Lichens accumulate heavy metals over the longer term, when the equilibrium between the dynamic uptake/loss mechanisms and the retention of accumulated heavy metals is achieved through prolonged exposure (Paoli, Pisani, Guttova, Sardella, & Loppi, 2011). The better retention of metals within lichen tissue over the longer term may have led to



the higher concentrations observed for the transplants exposed for 48 weeks, compared with the 12 week exposure periods for the seasonal data (Table 5.2).

#### 5.4.2 Effect of background concentrations on metal accumulation patterns

Lichen samples collected for transplantation experiments can show high variability in metal composition before exposure (background concentrations), even if they are collected from the same area (Fрати et al., 2005; Paoli et al., 2011). Some metals in the current study recorded elevated concentrations in the background samples across the seasons, which were all collected from within a 500 m<sup>2</sup> radius in a supposedly unpolluted site. These results indicated that the control site, from which lichens were harvested for transplantation, was recording a pollution influence that was possibly related to the Worsley alumina refinery. The proximity of this site to the refinery was not considered during the site selection at the beginning of this project and the area downwind from the refinery was not indicated as a polluted area in the air dispersion modelling maps for the Collie air-shed (GHD, 2009).

Factors such as substrate heterogeneity, micro-environment variability and the influence of non-pollution sources could also explain the elevated concentrations in the background samples (Policnik et al., 2004). The difference in concentrations between background *in situ* samples and transplant bags exposed at the control sites, which was used as a measure to determine the variability introduced by the transplantation approach, showed significant seasonal differences only for lithogenic metals such as Pb, Mn, Ni and Zn (Appendix 5.3), suggesting that the seasonal variation could be related to soil contamination and not industrial emissions (Fрати et al., 2005). However, the elevated concentrations in the background *in situ* samples for most metals (Figure 5.8) indicated that the control sites chosen for lichen collection had a pollution influence and therefore was not representative of an unpolluted area recommended for lichen collection for transplant experiments (Fрати et al., 2005). This pollution effect may have had a substantial influence on the metal accumulation values recorded in the lichen transplant bags.

#### 5.4.3 Exposed-to-control ratios (EC ratios) for lichen transplants

The exposed-to-control ratios (EC ratios) for all seasons showed very little loss of heavy metals in transplanted lichens (Table 5.3), demonstrating that the lichen *Usnea inermis* is a good accumulator of metals, even over short exposure periods. Other studies have observed similar EC ratios in fruticose lichen species and have found good agreement in

repeated surveys (Fрати et al., 2005). However, the lower heavy metal concentrations in *Usnea inermis* transplants in the current study indicate that further investigations are required to confirm its suitability for biomonitoring. Furthermore, metal leaching was observed in a few samples, particularly in the summer season for Mn, across all exposure categories (Table 5.3). The loss of metals in summer for Mn was mainly because of the elevated concentrations of this metal observed in the background samples (Figure 5.8).

The exposed-to-control ratio for Pb recorded the highest values across the exposure categories (Table 5.3) and were approximately four times higher than those reported for *Usnea* species in northern Italy (Bergamaschi et al., 2007), demonstrating a high affinity for Pb in *Usnea inermis* transplants. Brown and Brown (1991) reported that this different affinity was associated with the different competitive capacities of metals to cell-wall exchange sites, where Pb demonstrated a high affinity, which was also confirmed by Antonelli *et al.* (1998). Similar mean Pb concentrations, observed for *in situ* lichens in Chapter 4 (Table 4.2), further confirm the higher accumulation of Pb by *Usnea inermis*.

However, this interpretation is contradicted when the transplant effect was corrected by subtracting the accumulation at the control sites (as described in 5.2.6), which recorded leaching of Pb by lichen transplants (Figure 5.3), especially in the 48 week exposure period (Figure 5.4). Discrepancies between the two correction techniques were also found for Mn, where the EC ratio showed loss of metals in summer (Table 5.3), while the correction of the transplant effect showed higher accumulation of Mn in summer (Figure 5.3). The EC ratio only accounts for the variation introduced by the background concentrations, while the correction for the transplant effect also eliminated any changes in accumulation rates related to the bagging technique and the removal of lichens from their native environment. This could explain the contradicting results obtained from the two different approaches.

#### 5.4.4 Distance to the closest pollution source

The influence of distance to the closest pollution source on metal accumulation in lichens for all four seasons showed no clear patterns. This was possibly because the Collie air-shed is affected by several point-source emitters, making it difficult to identify the contribution of individual emission sources. Furthermore, the scatter plots for the 48 week transplants showed an unexpected pattern, recording lower

accumulation of metals at the high exposure sites that were closer to the emissions sources, and higher accumulation at the low exposure and control sites that were further away from the emissions sources (Figure 5.9). It appears that the transplants at the high exposure sites may have lost their ability to accumulate metals because of the effect of one or more pollutants from the emission sources. When lichens lose their vitality, metal uptake processes will be affected, and previously accumulated metals can be lost from their thalli over the longer term (Anicic, Tasic, et al., 2009). This may explain much lower concentrations recorded in the 48 week transplants at the sites with a high pollution intensity (Figure 5.4 and 5.9). The higher concentrations at the control sites when compared with the low exposure sites could be because the control sites were located closer to the Worsley alumina refinery (Figure 5.1), which was not considered as a potential emission source affecting the Collie air-shed during the site selection for the current research. The lichen transplants at these sites were possibly having an influence from the refinery emissions. The Chapter 4 *in situ* lichen biomonitoring results did not reflect the influence of the Worsley alumina refinery because of the expansion work at the refinery during the time of the *in situ* lichen biomonitoring study in the years 2010 and 2011, resulting in an increase in production capacity in 2012 during the transplant experiment (BHP Billiton, 2013).

These findings suggest that a 48 week exposure period might be too long for the lichen transplant approach in the Collie area, especially if transplants are exposed at sites with high pollution signals. Exposure periods of 24 – 32 weeks (6 - 8 months) over the wet seasons (winter and spring), might provide more effective results with minimal vitality-loss in lichen transplants. The time dependence on the level of metal saturation in *Usnea inermis* lichens requires further investigations to make accurate recommendations for the best exposure periods for transplant experiments in Collie.

#### 5.4.5 Limitations and future recommendations

The distribution of study sites for the transplant experiment (Figure 5.1) did not show good spatial resolution as the *in situ* lichen biomonitoring study (Chapter 4, Figure 4.1), since only 15 sites were selected and classified based on the results of Chapter 4. With the overlap of only five sites, direct comparisons between the two studies was difficult. Furthermore, replicate samples were not analysed from each study site in the transplant experiment, which might have provided more accurate heavy metal accumulation values for the study. The accuracy of the analytical method is probably not as important as the

need to sample more sites and thus improve our understanding of the spatial distribution of heavy metals in the region.

No visible signs of bleaching or discoloration were observed in any of the transplanted lichens at the end of their exposure periods. However, the study did not measure parameters such as pigment degradation and changes to electrical conductivity which might have indicated lichen vitality loss (Godinho, Freitas, & Wolterbeek, 2004). Such measurements would have been useful to confirm whether the lower concentrations at the high exposure sites for the one-year transplants were related to loss of lichen vitality. It is therefore suggested that future lichen biomonitoring studies should measure the vitality of transplanted lichen thalli by either measuring chlorophyll content or by measuring the electrical conductivity of the leachate of the lichen material (Godinho, Freitas, & Wolterbeek, 2004).

The main drawback of this study was the different concentrations recorded in background *in situ* lichen samples, with several elevated concentrations that suggested a pollution influence and not just natural variability, at the control sites where lichens were collected for transplanting (Figure 5.8). This pollution effect before exposure possibly had a substantial influence on the metal accumulation values recorded in the lichen transplant bags, even if they were corrected for the background variability. This means that a new location for control sites for lichen collection should be found for any future lichen transplant studies in Collie.

## **5.5 Conclusions**

The transplant approach using *Usnea inermis* lichen bags revealed low heavy metal concentrations in the Collie air-shed. Despite the low concentrations, the dispersion patterns associated with one or more industrial point-sources in the area show that the Collie air-shed is influenced by industrial emissions. The transplant study also identified that the control sites at the Wellington National Park were affected by industrial emissions, possibly those from the Worsley alumina refinery. The study also indicated that exposure periods of 24 – 32 weeks (6 – 8 months) across the wet seasons would provide better results for lichen transplant experiments in Collie.

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## **Chapter 6 – General Discussion**

In this thesis the suitability of three lichen biomonitoring approaches for measuring the dispersion of heavy metal emissions from industrial point-sources in Collie, south-western Australia was evaluated. Studies involved a lichen community composition study (Chapter 2), heavy metal analysis of *in situ* lichens (Chapter 4) and heavy metal analysis of transplanted lichens (Chapter 5). The influence of gaseous emissions on lichens was also evaluated (Chapter 3), to determine any confounding effects on the heavy metal concentrations observed. In this synthesis chapter, emphasis is placed on temporal and spatial variations arising from the different biomonitoring techniques, strengths and weaknesses of each technique and the suitability of each for routine monitoring in a regional industrial setting in a native forest ecosystem characteristic of Western Australia.

### **6.1 Low heavy metal concentrations in the Collie air-shed**

Analysis of both *in situ* lichens (Chapter 4) and those in transplant bags (Chapter 5) showed low concentrations of heavy metals, suggesting that heavy metal emissions from the industrial sources in Collie were not substantial. However, these findings were unexpected, since Collie houses several coal-fired power stations the emissions of which are known to contain heavy metals. Several explanations were explored. The combined production capacity of the three coal-fired power stations in Collie was 1570 MW during the time of the study, comparable to the production capacity of other international locations where lichen heavy metal biomonitoring work has been carried out; for example the Soma coal-fired plant (1034 MW) in Turkey (Gur & Yaprak, 2011) and the Sines coal-fired power station (1180 MW) in Portugal (Freitas, 1994). However, the power stations in Turkey and Portugal did not have efficient dust collection mechanisms to reduce atmospheric emissions (Bellona Europa, n.d; Direskeneli, 2009), while both Collie and Bluewaters power stations in the current study area had cleaner technologies designed to reduce atmospheric emissions (EPA, 2003; Griffin Energy Pty Ltd, 2004, 2005), this may be an explanation for the lower metal concentrations recorded compared with other international locations.

The quality of the coal being burnt at coal-fired power stations can greatly influence the concentrations of pollutants in the atmosphere; therefore, ambient concentrations can

vary across different geographic areas (US EPA, 1993). For instance, the coal used in the Soma coal-fired power station was poor-quality lignite coal and is known to release more pollutants (Uslu & Gökmeşe, 2010), compared with the coal that is mined in Collie. Coal is broadly grouped into four categories; anthracite, bituminous, subbituminous, and lignite, and the coal fields in Collie produces subbituminous coal (Sappal, 1995) which has lower heating values, higher volatile matter, higher moisture and oxygen content, and low carbon and sulfur content. Burning of bituminous and subbituminous coal is much quicker and requires less energy; however, the gaseous emissions are much lower in subbituminous coal compared with bituminous coal, owing to its unique properties (US EPA, 1993). The subbituminous coal in Collie, therefore, releases less pollutants during the burning process, compared with other studies that used either bituminous or lignite coal, which might be another explanation for the low heavy metal concentrations recorded in the current lichen biomonitoring study.

Using lichens growing on dead stumps and logs rather than live trees may also have influenced the heavy metal concentrations recorded in the current study. Trees growing in the jarrah forests shed their bark regularly, so do not retain a rich lichen cover, which is why this study sampled lichens on fallen logs and old tree stumps for heavy metal analysis. Canopy leachate of trapped aerosol can introduce more pollutants to lichens growing on live trees (Hauck, 2011), compared with lichens growing on fallen logs and tree stumps in open canopy jarrah forests. Most lichen biomonitoring studies in other parts of the world have used live trees as substrates for lichen collection, which might have led to higher metal absorption levels, providing another possible explanation for the lower concentrations recorded in this study when compared with other studies in Turkey and Portugal which had similar emission levels (Freitas, 1994; Gur & Yaprak, 2011).

## 6.2 The influence of industrial sources and the inability to separate individual sources

The spatial dispersion maps from the *in situ* lichen analysis (Chapter 4) showed areas downwind from the coal mines and coal-fired power stations had higher heavy metal concentrations compared with other areas. The results from both *in situ* and transplant lichen studies (Chapters 4 and 5) also showed an impacted area downwind from the Worsley alumina refinery. These impact areas were also identified in the diversity indices maps in Chapter 2, where lower values for lichen diversity and abundance data were recorded at these locations. These findings confirm that industrial sources in Collie

were contributing pollutants to the air-shed. However, the experimental design and methodologies in the current study did not provide a means for identifying specific point-sources.

Biological monitoring can identify whether a problem exists, and can provide useful baseline data for further investigations to obtain more accurate measurements of ambient pollution levels (Siontorou & Batzias, 2011). A major disadvantage of biological monitoring is the inability to separate all processes that act simultaneously, and thus to isolate the effects of one particular source or pollutant (Batzias & Siontorou, 2006). In Collie, where several point-sources exist in close proximity, a key issue was the inability to separate the effects from individual sources, as well as the effects from coal mining versus coal power generation. The fallout of pollutants was mapped by considering the distance to the closest pollution source; however, since two or more pollution sources were in close proximity to each other, the fallout relationship with the closest point-source may have shown an interaction effect from one or more point-sources (Elsinger, Burrell, DeBruyn, Tanasichuk, & Timoney, 2007). Stable isotope analysis would allow the sources and pathways of metal pollution to be identified more clearly.

### 6.3 Pollution tolerance classes for lichens in Collie

The lichen community study (Chapter 2) recorded a higher distribution of known pollution-tolerant lichen species in the study area thus providing a basis for the classification of pollution-tolerant classes for lichens in jarrah forests of south-western Australia (Table 2.8), by comparing with pollution-tolerant classes established in other international locations (Ryan 1990, USFS, n.d.). Classification of lichens based on their response to air pollution has not been carried out for Australian lichens; the current study provided baseline data. Further investigations involving repeated surveys and more sampling sites would enable the confirmation of pollution-tolerance levels of lichen species found in the jarrah forests of WA and are essential before further biomonitoring studies are contemplated. Other processes needing further study include the natural succession of lichens and habitat competition amongst lichens, particularly post-fire, when new substrates and habitats are created. Over-time, different lichen groups will become established and may replace or even eliminate certain other species (Cranfield, Robinson, Williams, & Tunsell, 2011). The effect of fire on epiphytic communities in jarrah forests has not been studied to determine lichen succession and

adaptation patterns and their influence on community composition patterns (Cranfield et al., 2011). However, fire episodes are probably not so frequent in the jarrah forests as to have any direct influence on the establishment of pollution-tolerant lichen species in Collie from the production of aerosols from smoke plumes.

#### 6.4 Ecological importance of lichen community composition studies

Lichens are important components of forest ecosystems and their contribution to terrestrial diversity is substantial. Understanding their distribution, therefore, is important from an ecological perspective (Turner, Kirkpatrick, & Pharo, 2006; Will-Wolf, McCune, & Esseen, 2006). The study of lichen community composition provided a basis for understanding the response of forest ecosystems to air pollution; such data are useful for forest conservation and restoration in areas associated with industrial pollution. This is particularly important for managed forests, where harvesting and controlled burning practices can alter the diversity of forest habitats, resulting in a lower lichen-dominated, epiphytic diversity (Aragon, Martinez, Izquierdo, Belinchon, & Escudero, 2010). The interaction of forest management practices and atmospheric pollution can have added effects on lichen communities leading to higher disturbance, and there is a critical need to monitor and understand the level of disturbance to lichen communities in such situations. Research on the response of lichen communities found in open-canopy structures such as the jarrah forests of south-western Australia, is lacking in the literature. The higher light intensity and dust deposition in more open woodlands such as the jarrah forest can hold implications for lichen communities, especially if silvicultural practices have introduced more gaps in the canopy (Aragon et al., 2010). Although some studies have assessed the effects of harvesting and fragmentation on lichen communities in Western Australia (Cranfield et al., 2011), no studies have looked at the effect of atmospheric pollution on lichen community composition in this region. Therefore, the current study provided essential baseline information for future research on the effect of industrial pollution on lichen communities in WA.

#### 6.5 Lichen *Usnea inermis* as a biomonitor

This research identified the presence of suitable lichen species for heavy metal biomonitoring in the Collie area (Chapter 2). The lichen *Usnea inermis* was selected because it was ubiquitous in the jarrah forests of Collie, and its fruticose thallus facilitated sample collection, analysis and transplantation. *Usnea* spp. have been used as

biomonitors in other Mediterranean environments (Conti et al., 2012; Conti et al., 2009) and was identified as a pollution-tolerant lichen in Collie (Chapter 2, Table 2.8). This species was used as a biomonitor in both the *in situ* lichen analysis (Chapter 4) and the transplant study (Chapter 5) and proved to be effective for the role of determining heavy metal dispersion patterns in the Collie area. Although not explored as a biomonitor in the current study, the lichen *Hypogymnia subphysodes* was also identified as a potentially suitable species because of its foliose thallus structure, good pollution tolerance, and abundance in the jarrah forests in Collie. This species could be employed in future biomonitoring studies and simultaneous sampling of more than one lichen species would improve the reliability of the biomonitoring approach (Bergamaschi, Rizzio, Giaveri, Loppi, & Gallorini, 2007; Cercasov, Pantelica, Salagean, Caniglia, & Scarlat, 2002).

#### 6.6 Importance of season for pollution biomonitoring in Collie

Metal uptake by lichens in this study was favoured by cool temperatures and high rainfall, with higher metal uptake during periods of wet deposition. This trend was observed in both the *in situ* and transplanted lichen studies, where higher rainfall in autumn for *in situ* lichens (Chapter 4) and wet, winter conditions for transplant lichens (Chapter 5) probably led to the high concentrations of metals found during these seasons. Wet weather conditions support active growth in lichens, with increased metabolic activities resulting in efficient uptake of elements (Nash III & Gries, 1995). Occult precipitation (fog and dew) is also an important process that can increase the metal uptake by lichens, and it is formed under stable conditions such as the cold winter seasons in Collie. It may lead to pollutant concentrations orders of magnitudes higher than normal precipitation (Nash III & Gries, 1995). However, no data was available on fog and dew to confirm their influence on the heavy metal concentrations observed.

The transplant study (Chapter 5) also clarified that dry, summer conditions may result in an underestimation of industrial emissions of heavy metals in the Collie area, due to inefficient uptake during dry weather conditions. Autumn may also not be the ideal season for lichen biomonitoring, as the transplant study (Chapter 5) recorded lower concentrations of heavy metals compared with the other seasons, possibly due to the sudden hydration of lichens, after the dry summer conditions, that could have caused leaching of metals from the autumn transplants (Brown & Brown, 1991). Furthermore, the rainfall patterns in autumn were different in the two sampling years, where in 2010

during the *in situ* lichen biomonitoring (Chapter 4), Collie experienced higher than average rainfall in autumn, while in 2011 when the transplant study (Chapter 5) was carried out, rainfall was low in autumn. These conditions may have affected the accumulation of heavy metals by *Usnea inermis*, leading to higher and lower concentrations of heavy metals in the *in situ* and transplant biomonitoring experiments respectively. Clearly, differences in rainfall should be accounted for during biomonitoring studies (Frati, Brunialti, & Loppi, 2005). The large fluctuations in temperatures observed in autumn could also have affected the accumulation rate in lichens (Backor & Loppi, 2009). These findings indicate that seasonal effects need to be taken into account when using lichen biomonitoring to measure heavy metal pollution in Collie and it is recommended that the wetter and cooler winter conditions be used for long-term biomonitoring campaigns in the Collie area.

#### 6.7 The need for field measurements to validate predictive modelling data in Collie

The results of the current biomonitoring study showed different dispersion patterns for industrial emissions to those generated by The Air Pollution Model (TAPM) for the Collie area. This model considered both existing and proposed emissions sources, including the expansion of the Worsley alumina refinery that happened during the current study (GHD, 2009). However, the TAPM outputs are for short-term atmospheric loads and do not account for long-term trends. Differences were seen between the *a priori* classifications based on TAPM modelling and current study findings, where a majority of the ‘low exposure’ sites showed ‘high pollution’ signals (Chapters 2 & 4). Such areas were identified downwind from the Worsley alumina refinery, where the lichen populations in these ‘low exposure’ sites were scarce (Chapter 2), suggesting that the *a priori* classification using TAPM data was not appropriate for site selection. The TAMP modelling also did not show the Wellington National Park area as having a metal or gaseous pollution influence from the point-sources in Collie (Chapters 3, 4 & 5).

Although government regulations require industries to monitor and manage their atmospheric emissions, many agencies only make use of indirect methods as their monitoring tools, i.e. dispersion modelling. Because of the differences observed in the current study, there is a need to validate predictive modelling using field measurements, particularly when making management decisions on future expansions to existing industries in Collie. The use of precipitation collectors for direct measurement of heavy

metals may not be feasible for the continuous monitoring of pollutants on a large enough spatial scale, because of the high costs associated with this approach (Nelson, Shah, Strezov, Halliburton, & Carras, 2010). The lichen biomonitoring approach, established in this study, could provide a more cost-effective method for such continuous analysis for the Collie area. Mosses have also been suggested as effective heavy metal biomonitors (Aboal, Fernandez, Boquete, & Carballeira, 2010), and could also be explored for pollution monitoring for the Collie area. However, because of the seasonally dry Mediterranean climate in Western Australia, moss abundances would be low in the jarrah forests and therefore unsuitable for a biomonitoring role. Jarrah forests are also known to have low moss diversity when compared with lichens (Cranfield et al., 2011).

#### 6.8 Confounding effects on lichen heavy metal biomonitoring

Biomonitoring often reflects the cumulative effects of multiple stressors and therefore it is not possible to separate the effects of several pollutants occurring simultaneously, because of the synergistic interactions occurring in nature (Batziias & Siontorou, 2006; Siontorou & Batziias, 2011). The influence of such integrated responses can be determined by direct measurements for certain pollutants, particularly NO<sub>2</sub> and SO<sub>2</sub>, which have a strong influence on lichens (Batziias & Siontorou, 2006). This was determined in Chapter 3, where the Radiello<sup>®</sup> passive sampling results recorded low concentrations for both NO<sub>2</sub> and SO<sub>2</sub> pollutants in the Collie air-shed. However, clear dispersion patterns associated with point-sources indicated that there was some influence from gaseous pollutants emitted by industrial sources in the Collie air-shed. Seasonal concentrations and meteorological data in Chapter 3 also indicated that wet deposition is a key mechanism for the removal of these pollutants from the Collie atmosphere. Although the gaseous concentrations detected by the Radiello<sup>®</sup> samplers were low, the influence of these gaseous pollutants on lichen communities cannot be ignored, since lichens absorb nutrients from wet deposition (Chapters 4 and 5). Because pollutants in wet deposition were not detected by the Radiello<sup>®</sup> samplers, the passive sampling results could be an underestimation and may not give a true indication of influence from NO<sub>2</sub> and SO<sub>2</sub> on lichen communities in Collie. The establishment of SO<sub>2</sub> tolerant lichen species in the area (Chapter 2) also confirms that there is some influence from these gaseous pollutants on lichen communities in Collie.

## 6.9 Biomonitoring recommendations for Collie

Studying lichen community composition patterns (Chapter 2) provided information on the pollution status of the Collie air-shed. However, this technique required training in lichen identification and involved many hours in the field, making it unsuitable for routine monitoring. Furthermore, changes in community composition have a suggested time-delay and may not provide data on short-term emission impacts. So, while the lichen community study demonstrated that this method could be used to identify impacted areas, it is suited for monitoring long rather than short-term effects from industrial emissions. The analysis of *in situ* and transplant lichen tissues provide information on changes in pollution concentrations at shorter time scales (Branquinho et al., 2008), making these more practical for routine monitoring. However, there were several limitations in using the transplant approach. Firstly, when looking at metal accumulation by lichen transplants, the background concentrations (before exposure) were high for some heavy metals and showed seasonal variations, resulting in variable accumulation, particularly in the autumn season. Secondly, no clear patterns associated with pollution sources were found for the longer exposure periods (48 week transplants) and lower concentrations were recorded at the high exposure sites, indicating the possible loss of lichen vitality and leaching of pollutants from tissue (Fрати et al., 2005). Thirdly, the pollution signals recorded from the ‘control’ sites in this study, from which lichens were collected for transplantation, indicated that the lichen transplants had already been exposed to the pollutants before transplantation, and were therefore not representative of lichens collected from ‘pristine’ areas (Fрати et al., 2005). The ‘remembrance time’ of lichens used for transplantation may also have affected the metal accumulation rates at the new sites in which they were exposed (Godinho, Verburg, Freitas, & Wolterbeek, 2009). Therefore, it is suggested that the *in situ* lichen biomonitoring approach (Chapter 4) has the most potential for future biomonitoring studies in Collie. The relatively low concentrations of heavy metals in the Collie area make spatial analysis using this approach feasible. Spatial analysis of *in situ* lichens may not be suitable in other areas with very high pollution which lack native lichens, and in that case the transplantation approach would have to be used.

The use of lichens as a tool to monitor air pollution provides preliminary data on the level of pollutants in an air-shed, the identification of pollution sources, areas influenced by the pollutants, as well as providing a method for mapping the dispersion of pollutants in an affected area (Gur & Yaprak, 2011). Although the use of lichens may



not always be an ideal substitute for direct measurement of pollutants emitted by industries, it provides integrated and meaningful measures on the ecological consequences of pollutants. Such an approach may assist in addressing environmental concerns more effectively than direct measurement approaches (Batziias & Siontorou, 2006) since the impacts of the pollution exposure can be demonstrated.

#### 6.10 Concluding remarks

The Australian biota is facing a significant threat from the rapid mining and industrial advancements that have occurred in recent years. Emissions associated with coal power generation need special attention, particularly because coal combustion releases toxic and persistent heavy metals at elevated concentrations in the atmosphere. This direct confirmation of pollutant impacts and their spatial dispersion can be implemented through the use of lichen biomonitoring. Such approaches avoid the high costs associated with direct measurements using precipitation collectors. The *in situ* biomonitoring approach recommended in this study will allow for good spatial resolution and routine monitoring of industrial emissions, thus making the regulatory and compliance tasks more feasible for both industries and government agencies.

## 6.11 References

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## **Appendices**

## Chapter 2

Appendix 2.1: Percentage cover values averaged for each study site for the lichen species recorded in the community composition study; bold numbers indicate the most abundant lichen species for each study site)

Site ID	<i>Thy scu</i>	<i>Usn ine</i>	<i>Hyp sub</i>	<i>Cla rig</i>	<i>Cla cer</i>	<i>Pan wil</i>	<i>Cla sch</i>	<i>Hyp aus</i>	<i>Hyp sca</i>	<i>Pun pse</i>	<i>Ram sp.</i>	<i>Cla agg</i>	<i>Och sp.</i>	<i>Can xan</i>	<i>Cal sp.</i>	<i>Cal eli</i>	<i>Par con</i>	<i>Xan eli</i>	<i>Tep ale</i>	<i>Thy hoo</i>
Shotts 1	9.60	<b>19.70</b>	0.10	1.50	4.30				1.50		0.80	3.00				2.00				
Shotts 2	13.60	<b>40.50</b>	0.70	14.40	1.50		0.60		2.10							0.10		0.50		
Shotts 3	6.50	<b>30.80</b>	0.50	7.85	1.30	0.30	0.10		5.65											
Western 1	<b>24.00</b>	13.30	1.00	21.70	1.00		2.80			1.00	1.50	1.00	1.00						1.50	
Western 2	6.65	4.83	0.60	<b>14.00</b>			0.75		0.40	0.08				0.25		1.80	1.65			
Western 3	6.08	4.70	0.48	<b>6.81</b>	0.08		0.25		0.40	0.18	0.33			0.13						
Bristol 1	4.40	15.70	4.00	<b>16.20</b>		0.50		0.10	3.00		0.40	0.30				0.10		0.50	0.10	0.20
Wesfarmers 1	3.88	<b>15.15</b>	0.85	11.45	0.45	0.50		0.03	1.80	1.25	0.40		0.08			0.38	1.63			
Shotts 4	5.30	<b>46.00</b>		3.40	2.50		0.40		6.90		2.50	1.00				2.10				2.50
Shotts 5	7.85	<b>24.75</b>	0.53	7.38			0.78		4.38					1.00		0.25	1.00			
Shotts 6	6.43	<b>20.50</b>	0.18	8.05	1.50	0.25	0.48	0.88	0.28	1.25	0.20						0.88			0.15
Bristol 2	<b>18.00</b>	16.40	0.20	13.30	0.50	0.10	1.20	0.10	2.40		1.30							0.20		0.50
Western 4	10.20	14.60	1.10	<b>25.40</b>	3.00	0.10	1.00	0.50	1.00		0.60	3.00					10.00			0.40
Western 5	15.98	6.85	0.50	<b>26.83</b>	0.75	1.28	1.25		0.38		0.10		0.50						0.10	
Bristol 3	<b>26.48</b>	4.75	1.00	9.50			5.58		2.03											0.30
Shotts 7	6.60	<b>22.50</b>	2.90	21.80	0.50			0.50	8.00		0.30									0.90
Shotts 8	11.30	<b>15.70</b>	0.20	11.50		0.10	0.20		2.50		1.50					1.00				0.50
Shotts 9	2.84	12.40	3.68	<b>14.35</b>	1.00		1.00		1.02	0.08				0.30						
Fleays 1	0.50	3.60	0.70	<b>39.00</b>		3.00	0.30		2.10		0.10				0.80		0.30	0.10		
Fleays 2	0.60	8.50	4.00	<b>17.70</b>		7.30		0.20	0.80		1.00		0.10		0.40		7.50			
Proprietary 1	15.15	4.17	1.42	<b>19.07</b>	8.17	0.80	3.16	0.35	0.97		0.23		0.30						0.50	
Westralia 1	<b>43.00</b>	3.70	0.20	9.30		0.50	1.20		1.90								0.10	0.30		
Arklow 1	<b>34.15</b>	0.85	2.38	2.45	1.53		0.50		0.50											

Darrell 1	0.10	17.80	7.40	<b>19.00</b>		1.20			1.90					12.50		0.10
Darrell 2	5.50	13.25	1.78	<b>17.23</b>		0.30	0.75		0.65	2.00	0.05					
Arklow 2	<b>28.13</b>	0.13		2.00	0.13		0.33		0.13							
Bristol 4	3.90	15.00	14.80	<b>16.80</b>	0.10	1.90	0.50		2.30		3.10				0.10	0.10
Godfrey 1	2.55	<b>23.88</b>	8.25	9.23		0.30		0.45	0.95	16.25	1.45			0.10	10.18	
Leach 1	6.68	13.08	1.10	<b>14.20</b>	4.38	0.18	1.78	0.33	0.80	0.50		1.20		1.00		
Trees 1	2.10	<b>17.45</b>	1.85	11.88		0.90	1.50	0.30	0.75	4.15	0.70		0.20	0.05		5.30
Sherwood 1	0.88	6.30	10.75	<b>19.75</b>		2.13	0.75		2.00	2.25	1.50			1.00		9.25
Sherwood 2	3.78	9.50	10.13	<b>24.58</b>	0.20	12.00	0.20	0.05		2.80	1.30					2.00
Sherwood 3	3.23	12.88	<b>17.75</b>	15.00	0.20	0.53		0.25	0.65	9.33		0.20				
Gervasse 1		2.05	27.00	<b>29.35</b>	10.18	0.80			1.40							
Gervasse 2	2.00	<b>21.50</b>	13.80	18.60	0.15	8.60	0.50		1.40		0.90				1.50	0.50
Gervasse 3	0.30	8.40	<b>18.40</b>	17.30		3.70	1.50		1.10		2.00		0.20		0.10	0.20 0.10
Sites with Highest % Cover	6	12	2	16												

*Thy scu* - *Thysanothecium scutellatum*; *Usn ine* - *Usnea inermis*; *Hyp sub* - *Hypogymnia subphysodes*; *Cla rig* - *Cladonia rigida*; *Cla cer* - *Cladonia cervicornis* var. *Vetricillata*; *Pan wil* - *Pannaparmelia wilsonii*; *Cla sch* - *Cladia schizopora*; *Hyp aus* - *Hypocenomyce australis*; *Hyp sca* - *Hypocenomyce scalaris*; *Pun pse* - *Punctelia pseudocoralloidea*; *Ram sp.* - *Ramboldia sp.*; *Cla agg* - *Cladia aggregate*; *Och sp.* - *Ochrolechia sp.*; *Can xan* - *Candelariella xanthostigmoides*; *Cal sp.* - *Calicium sp.*; *Cal eli* - *Caloplaca elixii*; *Par con* - *Parmelina conlabrosa*; *Xan eli* - *Xanthoparmelia elixii*; *Tep ale* - *Tephromela alectoronica*; *Thy hoo* - *Thysanothecium hookeri*;

Appendix 2.2: Ecological diversity indices values for individual study sites

Site Name	Shannon-Wiener (H)	Simpson's Index (D)	Species Richness (S)	Species Evenness (E)	IAP	Percentage Cover
Shotts 1	1.58	3.49	9	0.72	29	42.50
Shotts 2	1.27	2.68	9	0.58	39	<b>74.00<sup>a</sup></b>
Shotts 3	1.27	2.59	8	0.61	35	53.00
Western 1	1.64	3.93	11	0.69	36	69.80
Western 2	1.58	3.55	10	0.68	40	31.00
Western 3	1.47	3.56	10	0.64	40	<b>19.41<sup>b</sup></b>
Bristol 1	1.59	3.74	<b>13<sup>a</sup></b>	0.62	38	45.50
Wesfarmers 1	1.66	3.72	<b>13<sup>a</sup></b>	0.65	47	37.83
Shotts 4	1.39	2.37	10	0.60	46	72.60
Shotts 5	1.45	3.06	9	0.66	40	47.90
Shotts 6	1.53	3.16	<b>13<sup>a</sup></b>	0.60	44	41.00
Bristol 2	1.54	3.77	12	0.62	41	54.20
Western 4	1.81	4.64	<b>13<sup>a</sup></b>	0.71	48	70.90
Western 5	1.35	2.89	11	0.56	40	54.50
Bristol 3	1.36	2.90	7	0.70	36	49.63
Shotts 7	1.53	3.73	9	0.70	38	64.00
Shotts 8	1.54	3.84	10	0.67	35	44.50
Shotts 9	1.51	3.50	9	0.69	41	36.66
Fleays 1	0.94	1.65	11	0.39	38	50.50
Fleays 2	1.74	4.51	11	0.72	42	48.10
Proprietary 1	1.73	4.26	12	0.70	47	54.28
Westralia 1	0.98	1.85	9	0.45	32	60.20
Arklow 1	0.80	1.52	7	0.41	27	42.35
Darrell 1	1.54	4.07	9	0.70	40	60.30
Darrell 2	1.46	3.37	9	0.66	43	41.50
Arklow 2	<b>0.38<sup>b</sup></b>	<b>1.19<sup>b</sup></b>	<b>6<sup>b</sup></b>	<b>0.21<sup>b</sup></b>	<b>21<sup>b</sup></b>	30.83
Bristol 4	1.70	4.52	11	0.71	48	58.60
Godfrey 1	1.79	4.92	11	0.75	45	73.58
Leach 1	1.81	4.60	12	0.73	49	45.20
Trees 1	1.82	4.42	<b>13<sup>a</sup></b>	0.71	51	47.13
Sherwood 1	<b>1.88<sup>a</sup></b>	<b>4.93<sup>a</sup></b>	11	<b>0.79<sup>a</sup></b>	45	56.55
Sherwood 2	1.76	4.57	11	0.73	38	66.53
Sherwood 3	1.63	4.48	10	0.71	49	60.00
Gervasse 1	1.24	2.95	<b>6<sup>b</sup></b>	0.69	34	70.78
Gervasse 2	1.70	4.46	11	0.71	46	69.45
Gervasse 3	1.61	3.89	12	0.65	<b>58<sup>a</sup></b>	53.30
<b>Mean</b>	<b>1.49</b>	<b>3.53</b>	<b>10.22</b>	<b>0.64</b>	<b>40.72</b>	<b>52.73</b>
<b>SD</b>	<b>0.31</b>	<b>0.98</b>	<b>1.93</b>	<b>0.11</b>	<b>7.21</b>	<b>13.45</b>

N = 36, <sup>a</sup> = maximum value, <sup>b</sup> = minimum value



Appendix 2.3: Lichen community study data based on different growth forms; distance to closest pollution source; site characteristics and forest management data (n=36)

Site ID	Total P% Cover Based on Growth Form				Closest PS		Site Characteristics				Forest Management Variables <sup>‡</sup>		
	Fruticose	Squamulose	Foliose	Crustose	Distance (Km)	Closest PS	% Canopy Cover	Tree Density (km <sup>-2</sup> )	Log Density (km <sup>-2</sup> )	Av. Trunk Dia.	Harvest	Fire History	Block Size (Ha)
Shotts 1	11.6	26.4	4.5	0.0	2.1	Muja	10	19.4	5.0	42.3	1970-79	-	6140.22
Shotts 2	13.7	44.8	15.0	0.5	3.4	Muja	20	35.0	6.9	35.3	1940-49	1990-1991	6140.22
Shotts 3	6.8	38.3	8.0	<b>0.0</b>	4.5	Muja	15	32.5	10.0	40.2	1980-89	1990-1991	6140.22
Western 1	24.0	16.8	26.5	2.5	5.7	Muja	20	30.6	9.4	36.8	1970-79	1991-1992	5106.19
Western 2	8.5	6.1	14.8	1.7	5.8	Collie	25	25.6	1.9	51.2	1970-79	2005 Sp	5106.19
Western 3	6.1	6.1	7.2	<b>0.0</b>	7.3	Muja	15	42.5	1.9	41.6	1970-79	2005 Sp	5106.19
Bristol 1	5.0	23.1	16.5	0.9	10.7	BW	35	31.9	7.5	31.8	1980-89	2001 Sp	5705.71
Wesfarmers 1	4.8	18.7	12.7	1.7	6.0	Muja	15	21.9	2.5	37.2	1930-39	-	-
Shotts 4	7.4	<b>57.9</b>	4.8	2.5	1.9	Muja	20	29.4	5.0	28.1	1970-79	1990-1991	6140.22
Shotts 5	8.1	30.7	8.2	1.0	4.7	Muja	10	42.5	3.8	31.2	1970-79	1983-1984	6140.22
Shotts 6	6.7	22.7	9.8	1.9	6.7	Muja	20	130.0	5.6	34.5	1990-99	2008 Sp	6140.22
Bristol 2	18.1	20.8	14.5	0.8	10.4	Muja	20	30.0	-	27.0	1980-89	1990 Sp	5705.71
Western 4	10.3	20.3	29.4	10.9	6.7	Muja	15	43.8	8.1	38.7	1970-79	2004 Sp	5106.19
Western 5	17.3	8.6	28.1	0.6	8.2	Collie	10	26.9	4.4	36.1	1980-89	2007 Sp	5106.19
Bristol 3	26.5	7.8	15.1	0.3	9.7	BW	20	54.4	6.3	37.3	1960-69	2001 Sp	5705.71
Shotts 7	6.6	34.2	21.8	1.4	4.0	Muja	25	26.9	7.5	33.1	2000-2009	1987-1988	6140.22
Shotts 8	12.4	19.9	11.7	0.5	6.5	Collie	25	42.5	5.6	36.6	1970-79	1985-1986	6140.22
Shotts 9	2.8	18.4	15.4	<b>0.0</b>	3.5	Collie	25	79.4	6.9	30.5	1970-79	1995-1996*	6140.22
Fleays 1	3.5	6.5	<b>40.1</b>	0.4	9.6	Muja	15	35.6	10.6	42.2	1950-59	2005-2006	5603.72
Fleays 2	7.9	14.3	18.1	7.8	8.0	Collie	20	35.0	-	35.5	2000-2009	2006 Sp	5603.72
Proprietary 1	16.0	15.0	22.2	1.2	3.9	BW	20	16.9	3.8	51.2	1970-79	2003 Au	3273.38
Westralia 1	<b>43.5</b>	5.8	10.5	0.4	10.7	BW	25	35.6	-	44.7	1920-29	2009 Sp	1349.71
Arklow 1	34.2	5.3	3.0	<b>0.0</b>	10.6	BW	25	46.3	-	30.2	1970-79	2004 Sp	4932.13

Darrell 1	1.3	27.4	19.0	<b>12.6</b>	6.3	BW	30	61.3	3.1	50.3	1930-39	2005 Sp	3198.62
Darrell 2	5.8	15.7	20.0	<b>0.0</b>	10.1	BW	30	68.8	-	49.5	1940-49	2002 Sp	3198.62
Arklow 2	28.1	<b>0.4</b>	<b>2.3</b>	<b>0.0</b>	12.5	BW	30	63.1	-	33.4	1970-79	2004 Sp	4932.13
Bristol 4	5.8	35.3	17.3	0.2	15.9	BW	30	30.0	11.3	40.2	1970-79	2000 Sp	5705.71
Godfrey 1	3.0	34.5	25.5	10.6	14.0	Collie	25	55.6	-	43.5	2000-2009	2002 Sp	11851
Leach 1	6.9	20.4	17.7	0.3	15.5	Collie	35	24.4	3.1	42.2	1970-79	1994-1995	7450.67
Trees 1	3.0	20.8	17.5	5.8	15.0	Collie	25	37.5	5.0	43.9	1940-49	2004 Au	6241.6
Sherwood 1	3.0	20.6	23.8	9.3	19.3	Muja	25	21.3	3.8	57.6	1950-59	2002 Sp	4409.58
Sherwood 2	15.8	21.1	27.6	2.1	18.8	Muja	30	46.3	3.8	51.5	1950-59	2002 Sp	4409.58
Sherwood 3	3.8	31.5	24.5	0.3	20.2	Muja	25	38.1	7.5	36.9	1940-49	1999-2000*	4409.58
Gervasse 1	<b>0.8</b>	40.6	29.4	<b>0.0</b>	25.5	BW	70	46.9	5.0	43.0	1920-29	2000 Sp	6445.21
Gervasse 2	10.6	37.8	19.1	2.0	24.5	BW	60	66.9	-	47.0	1930-39	1988-1989	6445.21
Gervasse 3	4.1	29.9	18.8	0.5	24.9	BW	60	43.8	8.1	49.2	1950-59	1999 Sp	6445.21

Total % - Total percentage lichen cover data; IAP – Index of Atmospheric Purity; PS – Power Station (BW – Bluewaters); Harvest – Year last harvested; Fire History – Year of last prescribed burn with Season (Sp - Spring, Au - Autumn Season, \*Denotes Wildfire); Block Size – Forest Fragment Size; † - All data obtained from the Department of Environment and Conservation; Site characteristics were measured within a block of 40 x 40 m for each site and average values considered

Appendix 2.4: Spearman's correlation results for diversity indices with the distance to the closest mine, closest power station and the Worsley alumina refinery

		<b>Closest Mine</b>	<b>Closest PS</b>	<b>Worsley Refinery</b>
Shannon-Wiener Index	Correlation Coefficient	<b>0.334</b>	0.247	0.210
	Sig. (2-tailed)	0.047	0.147	0.220
Simpson's Index	Correlation Coefficient	<b>0.407</b>	<b>0.349</b>	0.121
	Sig. (2-tailed)	0.014	0.037	0.483
Species Richness	Correlation Coefficient	0.069	0.189	0.217
	Sig. (2-tailed)	0.690	0.269	0.204
Species Evenness	Correlation Coefficient	<b>0.496</b>	0.264	0.190
	Sig. (2-tailed)	0.002	0.120	0.266
IAP	Correlation Coefficient	<b>0.365</b>	0.223	0.163
	Sig. (2-tailed)	0.029	0.192	0.341
Lichen % Cover	Correlation Coefficient	0.069	0.189	0.217
	Sig. (2-tailed)	0.690	0.269	0.204

Bold numbers indicate statistically significant correlations at P<0.05

### Chapter 3

#### Appendix 3.1: Quality assurance test (duplicate analysis) results for calibration standards for NO<sub>2</sub> analysis

<b>Sample ugL<sup>-1</sup></b>	<b>Sample ugL<sup>-1</sup></b>	<b>Duplicate ugL<sup>-1</sup></b>	<b>Relative % Difference %</b>
Calibration Standard 50	52.4	55.5	5.9%
Calibration Standard 20	21.5	20.1	7.0%
Calibration Standard 5	4.9	4.9	0.0%
Calibration Standard 0	-0.5	-0.5	0.0%

#### Appendix 3.2: Quality assurance test (duplicate analysis) results for calibration standards for SO<sub>2</sub> analysis

<b>Sample ug/L</b>	<b>Sample ug/L</b>	<b>Duplicate ug/L</b>	<b>Relative % Difference %</b>
Calibration Standard 2	2.1	2	5.0%
Calibration Standard 1	1.1	1.1	0.0%
Calibration Standard 0.5	0.48	0.49	2.1%
Calibration Standard 0.2	0.19	0.17	11.8%
Calibration Standard 0	-0.1	-0.1	0

Appendix 3.3: Source characteristics and emission rates for existing pollution sources in Collie (GHD, 2009)

	<b>Muja A</b>	<b>Muja B</b>	<b>Muja C</b>	<b>Muja D</b>	<b>Collie A</b>	<b>Bluewaters I</b>	<b>Bluewaters II</b>	<b>Worsley PS</b>
MGA94 Easting (m)	435,785	435,734	435,636	435,525	431,227	428,126	428,202	413,242
MGA94 Northing (m)	6,298,979	6,299,001	6,299,074	6,299,109	6,310,439	6,311,651	6,311,609	6,322,257
Stack height (m)	98	98	151	151	170	100	100	76
Stack tip diameter (m)	3.94	3.94	5.91	5.91	5.23	4.00	4.00	4.00
Exit velocity (m/s)	19.0	19.0	20.4	19.0	24.4	24.0	24.0	23.7
Exit temperature (°C)	200	200	133	133	152	131	131	130
<b>Emission rate (g/s)</b>								
Sulphur dioxide	269	269	779	779	515	230	230	315
Oxides of nitrogen	256	256	399	399	158	112	112	110
Nitrogen dioxide	25.6	25.6	39.9	39.9	15.8	11.2	11.2	11.0
Particulate matter as PM <sub>10</sub>	538	538	13.8	13.8	6.1	9.4	9.4	2.9
Particulate matter as PM <sub>2.5</sub>	140	140	5.9	5.9	2.6	5.5	5.5	1.6
Carbon monoxide	5.7	5.7	16.4	16.4	126	112	112	11
Ammonia	0.0064	0.0064	0.0183	0.0183	0.0119	0.0085	0.0085	0.0079

Appendix 3.4: Compliance with NEMP Standards based on existing, approved and proposed industrial point-sources in Collie (GHD, 2009)

Pollutant	Averaging period <sup>22</sup>	Criteria	Basis	Predicted maximum concentration ( $\mu\text{g}/\text{m}^3$ )		
				Across model domain	% of criteria	Compliance
Sulphur dioxide	1-hour	572	Air NEPM	2,934.9	513%	✘
	24-hour	229	Air NEPM	211.4	92%	✔
	Annual	57	Air NEPM	12.0	21%	✔
Nitrogen dioxide	1-hour	247	Air NEPM	321.3	130%	✘
	Annual	62	Air NEPM	11.8	19%	✔

## Chapter 4

### Appendix 4.1: ICP –MS Instrument specifications and operational conditions

**Full Name:** Inductively coupled plasma mass spectrometer

**Manufactory:** Perkin Elmer

**Model:** Elan DRC II

#### Operational conditions:

RF power: 1500 watts

Nebulizer gas flow: 0.85-1.05 L/min

Plasma gas flow: 15 L/min

Auxiliary gas flow: 1.2 L/min

Ion lens voltage: 4-10 volts

Quadrupole rod offset : 0 volts

Detector voltage: pulse stage: 800 volts

Analogue stage: -1700 volts

**Internal standards:** 5 ugL<sup>-1</sup> In 115 & Ir 193

**Conc. of calibration standards:** 5, 10 and 20 ugL<sup>-1</sup>

**Correlation coefficient (R<sup>2</sup>):** >0.99

**Laboratory control sample:** EPA Method 200.7 Standard 6 Solution A

Cat. # ICP-200.7-6.

LOT # 1008403

Appendix 4.2: Elemental spike recovery (percentage) for metals in blank spikes and sample spikes averaged for both sampling campaigns

<b>Metal</b>	<b>Blank Spike 1</b>	<b>Blank Spike 2</b>	<b>Blank Spike 3</b>	<b>Sample Spike 1</b>	<b>Sample Spike 2</b>	<b>Sample Spike 3</b>
	<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>
<b>As</b>	89.4	86.9	87.6	92.2	92.9	92.1
<b>Cd</b>	92.5	94.4	93.4	91.7	95.7	88.4
<b>Cr</b>	84.6	87.6	88.7	90.7	97.0	90.7
<b>Cu</b>	86.5	88.2	83.8	87.4	89.3	88.9
<b>Pb</b>	100.6	98.5	96.8	97.0	99.2	100.4
<b>Mn</b>	94.3	94.9	97.2	92.9	55.6	71.3
<b>Hg</b>	66.4	69.9	62.1	65.9	87.2	78.7
<b>Ni</b>	89.0	90.0	89.9	90.4	88.6	88.5
<b>Zn</b>	91.0	90.9	99.3	95.5	99.4	86.3

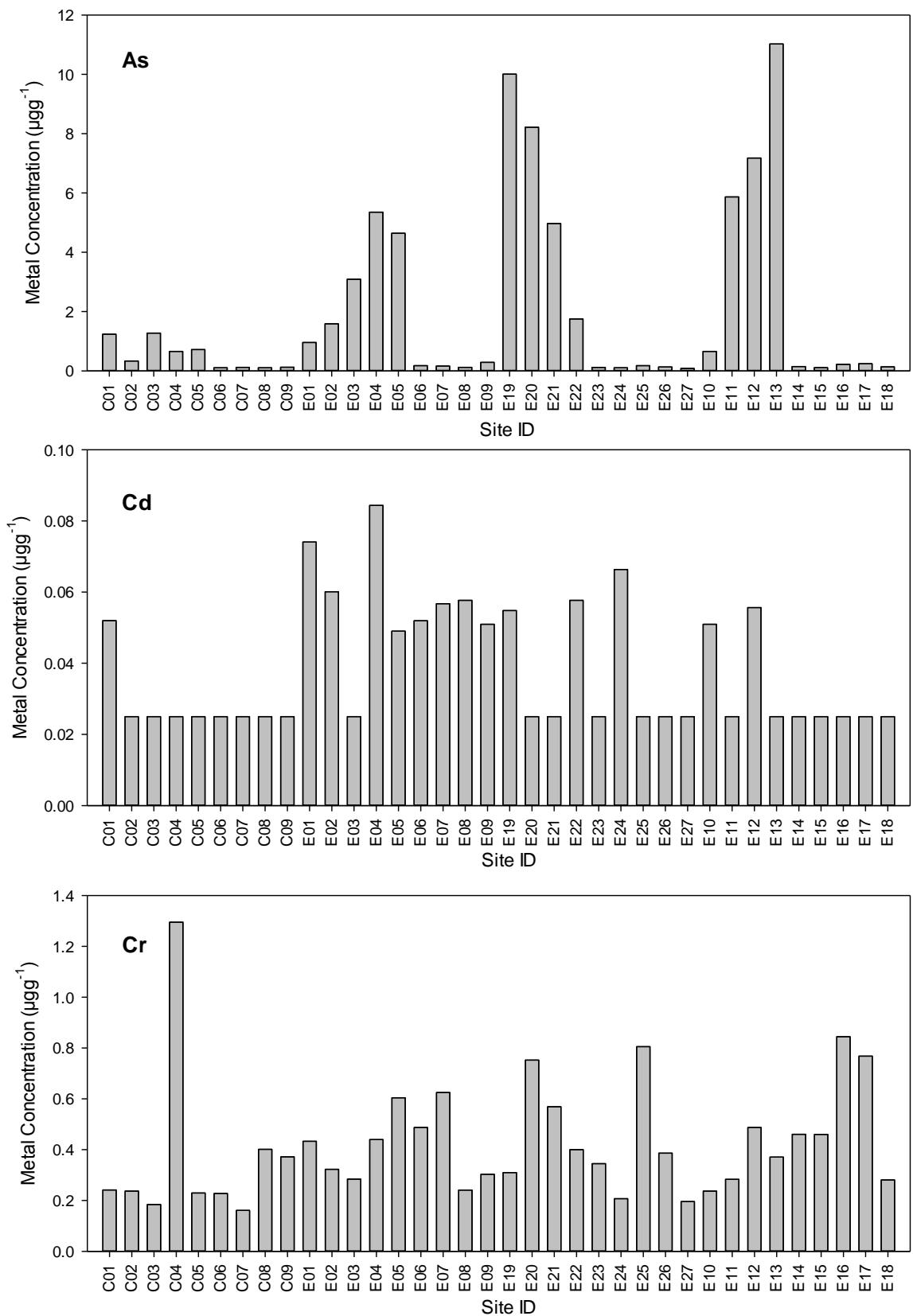


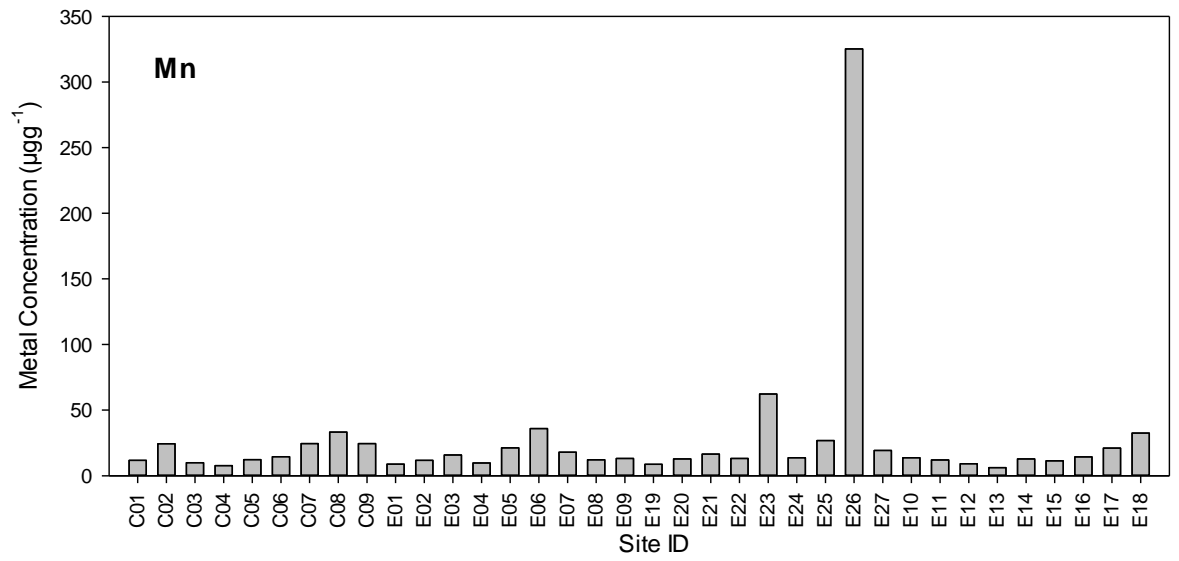
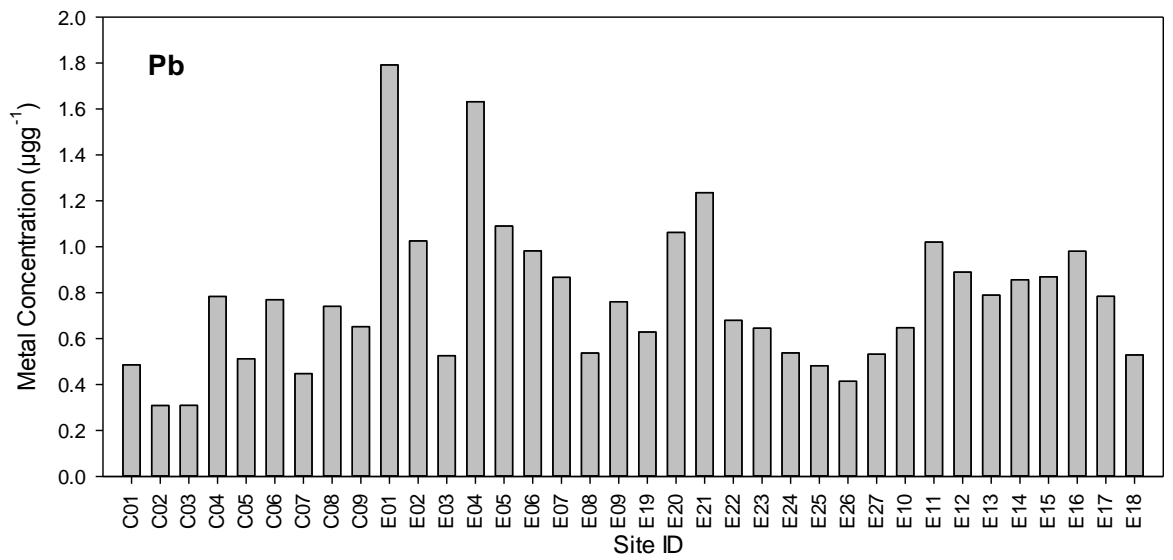
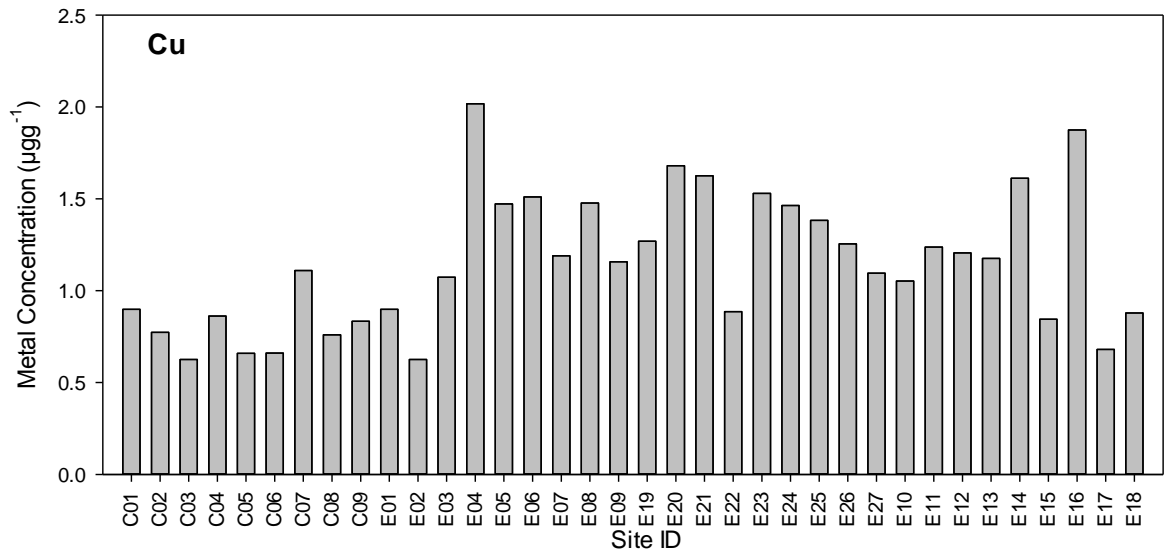
Appendix 4.3: NMI quality assurance report for all sample analysis batches for both spring and autumn sampling campaigns

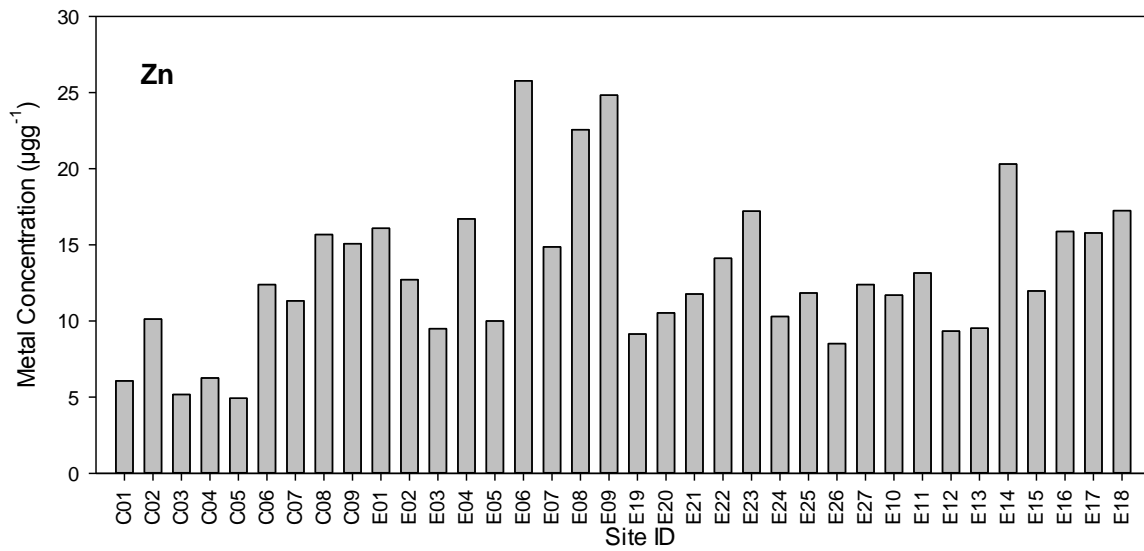
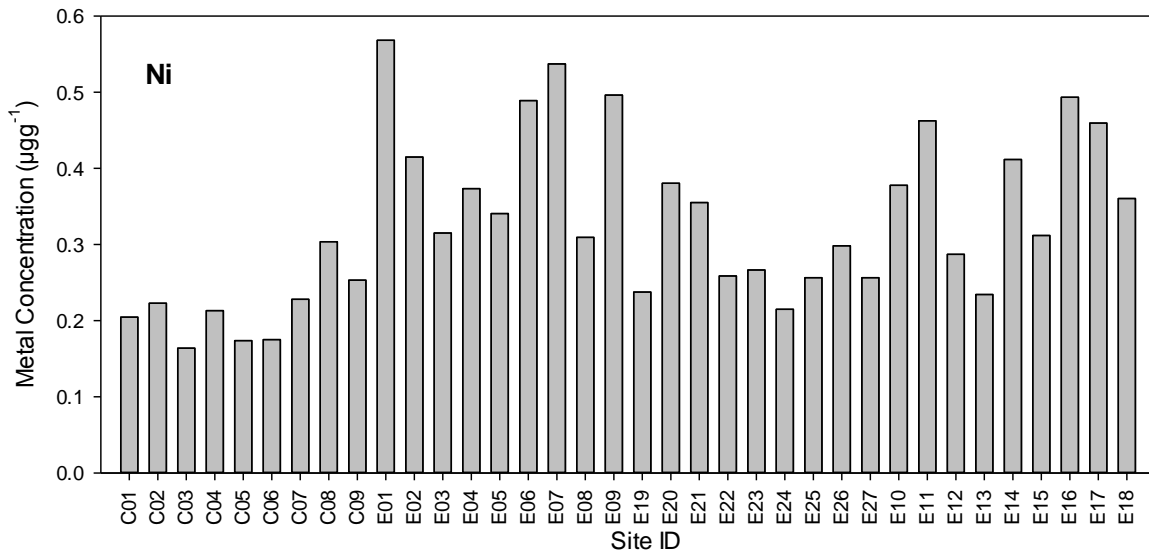
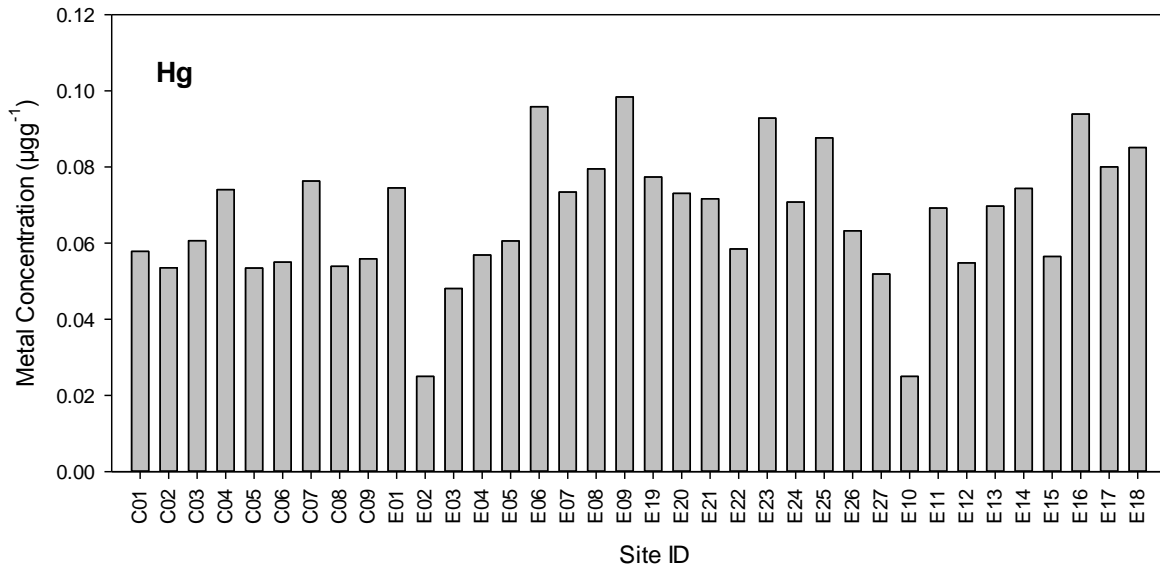
Analyte	Reporting Limit	Blank	Spring Campaign - Batch 1					Spring Campaign - Batch 2					Autumn Campaign				
			Duplicates			Recoveries		Duplicates			Recoveries		Duplicates			Recoveries	
			Sample	Dup.	% Diff.	Lab Control	Matrix Spike	Sample	Dup.	% Diff.	Lab Control	Matrix Spike	Sample	Dup.	% Diff.	Lab Control	Matrix Spike
µg/L	µg/L	µg/L	µg/L	%	%	%	µg/L	µg/L	%	%	%	µg/L	µg/L	%		%	
Arsenic	0.5	<0.5	5.6	5.2	7	110	#	86	88	2	95	98	3.9	3.9	0	94	104
Cadmium	0.5	<0.5	<0.5	<0.5	ND	89	89	89	91	2	99	95	<0.5	<0.5	ND	95	101
Chromium	0.5	<0.5	4.7	5	6	82	98	80	79	1	97	107	11	11	0	99	101
Copper	0.5	<0.5	22	21	5	117	98	100	100	0	107	98	18	18	0	93	99
Lead	0.5	<0.5	17	17	0	89	101	93	95	2	96	103	18	17	6	97	98
Manganese	0.5	<0.5	100	100	0	97	106	110	110	0	97	98	300	290	3	99	97
Mercury	0.5	<0.5	<0.5	<0.5	ND	92	83	58	62	7	92	#	0.95	0.77	21	95	101
Nickel	0.5	<0.5	6.8	7	3	93	92	94	94	0	101	95	8.9	8.8	1	98	106
Zinc	0.5	0.86	210	210	0	103	97	280	280	0	103	105	120	120	0	90	105

ND - Not Determined; NA - Not Applicable; # - Spike level is less than 50% of the sample's concentration, hence the recovery data is not reliable

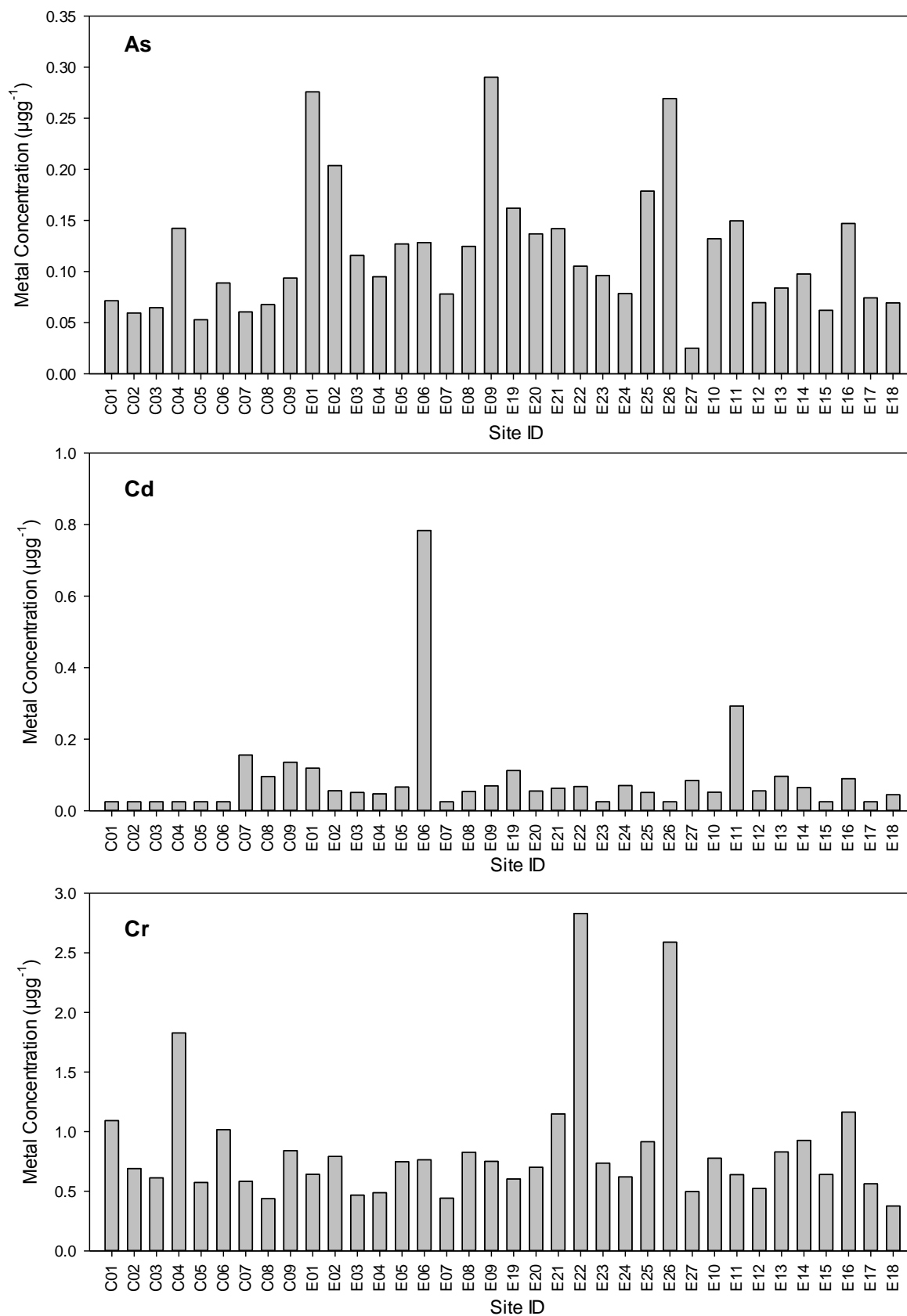
Appendix 4.4: Graphical representation of mean heavy-metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) for each study site for the spring sampling season

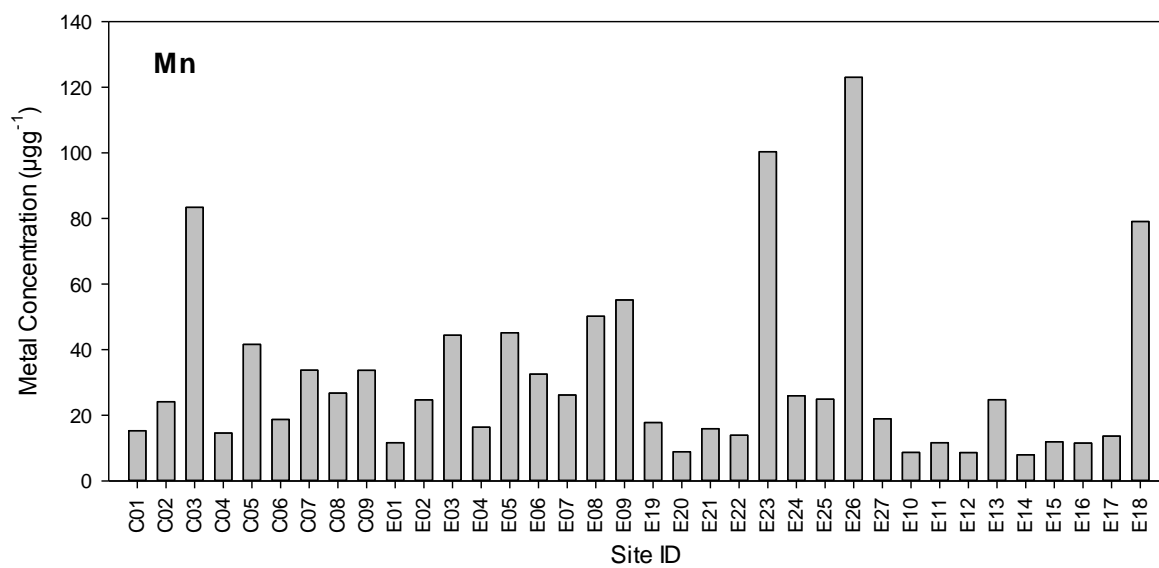
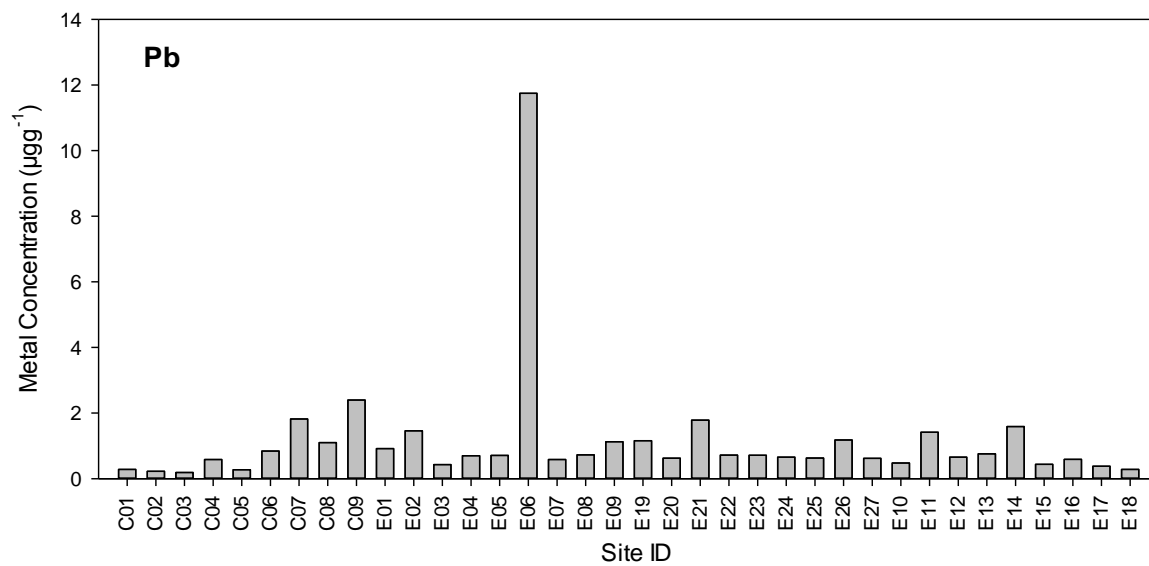
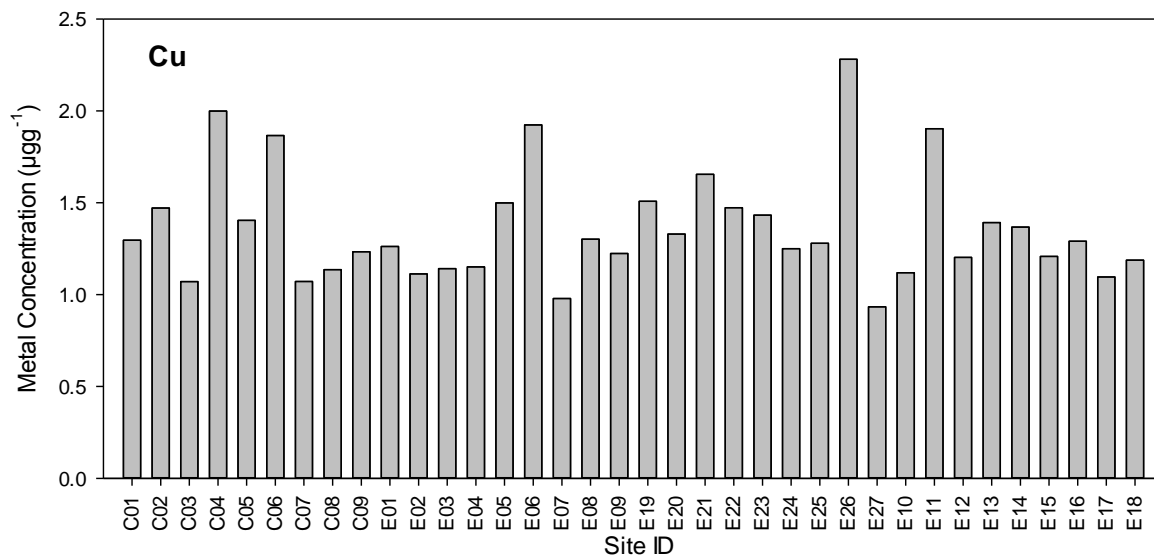


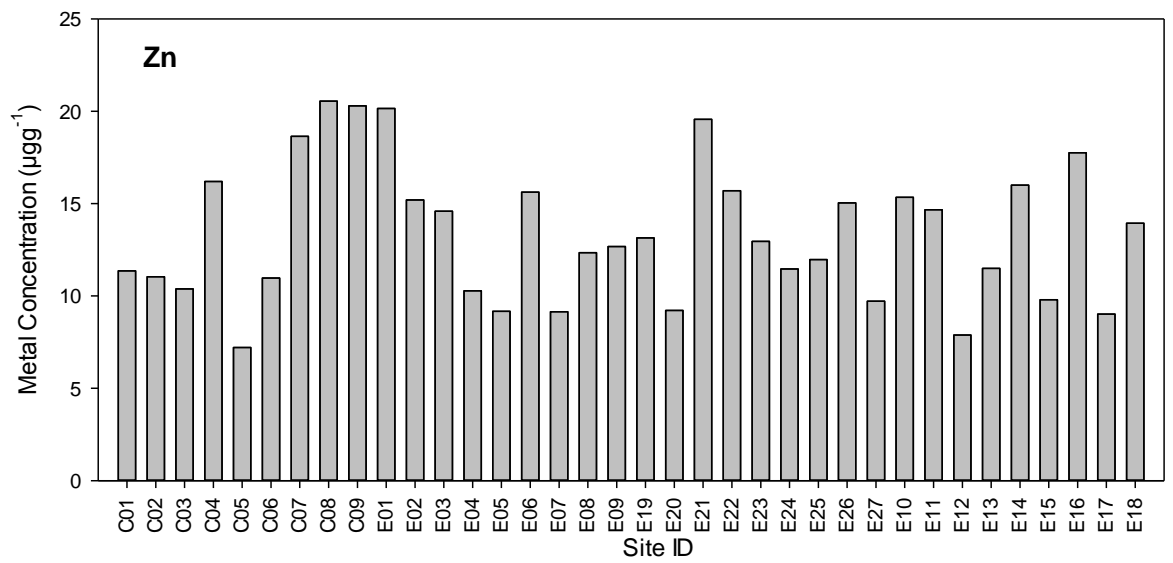
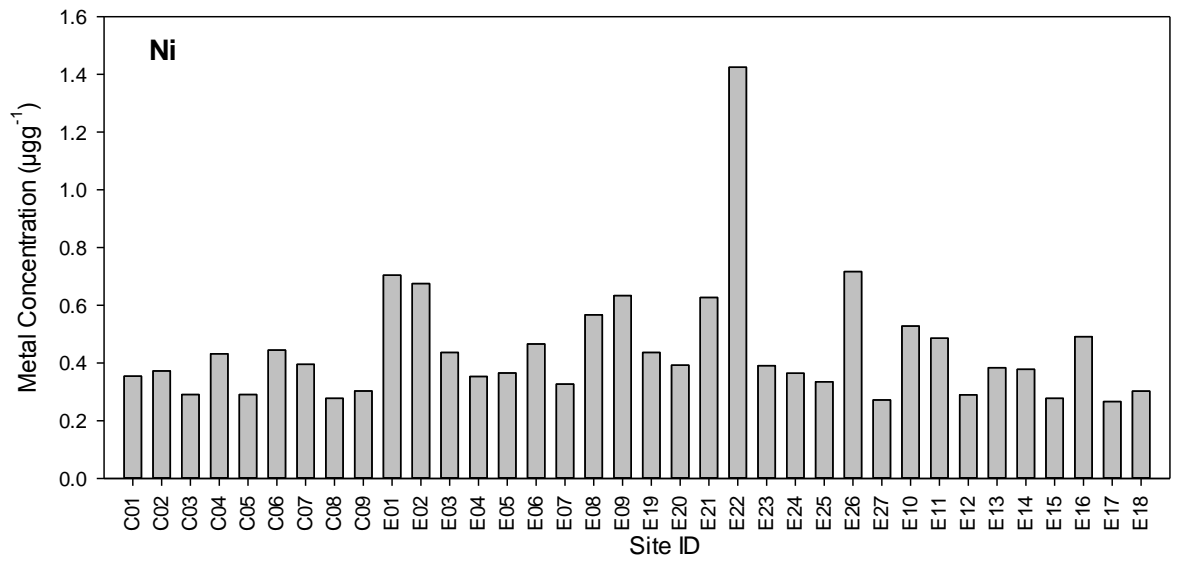
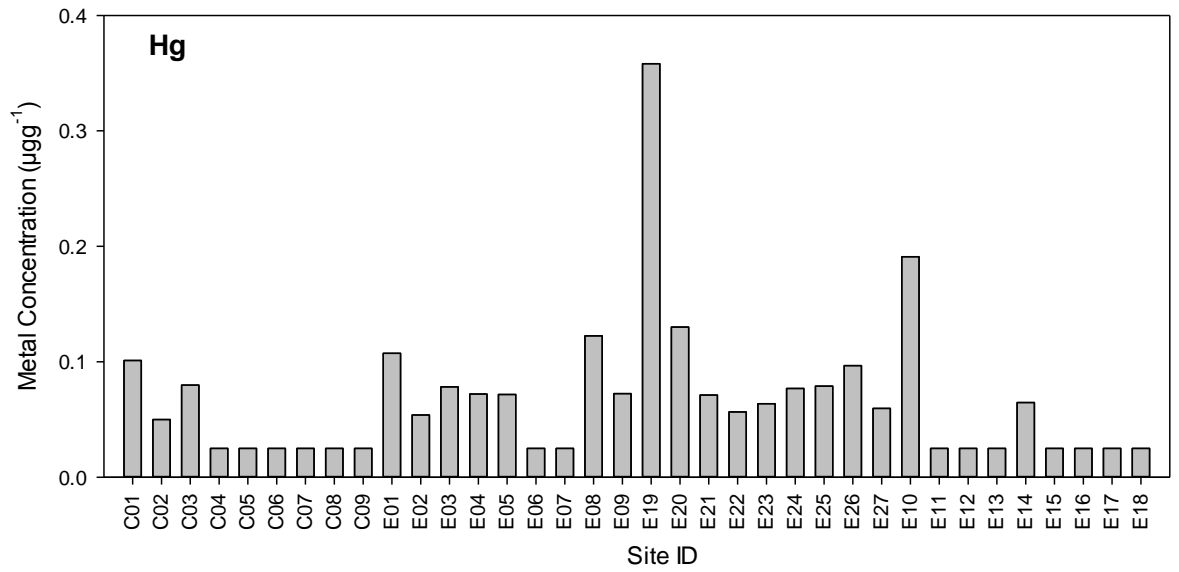




Appendix 4.5: Graphical representation of mean heavy-metal concentrations ( $\mu\text{g g}^{-1}$  dry weight) for each study site for the autumn sampling season







Appendix 4.6: Air quality standards/guidelines for heavy metals issued by various international agencies

Metal	Standard	Averaging Period	Short term		Long term	
			(in $\mu\text{gm}^{-3}$ )	(in ppb)	(in $\mu\text{gm}^{-3}$ )	(in ppb)
Arsenic	OAQC <sup>a</sup>	24 hour av.	0.3	$1 \times 10^{-7}$	0.01	$3 \times 10^{-9}$
Cadmium	TCEQ <sup>b</sup>	Short term health	0.1	$2 \times 10^{-8}$	0.01	$2 \times 10^{-9}$
Chromium	WHO <sup>c</sup>	(not given) <sup>1</sup>	0.13	$6 \times 10^{-8}$	0.012	$5 \times 10^{-9}$
Copper	OAQC	24 hour av.	50	$2 \times 10^{-5}$	1	$4 \times 10^{-7}$
Lead	OAQC & EU <sup>d</sup>	24 hour	0.53	$6 \times 10^{-8}$	0.154	$2 \times 10^{-8}$
Manganese	TCEQ	Short term health	2	$9 \times 10^{-7}$	0.2	$9 \times 10^{-8}$
Mercury	OAQC	24 hour av.	2	$2 \times 10^{-7}$	-	-
Nickel	TCEQ	Short term health	1.1	$5 \times 10^{-7}$	0.059	$2.5 \times 10^{-8}$
Zinc	OAQC	24 hour av.	120	$4.5 \times 10^{-5}$	5	$2 \times 10^{-6}$

<sup>a</sup>Ontario Ministry of the Environment Ambient Air Quality Criteria; <sup>b</sup>Texas Commission on Environmental Quality;  
<sup>c</sup>World Health Organization Guidelines for Air Quality; <sup>d</sup>European Commission Air Quality Standards;



Appendix 4.7: Selected references of heavy metals ( $\mu\text{g/g}$  dry weight) studied on lichen species from different geographical areas (mean values and concentration ranges given)

Country	Pollution Source	Lichen Species	Analytical Method	As	Cd	Cu	Pb	Mn	Ni	Zn	Reference
Kosovo	Depleted uranium (ammunition) repository (n=5)	Various species	ICP-MS	3.05 0.91–11.61	1.08 0.41–3.48	37.73 11.4–188.6	48.67 9.1–282.1	–	13.51 3.2–48.7	149.53 8–272	Di Lella et al. (2003)
Russia – Norilsk	Heavy metal smelting complex (n=90)	<i>Cetraria cucullata</i>	ICP-MS <sup>#</sup>	0.22 0.11–0.44	0.12 0.0–0.38	1.52 1.12–12.79	1.26 0.78–5.79	–	1.52 0.83–10.20	20.5 9.73–29.6	Allen-Gil et al. (2003)
Central Italy	Geothermal fields & abandoned sulphide ore smelting plants (n=84)	<i>Parmelia caperata</i>	ICP-AES	0.36 0.12–1.27	0.26 0.06–0.69	5.77 3.94–9.17	3.88 0.68–11.20	–	2.65 1.03–8.00	34.7 25.90–57.70	Bargagli et al. (2002)
Manisa, Turkey	Coal-fired power plant (n=44)	<i>Xanthoria parietina</i>	ICP-MS*	12.8 5.2–30.2	0.4 0.16–1.53	17.3 7.2–34.2	22.01 7.68–45.27	–	14.2 5.9–34.0	104.4 50.0–239.9	Gur & Yaprak, (2011)
Eastern Macedonia	Copper mine (n=50)	<i>Hypogymnia physodes/Evernia prunastri</i>	ICP-AES & ETAAS*	0.74 0.10–3.8	0.11 0.05–0.38	7.9 1.5–130	4.3 0.61–120	51 14–150	2.6 1.5–10	20 10–39	Balbanova et al. (2012)
Northern Canada	Remote region (n=12)	Various species	ICP-MS & INAA	0.42 0.15–1.00	0.15 0.06–0.24	3.61 0.73–15.80	6.44 0.76–19.70	–	3.25 1.84–6.68	22 15–44	Chiarenzelli et al. (2001)
Greenland	General localities (n=4)	<i>Cetraria nivalis</i>	Not given	<0.14 <0.1–0.16	0.09 0.05–0.16	0.79 0.45–1.32	1.72 0.32–4.29	–	<1 <1–1.8	17.65 6.48–36.9	Riget et al. (2000)
Singapore	Urban areas (n=6)	<i>Dirinaria picta</i>	ICP-MS	1.3 0.45–2.06	0.2 0.14–0.28	32.88 11.75–45.13	11.86 2.83–16.59	–	5.83 2.52–11.54	65.58 44.17–83.15	Ng et al. (2005)
Wellington, New Zealand	Urban streets to very rural roads (n=3)	<i>Xanthoparmelia scabrosa</i>	ICE-OES	–	0.21–0.84	7.52–60.67	26.3–201.9	74.2 70.4–79.4	8.07 7.87–8.31	130 93.2–189	Bennett et al. (2004)
Argentina	Pristine environment (n=71)	<i>Usnea barbata</i>	ICP-MS	0.734 0.04–2.49	0.174 0.02–1.53	2.46 0.83–8.83	2.215 0.2–19.3	143 43–656	0.827 0.23–2.54	41.917 17.22–153.9	Conti et al., (2009)
<b>Western Australia**</b>	<b>Coal-fired power stations (n=36)</b>	<b><i>Usnea inermis</i></b>	<b>ICP-MS*</b>	<b>0.24</b> <b>0.03-11.02</b>	<b>0.04</b> <b>0.03-0.78</b>	<b>1.21</b> <b>0.63-2.83</b>	<b>0.73</b> <b>0.19-11.75</b>	<b>19.97</b> <b>6.14-325.17</b>	<b>0.36</b> <b>0.16-1.42</b>	<b>12.5</b> <b>4.93-25.77</b>	<b>This study</b>

\*Geometric mean values are presented; \*\* Results averaged for both campaigns; <sup>#</sup> A combination of ICP-MS, graphite furnace, flame and cold-vapor atomic absorption used

Appendix 4.8: Emissions of metals and metal compounds from industries and other activities in Collie for the year 2010/2011 as reported by the National Pollution Inventory (Total emissions in air kgs\*)

Source	As	Cd	Cr	Cu	Pb	Mn	Hg	Ni	Zn
Electricity Generation	89	120	370	300	320	740	110	700	870
Coal Mining	19	-	160	130	350	1,900	-	65	870
Metal Manufacturing <sup>1</sup>	28	9	736	220	340	410	370	210	540
Paved/Unpaved Road <sup>2</sup>	490	760	-	3,100	30,000	34,000	520	2,300	21,000
Windblown Dust	11	10	56	64	430	1,200	6.9	70	2,800
Other	-	18	9.95	-	-	-	3.7	-	-
<b>Total Emissions</b>	<b>640</b>	<b>930</b>	<b>1366</b>	<b>3,800</b>	<b>32,000</b>	<b>38,000</b>	<b>1,000</b>	<b>3,300</b>	<b>26,000</b>

\*Air kgs - Kilograms of emissions released to the atmosphere for the entire year; <sup>1</sup>Basic non-ferrous metal manufacturing; <sup>2</sup>Doesn't include exhaust and other emissions directly associated with motor vehicles

Appendix 4.9: Emissions of metals and metal compounds in Collie from the key point-source industrial emitters in the area for the year 2010/2011 as reported by the National Pollution Inventory (Total emissions in air kgs)

Facility	As	Cd	Cr*	Cu	Pb	Mn	Hg	Ni	Zn
Worsley alumina	28	9	736	220	340	410	370	210	540
Bluewaters 1&2	5.3	51	121	42	0.34	120	0.068	290	600
Ewington Mine	8.8	0.32	100	85	310	1100	0.9	37	540
Muja Mine	2.8	-	-	-	-	110	-	-	55
Premier coal operations	7.4	0.23	62	48	36	620	0.51	28	280
Collie power station	40	28	105.5	130	150	260	40	140	220
Muja power station	44	44	210.2	130	180	360	67	260	43
<b>Total Emissions</b>	<b>136.3</b>	<b>132.55</b>	<b>1334.7</b>	<b>655</b>	<b>1016.3</b>	<b>2980</b>	<b>478.48</b>	<b>965</b>	<b>2278</b>

\*Includes Cr III and IV and their compounds

## Chapter 5

### Appendix 5.1: ICP-MS analysis quality assurance report for transplant samples

Analyte	Reporting Limit (ug/L)	Blank (ug/L)	RPD*	% Recoveries	
				Lab Control	Matrix Spike
Arsenic	0.5	<0.5	0	105	102
Chromium	0.5	<0.5	0	105	94
Copper	0.5	<0.5	10	104	110
Lead	0.5	<0.5	0	108	98
Manganese	0.5	<0.5	5.1	98	119
Nickel	0.5	<0.5	10	101	116
Zinc	0.5	0.86	4.7	98	106

\*Relative percent difference for duplicate samples

### Appendix 5.2: Elemental spike recovery (percentage) for metals in blank spikes and sample spikes used for quality assurance

Metal	Blank Spike 1	Blank Spike 2	Blank Spike 3	Sample Spike 1	Sample Spike 2	Sample Spike 3
<b>As</b>	87.5	88.4	93.8	98.6	97.2	89.9
<b>Cr</b>	78.0	80.2	76.5	85.8	98.0	91.2
<b>Cu</b>	84.6	94.2	97.2	87.4	93.0	98.4
<b>Pb</b>	93.6	88.2	85.0	96.8	89.6	97.2
<b>Mn</b>	95.2	99.8	100.4	101.2	102.1	99.8
<b>Ni</b>	96.3	95.7	94.5	89.9	96.2	91.5
<b>Zn</b>	99.1	97.2	93.2	97.3	99.4	96.1

Appendix 5.3: Transplantation effect on *Usnea inermis* lichens: the difference in concentration for transplants at the control sites and the background in situ concentrations before exposure showing the seasonal variation introduced by the transplantation approach (N=5; ANOVA, As: F 3,16 = 2.9, P=0.068; Cr: F 3,16 = 0.11, P=0.95; Cu: F 3,16 = 2.79, P=0.07; Pb: F 3,16 = 23.5, P=0.00; Mn: F 3,16 = 18.35, P=0.00; Ni: F 3,16 = 4.03, P=0.026; Zn: F 3,16 = 12.3; P=0.00; lowercase letters indicate Tukey's *post hoc* difference between seasons at P<0.05)

