

**EFFECTS OF
ATMOSPHERIC POLLUTANTS
ON EPIPHYTIC TERRESTRIAL ALGAE**

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Abstract

Unlike their counterparts lichens and bryophytes, the role of epiphytic terrestrial algae as indicators of atmospheric pollution is not widely explored by biologists. This thesis investigates the relationship between the two factors using green algae due to their high degree of exposure to pollutants and their potential for rapid response to environmental change. We looked into how contemporary atmospheric pollutants influence the growth, abundance and diversity of epiphytic terrestrial algae in the UK. Approaches included surveying algal abundance along a local (150m) transect adjacent to a point source of ammonia pollution, and a similar survey along a much longer transect (50km) spanning urban and rural locations. Controlled application of nitrogen and sulphur gaseous pollutants was also undertaken, initially on a small scale using quadrats on tree trunks, and on a larger scale using the facility operated by the Centre for Ecology & Hydrology at Whim bog, south of Edinburgh. The response of selected algae to the same chemicals under laboratory conditions was also investigated.

Through the five separate but related field and laboratory experiments, it is concluded that nitrogen (N) deposition is probably the most important factor controlling the growth of green epiphytic algae as opposed to other pollutants such as sulphur dioxide. Local high N deposition is markedly stimulatory to the growth and sustainability of *Desmococcus olivaceus*. It also contributes to the dominance of this and other nitrophilous algae and suppresses acidophyte species. Algae exposed to wet N deposition show greater species diversity compared to dry N deposition. In general *Desmococcus* spp. were much more tolerant of pollution than *Trentepohlia* spp.

Long-term experiments at both Whim bog (Edinburgh, Scotland) and Silwood Park (Ascot, England) showed that a reduced form of N was more stimulatory to algal growth than oxidized N. In contrast, over short-term exposure periods, oxidized N in general was more beneficial to algal growth than the reduced form. The same contrasting result between short and long term exposure was also observed where bisulphite, as a proxy for sulphur dioxide, did not show any toxicity to algae under the short-term study but was damaging when exposed over a longer period.

DECLARATION OF OWN WORK

I confirm that this thesis:

Effects of atmospheric pollutants on epiphytic terrestrial algae

is entirely my own work, conducted under the supervision of Dr Simon Archer. Where any material could be construed as the work of others, it is fully cited and referenced, and or with appropriate acknowledgement. No part of this research has been submitted in the past, or is being submitted, for a degree or examination elsewhere. The input of my supervisor to the research and to the thesis was consistent with normal supervisory practice. I grant copyright of this thesis to Imperial College London.

January 2012

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Chapter 1

General Introduction

1.1 Atmospheric Pollution

1.1.1 History of Air Pollution

“As soon as I had gotten out of the heavy air of Rome and from the stink of the smoky chimneys thereof, which, being stirred, poured forth whatever pestilential vapours and soot they had enclosed in them, I felt an alteration of my disposition.” – Seneca, a Roman philosopher (AD 61). Extracted from Boubel *et al.*, (1994).

In the early days of human civilisation before the Industrial Revolution, nomadic migration periodically allowed humans, to get away from the stench of the animal, vegetable and human wastes generated by their existence. Long after that, when humans were more civilized, they started to use fire for many purposes. Smoke from fireplaces caused by wood burning was reported to force the wife of King Henry II of England, Eleanor of Aquitaine, to move from Tutbury Castle due to the unendurable air pollution caused by wood burning. 100 years after that, coal burning was prohibited in parts of London (Boubel *et al.*, 1994). Coal burning was not finally stopped in London until after the great smogs of the early 1950s.

During the Industrial Revolution, steam (initially from wood burning but later also from coal) was used to provide power to pump water and to move machinery. Later in the 19th century, the burning of oil and coal from stationary power plants, locomotives, marine vessels, and furnaces produced heavy smoke and ash. During the 20th century, the severity of air pollution increased as the cities and factories grew. Therefore a search for protecting the environment has led, amongst other measures, to the building of natural gas pipelines (gas being a cleaner-burning fuel), replacing coal and oil as heating and industrial fuels (Boubel *et al.*, 1994).

In 1952, Great Britain was hit by a major air pollution disaster which led to the passage of the Clean Air Act in 1956. Starting from here, more attention was paid to air pollution and its control. A wide variety of air pollution measuring instruments became available at this time and air quality monitoring systems became operational (Boubel *et al.*, 1994). This positive action is still continuing to date and has been spreading to many countries in Europe and across the Atlantic.

1.1.2 General influence of gaseous atmospheric pollutants to ecosystems

Aside from particulate matter atmospheric pollutants consist of various harmful gases such as sulphur dioxide, nitrogen dioxide, nitrous oxide, nitric oxide and ammonia. When these chemicals accumulate in high concentrations, they will have adverse effects not only on humans and animals but also on plants, both higher (vascular) and lower (non-vascular) plants. Epiphytes such as bryophytes, lichens and algae are considered as lower plants. These epiphytes experience the negative effect of atmospheric pollutants, firsthand, because of their lack of complex structures compared with higher plants. Apart from simple structural differences such as the

absence of a root system or of a cuticle and periderm, epiphytic lower plants acquire most of their nutrients directly from the atmosphere or from rainwater (Creese *et al.*, 2011). Furthermore, lower plants have high sorption capacity. Therefore, they can readily accumulate atmospheric pollutants in their cells.

Atmospheric pollutants especially increased nitrogen (N) have been reported to alter community ecosystems. In areas of high atmospheric N deposition, nitrophytic lichen species have increased in parallel with a decrease of acidophytic lichen species (van Dobben & ter Braak, 1998). The same phenomenon has been observed in heathland ecosystem. In The Netherlands, nitrophilous (nitrogen-loving) grass species have been reported to out-compete local species. Less N tolerant species were out-grown by plant species tolerant of high N (Buijsman, 1998; Egerton-Warburton & Allen, 2000). It was discovered that the susceptibility of species to particular concentrations of N determines their relative survival. Acidophyte species which are highly susceptible to N could not survive in high N ecosystems. Thus the nitrophyte species which are usually fast-growing have caused a decline in species richness of highly nitrogen polluted areas by outgrowing the acidophyte species (Tilman, 1997).

Since the 1980s, atmospheric pollutants have been associated with the deterioration of forest trees. Increased N has been reported to weaken the productivity of trees and has increased the mortality of trees in German forests. In Vermont (USA), coniferous forest is declining and is being replaced by fast-growing deciduous forests which have the capability to cycle N more rapidly (Ashmore *et al.*, 1988; Godish, 2004). Increased N has also been associated with reduced lignin in wood, permanent increase in foliage N, decreased root growth and increased nitrate leaching. It has been

reported in certain European countries that the composition of woody plants in mixed oak forests has changed in favour of nitrogen-loving species. Twenty out of thirty plant species in mixed oak forest which was subjected to high N deposition have increased in abundance. At the same time, herbaceous plant composition has shifted to species that favour high N content in soil (Jokela & Huttunen, 1990; Godish, 2004).

In Finland, algae on conifers have been reported to increase in abundance particularly in southern and central Finland where the N concentration is highest (Poikolainen *et al.*, 1998). It was no surprise that this increase has been associated with increased nitrogen and decreased sulphur. Agriculture and industry has resulted in artificial nutrient enrichment of waters and this eutrophication process has changed species composition, diversity and abundance of algal species. Watercourses in mountainous areas that once belonged to filamentous algae have now been invaded by other species that favour high nitrogen.

One study has shown that higher plants in a city that was exposed to higher concentrations of atmospheric pollutants are three to four times smaller in size compared with plants in rural areas. A study by Ashmore *et al.*, (1988) in the mid 1980s reported that improved plant performance was correlated with distance from the city centre. They found that this link was statistically significant and correlated with concentrations of nitrogen dioxide, sulphur dioxide and ozone.

Grasses and cereals exhibit slower growth after exposure to a mixture of pollutants. The sugar content of potato leaves has been reported to decrease due to pollution, as early as the first day of exposure (Petitte & Omrod, 1992). Plant injury has long been

associated with both acute and chronic exposure to atmospheric pollutants. Since mid 19th century, severe injury and destruction to vegetation has been reported as an effect of sulphur dioxide from metal smelters (Bell & Treshow, 2002; Godish, 2004). Smelter emissions have caused significant damage to agricultural crops in many countries. Exposure to sulphur dioxide and nitrogen dioxide, even for a short time can cause severe injury to plants. Tingey *et al.* (1971) observed a surprisingly high toxicity of plants after 4 hours of exposure to a mixture of sulphur dioxide and nitrogen dioxide.

1.2 Aims and objectives of this study

The aim of this study is to understand how contemporary atmospheric pollutants influence the growth, abundance and diversity of epiphytic terrestrial algae in the UK, algae being chosen due to their high degree of exposure to pollutants and their potential for rapid response to environmental change. This will be achieved by pursuing the following objectives:

- 1) To explore and determine appropriate sampling techniques for epiphytic terrestrial algae.
- 2) To assess the effects of atmospheric pollutants on the abundance and diversity of subaerial epiphytic algae along a gradient of air quality provided by a transect from central London into the countryside.
- 3) To examine the effect of pollutants on naturally occurring epiphytic algae around an intensive husbandry unit emitting appreciable quantities of ammonia.
- 4) To investigate the effects of various pollutants on epiphytic algae by the experimental application of dissolved pollutants, over an 18 month period in the field.

- 5) To examine the effect of gaseous and wet N application treatments on epiphytic algae in pre-existing field experiments at Whim Bog in southern Scotland.
- 6) To study the effects of pollutants on the physiology of selected epiphytic algae (*Desmococcus* sp. and *Trentepohlia* sp.) cultured from field populations.

1.3 Overview of the Thesis

This research project was undertaken to understand how contemporary atmospheric pollutants influence the abundance and diversity of epiphytic terrestrial algae in the UK. A survey looking into the diversity and abundance of epiphytic terrestrial algae has been carried out in the woods located at the Silwood Park campus (Ascot, Berkshire) of Imperial College. Algal samples were collected, identified and quantified. A large scale study has been carried out using a line transect, along a 70 km pollutant gradient that runs from Central London to the rural area of West Sussex. Algal populations were related to concentrations of major atmospheric pollutants at each station as monitored using passive sampler diffusion tubes and also to historic data.

To examine further the effect of an atmospheric pollutant on epiphytic terrestrial algae, one specific study has been conducted around an intensive husbandry unit which emits substantial amounts of ammonia. This work focused on the effect of ammonia on algae on oak trees within close proximity of the pollutant source. The study tests the hypothesis that epiphytic algae increase in abundance in response to the release of ammonia from intensive animal rearing units. Following that, a field experiment was conducted to investigate the effect of dissolved nitrogen compounds in the form of sodium nitrate and ammonium chloride and sulphur in the form of

bisulphite on two epiphytic algal species on oak and beech trees. Algae within 10 x 10 cm² quadrats were treated with pollutants of different concentrations on a weekly basis. This study aims to test the hypothesis that *Desmococcus* sp. and *Trentepohlia* sp. are tolerant of both nitrate and ammonium compounds. Another aim is to prove that the latter species is relatively intolerant of SO₂ pollution simulated by bisulphite solution.

In response to this field experiment, a short-term physiological study in a controlled environment has been carried out. The algae from the sites were cultured and exposed to the same treatments as in the field. A further study assessed the response of algae on a peat bog in southern Scotland to simulated high N deposition and sought to separate the effects of reduced versus oxidised forms of N. Algae from plots within the bog were sampled systematically to permit a statistical analysis of the effects of the wet and dry N deposition on populations of epiphytic algae.

1.4 Literature Review

1.4.1 Atmospheric Pollutants

Atmospheric pollutants are categorized into primary and secondary pollutants. Primary pollutants are those pollutants that are emitted directly into the atmosphere from a source, examples being carbon monoxide or hydrocarbons expelled from car exhaust. While secondary pollutants are air pollutants that form within the atmosphere, such as sulphuric acid that is formed from the oxidation of sulphur dioxide (Harrison, 2001).

There are five scales of air pollution namely local, urban, regional, continental and global (*Boubel et al.*, 1994). Local scale pollution is characterized by one or several large emitters, or a large number of small emitters. For example, carbon monoxide from vehicles causes high concentrations near roadways. Urban scale pollution, normally covering tens to thousands of square kilometres of affected air quality, originates from many locations (Hemond & Fechner-Levy, 2000). Atmospheric pollution at this scale is characterized by primary and secondary pollutants whose concentrations are correlated. For example, carbon monoxide emitted from transportation (primary pollutant- released direct from source) will react with free oxygen and produces ozone (secondary pollutant – product of chemical reactions). Both gases then contribute to air pollution, often on a city-wide scale.

At a regional scale, air pollution in a major metropolitan area may flow into the adjacent metropolitan or rural area. Smog and haze in one city could easily be transported to another city, causing severe health effects in both animals and plants and reduced visibility that could contribute to motor accidents. On a continental scale long-range pollutant transport takes place such that pollution in one country can affect those adjacent. For example, acid rain in Great Britain and parts of Western Europe was reported to have an impact on Scandinavia. Lastly, at a global scale, air pollution is considered to affect very large areas. Upper atmosphere ozone depletion and carbon dioxide build up are global issues where the negative impact embraces all countries.

Air pollution exists in a gaseous form (such as nitrogen dioxide) and in particulate form (such as soot particles and liquid droplets). Concentrations of both forms are

usually expressed in mass per unit volume ($\mu\text{g m}^{-3}$) or as a volume mixing ratio (ppm or ppb) (Hobs, 2000). Atmospheric pollution which has been made worse by a rising human population and increased use of resources, also occurs as a consequence of natural processes such as volcanic activity, plant and animal decomposition, ocean spray, soil erosion and mineral weathering (Hemond & Fechner-Levy, 2000; Godish, 2004).

Pollutant removal from the atmosphere is described as deposition and can be wet or dry. Wet deposition is a process where airborne gases and particles are accumulated and transferred through precipitation such as in rain, snow or hail and deposited onto the earth's surface (Harrison, 2001; Godish, 2004). Many toxic gases are removed from the atmosphere by dissolving into raindrops which will become acidified in the case of oxides of non-metals and may cause acid rain that brings many negative impacts to ecosystems.

Another type of atmospheric deposition is called dry deposition. Dry deposition is a process where gas and particulate substances are removed from the atmosphere and are directly transferred to the earth's surface through gravitational settling, impaction and absorption (Hemond & Fechner-Levy, 2000; Godish, 2004). This process is driven by a concentration gradient of the pollutants. Gravitational settling is significant in the removal of particulate atmospheric chemicals that are larger than 1 μm diameter, from the atmosphere. Impaction refers to a process where air containing particles collides with and sticks to an object (building or vegetation) whenever the air moves past stationary objects (Hemond & Fechner-Levy, 2000).

Absorption is a process where atmospheric gases are absorbed by liquid (water bodies) or solid (soil and vegetation) surfaces.

1.4.1.1 Types of pollutants

There are many types of atmospheric pollutants that significantly affect ecosystems.

The important ones are described as below:

i) Sulphur dioxide (SO₂)

Naturally, sulphur gases are emitted from volcanoes, wetlands and oceans (Hemond & Fechner-Levy, 2000). However, their loading in the atmosphere has been greatly enhanced by human activity such as the combustion of sulphur-containing coal and petroleum. The atmospheric lifetime of sulphur dioxide is relatively short because of its high affinity with water vapour (Godish, 2004; Hawkesford & De Kok, 2007). This gas in solution forms dilute sulphurous acid and on oxidation gives rise to sulphuric acid in fog, cloud and rain droplets.

During the Industrial Revolution, concentrations of sulphur dioxide in the atmosphere in some parts of the UK rose greatly. They continued to rise until around the year 1965 after which the sulphur dioxide concentration began to fall (**Figure 1.1**). After a series of regulations were introduced to rectify this air pollution problem, the concentration of sulphur dioxide rapidly decreased. Between 1970 and 1990, the use of coal as a source of heating was greatly reduced. In the seven years, 1990-1997, total UK emissions declined by more than half, because power stations were installed with flue gas desulphurization, natural gas was increasingly being used in power stations and a source of heating had been switched to electricity (Harrison, 2001).

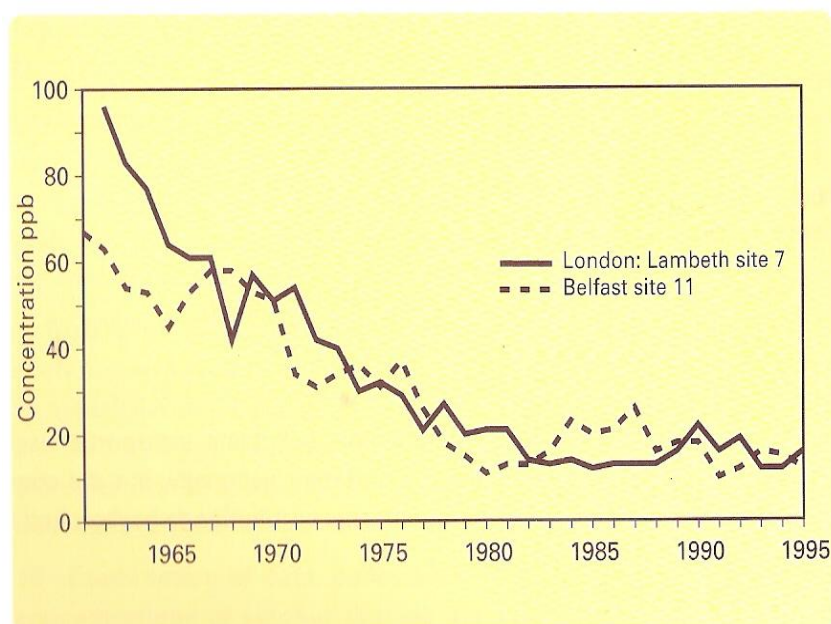


Figure 1.1 : Historic mean annual concentrations of sulphur dioxide post the Industrial Revolution and after the Clean Air Act 1965. Extracted from *The United Kingdom National Air Quality Strategy*, DEFRA, 1997.

ii) Oxides of nitrogen / nitrogen oxides (NO_x)

Nitrogen which constitutes 78 % of the atmosphere is a colourless, odourless and tasteless gas. Nitrogen in water and soils can be found in both oxidised and reduced forms mainly as nitrates, ammonium salts and nitrites. It is relatively unreactive under ambient conditions but due to combustion and certain biological process, it serves as a precursor molecule for the production of NO and NO₂. These two gases are in turn precursors for many atmospheric reactions (Godish, 2004).

Nitrogen must be converted to reactive N before it can be utilized. Reactive N includes inorganic reduced forms such as ammonia and ammonium, inorganic oxidized forms such nitric oxide, nitrogen dioxide and nitrate, and organic compounds such as urea and amines (Galloway *et al.*, 2003). Oxides of nitrogen are generated naturally from bacterial activity, wildfires and lightning. Their concentration in the atmosphere has been increased by industrial combustion processes and the use of

nitrogenous fertilizers. All combustion processes in air produce mixed oxides of nitrogen of which NO predominates. Reaction between NO and ozone will produce NO₂, the red-brown gas that is associated with adverse effects to human health and ecosystems (DoE, 1997).

The main source of NO_x (total oxides of nitrogen) in the atmosphere is from road traffic. Nitrous oxide (N₂O) is the most abundant nitrogen oxide in the atmosphere. Nitrous oxide has an average lifetime of 114 – 120 years and is 300 times more effective than carbon dioxide as a greenhouse gas (Schlesinger, 1997). The use of nitrogenous fertilizer has increased microbial nitrification and denitrification. Both processes naturally leak nitric oxide and nitrous oxide to the atmosphere, (Schlesinger, 1997). Excess N is deposited in terrestrial and aquatic ecosystems through wet (in the form of nitrate and ammonium in precipitation) and dry (in the form of ammonia) deposition (Galloway, 2003). Other oxides of nitrogen that are harmful to the environment are nitric oxide (NO) and nitrogen dioxide (NO₂). The ambient level of nitrogen dioxide is 38 µg m⁻³ (Harrison, 2001), a value that is often exceeded in areas where there is heavy road traffic and agricultural activities.

iii)Ammonia

Ammonia is the most abundant basic chemical substance in the atmosphere and is the third most abundant N compound (Koerkamp, 1994; Godish, 2004). Ammonia is present in the atmosphere as ammonia gas together with its reaction product ammonium following reaction with for example SO₂ and NO_x emissions (Asman *et al.*, 1988) In the UK, ammonia concentrations in rural areas are mostly around 2 µg m⁻³ (Allen *et al.*, 1988) while 1 µgm⁻³ was recorded over oceans and remote

mountains. However, average ammonia concentration in urban areas in the UK is $16 \mu\text{g m}^{-3}$. Areas with intensive animal husbandry units have recorded an average ammonia concentration as high as $50 \mu\text{g m}^{-3}$.

Ammonia emissions also depend on soil reactions. Soils that are rich in organic matter will have lower ammonia concentrations and thus lower ammonia emissions (Freney *et al.*, 1983). Ammonia in the form of ammonium ions dissolved in soil water is prone to be converted to nitrate by microbial action.

Ammonia is used commercially as an agricultural fertilizer. It is produced naturally by bacteria and cyanobacteria in the soil by converting atmospheric N to ammonia using nitrogenase enzyme, a process also achieved by *Rhizobium* bacteria living symbiotically in the roots of legumes and some other plants. This process is called nitrogen fixation. Nitrogenous fertilisers were historically based on guano and sodium nitrate deposits, and latterly on ammonium salts produced by the Haber-Bosch process.

iv) Carbon monoxide

Carbon monoxide is a toxic gas that is usually associated with petrol vehicles: about 98% of carbon monoxide emissions come from motor traffic. Besides that, this pollutant is also emitted from industrial combustion and biomass burning (NEGTA, 2001; Godish, 2004). Background carbon monoxide concentrations are increasing at 1% per year due to human activity. At present, the level of carbon monoxide in urban areas has declined due to the fitting of catalytic converters in motor vehicles and more efficient engine design leading to more complete combustion.

1.4.1.2 Source of pollutants

i. Natural sources

Atmospheric pollutants arise both from man-made and natural process. Natural sources include volcanic activities, accidental fires in forests and prairies, dust storms and ocean spray (Boubel *et al.*, 1994; Hobbs, 2004).

Volcanic eruption emits large volume of particulate matter and pollutant gases such as sulphur dioxide, methane, carbon dioxide and hydrogen sulphide. Deposits of volcanic ash can cause health problems in humans and animals, and contribute locally to the mortality of plants and vegetation. Accidental fires in forests are caused by lightning or due to extremely dry and hot weather that trigger forest fires. A large fire emits large quantities of smoke, carbon monoxide, carbon dioxide, oxides of nitrogen and ash. A small storm or road dust can result in suspended particulate matter that is one or two orders of magnitude above normal ambient air quality. Reduced visibility due to these events can cause severe motor accidents and affect air travel. Ocean sprays emit aerosols containing salt particles that are corrosive to metals, paints, rocks and building materials. This corroded material may itself become airborne and contribute to air pollution.

ii. Anthropogenic sources

Anthropogenic pollutants are emitted from a range of sources such as from transportation, industrial plant, stationary fuel combustion, waste disposal and many more. In automobile exhaust alone, over 400 gas-phase chemical species have been identified. Fuel combustion and use of nitrogenous fertilizers are among the main

contributors to increased mobilisation of N in the environment, in turn resulting in increased N in groundwater, surface water and the atmosphere.

High N emissions have been reported from petrol and diesel powered vehicles, fossil fuel-powered electricity generating stations and industrial boilers (Posthumus, 1982; Godish, 2004). Major sources of ammonia, another atmospheric pollutant are from anaerobic decomposition of organic matter, animal wastes, biomass burning and soil humus formation (Godish, 2004). Even though some increased N is attributed to agricultural runoff and wastewater treatment, a total of 40 % of nitrate N comes from the atmosphere.

1.4.1.3 Negative effect of pollutants

Atmospheric pollutants have caused many adverse effects to humans, animals and plants. Furthermore, these pollutants are also affecting many man-made structures such as buildings and statues.

i) Human and animal

Air pollution affects the respiratory, circulatory and olfactory systems of humans. Being the principal route of air pollution entry, the respiratory system which includes the lungs is easily affected by air pollution compared with other parts of the body. Different pollutants vary in their impact on human and animal health. However, most pollutants elicit the same responses when they attack the stability of the respiratory system.

Pollutant such as nitrates and nitrites are known to affect human health by for example decreasing the oxygen carrying capacity of the blood, lowering the function of the thyroid gland and causing a shortage of vitamin A. An exposure to high concentrations of nitrogen dioxide causes damage to lung tissue as this pollutant is an oxidizing agent. It initiates inflammation within the lung, affecting the small airways close to the site of gas exchange (Harrison, 2001). This pollutant also impairs the ability of the human body to fight infection. Increased nitrogen dioxide was shown to enhance the response to allergen challenge in asthmatic people (Harrison, 2001).

Sulphur dioxide is known to constrict mammalian airways after only a few minutes of exposure. This is due to its potent bronchoconstrictor activity which reduces lung function (Harrison, 2001). Some other pollutants need repeated or prolonged exposure to have any negative effect on human health. As would be expected, asthma patients are more at risk compared to healthy individuals (Harrison, 2001).

Another pollutant, carbon monoxide, binds to haemoglobin and reduces oxygen-carrying capacity of the blood. Cerebral and cardiac hypoxia occurs when exposed to high doses of carbon monoxide. Even though it is difficult to prove scientifically, an atmospheric pollutant has long been associated with cancer (Harrison, 2001).

Air pollution which causes acid rain can jeopardise ecosystem stability. An indirect effect of acid rain (mainly caused by compounds of N and S) has caused pH changes in many lakes and rivers and is reported to cause widespread damage to terrestrial and limnic ecosystems (Brasseur *et al.*, 2003). The acidification of lakes, rivers and estuaries has contributed to the changes of algal communities and mortality of pH

sensitive fish especially in soft water lakes in northeastern United States and southern Scandinavia (Hemond & Fechner-Levy, 2000). The alteration of pH to a more acidic range has caused a change in fish survival and may interfere with reproduction. In Sweden, thousands of lakes are no longer able to support fish due to the low pH (Boubel *et al.*, 1994).

Another negative effect of excessive nitrogen (and also phosphorus, another agricultural nutrient) input to fresh waters is eutrophication resulting from algal blooms. As the algae die the excess biomass breakdown consumes oxygen causing an anaerobic environment that results in the death of fish and other aerobic organisms.

ii) Plants and crops

Nitrogen concentrations in the atmosphere and on the ground have increased due to anthropogenic causes from agricultural and industrial activity. Over one-third of nitrous oxide emissions are caused by agriculture (Francesco *et al.*, 2010). Atmospheric pollutants have direct access to the cellular system of the leaf structure of a plant. Thus any disturbances to ambient air are likely to have an immediate effect on photosynthesis, transpiration and respiration of plants (Boubel *et al.*, 1994). The indirect pathway by which gaseous pollutants interact with plants is through the root system. Changes in soil nutrients can lead to indirect effects of atmospheric pollutants at both the single plant and vegetation levels.

Overt acute injury to plants from gaseous pollutants is revealed through symptoms such as chlorosis (reduced number of chloroplasts), glazing (injury to the epidermal layer) and flecking (spots on leaves) (Boubel *et al.*, 1994). Atmospheric pollutants

also cause leaf drop or early senescence, decreased yield of fruit trees and misshapen stems and leaves. Some plants, such as tobacco, are very sensitive to injury; indeed the speckling of some varieties of tobacco is used as an early warning of ozone accumulation. Physiologically, atmospheric pollutants have caused alteration in net photosynthesis, stomatal response and metabolic activity of plants.

Sulphur dioxide has been reported to cause death of terminal buds on deciduous plants. Hawkesford & De Kok (2007) and Godish (2004) stated that sulphur dioxide caused interveinal necrosis in broad-leaved species and injury at the tip of the leaf for narrow-leaved species. Chlorosis and premature needle drop was also reported in conifers exposed to sulphur dioxide (Godish, 2004).

A more subtle type of injury is caused by another type of pollutant: nitric oxide is reported to decrease defence mechanisms in plants. It has been reported that aphid infestation greater in urban areas where nitric oxide concentration is higher (Boubel *et al.*, 1994). This pollutant also has an inhibitory effect on photosynthesis and is known to act as an intercellular second messenger in both plants and animals (Boubel *et al.*, 1994).

1.4.1.4 Current air pollutant status

In the UK, overall emissions of NO_x in 2000 had fallen by 37 % compared to 10 years before that (DEFRA, 2004). This improvement has been due to better engine design in motor transport and also the fitting of three-way catalytic converters to vehicles. The reduction of NO_x concentration has continued until the present, even though there are still many areas in the UK which exceed the target level (**Table 1.1**).

Table 1.1 : National air quality target values of major air pollutants in the United Kingdom.

*** Indicates target values for the protection of human health. ** Indicates target values for the protection of vegetation and ecosystems.**

Source : http://www.apis.ac.uk/overview/regulations/overview_UK_NAQS.htm

Pollutant	Nitrogen dioxide	Sulphur dioxide	Carbon monoxide
Target concentration	40 μgm^{-3} *	125 μgm^{-3} *	10 mgm^{-3} *
	Annual mean	24 hour mean, not to be exceeded more than 35 times a year	Mean of maximum daily running of 8 hours
	30 μgm^{-3} **	20 μgm^{-3} **	-

Following the Clean Air Act 1965, sulphur dioxide concentration has decreased dramatically from almost 100 ppb in 1965, to below 20 ppb in 1995. These changes were made possible due to cleaner fuels such as natural gas that replaced coal as a power source (DoE, 1997). The current status of air pollution in the UK stands at 3.6 μgm^{-3} for sulphur dioxide. The annual mean of nitrogen dioxide in urban areas exceed 40 μgm^{-3} and the concentration within built-up areas is between 15-40 μgm^{-3} (DEFRA, 2011).

1.4.2 Algae

1.4.2.1 Algae in general

Algae do not represent a formal taxonomic group. The name is used informally to describe disparate groups that do not share common ancestry. Their classification constitutes a loose connection of divisions or phyla of different lineages and historically has been based on the nature of the photosynthetic pigments, food storage materials, type of cells and much more (Irvine & John, 1984; Graham *et al.*, 2009).

Algae are a heterogeneous assemblage of organisms that range from a single cell to giant macroalgae (seaweeds). They include some protists which are eukaryotes, and the name also extends to the blue-green algae or cyanophyta which are prokaryotes and properly regarded as totally separate from the eukaryotic algae. Most algae are photosynthetic, producing oxygen and live in aquatic environments (Lewin, 1962).

As primary producers in the ocean, algae contribute 40-50 % of the replenishment oxygen in the atmosphere. Algae are also known as the original source of fossil carbon in crude oil (Andersen, 2005). Algae inhabit diverse habitats, from seawater to freshwater, and for a few species, terrestrial locations such as tree trunks and masonry. Algae often occur in extremely hostile environments, due their ability to tolerate various kinds of stress (Rai & Gaur, 2001).

The major algal groups include the Chlorophyta, Phaeophyta, Rhodophyta, Chrysophyta, Bacillariophyta and a few others. In the context of this thesis, we focus only on Chlorophyta, mainly the Trebouxiophyceans such as *Desmococcus*, *Trebouxia*, *Chlorella* and *Trentepohlia* since this is the main algal group found on terrestrial substrata. According to Graham & Wilcox (2000), the Chlorophyta are estimated to consist of 17,000 species, the largest major group, although possibly this is because they are the most studied. These green algae, sometimes known as chlorophytes, contain photosynthetic pigments of grass-green coloration due to the predominance of chlorophyll *a* and *b* over the carotenes and xanthophylls (Smith, 1950). However, some chlorophytes such as *Trentepohlia* do not appear green to the naked eye, instead forming orange-red growths on terrestrial substrata (Graham & Wilcox, 2000).

Green algae include a variety of morphological, reproductive and ecological types. Morphologically, coccoid is the most common body type in microscopic algae. Some algae are filamentous, uniseriate (one row filament), biseriate (two row) or pluriseriate (more than two). The filaments can be branched or unbranched. Coenobia are algae with genetically defined number and pattern (Pickett-Heaps, 1975). Examples of morphologically variable green algae include *Pleurastrum* which is filamentous, *Desmococcus* that are sarcinoid or cell pocket-forming and *Prasiola* that form a sheet-like blade. Variability in terms of reproductive system include nonflagellate (nonmotile) unicells with nonflagellate autospores for reproductive cells (*Chlorella*). However, other nonflagellate unicells such as *Trebouxia*, generate flagellate zoospores to reproduce (Graham & Wilcox, 2000). Generally, algae reproduce mainly asexually but some can reproduce both sexually and asexually. Asexual reproduction in algae, allows rapid population growth (Prescott, 1982).

1.4.2.2 Algae and habitat quality

There are estimated to be 36,000 to 10 million species of algae in the world, where approximately 17,000 are Chlorophyta (Graham & Wilcox, 2000). In the UK, there are estimated to be 20,000 algal species (Biodiversity, 1995). However, Whitton *et al.* (1998) reported that there are only 5000 species in Great Britain and the Republic of Ireland. John *et al.* (2002) in his book reported a total of 2275 species of British freshwater algae. This number does not include diatoms which at the end of 20th century stood at 1652 species (Whitton *et al.*, 1998), although many phycologists consider this to be an underestimate (Anderson, 1992; Man & Droop, 1996).

In 1927, West & Fritsch produced what was then a comprehensive book on freshwater algae called *A Treatise on British Freshwater Algae*. The latest comprehensive guide to terrestrial algae was *The Freshwater Algal Flora of the British Isles*, a book edited by John *et al.* (2002). Other than that, freshwater algae in general and terrestrial algae in particular, have received little attention from phycologists, mainly because of complicated identification processes due to their nature that is highly varied. Up to date, the desmids have received the most attention from British workers probably because this algal group is the only one that has a dedicated monograph. A five-volume series was published by William and George West which described desmids complete with meticulous illustration (John *et al.*, 2002).

A very diverse range of habitats in the British Isles supports a wide variety of algal species. This is mainly due to the varied geology and geomorphology of the British Isles. Coupled with a maritime climate, this region provides ample habitats for algal growth. In mountainous areas where the water is soft with an often acidic pH, filamentous zygnematalean algae are abundant. Commoner unicellular algae such as *Chlorella* are abundant in lowland areas, most of them belonging to the Chlorococcales group (Vymazal, 1995).

Some algal groups such as the charophytes have been included in the Red Data book of threatened species (Stewart & Church, 1992). A total of 12 species of charophytes are considered as priority species for conservation (Biodiversity, 1995).

While studies on the effect of pollutants on lichens started as early as 1810 by Turner & Borrer, it was Nylander who concluded that lichens have a practical use as an

indicator of air quality (Hawksworth & Rose, 1976). As compared to the study of lichens, works and references on algae on trees are very limited. Hawksworth & Rose (1970 & 1976) in their study on lichens for pollution monitoring, stated that in Zone 1 where lichens were absent from tree trunks due to high pollution level, free-living algae namely *Pleurococcus viridis* were present at the base of trees on acid bark, such as oak, but extend up the trunk on basic or nutrient-enriched bark such as elm. To our knowledge, there are no studies which specifically examine the effect of pollutants on oak and similar trees in Britain. Bates (2001), Stapper (2006) and Davies (2007) are among researchers who have briefly mentioned the presence of free-living algae (*Desmococcus*) on trees in their work on lichens.

However, the role of aquatic algae in monitoring water quality has been established since 1969 by Palmer. Since then many studies have been conducted to study the role of algae in marine and freshwater bodies (Williams, 1972; Shubert, 1984; Hanninen *et al.*, 1993) and the influence of water quality on their populations. Subsequent studies have also examined algal ecology in soils including their involvement in maintaining fertility (Munawar and Munawar, 1987; McCormick and Cairns, 1994; Barinova *et al.*, 2011).

1.4.2.3 Habitats and substrata of terrestrial algae

Algae exist in varied habitats, from the extreme arctic to the hot and humid tropics. The substratum of terrestrial algae varies depending on their habitat, from grassland to dense forests, polluted soils or concrete walls (Graham *et al.*, 2009). Eukaryotic algae have been reported to grow on and under the surface layers of snowfields and icefields, and also beneath the surface of rocks in the Arctic and Antarctic. Algae are

much more conspicuous in wetter regions because they require moisture to survive. Terrestrial algae are only metabolically active when there is sufficient moisture. If the water supply is scarce, algae move to lower layers, deeper in their substratum to follow the water or become inactive until moisture returns. Most algae are microscopic and visible to the naked eye only when they exist in large colonies. Larger microscopic algae such as phytoplankton have been studied for a long time, partly because of their ecological importance and also because larger species are easy to sample.

Various terms are used to describe subaerial algae according to habitat and substratum (John *et al.*, 2002). Below are some examples of subaerial algae and their habitat:

- i. Epilithic = Algae growing on rocks.
- ii. Epipellic = Algae growing on silt.
- iii. Epipsammic = Algae growing on sand.
- iv. Epiphytic = Algae growing on plants.
- v. Epizoic = Algae growing on animals.
- vi. Epiphylic = Algae growing on leaves.

Most studies on terrestrial algae are focused on soil-dwelling species. Soil algae contribute to the fertility and stability of soil through carbon and nitrogen fixation, the release of organic compounds and reduced soil erosion (Graham *et al.*, 2009). In arid and semi-arid lands, terrestrial algae that form part of the biological soil crust play a role in maintaining soil fertility, water retention and preventing invasion of weeds.

When discussing subaerial algae, lithic algae are the most widespread. Among lithic algae are algae on the open surfaces of rocks (hypolithic algae), in cracks or fissures (epilithic algae), in the pore spaces of rocks (endolithic algae) and there are also algae that bore into rocks (euendolithic algae).

After soil algae and lithic algae, epiphytic algae are the next most widespread subaerial group. They grow on the surface of bark or leaves. Algae that form symbioses with fungi are known as lichens, and are the most common epiphytic algae (Kauppi, 1981; Purvis *et al.*, 2007; Liska & Herben, 2008). *Chlorococcus* and *Gloeocapsa*, coccoid algae with mucilaginous sheaths are the next most common after lichens. Coccoid and filamentous algae such as *Desmococcus*, *Chlorella*, *Klebsormidium* and *Trentepohlia* are common on tree bark in temperate and tropical ecosystems (Graham *et al.*, 2009). On wetter substrata such as in wetlands and mires, epiphytic algae such as *Desmidium* sp. and *Cylindrocystis* sp. are commonly associated with *Sphagnum* moss.

Algae that exist in a very large number at a particular time are referred to as algal blooms. They form mats of floating algae on the water surface and may dominate large areas. Some algal species are present in the water column throughout the year while others are present for part of the year only. Besides floating algae, there are benthic algae that remain attached to the bottom of lakes or river. Larger algae such as the filamentous *Spirogyra* are free floating or entangled around aquatic higher plants.

In the aquatic and terrestrial environment, physical factors such as temperature, pH, light intensity and nutrient concentration affect algal populations in ecosystems (Patrick, 1972; Starks *et al.*, 1981). Assuming that moisture, light and temperature are not limiting, a pH that is nearly neutral or slightly alkaline supports the most varied algal population (Lund, 1962), although overall green algae can tolerate a wide pH range (John *et al.*, 2002). Tropical rain forests that are characterised by high moisture and low light intensity within the canopy are reported to contain more epiphytic terrestrial algae than areas with lower moisture content and higher light intensity. In fact, it is generally believed that algae are more abundant on the north side of trees due to the lower light intensity and greater persistence of moisture.

1.4.2.4 The importance of algae

Palmer (1969) reported that algal assemblages could be a possible indicator for clean or polluted water. Ten years after that in 1979, he published a list of algae that indicate polluted water (*Euglena*, *Oscillatoria*, *Scenedesmus*, *Navicula*, *Chlamydomonas* and *Nitzschia*) and another list of algae to indicate that the water is clean (*Pinnularia*, *Staurostrum*, *Micrasterias*, *Surirella*, *Meridion*, *Stigeoclonium* and *Lemanea*). Following that, Cairns (1974) and Patrick (1971) also studied algae as an indicator for water quality. Many researchers followed suit and this led to an extensive literature on algae as an indicator for water quality (Palmer, 1977; Whitton and Kelly, 1995; Kieu *et al.*, 2001). In the late 20th century and early 21st century, scientists have started looking at terrestrial algae as a possible indicator for air quality (Brakenhelm & Qinghong, 1995; Poikolainen *et al.*, 1998; Stapper, 2006). The general conclusion from these studies is that areas with high nitrogen deposition support higher populations of subaerial algae compared to other areas.

Besides acting as a pollution indicator, algae play a very important role in global mineral cycles and in food chains as they are primary producer organisms. Ancient algae produced fossil fuel deposits and carbonate rock which together reduced atmospheric carbon dioxide in earlier geologic times (Graham *et al.*, 2009). Since algae are photoautotrophs, they release a large volume of oxygen from photosynthesis, into the atmosphere. This oxygen influences many other cycles such as those of carbon, nitrogen and sulphur. Besides oxygen, algae also produce enormous quantity of organic carbon which is useful to other organism as a source of food. For example algal phytoplankton, via zooplankton and invertebrates are the primary drivers of all marine and freshwater (lake) ecosystems.

Modern technology is now developing biofuels using algae. Algae have also been widely used in laboratory systems for many experiments in physiology, biochemistry and molecular biology. This is because algae, especially unicellular species, are easily accessible, easily cultivated and provide reliable data. There are many studies involving algae as important components in sewage and agricultural effluent treatments because of the ability of some species to remove pollutants (Graham, 2009). Some species such as *Chlorella*, *Euglena* and *Spirogyra* are able to absorb radioactive wastes and heavy metals (Bilgrami & Saha, 2004).

Algae are also interconnected with other organisms in biogeochemical cycles, aquatic and terrestrial food webs and symbiotic associations. Lately, algae have been proposed to help mitigate anthropogenic effects on atmospheric chemistry, thus helping to reduce drastic climate change (Graham *et al.*, 2009). For many years, algae

have been used as bioassay organisms to monitor the quality of water. In paleoecological studies, algae have been used to infer changes in water quality of lakes over time.

Algae have also been examined for commercial use in human nutrition (Bilgrami & Saha, 2004). In aquaculture, algae have been used to feed shellfish and other aquatic species. *Chlorella* is an example of an alga that has been consumed by humans as a complementary dietary supplement to boost health and for pharmaceutical purposes. Even more remarkably, algae have been part of space research where algae have been flown to space to study their role. Results showed that algae seem to suffer few adverse effects from many stresses including micro-gravity. Thus algae are considered as a possibility for life-support systems during spaceflight because of their ability to remove carbon dioxide and add oxygen (Graham *et al.*, 2009).

The above review reveals the paucity of information about terrestrial algae (other than lichenised species) in relation to environmental stresses. The current study aims to at least provide a starting point in terms of data and insight on the relationship between algae and air pollution. Among possible reasons for the shortage of data on algae on plants in general, and trees in particular, is the difficulty to examining them, the lack of awareness in terms of algal ability to respond towards pollutants or possibly their presence has been overshadowed by the focus of researchers on the much easier to study lichens.

Chapter 2

General Methods

The standard methods used in this study are described below. Details on the methods to specific experiments are included in the corresponding chapters.

2.1 Systematic Algal collection

Algal collection was made by scraping the algae from quadrats of tree bark with the exception of a study in Whim Moss (Scotland) where algae on twigs of *Calluna* were also collected. Quadrats size was of 15 x 15 cm or 10 x 10 cm depending on the availability of algae on-site. Number of algal cells per ml was adjusted according to quadrat size. Algae within each quadrat were brushed off the surface with a wetted cotton bud and then placed in a 100 ml sterile specimen tube containing 40 ml phosphate buffered saline solution of 0.01M or deionized water. Some algae were quite difficult to remove and a scalpel was then used to scrape the algae from the bark of the tree (*Quercus robur* and/or *Fagus sylvatica*). 1 ml of the solution was then transferred into a 1.5 ml centrifuge tube for quantification process. All specimen tubes and equipment were sterilized by heat or ethanol before use.

In the case of algal collection at Whim Moss, due to distance of the sampling site from the laboratory, algal slime samples were collected and placed in a labelled

plastic bag together with an ice pack before being transported to the laboratory. Collection of algae on the twigs of *Calluna vulgaris* was done by scraping the algae using a scalpel. On certain occasions where the twigs were very small, algae were removed from the substratum by placing them in a vial and shaking vigorously to loosen the algae from the twigs.

2.2 Microscopy

All samples were examined as soon after collection as possible. If it was not possible for logistic reasons, samples were stored in a refrigerator at 1-4°C to prevent post-sampling growth. A Brunel digital light microscope at 400 x magnification was used to aid the identification process and to count algal cells. A built-in camera attached to the microscope was used to capture algal images. Scope Image Advance software was used to facilitate managing the images.

2.3 Algal quantification

Algal samples were shaken vigorously to break down clumped algae and to separate filamentous algae into fragments. Algal cells were counted using a 'Scepter' handheld automatic cell counter (Millipore, UK). 1 ml of algal samples were pipetted into 1.5 ml centrifuge tube. Scepter tips were attached to the cell counter and dipped into the centrifuge tube containing a suspension of algal cells. The cell counter measured the number of algal cell per ml and data were uploaded into proprietary software (Scepter Data Upload and Storage Application). For some purposes quantification was manual; an algal sample was pipetted into the well of a haemocytometer and counted.

2.4 Algal identification

Some common microscopic algae can be identified by their macroscopic appearance but the majority require microscopic examination, even when they occur in visible mats. Identification to genus level was determined using the floras of John *et al.* (2003), López-Bautista *et al.* (2006), Milow & Aishah (2006) and the database for the world's algal listings (www.algaebase.org). Photomicrographs of the algae were compared to the images in these sources to facilitate identification. Images of a few 'difficult' species were sent to Dr Fabio Rindi of the National University of Galway, Ireland for further discussion and confirmation. Contact and several meetings were also made with Prof. David John of the Natural History Museum, London to discuss species identification.

2.4.1 Algae Species Description

i) *Desmococcus olivaceus*

Synonyms : *Desmococcus vulgaris*, *Pleurococcus naegelii*, *P. angulosus*, *P. vulgaris* , *Protococcus viridis*. Cells solitary or in indefinite clusters, often forming 2-3 or 4-celled cuboidal packets; cells spherical or angular, 9-20µm, sometimes giving rise to unbranched filaments. Parietal chloroplast with pyrenoid (**Figure 2.1**). Cosmopolitan; often common in shaded and polluted habitats where lichens are absent or sparse.

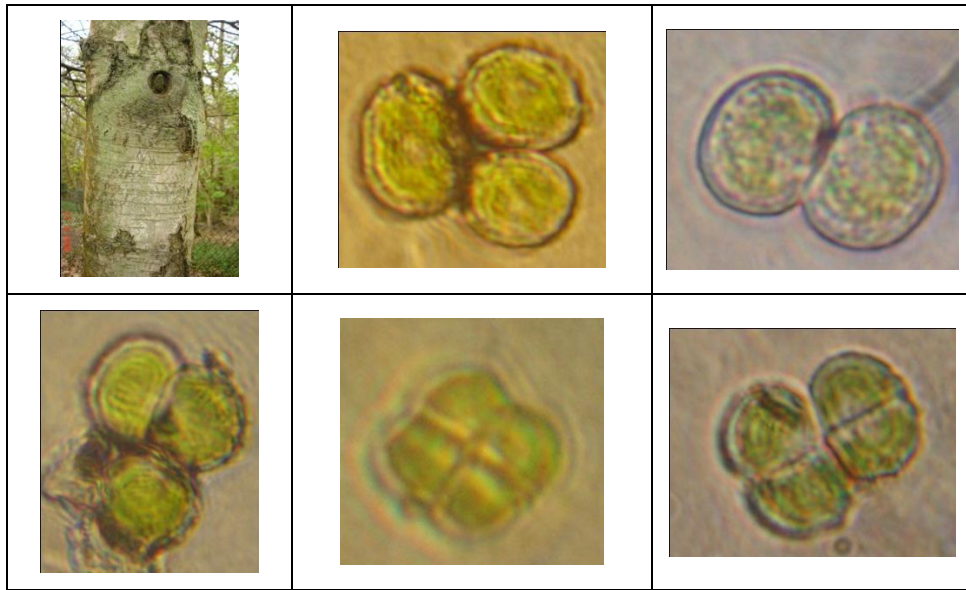


Figure 2.1 : *Desmococcus olivaceus* as seen on tree and under light microscope (400x magnification).

ii) *Trentepohlia abietina*

Forms orange tufts with unilateral or irregularly arranged branches, cells of the prostrate filaments spherical or swollen, 10-15µm in diameter, with those of the erect filaments cylindrical, 5-9µm wide, 10-30µm long, walls smooth (**Figure 2.2**).

Probably widely distributed. It is often confused with *Trentepohlia aurea*.



Figure 2.2: *Trentepohlia abietina* as seen on trees and under light microscope (400x magnification).

iii) *Trentepohlia umbrina*

Forms reddish to reddish-brown expansions of creeping filaments (**Figure 2.3**). Irregularly and openly branched, congested with filaments readily breaking into 1-or few-celled fragments; cells in intercalary position to ellipsoidal, 7-15 to 27-35 μm wide, 1 to 2 times longer than wide; ultimate branches terminate in a blunt and shortly cylindrical apical cell, 8-10 μm wide and 1 to 3 times longer than wide; walls smooth, often becoming rough; sporangia single and spherical, subspherical or ellipsoidal, terminal on curved stalks with a basal ring, or spherical to ovoid or a little curved, sessile and terminal, intercalary or lateral in position; gametangia intercalary, spherical and often becoming flask-shaped with a papilla. This species usually grows well in alkaline conditions, thus it is regarded as an indicator of alkaline pollution.



Figure 2.3 : *Trentepohlia umbrina* as seen on trees and under light microscope (400x magnification).

iv) *Apatococcus lobatus*

Cells single or forming 2-, 3- or 4-celled packets, often pressed, 6-12(-15) μm across, walls usually thickening with age. Chloroplast parietal without pyrenoid. Probably cosmopolitan, one of the commonest terrestrial algae and sometimes the principal

component of the widespread '*Pleurococcus-Protococcus* assemblage' that forms a pale green, powdery coating over tree bark.

Very evident in urban environments where atmospheric pollution results in the absence or reduced abundance of many pollution-sensitive lichens that often compete with algae. It is well developed in agricultural areas where fertilizers are abundant, leading to rapid growth of subaerial algae at the expense of lichens. Often most conspicuous in the spring and autumn when humid, damp and reasonable light conditions result in the optimal growth of many subaerial algae (**Figure 2.4**).



Figure 2.4 : *Apatococcus lobatus* as seen under light microscope of 400x magnification.

v) ***Trebouxia arboricola***

Synonyms: *Trebouxia humicola*. Cells 2-25µm wide, spherical, walls relatively thin; chloroplast hollow with a lateral notch and one or more pyrenoids (**Figure 2.5a**). Probably cosmopolitan, a common terrestrial alga on tree trunks. Often occurs with *Desmococcus* and *Apatococcus*.

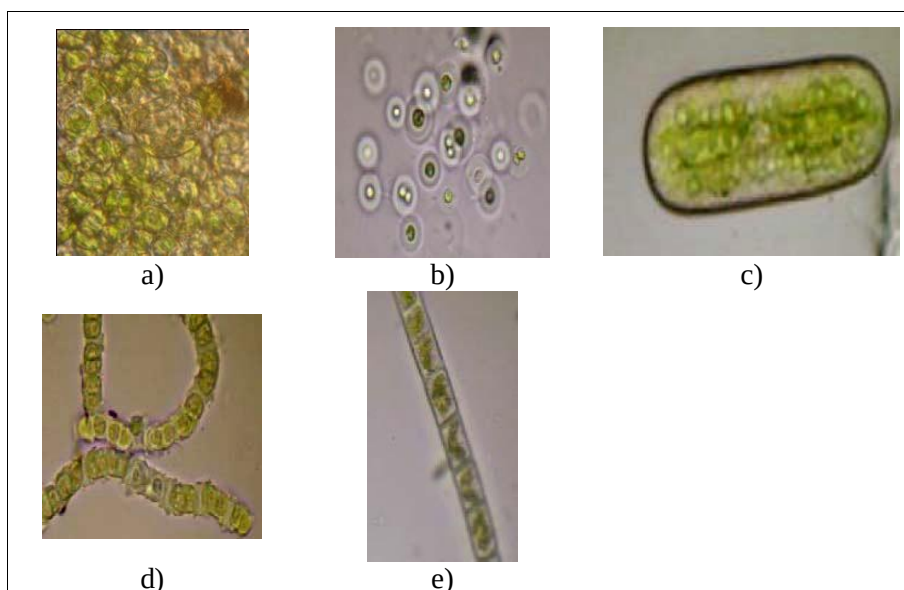


Figure 2.5 : Algal cells as seen under light microscope of 400x magnification.

a) *Trebouxia humicola*

b) *Chlorococcus* sp.

c) *Cylindrocystis* sp.

d) *Desmidium* sp.

e) *Spirogyra* sp.

vi) *Chlorococcus* sp.

Usually found in small colonies of cells and rarely as single cells. Aggregations of 2-16 cells. Cells subspherical, cells often form new sheath layer each time they divide. Less distinct layer in terrestrial forms. Sheath colourless or yellowish (**Figure 2.5b**).

vii) *Cylindrocystis* sp.

This green alga has cylindrical cells with broadly rounded apices, smooth walls and mucilage around it (**Figure 2.5c**). Contains star-shaped chloroplast with pyrenoid. Extensions of the chloroplast radiates from pyrenoid often becoming longitudinally extended into ridges. Cosmopolitan, widely distributed in acidic environments especially bog areas.

viii) *Desmidium* sp.

Cells joined in long, twisted filaments, some within a thick mucilage sheath (**Figure 2.5d**). Cells ranging from transversely narrow-oblong, oblong semi-elliptical to barrel-shaped. Restricted to acid waters.

ix) *Spirogyra* sp.

Green algae with long, unbranched, cylindrical cells (**Figure 2.5g**). Up to 30 times as long as broad, with outer mucilaginous sheath that gives them a distinctively slippery feel. Chloroplast parietal and in the form of spiral bands or ribbon-like, usually 1-16 per cell, sometimes closely coiled, sometimes almost straight, broad or narrow with smooth or wavy margins. Pyrenoids disc-like, regularly placed along length. Cosmopolitan.

x) Other species

In this study, diatoms and other species were not recorded in detail as they were only found in a very small number compared to other species. We feel that low species records are perceived to be insignificant to this study (**Figure 2.6**).

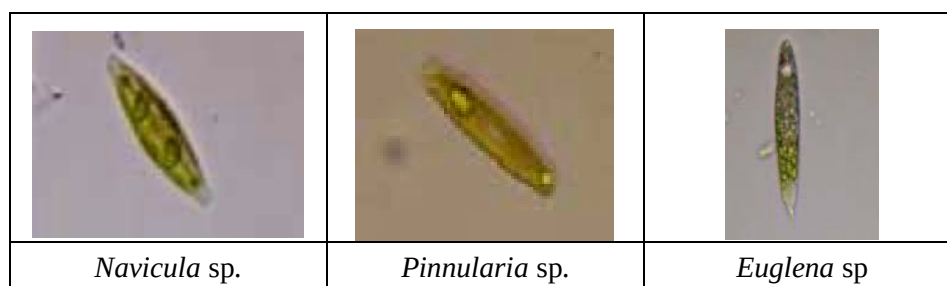


Figure 2.6 : Other algal species found in this study, mostly diatom. Magnification of 400x.

2.5 Pollutant concentration determination

Monitoring of nitrogenous gases was carried out to determine the concentrations of NH₃, NO_x, NO₂ and NO using open-ended passive diffusion tube samplers supplied

by Gradko UK. Tube samplers were first introduced by Palmes (Palmes et al., 1976) and have been widely used for many studies (Campbell, 1988; Bower et al., 1991; Campbell *et al.*, 1994; Batty, 2003). The tubes were designed for passively monitoring airborne gases and other molecules.

They consist of an acrylic tube with two closely fitted caps and small steel meshes between the tube and the cap. The sampler contains filters impregnated with phosphoric acid which absorbs gas-phase NH_3 as NH_4 , that can be easily measured spectrophotometrically by the indophenol blue method (Campbell, 1988; Bower *et al.*, 1991). The mean concentration of NH_3 during the exposure period was calculated using the exposure time and ammonium content (Andersen *et al.*, 2006). The detection limit of NH_3 analysis was $0.149 \mu\text{g NH}_4$. The levels of NH_3 were later obtained by ion chromatography with reference to a calibration curve derived from the analysis of standard ammonium solutions for NH_3 .

The mesh discs which detect NO_2 were soaked in 50%v/v triethanolamine (TEA)/acetone solution as an absorbent. Nitrogen dioxide and its derivatives were analysed using UV spectrophotometry. The concentrations of nitrite ions absorbed by the mesh were quantitatively determined by UV/visible spectrophotometry with reference to a calibration curve derived from the analysis of standard nitrite solutions.

Three replicate samplers were placed at each monitoring site, mounted vertically at 1.5m above ground. Tubes were exposed to ambient pollution by removing the plastic cap on the bottom end of the tube and left on-site for 3 weeks. All tubes were supplied and analysed by Gradko International, England.

2.6 Bark pH

Bark samples were collected 1 m above from the base of trees beneath the quadrat used for algal collection. Method for bark pH followed Kricke (2002) where thin slivers of tree bark were removed from the tree using a Surform-type wall-paper scraper. This removes only the outermost bark layer which has the closest association with the epiphytic vegetation. A total of 0.5 g of the surface tree bark was ground and soaked in vials with 10 ml deionized water. The vials were shaken vigorously and left for 30 minutes, then shaken in an automatic shaker for 8 hours. The supernatant solution was used for pH measurement. For determination of twig pH, the twigs were sealed with wax at both ends before being soaked. The vials were then shaken vigorously and left for 30 minutes and then shaken in an automatic shaker for another 20 minutes. Stoppered vials were used to prevent the ingress of atmospheric CO₂. Values of pH were determined using a Mettler Toledo MP 230 pH meter.

Detailed methods, whenever required are discussed in each subsequent chapter.

Chapter 3

Effects of NH₃ and other nitrogenous gases emitted from a pig and poultry farm on epiphytic terrestrial algae

3.1 Introduction

In addition to the well-documented gaseous emissions from industrial premises (see chapter 1) it is also clear that farms, especially intensive livestock units, can be a source of gaseous pollutants. Emissions to the atmosphere from poultry farms consist of dust, particulate matter, odours, endotoxins, methane, H₂S, CO₂, and nitrogenous compounds such as NH₃. Of these ammonia and other forms of N deposition can be a major source of pollution adjacent to pig and poultry farms (Bouwman *et al.*, 1997; Sommer *et al.*, 2001).

In the UK, 80% of NH₃ emissions come from livestock manure and N fertilizers (Asman, 1998; Sutton *et al.*, 2003). The gas is normally transferred by wet or dry deposition to terrestrial plants and other surfaces in the surrounding environment (van der Waluw *et al.*, 2011). In Europe, NH₃ emissions have increased by more than 50% over the last few decades (McCorry & Hobbs, 2001; Sutton *et al.*, 2003). 80% of the yearly increases come from intensification of livestock production (Misselbrook *et al.*, 1998), with 90% of it from animal wastes and fertilizers (Buijsman *et al.*, 1998). In the Netherlands, 85% of the total NH₃ emissions originates

from livestock farming (Koerkamp *et al.*, 1998), while in Germany 70-90% comes from this source (Flaig & Mohr, 1996).

Ammonia is a colourless gas, lighter than air and has a pungent odour. The gas is released during the breakdown of urea excreted by farm animals. It is only in the case of housed livestock that appreciable concentrations of the gas accumulate. According to Koerkamp (1994) and Sutton *et al.* (1998), this reactive gas is deposited and absorbed by land and water surfaces, usually close to the source (dry deposition). Under some circumstances, some ammonia may reach higher levels in the atmosphere and be blown long distances before being deposited in rainfall (wet deposition). The greatest concentrations of ammonium-N in rain are found in the south and east of the UK which also has the greatest number of intensive livestock farms.

Besides that, NH_3 is highly water-soluble and this gas is classified as a particulate precursor - readily reacting with other substances in the atmosphere to form ammonium hydroxide and other molecules. Ammonia is referred to as a reduced form of nitrogen, and can be readily released from solution by volatilization. Animal wastes contain a significant amount of nitrogen in the form of microbial protein in the faeces and uric acid in urine. Both are readily broken down to urea (**Figure 3.1**). During this process, ammonia is released by volatilization aided by the exothermic nature of the microbial metabolism responsible. Livestock manures are known to be the major sources of ammonia emissions. Release is increased by the use of N in fertilizers or high protein diet in animal feeds. If the N is not metabolized into animal protein, the amount of N in dung and urine will be increased.

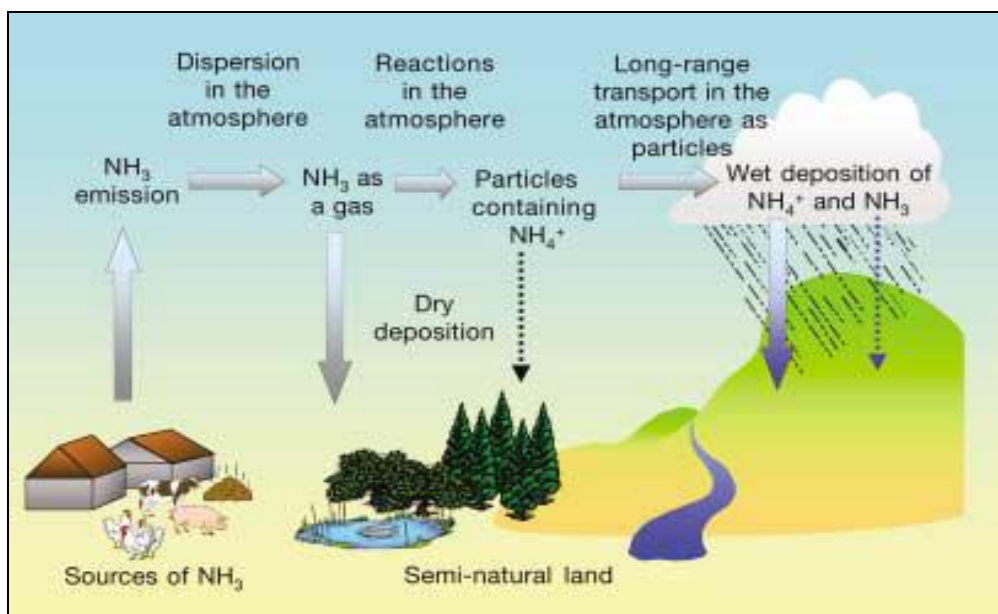


Figure 3.1: Ammonia flows in the atmosphere, adapted from (<http://www.defra.gov.uk/environment/quality/air/airquality/publications/ammonia/documents/ammonia-in-uk.pdf>) showing the movement of NH₃ through wet and dry deposition.

3.1.1 Effects of NH₃ and other nitrogenous gases on the ecosystem community

NH₃ and NH₄ deposition is currently above the critical load of N in many parts of the UK including upland and lowland heath, upland bog, semi-natural grassland and some woodlands. Over the last 20 years, many studies have provided evidence of an adverse impact of N deposition on vegetation (Koechy & Wilson, 1988; Fangmeier *et al.*, 1996; Misselbrook *et al.*, 1998; Sutton *et al.*, 1998). An area of high NH₃ emissions has been reported to alter plant species composition of communities and ultimately change ecosystems (Roelofs *et al.*, 1996; Van Dobben & Ter Braak, 1998; Ruoss, 1999; Wolseley & James, 2002; Sutton *et al.*, 2003).

Apart from the offensive odour emanating from intensive husbandry units, substantial amounts of NH₃ can cause eutrophication of watercourses and changes in the pH (Koerkamp *et al.*, 1998; Pain *et al.*, 1998; Wathes, 1998; Erisman *et al.*, 2003). N deposition is known to cause subtle changes to the frequency and density of plant

species. In an area where the natural flora is adapted to limited N, increasing N-deposition causes native plants to be outnumbered by those that thrive on higher nitrogen inputs. Eutrophication increased the growth of plants thriving on a limited N supply. At the same time, species that cannot cope well with increasing N are replaced by N-loving species.

Some studies have shown an appearance of invasive species in semi-natural ecosystems. For example, some parts of heathland have been taken over by grass when N deposition increases. The loss of moss-dominated heathland is also reported to be caused by an increase of N. In terms of conservation, this situation may lead to loss of probably important species. According to Saggar *et al.*, (2004), ecological succession can be reversed due to NH_3 pollution.

The domination of N-loving species (nitrophytes) at the expense of acidophytes or original vegetation (Sutton *et al.*, 1993; Woodin & Farmer, 1993; Pitcairn *et al.*, 1995) was more apparent on acid-bark trees, where nitrophytes were reported to be absent or very rare. Enhanced N also triggered plant sensitivity to stresses such as frost, drought and insect damage (Posthumus, 1982). Thus this will also contribute to reduced stability of plant communities.

Besides eutrophication, NH_3 emissions also contribute to acidification of soil and surface waters. NH_3 is deposited on soil, oxidized to nitrate and increases soil acidity. Acidification begins when oxides of nitrogen and sulphur (NO_x SO_x) are converted to nitric and sulphuric acid, as also happens in acid rain formation. In the short term NH_3 can neutralise sulphuric acid converting it to ammonium bisulphate. However, this

process only happens if the NH_3 concentration is less than twice that of the sulphuric acid. If the ammonia concentration is greater, it will react with other acid vapours preferentially (Patterson & Adrizal, 2005).

Since excess ammonia can lead to serious environmental impact, many European countries such as the UK, Denmark, Sweden and Germany have passed regulations limiting the level of NH_3 emission from livestock houses (Sommer *et al.*, 2009). Under the Gothenburg Protocol, participating countries are committed to bring NH_3 emissions within national ceilings (Angus *et al.*, 2006). Sensitive areas (within 300 m from source) are classified according to their critical load and farms are not permitted to increase their NH_3 emissions when there are any changes in production (Sommer *et al.*, 2009).

Besides NH_3 , other N-sources emitted from pig and poultry farms are NO_x and N_2O . Even though the impact of NH_3 as the major pollutant is well documented, the effect of other gases should also be taken into consideration. NO_x is known to trigger ozone promotion and acid rain which has caused the death or decline of forests in many parts of Europe. The other important oxide, N_2O which can contribute to ozone depletion (Williams *et al.*, 1992; Saggar *et al.*, 2004), is emitted predominantly by solid manure heaps and other nitrogenous fertilizers (Williams *et al.*, 1992; Bouwman, 1997; Chadwick *et al.*, 1999).

N_2O naturally exists in the atmosphere at very low concentrations, typically about 310ppb. However, because N_2O has a long atmospheric half-life of 150 years its contribution to global warming could be as much as two times higher than that of CO_2

(Watson *et al.*, 1996). Excess NH_3 deposition on soil can also contribute to the increase of N_2O emissions (Williams *et al.*, 1992; Granli & Bückman, 1994; Bouwman, 1997). Williams *et al.* (1992) in their study concluded that N_2O deposition was associated more with denitrification than nitrification. However whichever of these two processes is dominant (thereby influencing N_2O production) can change very rapidly, depending on soil properties, drainage in soil, climatic properties and level of organic matter (Groffman, 1991; Saggar *et al.*, 2004).

3.1.2 Effects of N-deposition on lower plants

NH_3 is an important nutrient source for lower plants such as algae, lichens and bryophytes (Downing & Rigler, 1984; Nielsen *et al.*, 1997). Lacking a waxy cuticle or stomata these organisms are unable to regulate NH_3 intake in the way that higher plants can (Reiners & Olson, 1984). The uptake of NH_3 by lower plants is enhanced by moisture and dew (Apsimon *et al.*, 1987). There are several studies reporting the effect of NH_3 emissions on the growth and distribution of lichens (Hawksworth & Rose, 1970; Nimis *et al.*, 1990; Söchting, 1995; Wolseley *et al.*, 2002; Krupa, 2003) and bryophytes (Beltman *et al.*, 1995; Kooijman & Bakker, 1995; Pitcairn *et al.*, 1995; Paulissen, 2004). But there seem to be no parallel reports on the effects of ammonia emissions on epiphytic terrestrial algae.

Nitrogen and phosphorus have been determined as primary limiting nutrients to algal growth (Schindler, 1971). A suppressed supply of N caused a decline in algal growth but at the same time, excess NH_3 can affect the density and diversity of algae (Vymazal, 1995; Wear *et al.*, 1999; Thornber *et al.*, 2008). Besides NH_3 , NO_x which is responsible for the formation of acid rain also contributes to algal growth. Any

form of nitrogen in excess accumulating in fresh waters can cause algal blooms and subsequent eutrophication. As the algae decay, this rapidly depletes oxygen in the water to the detriment of all aerobic life forms (Handy & Poxton, 1993).

3.1.3 Hypotheses

Considering terrestrial epiphytic green algae only, the current study postulates that :

- 1) N-deposition plays an important role in determining species diversity; that N will promote the survival of nitrophyte species and suppress acidophytes.
- 2) NH_3 emitted from an N source (livestock farm) as dry deposition is deposited locally and concentrations decline rapidly with distance from the livestock farm.

3.1.4 Aims and objectives

The aims of this study are as follows :

- 1) To assess the relationship between distance from an N source (pig and poultry farm) and algal density.
- 2) To determine which algal species are affected by ammonia and other nitrogenous gases within close proximity of a pig and poultry farm.
- 3) To determine which pollutant has the greatest effect on algal density.
- 4) To investigate which N-gases exert the greatest effect on the growth of epiphytic algae.
- 5) To examine the role of aspect and bark pH in contributing to algal density.
- 6) To access the effect of pollutants on bark pH.

3.2 Materials and Methods

3.2.1 Site Description

This study was conducted at a rural area in a 200 acre farm located on the western side of a large woodlands in Berkshire, south east England (**Figure 3.2**). The farm which is situated at the edge of an intensely farmed irrigated region, has been developed progressively since the 1790s notably by the construction and extension of farm buildings (www.pastscape.org. English Heritage monument records). It was used as a dairy unit originally and only after April 1997 was the farm converted to a pig and poultry farm. The farm consists of approximately 100 sows, 200 piglets, 650 fattening pigs and 1200 chickens. The farm emits strong smells of ammonia.



Figure 3.2: Location showing the pig and poultry farm and 3 sites following the south westerly wind direction. A= 5 m, B= 35 m, C= 150 m (downwind sites), D= 400 m (upwind as a control site).

The area is semi-arid with minimum mean annual temperature of 17.8 °C and maximum of 23.5 °C. Mean annual rainfall for this area is 624 mm. Four sites along a south-west to north-east axis, corresponding to prevailing wind direction were selected along a transect at 5 m, 35 m, 150 m downwind and 400 m upwind as a control site. At each site, 3 *Quercus robur* (oak trees) trees were selected for biomonitoring.

3.2.2 N Gaseous Monitoring

N gaseous monitoring was conducted using open ended passive diffusion tube samplers (see Chapter 2).

3.2.3. Systematic Algal Collection

Systematic collection of algae was carried out along the line transect through the woodland adjacent to the pig and poultry farm. Three mature *Quercus robur* trees at each site were sampled with a 15 x 15 cm quadrat, at 1.5 m above the ground, in line with the passive samplers. The quadrats were placed at aspects of 130° SE, 210° SW and 310° NW. Algae collected from trees followed general methods (See Chapter 2).

3.2.4 Bark pH analysis

Bark pH determination followed general method (See Chapter 2).

3.2.5 Data Analysis and Statistics

Data were analysed using SPSS, MINITAB and R statistical software. Samples were tested for equal variance using Anderson-Darling normality test. The relationships between different variables were explored using Pearson's correlation coefficient and

linear regression. ANOVA and t-tests were used in many cases. Tukey's Pairwise Comparison was used to test for differences between sites. Non-normally distributed data were analysed using the Friedman test which uses ranking order, to investigate the role of aspect in affecting algal growth (Crawley, 1993, 2005; Grafen, 2002; Dytham, 2003).

3.3 Results

3.3.1 Algal Species

Algae recovered from all the quadrats revealed that almost 100 % were of the green alga, *Desmococcus* sp. Another algal species namely *Trebouxia* was also found but only in a very small quantity. Thus we considered *Trebouxia* as unimportant in this study.

3.3.2 The relationship between distance from source and algal density

Algal density was negatively correlated with distance from the farm (Pearson correlation coefficient, $R = -0.783$, $r = 88\%$, $p = 0.003$). Algae recovered from the quadrat showed obvious differences in terms of density with distance (**Figure 3.3**). In this study, data showed a significantly higher algal population nearer to the farm (1-way ANOVA, $F_{3,8} = 29.54$, $p = 0.001$). Numbers of algae were significantly higher nearer the source at 5m and 35m compared to other sites.

The highest density of algae was recorded 5 m from the farm, with 3166 ± 160 cells/ml. This equates to 563 cells/cm^2 of bark. This was followed by the site 35 m from the source with 2001 ± 397 cells/ml, equivalent to 355 cells/cm^2 . Further away from the farm at 150 m, algal density was reduced drastically to 332 ± 287 cells/ml

(59 cells/cm²). As expected, the control site which was located 400 m upwind from the farm showed the lowest number of algae at 194 ± 87.9 cells/ml (34 cells/cm²).

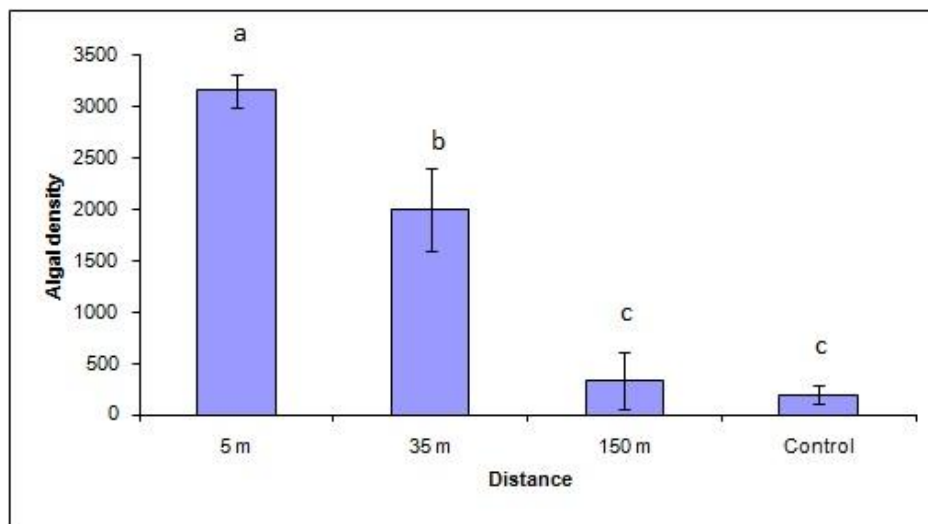


Figure 3.3: Variation in algal density (cells per ml of washing) with distance from the source (farm). The values are the means $\pm$ SE bars. Values ascribed a different letter differ significantly at $p < 0.05$. Data show a clear reduction of algae at 150 m from the source.

Tukey's pairwise comparison was run to test between sites. It showed that there were significant differences between the sites at 5m, 35m and 150 m from the farm. As expected, there was no significant difference between the farthest site (150 m) and the control site. All data were found to be normally distributed, using the Anderson-Darling Normality Test.

3.3.3 Pollutant concentrations in the locality of the farm

Atmospheric ammonia concentrations declined markedly with increasing distance from the farm (**Figure 3.4a**). NH₃ concentrations were negatively correlated with distance from the farm and this is the only pollutant which showed a clear reduction in terms of concentration. At 5 m, the concentration was $18.34 \pm 1.38 \mu\text{gm}^{-3}$. The concentration continued to decrease quite drastically at 35 m to $9.72 \pm 1.69 \mu\text{gm}^{-3}$. At

150 m from the farm, the NH_3 concentration almost reached the background value at $5.10 \pm 1.78 \mu\text{g m}^{-3}$. NH_3 concentration at the control site, which was located at 400 m upwind was $3.74 \pm 1.11 \mu\text{g m}^{-3}$.

Other atmospheric pollutants such as nitrogen dioxide, total nitrogen oxides and nitric oxide fluctuated with distance but showed no obvious pattern (**Figure 3.4b-d**).

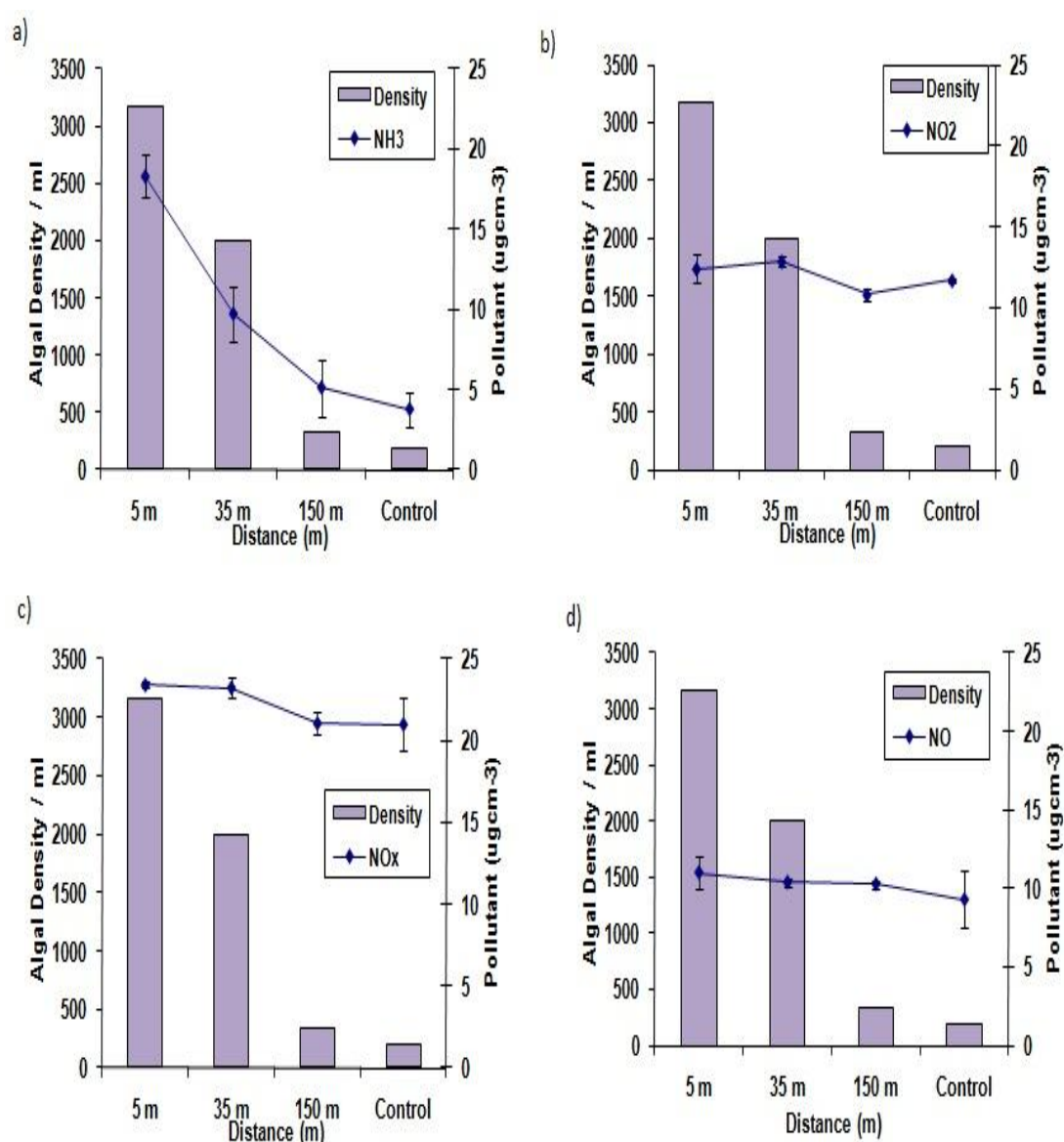


Figure 3.4: Variation in algal density and concentration of gaseous pollutants with distance from the Farm.

a) Ammonia

b) Nitrogen dioxide

c) Nitrogen oxides

d) Nitric oxide

3.3.4 The relationship between algal density and pollutant concentrations

Out of four pollutants tested in this study, only NH_3 and NO_x were found to have a significant correlation with algal density (**Figure 3.5a and 3.5c**). NH_3 concentration showed a strong positive correlation with algal density ($p < 0.001$, $r = 0.912$). The relationship between NO_x concentration while significant was weaker ($p < 0.05$, $r = 0.631$). Neither NO_2 nor NO was significantly correlated with algal density (**Figure 3.5b and 3.5d**).

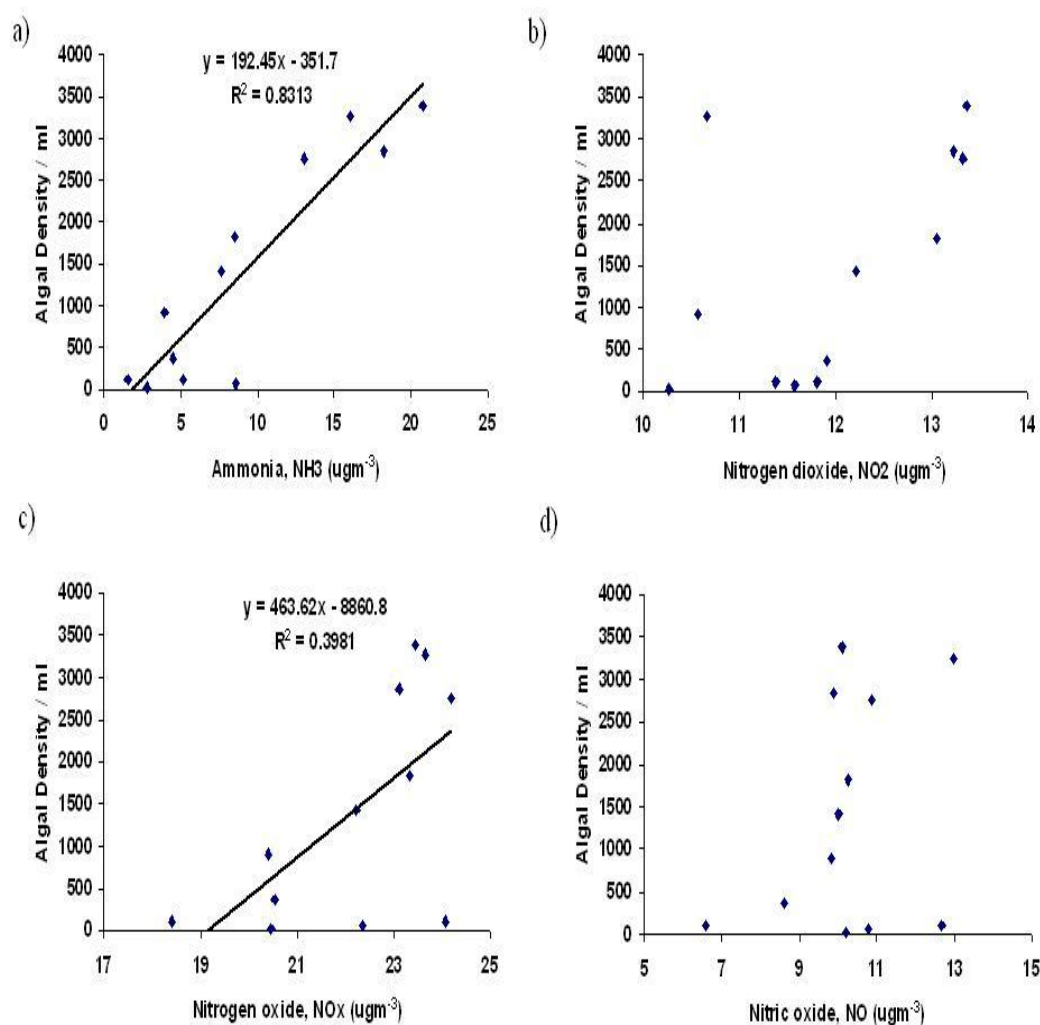


Figure 3.5: Correlations between algal density and atmospheric pollutants.

a) Ammonia

b) Nitrogen dioxide

c) Nitrogen oxide

d) Nitric oxide

3.3.5 The role of pollutants in affecting the bark pH

Bark pH was positively correlated with NH_3 concentrations ($R=0.768$, $p=0.004$) and negatively correlated with distance from the pig and poultry farm (1-way ANOVA, $F_{3,8} = 13.35$, $p = 0.002$). Bark pH ranged from 6.12 – 6.18 at 5 m and 35 m and then fell to 4.3 – 4.5 at sites away from the farm (**Figure 3.6**).

Tukey's pairwise comparison showed that there was no significant difference between bark pH at 5 m and 35 m. Both sites were located close to the farm. Similarly, there were no significant differences between the sites further away from the farm, at 150 m and the control site.

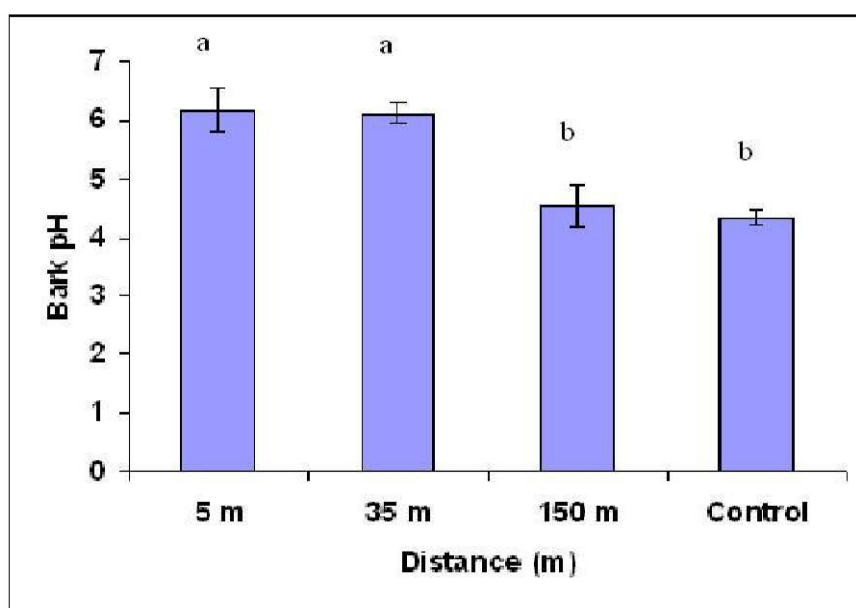


Figure 3.6: Variation of bark pH in relation to distance from the farm. The values are the means $\pm$ SE bars. Values ascribed different letters are significantly different at $p < 0.05$.

In terms of a relationship between bark pH and pollutant concentrations, NH_3 showed a positive correlation while NO_x showed a negative correlation (**Figure 3.7a and 3.7c**). NH_3 was found to have a strong relation with bark pH ($p<0.05$, $r = 0.768$). At the highest concentrations of ammonia, bark pH reached up to almost pH 6.6. When

the NH_3 concentration was at its lowest, bark pH tended to be more acidic, as low as pH 4.0 (**Figure 3.7a**).

On the other hand, NO_x showed a mild negative correlation with bark pH ($p < 0.05$, $r = -0.587$). Bark pH was more acidic at higher concentrations of NO_x as compared to lower concentrations (**Figure 3.7c**). NO_2 and NO showed no significant correlation with bark pH (**Figure 3.7b and 3.7d**).

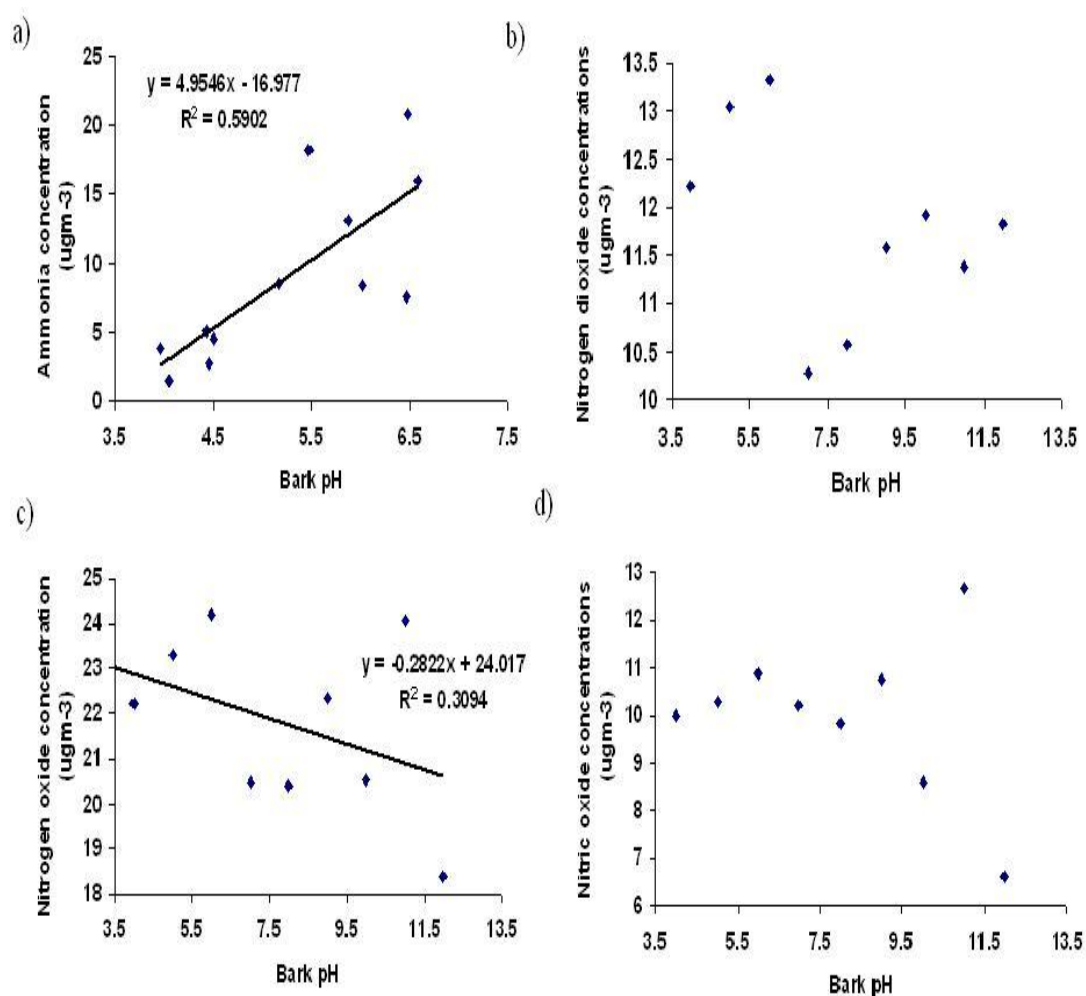


Figure 3.7: Correlation between bark pH and atmospheric pollutants.

a) Ammonia

b) Nitrogen dioxide

c) Nitrogen oxide

d) Nitric oxide

3. 3.6 The role of aspect in affecting algal density

The ranking order of algal density in relation to aspect is 210° SW > 130° SE > 310° NW. Algal density was highest at 210° SW with 1445 ± 377 cells/ml and lowest at 310° NW with 1383 ± 384 cells/ml (**Figure 3.8**), with the 130° SE value intermediate at 1442 ± 430 cell/ml. These values are well within the margin of experimental error and were not statistically significant. Thus, this particular study showed that aspect does not affect algal density ($p=0.779$, $S=0.50$). Data were analysed using the Friedman test for non-parametric data.

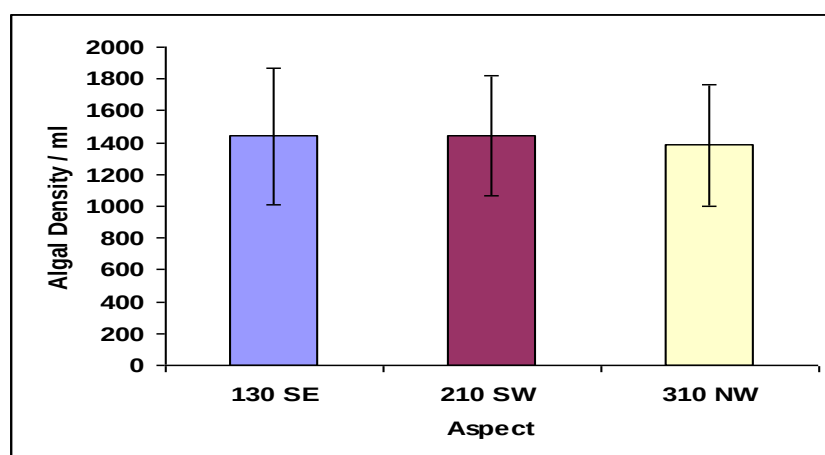


Figure 3.8: Variations of algal density in relation to aspect.

3.4 Discussion

3.4.1 Algal density within close proximity of a pollutant source

Algal density was greater closer to the source of pollution (**Figure 3.3**). A strong positive correlation between NH_3 and algal density explains the relationship between these two ($p < 0.001$, $r = 0.912$). As concentrations of NH_3 increase, algal density does so also (**Figure 3.5a**). There is a 72 % reduction in population density at 150 m, and 80 % at 400 m from the source. In a similar situation Fowler *et al.* (1998) reported a reduction of 98% in the first 200 m from the source and Pitcairn *et al.* (2002) reported a sharp decrease in the first 200 m and a 95% reduction at 650 m from an ammonia

source. The number of algae was highest when the NH_3 concentration was at its peak, which is expected since dissolved inorganic nitrogen such as ammonia, nitrate and nitrite are known usually to affect the distribution, productivity, and abundance of epiphytic algae (Ryther and Dunstan, 1971; Aneja *et al.*, 2003; Thornber *et al.*, 2008). Increased levels of N with decreasing distance from the pig and poultry farm (**Figure 3.4a**) is in agreement with Kauppi (1980), Pitcairn *et al.*, (1995), Söchting (1995), and Ruoss (1999). Higher N concentrations have often been reported to increase the rate of algal growth (Doering *et al.*, 1995; Taylor *et al.*, 1999; Thornber *et al.*, 2008). The background level of ammonia 400 m upwind is $3.74 \mu\text{gm}^{-3}$. This is in good agreement with work carried out in the Netherlands by Buijsman *et al.* (1998) where mean concentrations in background data ranged from 2-4 μgm^{-3} . This is similar to other regions with agricultural activities in the UK, Austria and Switzerland. Sutton *et al.* (2001), Löflund *et al.* (2002) and Thoni *et al.* (2004) however reported a lower annual mean NH_3 concentration of $<1 \mu\text{gm}^{-3}$ in regions without agricultural activity.

These data showed that *Desmococcus* sp. is a nitrophilic species and because NH_3 concentrations are high, other algal species could not compete and being less adapted to the conditions decline in number. Eventually, *Desmococcus* sp. outgrew other algal species due to the high NH_3 concentrations. Even though we also found *Trebouxia* in the quadrat, the quantity was very small, and we considered it as unimportant. That high rates of NH_3 deposition would result in nitrophilic species dominating was suggested by Pitcairn *et al.* (1998) and by van Herk (1999) who speculated that an area with high nitrogen deposition will result in nitrogen-tolerant species and a lack of N sensitive species. Also, they observed that species diversity within 50-300 m of the emission source is adversely affected with most being ‘weed

species' and the total number of species was reduced (Pitcairn *et al.*, 1998). *Desmococcus olivaceus* which is a known nitrophilous species was abundant in close proximity to the poultry and pig farm. According to Carfrae *et al.*, (2007) and Dupre *et al.*, (2010), nitrophilous epiphytes are positively correlated with NH_3 which at the same time, decreases acidophilous epiphytes.

In the present study, NH_3 concentrations close to the farm were 18 times higher than the critical level ($1\mu\text{gm}^{-3}$). The critical level is defined as the concentration in the atmosphere above which direct adverse effects on recipients such as plants, ecosystems or materials, may occur (Posthumus, 1988). Compared to other nitrogenous gases, NH_3 is the main source of dry deposition in the immediate vicinity of intensive animal husbandry units (van Herk, 2003; Frati *et al.*, 2008).

NH_3 concentrations at the closest station to the source (5 m) were higher than at the other stations (35 m, 150 m, control site) at $18.34 \pm 1.38 \mu\text{gm}^{-3}$. In line with theory, the number of algae at this station was highest at this point with 3166 ± 160 cells/ml (**Figure 3.4a**). As NH_3 declined with distance to $9.72 \pm 1.69 \mu\text{gm}^{-3}$, algal density also decreased to 2001 ± 397 cells/ml, but the difference was not significant. However, the difference between stations close to the source (5 m and 35 m) and those further away from the source (150 m and control) was significant. This result showed that at a distance below 35m from the source, NH_3 concentrations are sufficiently high to have a very significant effect on the algal density on tree trunks within this zone.

Gaseous ammonia is known to dissipate very quickly into the atmosphere within short distances of a source and algal density was also found to decrease accordingly. Fowler *et al.* (1998) and Sommer (2009) also observed the same pattern where NH_3 concentrations declined sharply at increasing distances from the pollutant source. Observation made by Fowler *et al.* (1998) showed that 60% of NH_3 emitted from a farm was deposited within 50 m and levels were close to background concentrations, 276 m from the source. This is further corroborated by Skiba *et al.* (2006) who observed an increase of NH_3 concentrations of up to 40 times close to the source as opposed to the background site.

At 150 m from the farm, algal density was significantly lower than at 5 m and 35 m (**Figure 3.3**). With NH_3 concentrations dropping to $5.10 \pm 1.78 \mu\text{gm}^{-3}$, algal density declined to 332 ± 287 cells/ml (**Figure 3.4a**). Also, algal density at 5 m and, 35 m differed significantly from that at the control site. Located 400 m upwind from the farm, with little or no effect of NH_3 , algal density at the control site was only 194 ± 87.9 cells/ml (**Figure 3.3**). The fact that the 150 m downwind site differed only marginally from this value is evidence for the rapidly dissipating nature of ammonia as a pollutant. This finding is in line with research conducted by Sommer *et al.* (2009) which concluded that at 150-200 m from the pollutant source, the farm under study was only marginally affected by NH_3 emitted from their chickens. The control site in the present study is in the upwind direction, but it has to be remembered that this is the direction of the prevailing not universal wind. Compared to our Transect and Nitrogen Deposition Study (Details in Chapter 4 and 5 of this thesis), background NH_3 is in the range of 2-6 μgm^{-3} with an average of average of 4 μgm^{-3} . Thus we consider that our control site provides representative background data for this study.

Apart from NH_3 , other N-containing gaseous pollutants detected in this study showed only a mild fluctuation with distances (**Figure 3.4b-d**). No apparent patterns were observed for NO_2 , NO_x or NO suggesting that the values detected were influenced by sources other than the farm and constituted the ‘background’ typical of this locality. None of them had any significant relation to the number of algae, except for NO_x (**Figure 3.5c**). Though only showing a mild positive correlation ($p < 0.05$, $r = 0.631$), it is possible that NO_x is influencing algal density. According to Sutton *et al.* (1993), areas of high deposition face a change in vegetation due to increased deposition.

3.4.2 Effects of bark pH and aspect in influencing algal density

Pollutant concentrations especially of NH_3 are believed to play a role in determining bark pH (van Herk, 2003). In this study, pH ranged from 4.3 to 6.2. Barkman (1988) reported in his work that the typical pH range for *Quercus* sp. is between 3.7 and 5.0. Van Herk (2001) reported a slightly different pH range, from 3.7-4.4 in a forest environment, 3.8-5.0 in urban areas and 5.6-6.4 in intense agricultural areas. In the present study as NH_3 concentrations increased, the pH also increased (**Figure 3.7a**). Close to the farm where NH_3 concentrations were highest, bark pH was at its highest as well. Further away from the farm where NH_3 concentrations were lower, the value of bark pH was also its lowest (**Figure 3.6**).

The higher density of algae close to the farm may be a result of pH, but could also be due to ammonia as a source of N nutrition. As the ammonia alters the bark pH, this situation provides a better environment for algal growth. Green algae as compared to other algal groups normally grow better at a higher pH. Moss (1973) stated that the

green alga *Desmidium swartzii* would not survive when pH was less than 4. In another study long exposure to NH₃ promoted nitrophilous species of lichens and this was thought to be mainly due to elevated bark pH, especially within 2-3km of the pollutant source (van Herk, 2001; van Herk *et al.*, 2003). Due to the alkaline properties of NH₃, the pH increased and ultimately helped in shifting the species composition (van Herk, 2001).

The growth of lichens and bryophytes is known to be affected by aspect or orientation (Plitt & Pessin 1924; Barkman 1988, 1990) probably because it affects the amount of light received. Also, aspect affects humidity, which is known to influence growth (Ferris-Kaan, 1995; Ferris and Carter, 2000). On the other hand, Buckley *et al.*, (1997) stated that light intensity does not necessarily have strong influences on vegetation.

In northern temperate areas, lichen growth is usually more prolific on the northern side of trees where the area is shielded from the desiccating effects of direct sunlight (Brodo, 1973; Barkman, 1990; Stubbs, 1989; Ferris & Carter, 2000). In the current study, the highest number of algae, but by an insignificant margin, were on the southwest side of trees, as also found by Larrson (2011). According to Barkman (1990) and Rubiano (1988), lichen coverage is higher when the site is protected from local winds. In the current study the near uniform distribution of algae around the trunk of trees suggests that aspect is a minor determinant of growth, and that other over-riding influences are at work.

3.5 Conclusions

This study follows the pattern of many other studies carried out at livestock farms over the past two decades, evaluating the involvement and effect of atmospheric pollutants from livestock farms to the adjacent area. The hypothesis that N-deposition plays an important role in determining species diversity was proved to be correct from the results presented from this current work. N deposition was found to aid healthy growth of *Desmococcus olivaceus*, the dominant algal species present at the sites. The occurrence of only a single dominant species of epiphytic algae on *Quercus robur* within close proximity of the farm suggests that local high N deposition is contributing to the dominance of nitrophilous species and is altering the composition of the community ecosystem. It is generally believed that other algal species could not tolerate high N and had been suppressed by N-thriving species such as *D. olivaceus*. In other words, a high N content in the atmosphere and at the plant surface triggered the survival of nitrogen-loving species and suppressed the acidophyte species normally dominant in the habitat.

The data in this chapter strongly suggest a direct link between ammonia concentration and the population density of a single algal species, the link being either nutritional or mediated via microhabitat pH. Although other gases such as NO_x also showed a degree of correlation with algal density, the concentration of ammonia is likely to be the over-riding factor.

In terms of conservation effort, attention should be paid to any area of high nitrogen deposition to prevent further loss of diversity of algal species in such habitats.

Chapter 4

Effects Of Atmospheric Pollutants On The Distribution Of Epiphytic Terrestrial Algae Along a Pollutant Gradient Transect In South-East England

4.1 Introduction

The algae comprise many different groups of photosynthetic organisms ranging from unicellular to multicellular forms. Algae can be found almost everywhere, from marine to freshwater and terrestrial ecosystems (John *et al.*, 2003). The taxonomy of terrestrial algae is still rather poorly known (Poikolainen *et al.*, 1998). According to Broady (1996), the diversity of terrestrial algae is not fully known as few collections have been made from all the suitable habitats. Furthermore, techniques used in earlier work were inadequate for recognition of the total algal flora present. In terms of the habitats occupied, subaerial algae are divisible into epiphytic (on living plants), epiphyllous (on leaves), epiphellous or corticolous (on bark, stems or trunks), epizoic (on animals), lithophilous (inhabiting stone, brick or concrete), epixylous (inhabiting dead wood such as poles, posts and buildings) and epimetallous (growing on metal surfaces) (Schlichting, 1975).

Algae which inhabit and are attached to higher plants are known as epiphytic terrestrial algae (Schlichting, 1975). John *et al.*, (2002) in their book, stated that Ettl & Gartner (1995) referred to algal species living on tree bark as aero-terrestrial or subaerial algae. They often form an ubiquitous powdery green layer on tree bark and are usually referred to as the '*Pleurococcus-Protococcus*' algal community. This assemblage is composed mainly of the green algae *Desmococcus* and *Apatococcus* (Lopez-Bautista *et al.*, 2006). Members of these two genera grow as solitary cells or small cell packets, although *Desmococcus* is capable of producing short filaments under very moist conditions (Wehr & Sheath, 2003; see also chapter 2). A further common epiphytic terrestrial alga is *Trentopohlia*, which appear as orange or red patches on tree trunks (Rindi *et al.*, 1999).

Algae are widely used for biomonitoring purposes (Bolier, 1985; Gregor & Munawar, 1989; Melville & Pulkownik, 2006; Keogh *et al.*, 2007). However, most of the species used in this way are freshwater or marine algae. Freshwater algae such as *Scenedesmus* have been used for the detection of water pollution for many years (Olguin, 2000; Olguin *et al.*, 2004).

4.1.1 Effects of atmospheric pollution on terrestrial algae

The abundance of terrestrial epiphytic green algae has been noticed to increase in polluted areas (Hanninen *et al.*, 1993). The increase of algal growth usually occurs simultaneously with the decrease or disappearance of lichens. Hallingback (1991) found that the abundance of algae increases on the periphery of a city, but diminishes towards the centre. Thus the abundance of epiphytic algae seems to respond to

gradients of air quality. Typically, the abundance first increases with increasingly polluted air, but then decreases again in the most polluted areas.

During the era of high atmospheric SO₂ pollution in western societies, subaerial algae came to be regarded as the most pollutant-tolerant epiphytes, compared with lichens and bryophytes. *Pleurococcus viridis* (currently known as *Desmococcus olivaceus*) was employed as an indicator for the most SO₂-polluted zone, with SO₂ concentration of > 170 µg m⁻³ (Hawksworth & Rose, 1970). Supporting evidence that sub-aerial algae are pollutant-tolerant was reported by Bates *et al.* (1990) in a study of lichen epiphytes on oak trees along a transect in south east England from rural Sussex into London. They reported that *Desmococcus viridis* was increased in abundance on moving from rural to built-up localities. In a continuation of the same study of lichen epiphytes on oak along the same transect into London, Bates *et al.* (2001) noted that a green alga, presumably *Desmococcus viridis*, was the most SO₂-tolerant epiphyte.

Later, with diminishing SO₂ concentrations in Europe, the algae continued to show higher cover values. Bates *et al.* (2001) and Stapper (2006) speculated that the abundance of green algae might be connected with high levels of atmospheric nitrogen in the form of NO_x and NH₃. Davies (2007) observed an increase in abundance of the filamentous green alga, *Trentepohlia*, on tree bark in south east England. In both Dortmund and Bonn, Germany, *Klebsormidium crenatum* has been observed on roadside trees where nitrogen concentrations are elevated (Stapper, 2006). At the same time, fast growing foliose lichens like *Parmelia sulcata* and *Physcia tenella* appear to have become overgrown rapidly by algal filaments,

particularly at sites with a strong influence of pollutants from motor traffic (Stapper, 2006).

More recently, increased N deposition has resulted in changes in vegetation, especially in areas of high deposition, where nitrophyte plant species have increased at the expense of acidophyte species (Sutton *et al.*, 2001). In Germany, in the early 1990s, pollution-sensitive lichens and bryophytes were reported as recolonising formerly heavily-industrialized areas. This area which is experiencing declining sulphur dioxide levels, showed that nitrophytic species now dominate over the acidophytes that were formerly predominant. Bates *et al.* (2001) reported that algae have outgrown and eliminated *Lecanora conizaeoides* in N-enriched environments, which probably indicates that algae are increasing in abundance when N deposition is high and S deposition is low.

In Sweden, Brakenheilm & Qinghong (1995) found significant positive correlations between the thickness of epiphyllous algal colonies on needles of Norway spruce (*Picea abies*) and atmospheric depositions of S and N. While in Finland, Poikolainen *et al.* (1998) reported that algae had become considerably more abundant over the period 1985 to 1995 and that this was a continuing trend. They noted that green algae were most abundant on conifers in Southern Finland where nitrogen deposition is also highest. Søbchting (1997), working in Denmark, also studied epiphyllous algal cover on spruce needles and confirmed that cover had increased in recent years in Denmark and neighbouring countries.

4.1.2 Air Quality in the UK

The harmful effects of SO₂ on lichens and their algae have long been known (Hawksworth & Rose, 1976). More recently, increased N deposition has resulted in changes in vegetation, especially in areas of high deposition, where nitrophyte plant species have increased at the expense of acidophyte species (Sutton *et al.*, 1993).

Sulphur dioxide was the most important atmospheric pollutant affecting plants until the mid 20th century in the UK. Although this has since greatly diminished, plants still face significant levels of nitrogen deposition and an array of other atmospheric pollutants such as ozone (Bell & Ashenden, 1997; Purvis *et al.*, 2003; Purvis *et al.*, 2007).

Many studies have assessed the effects of SO₂ and N pollutants on lower plants, including lichens, bryophytes and fungi (Hawksworth & Rose, 1970; Hawksworth & McManus, 1989; Brakenheilm & Qinghong, 1995; Bates *et al.*, 1996; Haapala, *et al.*, 1996; Søbchting, 1997; Poikolainen *et al.*, 1998; Brunialti *et al.*, 2002; Batty *et al.*, 2003; Newsham 2003; Larsen *et al.*, 2007). South-east England is one area where the recent remarkable decline in SO₂ pollution and its effects on lower plants have been clearly demonstrated (Rose & Hawksworth, 1981; Batty *et al.*, 2003).

Before the 20th century, most air pollution problems arose from the burning of wood, coal and other raw materials without emission controls. In the 20th century, increased use of the automobiles also increased emissions of nitrogen oxides and reactive organic gases. Prior to the 1980s, most energy in the UK was obtained from coal. However, coal consumption fell by 57% between 1980 and 1998. By 1998, the UK

was obtaining 37% of its energy from oil, 34% from natural gas, 14% from coal, 12% from nuclear power, 1% from hydroelectric power and 2% from other renewable sources (Jacobson, 2002). Even though natural gas still releases CO₂ and NO₂ when burned, the releases are smaller compared with those from other combusted fuels. Thus natural gas is regarded as the cleanest of the fossil fuels.

In 1956, the United Kingdom Clean Air Act was passed in response to a devastating winter smog event in London that killed 4000 people in 1952 (Wellburn, 1994). This Act controlled both household and industrial emissions of pollutants. However, it only dealt with black and dark smoke, not with sulphur dioxide. The Act resulted in many smokeless zones being imposed in London and other cities, and the relocation of many power plants to rural areas. In 1968, the Clean Air Act required industries burning fossil fuels to build tall chimneys so that their emissions would not deposit to the ground locally. In 1993, catalytic converters were required in all new gasoline-powered vehicles.

In the intervening period between the late 1960s and the 1990s there were many socio-economic changes, such as the collapse of traditional heavy manufacturing industry and the contraction of the coal mining industry which led to a great reduction in SO₂ emissions. In 1997, a plan called the United Kingdom National Air Quality Strategy was published. Under this strategy, ambient limits for ozone, nitrogen dioxide, sulphur dioxide, carbon monoxide and other pollutants were set (Jacobson, 2002). In 1990, the UK emitted an estimated 2.73 million tonnes of oxides of nitrogen, making it the third largest source of NO_x in Europe (Bell & Ashenden, 1997). However, both NO_x and NH₃ emissions have declined in recent years.

4.1.3 Aims and objectives

The objectives of the study recorded in this chapter are as follows:

- 1) To investigate the relationship between algal percentage cover and number of cells per unit area (population density).
- 2) To assess the effect of substratum, bark pH and slope angle on algal density.
- 3) To assess how contemporary atmospheric pollutants influence the abundance and diversity of epiphytic terrestrial algae along a gradient of air quality along a transect from the countryside of south-east England into London.

4.2 Materials and Methods

4.2.1 Site Descriptions

The study of epiphytic algae along an air quality gradient, involved a 68-km transect running from Kensington Gardens in Central London to Hesworth Common in rural Sussex (**Figure 4.1**). The intermediate stations were Putney Heath, Epsom Common, Holmwood Common and Ebernoe Common. All but the last station were originally established in 1979 for a study of the effects of atmospheric pollutants on epiphytic lichens (Bates *et al.*, 1990; Batty *et al.*, 2003). Each sampling station supports areas of relatively free-standing and reasonably mature oak trees although there are variations in canopy closure which may influence the types of epiphytes present. Details of the study sites are given in **Table 4.1**.

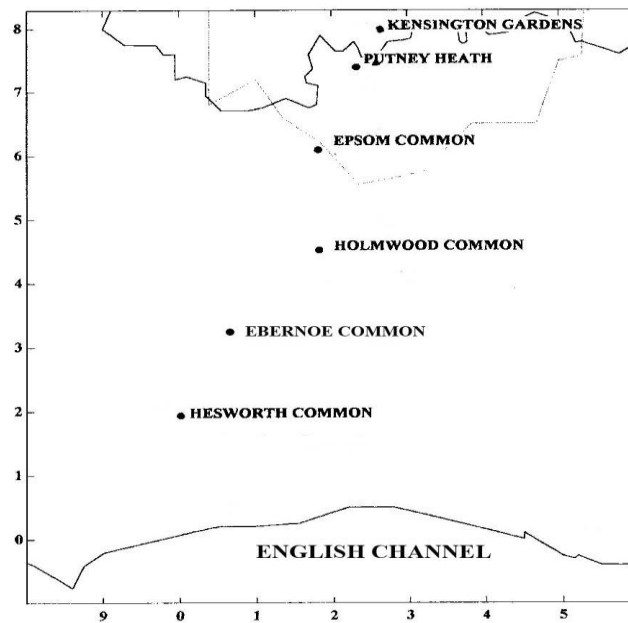


Figure 4.1. Sites for transect study at six stations along pollution gradient (modified after Bates, et al., 2001).

Table 4.1. Details of sampling stations.

Region	Station	Description	Grid Reference	Distance from City Centre (km)
City Centre	Kensington Gardens	Free-standing trees in extensive city park	TQ 265 799	0
Inner suburbs	Putney Heath	Free-standing trees in grass, surrounded by town dwellings.	TQ 233 740	7
Urban fringe	Epsom Common	Free-standing trees in grass between pond and woodland.	TQ 182 609	20.5
Rural Surrey	Holmwood Common	Free-standing trees in grass between pond and woodland.	TQ 183 453	35.5
Rural Sussex	Ebernoe Common	Free-standing trees at edge of large area of ancient woodland.	TQ 700 319	58.9
Rural Sussex	Hesworth Common	Free-standing trees at edge of common.	TQ 002 194	68

4.2.2 Algal sampling

The study of epiphytes along a transect from Central London to rural Sussex involved the collection of algae from 20 trees at each of the six stations. A single tree species (*Quercus robur*) was selected in this study to reduce variation caused by bark-related variables (See Appendix).

Trees were selected using a random walk technique. From a randomly chosen point at the station, a random compass bearing was taken. The first tree encountered in that direction was sampled. Another random compass bearing was taken after that and this process were repeated until 20 trees had been selected at each station. Three quadrats of 15 x 15 cm were placed at 1.5 m height on the trees. Method for algal collection followed Chapter 2 in this thesis.

4.2.3 Species identification

Algal species identification followed general methods (See Chapter 2).

4.2.4 Bark pH

Determination of bark pH followed general methods (See Chapter 2).

4.2.5 Determination of Atmospheric Pollutants

Concentrations of atmospheric pollutants such as SO₂, NO_x, NO₂ and NH₃ at each station on the London-Sussex transect were monitored using passive sampler diffusion tubes. See Chapter 2 for details.

4.2.6 Data Analysis

Data were analysed using MINITAB and R statistical software. Samples were tested for equal variance using the F-test, normality test and also t-tests. The relationships between different variables were explored using Pearson's correlation coefficients and linear regression (Crawley, 1993, 2005; Bailey, 1995; Quin, 2002; Hawkins, 2005).

4.3 Results

4.3.1 Diversity and abundance of epiphytic algae on the pollution gradient transect

4.3.1.1 Diversity and abundance of algae

Four major species of epiphytic terrestrial algae were recorded in this transect study, namely *Chlorella* sp., *Desmococcus olivaceus*, *Apatococcus lobatus* and *Trentepohlia abietina*. All of them were green algae from Division Chlorophyta. Mean algal density among the stations ranged from 57 - 157 x 10² cells/ ml which is equivalent to 10 – 28 x 10² cells/cm². The highest algal density was recorded at Epsom Common and the lowest at Kensington Gardens. The overall mean density for the stations was 99 x 10² individual cells per ml, which equates to 18 x 10² cells/cm².

Among the common species, *Chlorella* sp. (**Figure 4.2a**) was relatively abundant at the four more rural stations but declined to zero at the City Centre. *Desmococcus olivaceus* (**Figure 4.2b**) achieved its highest densities in the outer suburb and inner suburb stations, and was equally but less frequent at the City Centre as at the more rural stations. *Apatococcus lobatus*, was absent at the city centre but present at the other stations (**Figure 4.2c**). *Trentpohlia abietina* (**Figure 4.2 d**) was recorded in some abundance at only one of the rural stations.

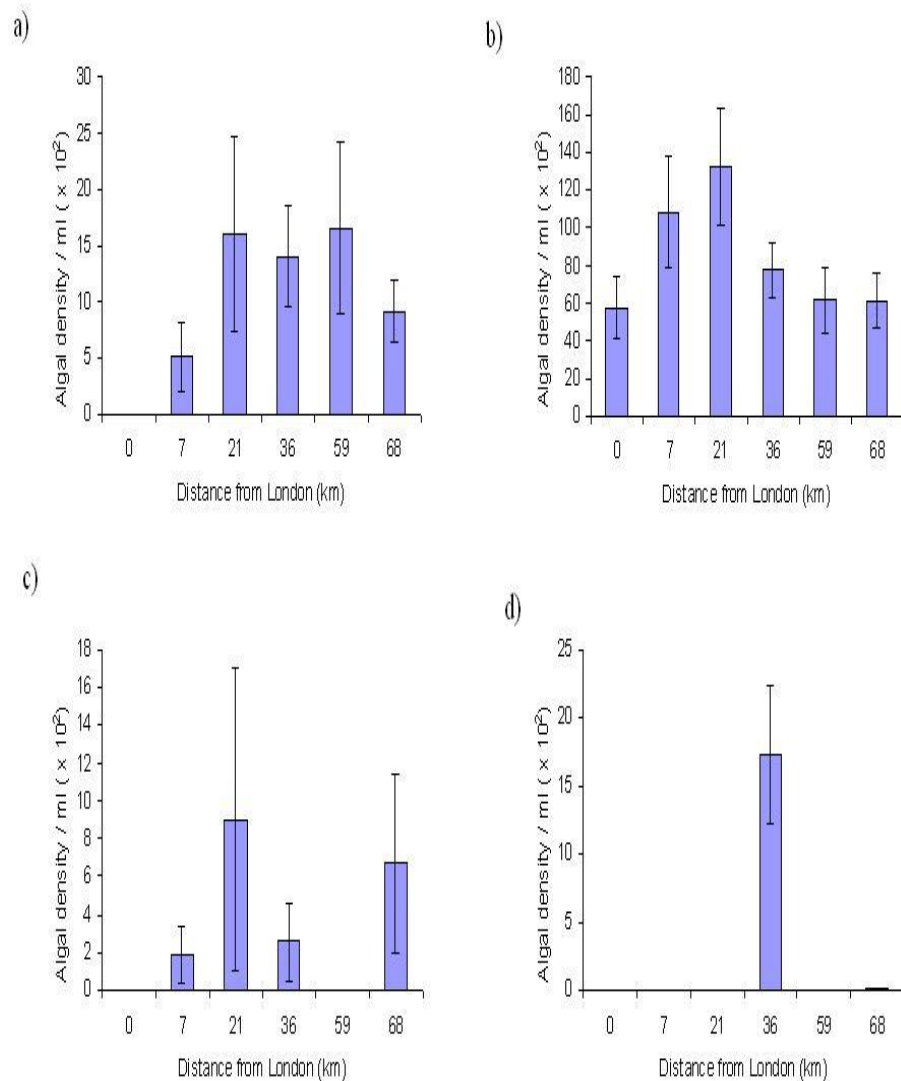


Figure 4.2 Variation in the densities of major algal species along the transect. Data points are means of 20 trees and their standard errors.
a) *Chlorella* sp., b) *Desmococcus olivaceus*, c) *Apatococcus lobatus*, d) *Trentepohlia abietina*.

The main feature is an increase in abundance of algae upon entering the built-up area, with an eventual decline (but not to zero) in the city centre. This pattern strongly suggests that growth of algae especially of *D. olivaceus* is favoured by some component of the urban environment, presumably an atmospheric pollutant, but possibly has something else, such as dust deposition or the elevated temperature.

Apatococcus lobatus showed a more rurally-based and erratic distribution pattern, but the orange-coloured *Trentepohlia* spp., which appears to have increased greatly in recent years with declining SO₂ levels (e.g. Davies *et al.*, 2007), did not feature strongly in this dataset for reasons unknown. The abundance of *T. abietina* at one of the rural stations does not oppose the hypothesis that it is relatively intolerant of one or more of the major atmospheric pollutants. *Apatococcus lobatus*, like the rarer *Chlorella* sp., was absent from the city centre but present at the other stations.

4.3.1.2 Variations of concentrations of atmospheric pollutants along the transect

Atmospheric pollutants measured in this study showed similar patterns with higher concentrations in the city centre, then a decline in the suburban areas and, for ammonia, increasing towards the rural area. High nitrogen deposition in the city centre was most probably due to traffic and in the rural area, it was probably due to agricultural emissions. The concentrations of NO₂ at all sampling stations did not exceed the air quality objective of 40 µg m⁻³ and this could be due to the location of the diffusion tubes which were in commons or parks.

Figure 4.3 shows mean concentrations of the major atmospheric pollutants (SO₂, NH₃, NO₂, NO_x and NO), measured by diffusion tubes in July 2008 and May 2009, as a function of distance from Central London. SO₂ concentrations were extremely low and showed no obvious trend with respect to the proximity of the city centre (**Figure 4.3a**). Thus, average SO₂ concentration was about the same at Ebernoe Common in rural Sussex as in Kensington Gardens, Central London. Except at Holmwood Common, SO₂ concentrations were slightly lower in May 2009 than in July 2008.

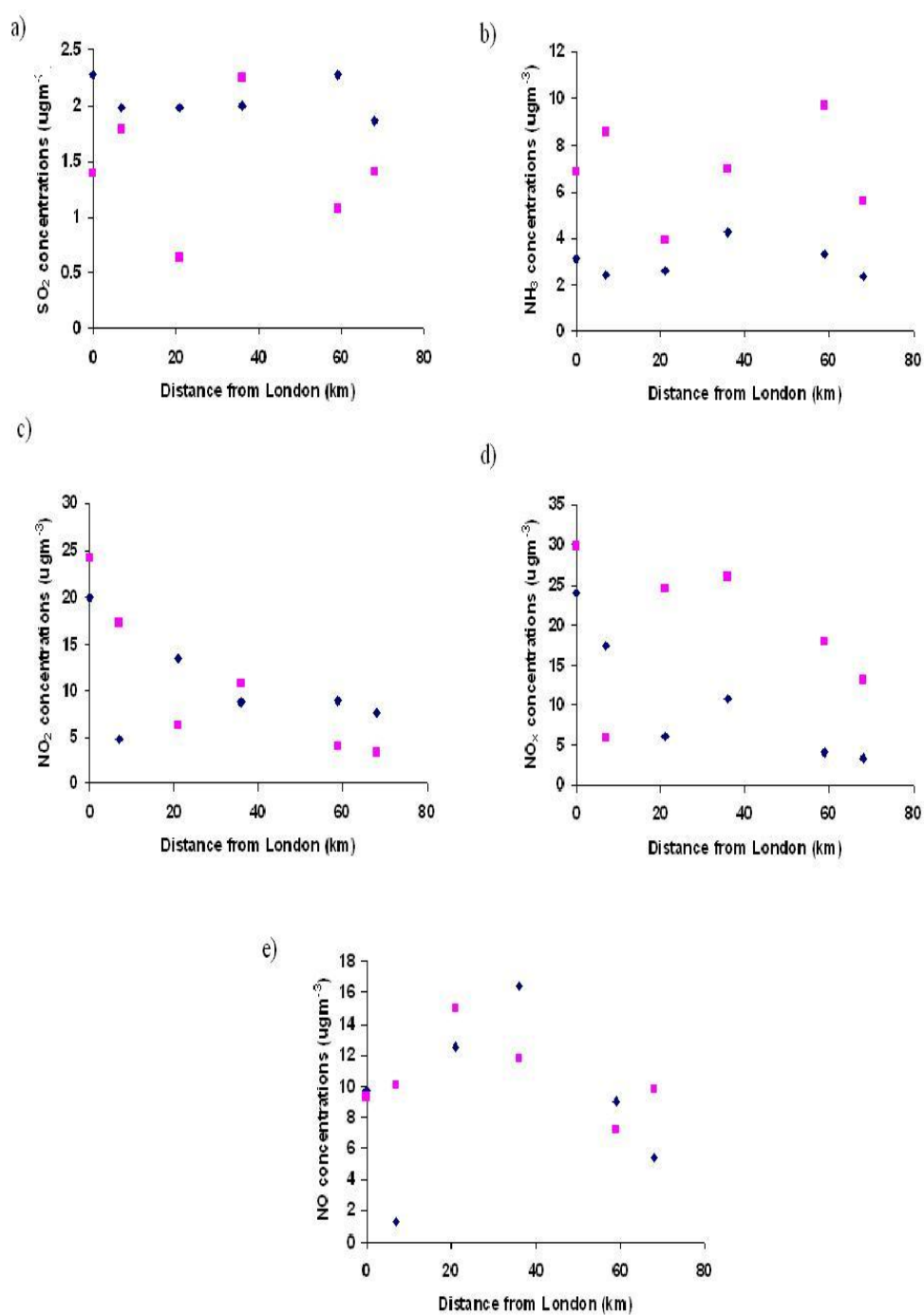


Figure 4.3: Variation of atmospheric pollutants measured in July 2008 (♦) and May 2009 (■) at the transect stations.

a) SO_2

b) NH_3

c) NO_2

d) NO_x

e) NO

Overall, NH_3 concentration also showed no obvious trend with position on the transect. The average NH_3 concentration for the urban sites was very similar to that

for the rural stations. NH_3 concentrations were consistently lower in May 2009 than in July 2008 (**Figure 4.3b**). NO_2 (**Figure 4.3c**) and NO_x (**Figure 4.3d**) showed relatively clear trends of decreasing mean concentrations with increasing distance from Central London. A similar trend was not present for NO which showed wide fluctuations among stations and sampling dates (**Figure 4.3e**).

4.3.1.3 Relationship between individual pollutants and algal density

Figures 4.4 to 4.8 show the densities of epiphytic algae inhabiting the sites along the transect, plotted against the mean pollutant concentrations recorded at each transect station. None of the species exhibited a significant linear trend with respect to SO_2 concentrations (**Figure 4.4a-d**). No significance is attached to this result.

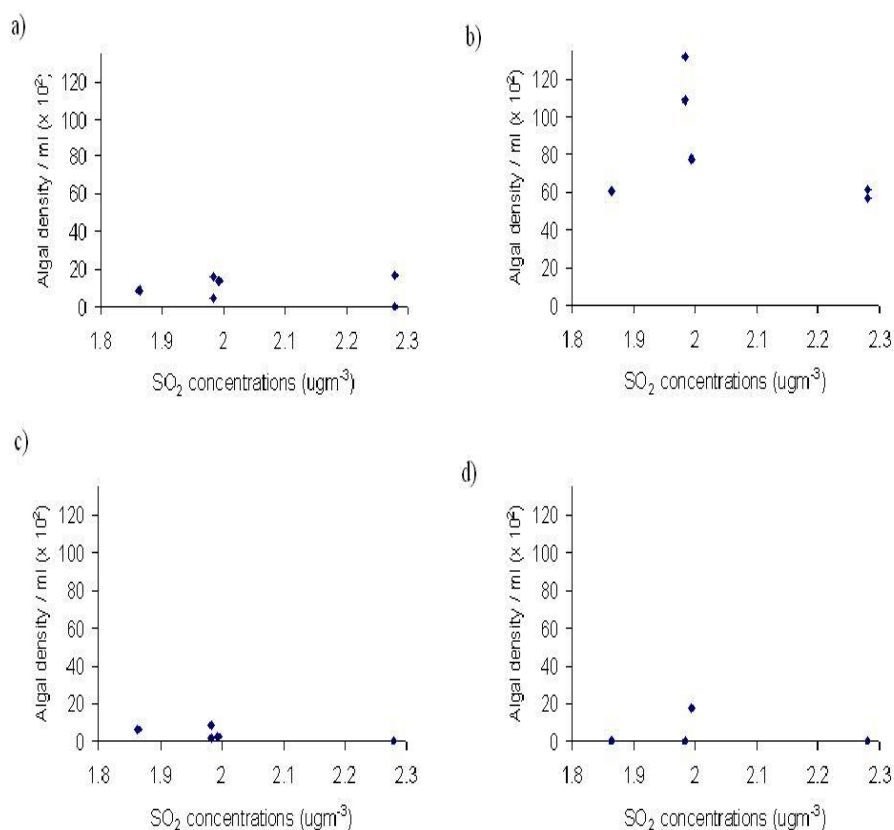


Figure 4.4 : Algal density plotted against the mean concentration of SO_2 .

a) *Chlorella* sp. ($r = -0.164$, $p = 0.756$) b) *Desmococcus olivaceus* ($r = -0.432$, $p = 0.392$)
c) *Apatococcus lobatus* ($r = -0.744$, $p = 0.09$) d) *Trentepohlia abietina* ($r = -0.202$, $p = 0.701$)

In contrast to SO₂, there was a significant positive correlation between the density of *Trentepohlia abietina* and the mean concentration of NH₃ (**Figure 4.5d**).

Concentrations of NH₃ were low and tended to be highest at the rural stations suggesting a predominantly agricultural source (Loppi & DeDominicis, 1996; Ruisi *et al.*, 2005; Wolseley *et al.*, 2006). No other clear trends were observed in algal densities with respect to mean ammonia concentrations at the transect stations.

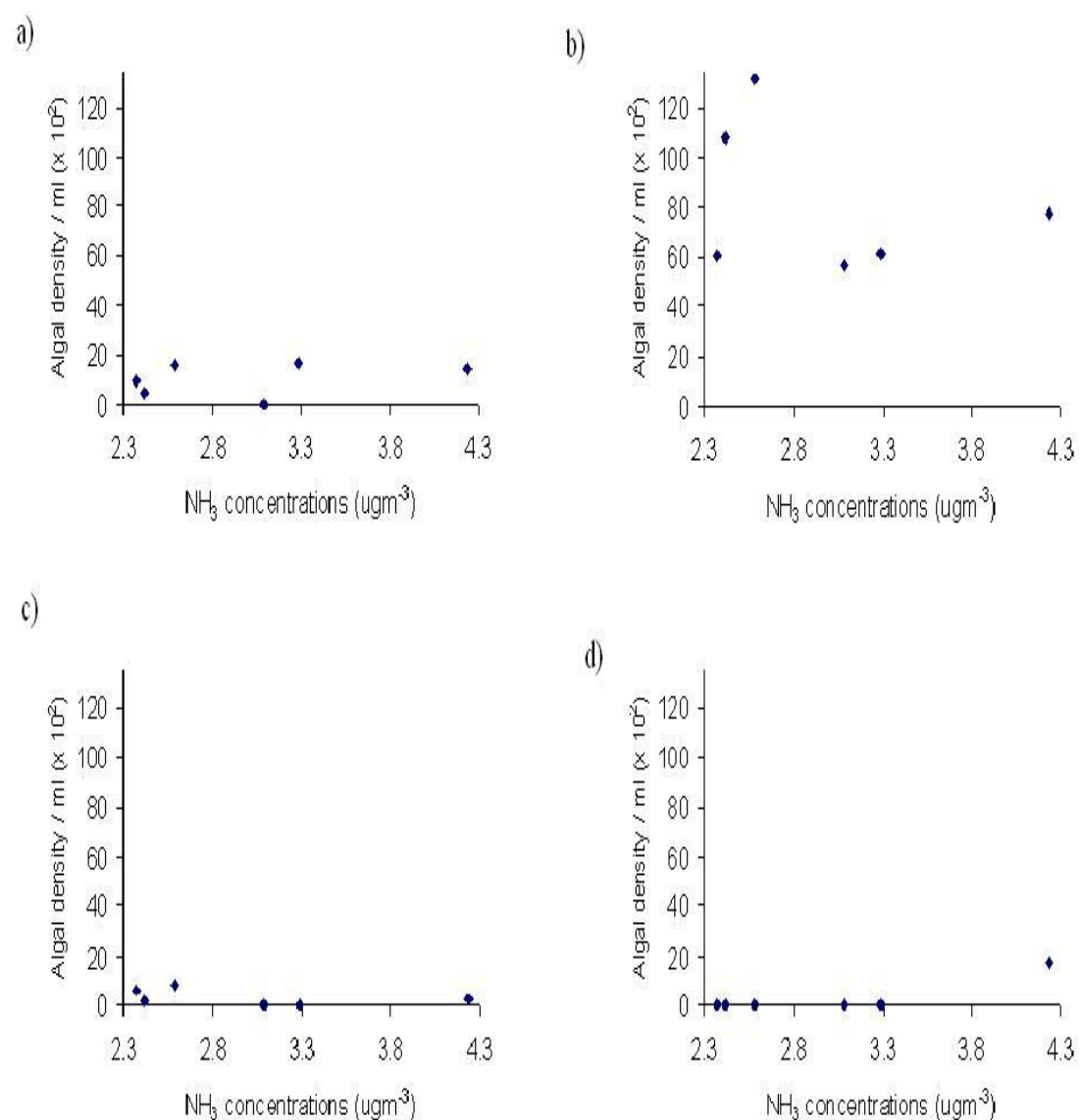


Figure 4.5: Algal density plotted against the mean concentration of NH₃.
a) *Chlorella* sp. ($r=0.291$, $p=0.576$) b) *Desmococcus olivaceus* ($r=-0.337$, $p=0.514$)
c) *Apatococcus lobatus* ($r=-0.443$, $p=0.379$) d) *Trentepohlia abietina* ($r=0.853$, $p=0.031^*$). * $p \leq 0.05$

None of the epiphytic algae shows a significant linear trend with respect to NO_2 (**Figure 4.6**) or NO (**Figure 4.8**) concentrations. However, there was a positive correlation between *Chlorella* sp. and NO_x (**Figure 4.7a**). Other types of response pattern may be present but it is not possible to demonstrate this statistically.

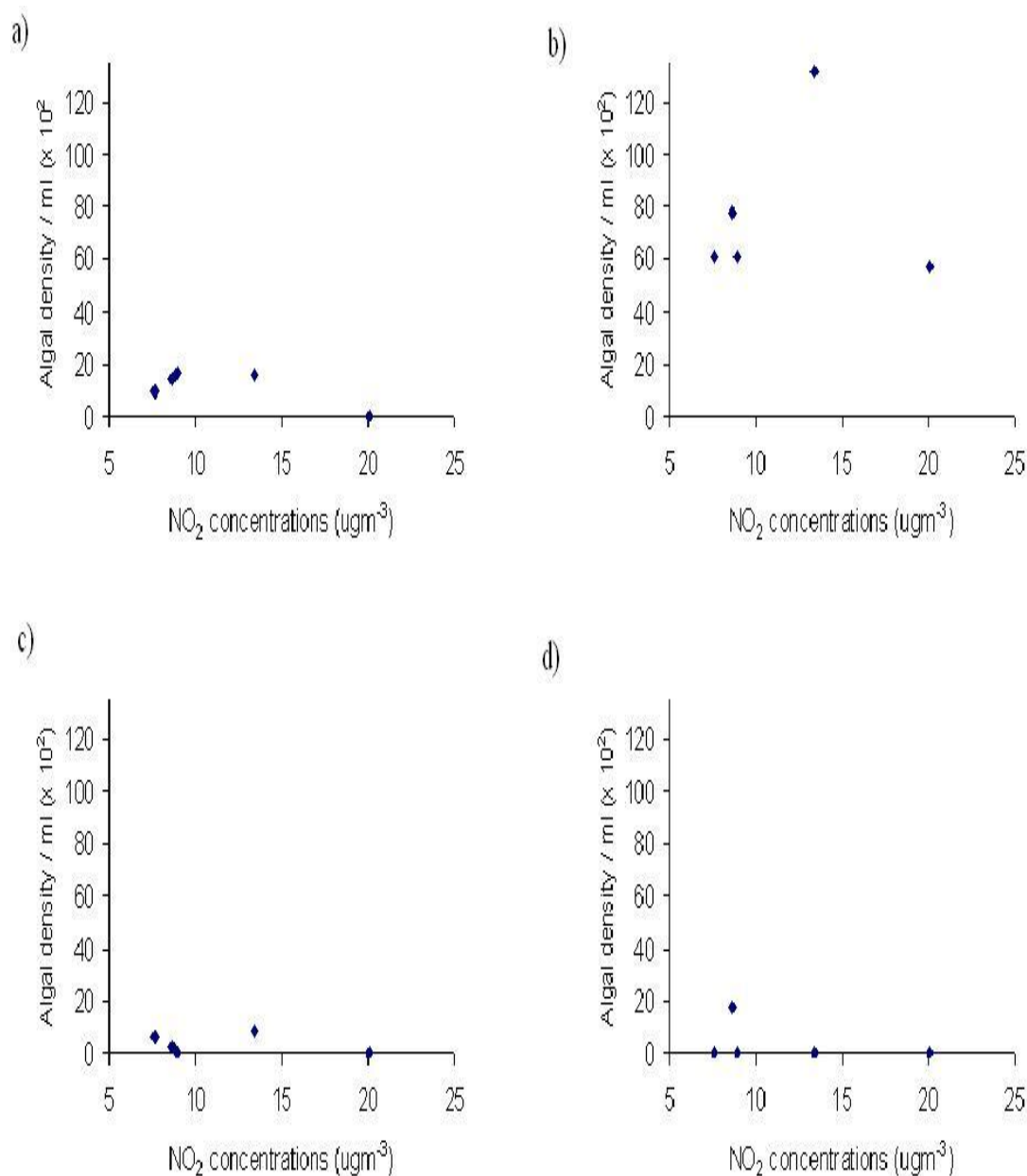


Figure 4.6: Algal density plotted against mean concentrations of NO_2 .

a) *Chlorella* sp. ($r=-0.359$, $p=0.485$) b) *Desmococcus olivaceus* ($r=-0.174$, $p=0.741$)
c) *Apatococcus lobatus* ($r=-0.096$, $p=0.857$) d) *Trentepohlia abietina* ($r=-0.174$, $p=0.741$).

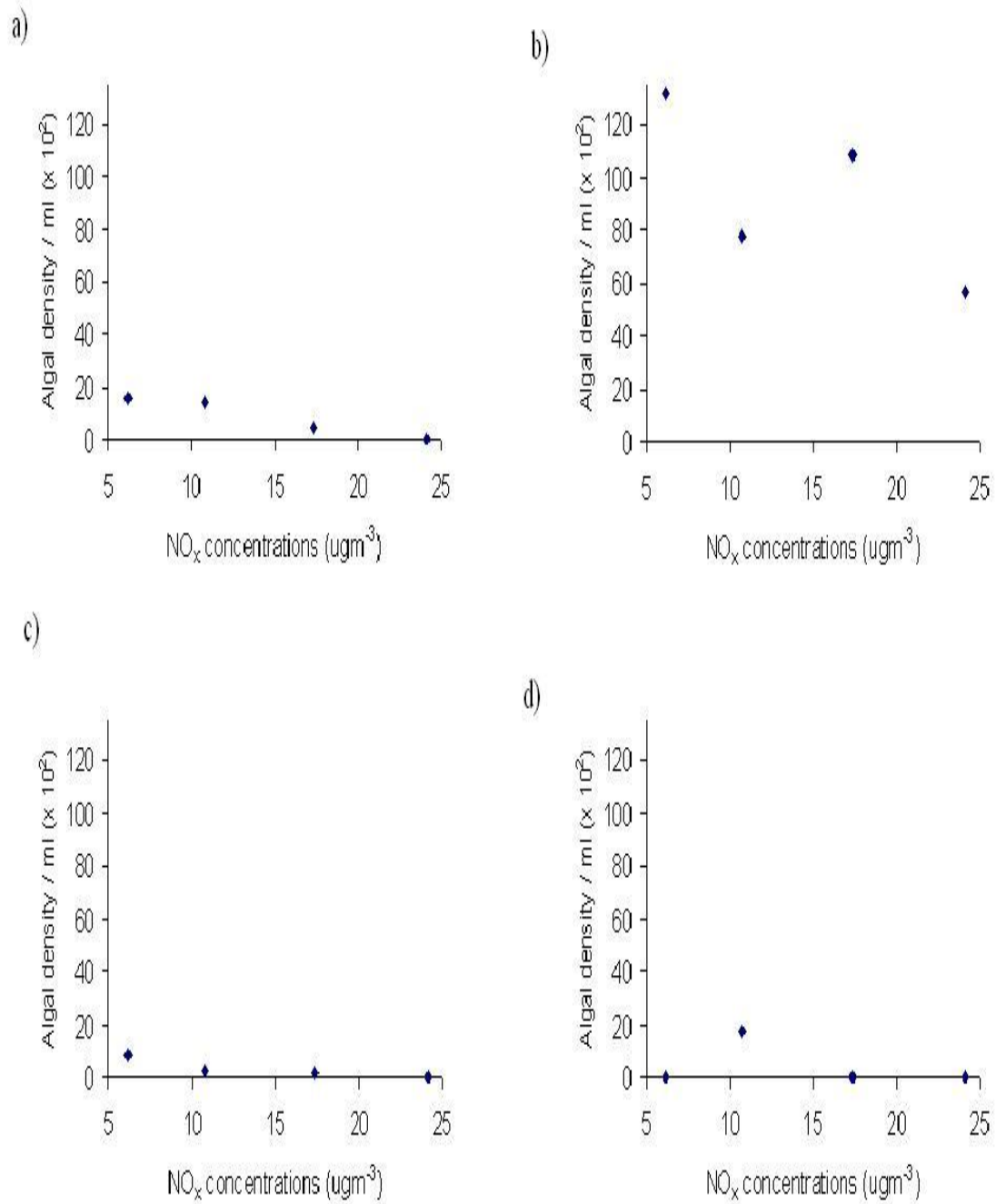


Figure 4.7: Algal density plotted against mean concentrations of NO_x .
a) *Chlorella* sp. (r=-0.844, p=0.035*) b) *Desmococcus olivaceus* (r=-0.077, p=0.844)
c) *Apatococcus lobatus* (r=-0.539, p=0.270) d) *Trentepohlia abietina* (r=-0.013, p=0.980).
* $p \leq 0.05$

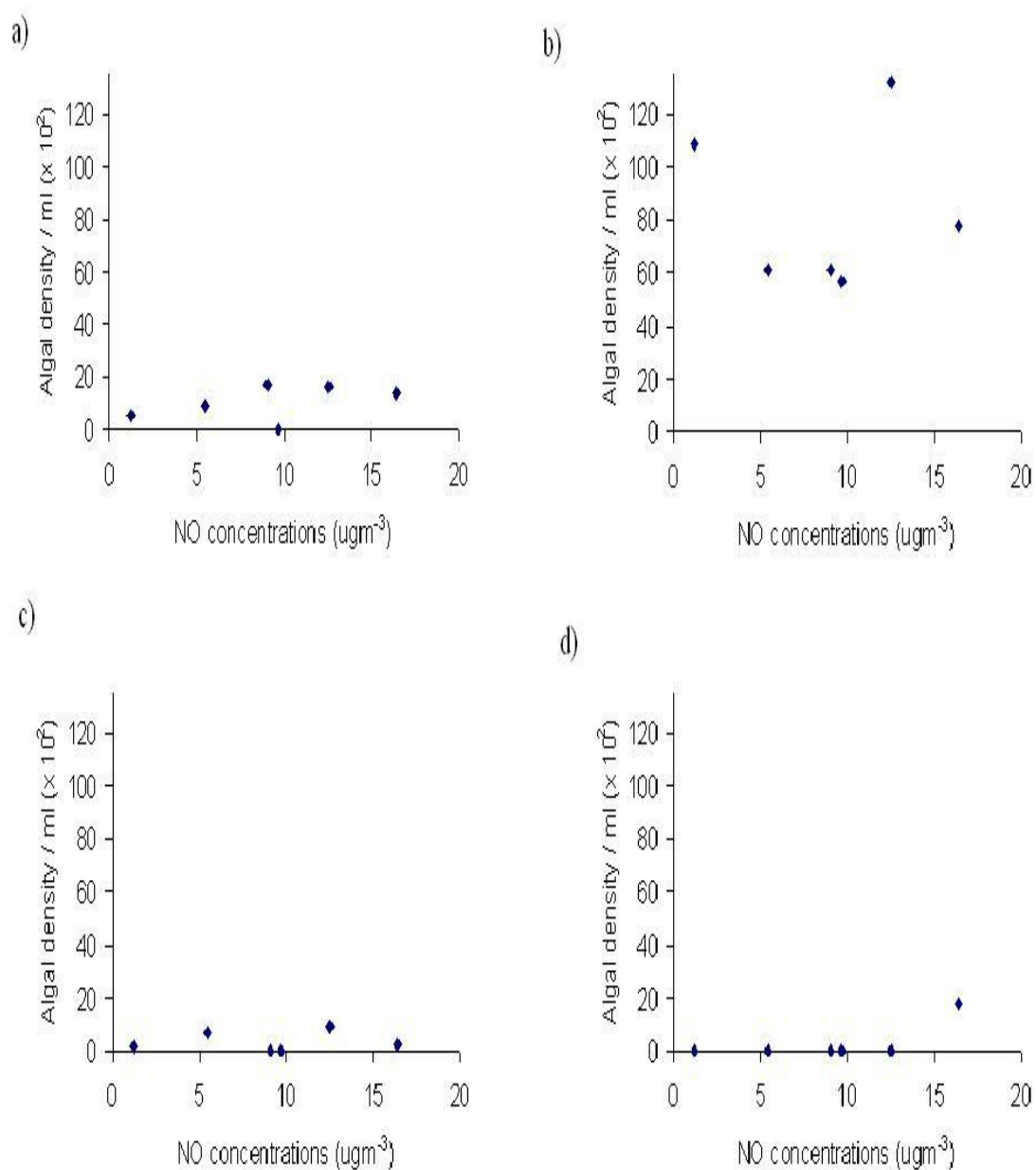


Figure 4.8: Algal density plotted against mean concentrations of NO.
a) *Chlorella* sp. (r=0.485, p=0.330) b) *Desmococcus olivaceus* (r=-0.006, p=0.991)
c) *Apatococcus lobatus* (r=0.117, p=0.825) d) *Trentepohlia abietina* (r=0.677, p=0.140).

4.4 Discussion

4.4.1 Algal distribution on the transect from Central London to rural Sussex

A similar type of epiphytic algal flora was found on the oaks at the transect sites as on those in the Silwood Park survey (See Appendix). Interestingly, despite the lack of any correlation between cover and density assessments of algal abundance in the Silwood study, the distribution of the common alga *Desmococcus olivaceus* along the transect based on the more precise density data is very similar to that reported on the basis of visual cover estimates by Bates *et al.* (1990, 2001). The main feature (**Figure 4.2b**) is an increase in abundance of this species on entering the built-up area, with an eventual decline (but not to zero) in the City Centre. This pattern strongly suggests that growth of *D. olivaceus* is favoured by some component of the city environment, presumably an atmospheric pollutant but possibly something else such as dust deposition or the elevated temperature. *Chlorella* sp. showed a more rurally-based distribution pattern, but the orange-coloured *Trentepohlia* spp., which appear to have increased greatly in recent years with declining SO₂ levels (e.g. Davies *et al.*, 2007), did not feature strongly in this dataset for reasons unknown. The abundance of *T. abietina* at one of the rural stations does not oppose the hypothesis that it is relatively intolerant of one or more of the major atmospheric pollutants.

Among the various pollutants measured in the transect study, only NO_x showed the expected steady decline from high values at the City Centre station (Kensington Gardens) to low levels in the countryside. The pattern of NO₂ decline along the transect was determined in detail at a slightly earlier period by Batty *et al.* (2003). This gas is almost certainly derived predominantly from motor vehicles present at highest density in the centre (Davies *et al.*, 2007). SO₂ concentrations were extremely

low confirming that this is no longer a significant factor for epiphyte colonization of trees. Concentrations of NH_3 were also low and tended to be highest at the rural stations suggesting a predominantly agricultural source (Loppi & DeDominicis, 1996; Ruisi *et al.*, 2005; Wolseley *et al.*, 2006).

Only in one case was a significant linear relationship demonstrated between the densities of an alga and the mean concentrations of a pollutant along the transect (**Figure 4.7a**). The lack of significant correlations may possibly be a result of more complex (e.g. bell-shaped) responses of the algae to each pollution gradient. It is also likely that lack of uniformity, for example proximity to major roads, among the transect stations, has confounded the responses of the algae to pollutants. Several studies elsewhere in Europe (Thomsen, 1992; Brakenhielm & Qinghong, 1995; Poikolainen *et al.*, 1998) have concluded that sub-aerial algae are most abundant where nitrogen deposition is greatest. This is in agreement with the conclusion of Hanninen *et al.*, (1993) that nitrogen is the most important factor restricting the growth of green algae (see also Chapter 3) and Zhai *et al.*, (2009) recently concluded that atmospheric nitrogen accounts for 40–100% of the required amounts of this element for algal growth. However, atmospheric N pollutants may also be harmful to some algal species (Mansfield, 2002).

A major challenge for future work is identifying the cause of the increase in abundance of *Desmococcus olivaceus* in the city compared to rural sites. It is likely that one of the *Trentepohlia* spp., apparently restricted to less polluted sites, would make a useful comparison. The field experiment at Silwood Park (Chapter 5) has compared the responses of these two algae to a range of simulated pollutant

applications. The pattern of abundance of *D. olivaceus* has apparently remained remarkably stable over many decades during which SO₂ concentrations declined greatly and major changes in the epiphytic lichen flora occurred. It is possible that genetic change has permitted this to occur. Therefore it is also imperative that some molecular sequencing work is undertaken with *D. olivaceus* to ascertain whether all populations are identical (and remarkably physiologically adaptable) or whether there is evidence of genetic adaptation to the different environments that this species has occupied both in space and time.

4.5 Conclusions

This study showed an increase in abundance of *Desmococcus olivaceus* in the city compared to rural sites. While *Trentepohlia* spp. is more likely to be restricted to less polluted sites. SO₂ concentrations were extremely low (typically 2 µg cm², compared with industrial era peak values of more than 170 µg cm², confirming that this is no longer a significant factor for epiphyte colonization of trees. Several studies elsewhere in Europe (Thomsen, 1992; Brakenhielm & Qinghong, 1995; Poikolainen *et al*, 1998) have concluded that sub-aerial algae are most abundant where nitrogen deposition is greatest. This is in agreement with the conclusion of Mansfield (2002) and Goransson *et al*,(2007) that nitrogen is now probably the most important factor controlling the growth of green algae.

Chapter 5

Effects of Long Term Deposition of oxidised and reduced forms of Nitrogen on Epiphytic Terrestrial Algae in an Ombrotrophic Bog

5.1 Introduction

Changes in ecosystems have resulted from anthropogenic sources such as agricultural activities and combustion of fossil fuels. These activities have been identified as among the major sources of NH_3 emissions (Krupa, 2003; Prendergast-Miller *et al.*, 2009). Negative impacts on native flora can be seen mainly in semi-natural ecosystems such as ombrotrophic bogs. An ombrotrophic bog is a habitat where soil or vegetation receives all its water and nutrients from precipitation, rather than from streams or springs. It is a home to organisms which are tolerant of acidic, low-nutrient environments (Charman, 2002). The effects of nitrogen addition on ombrotrophic bogs are relatively under-investigated, although such peatlands represent important ecosystems, especially in Scotland (Lindsay, 1993).

Even though recent data show that emissions of oxidised N compounds have declined over the last decade, the rate of reduction is very slow and in many ecosystems, biologically available nitrogen continues to increase (Vitousek *et al.*, 1997) and the critical load for N has already been exceeded (Fowler *et al.*, 2005; Solga *et al.*, 2005). At the same time, emission and deposition of ammonia has not declined and in many

habitats has become the main contributor in changing the ecosystem (Phuyal *et al.*, 2008).

The effects of N deposition include decreasing diversity both of lower and higher plant populations (Pitcairn *et al.*, 1995; Krupa, 2003; Wolseley *et al.*; 2006). Some grass species have been known to increase under long term exposure to enhanced N deposition and may come to dominate the habitat (Stevens *et al.*, 2004; Dupre *et al.*, 2010). Studies on the effects of N deposition have usually focused on the above ground effects such as biomass production, tissue N concentration and plant species composition (Pitcairn *et al.*, 1998; Leith, 2001; Krupa, 2003). One of the most commonly studied plant species has been *Calluna vulgaris* (Aerts, 1990; Prins *et al.*, 1991; Sheppard *et al.*, 2008).

In a habitat such as ombrotrophic bog, where productivity is very low or non-existent, cryptogamic plants are typically found in abundance. Most of the nutrients are obtained from the rainwater, even though some algae and bryophytes retrieve nutrients from the substratum (Ayres *et al.* 2006). Some particular species of bryophytes and algae have shown that they thrive in a changing N environment which favours nitrophilic species (Aldous, 2002; Stevens *et al.*, 2004). These nitrophilic species have evolved to live in low nutrient conditions (Grime, 1979). Short-term N exposure to bryophytes has shown that in N limited areas, the bryophytes increase their growth but this decreases when exposed to higher N deposition. The decreasing growth however may start to increase again after long-term exposure to high N (Gunnarsson & Rydin, 2000; Wiedermann *et al.*, 2007). This means that some species of algae and bryophytes are more susceptible to high N while others are not.

Hence nitrophilic species may grow in abundance while the non-tolerant species will decrease. However, if the exposure to high N continues for a longer period, the suppressed species (low tolerance of high N) will eventually evolve some kind of resistance or adaptive mechanism that allow them to continue their existence in this ecosystem. However, they will not be able to outcompete the growth of nitrophilic species.

5.1.1 Effects of Dry and Wet Nitrogen Deposition on Flora and Fauna in Ombrotrophic Bogs

Several studies have shown evidence of both negative and positive effects of wet and dry N deposition. Ombrotrophic bogs are dominated by *Sphagnum* moss, which acts as the ecosystem engineer of the peatland. Any threat to *Sphagnum* species will affect the entire low pH ecosystem (Gunnarsson, 2004). Over the past few years, researchers have noticed an expansion of algae in sites with introduced N. The algae start as small patches and continue to expand over the course of years. Increased algal cover has been shown to aggravate the damaging effect of N deposition on *Sphagnum* sometimes causing serious necrosis (Limpens *et al.*, 2003).

In a 3-year N exposure oxidized N was more damaging to *Hypnum* than reduced N (Carfrae *et al.*, 2007) but the authors suggested that responses might be reversed in a longer-term experiment. Kooijman & Bakker (1995) reported negative responses to enhanced N deposition, in particular exacerbating the effects of drought on the system.

In another study, Sheppard *et al.*, (2008) reported on the effects of dry N deposition on *Calluna vulgaris*. After more than four years of N treatments, mature *Calluna* nearest the NH₃ source became bleached. Enhanced N deposition has also been found to increase sensitivity of *C. vulgaris* to drought, frost and winter desiccation. At concentrations of >8 mg m⁻³, ammonia significantly enhanced foliar N concentrations. Similarly, enhanced N deposition increased spring frost damage and increased the incidence of pathogen outbreaks. However, no significant visible damage was shown by *Calluna* treated with reduced and oxidised N deposited via rainfall.

An interesting finding was reported by Phuyal *et al.* (2008) regarding effects of NH₄ and NO₃ on bryophytes, namely *Sphagnum* sp. and *Hypnum* sp. Phosphatase activity in *Sphagnum* was significantly enhanced by treatments. However, when exposed to NH₄ and NO₃ with additions of P and K, phosphatase activity was decreased (Skinner *et al.*, 2006). Phosphatase activity in *Sphagnum* was reported to be positively correlated with tissue N, but was negatively correlated with tissue P concentrations. In the same study, a negative relationship between shoot P concentration and phosphatase activity was observed for *Hypnum*. As in *Sphagnum*, phosphatase activity in *Hypnum* also decreased significantly with the addition of P and K. *Hypnum* seemed to be adapted much better to high N inputs than the more sensitive *Sphagnum* (Press *et al.*, 1986).

Phuyal *et al.* (2008) also concluded that enhanced nutrient supply significantly altered the nutrient recycling behaviour of bryophytes, even at modest doses. Similar relationships have been reported in *Racomitrium lanuginosum* and *Plantago lanceolata* (Pearce *et al.*, 2003).

A study on the effects of N deposition on enchytraeid worms (*Oligochaeta*) reported that abundance and diversity were not affected by $\text{NH}_3\text{-N}$ despite increases in peat pH and mineral N (Prendergast-Miller *et al.*, 2008). The authors speculated that a treatment duration of only three years was not enough to change litter quality and so did not alter enchytraeid distribution. Thus enchytraeids appear not to be sensitive indicators of NH_3 fumigation.

5.1.2 Algae in Bog Habitats

Bogs are usually low in pH, about 4-5.5. They are poor in nutrients, have high levels of dissolved organic matter from decomposed peat (Mofidpoor *et al.*, 2009). Detailed studies of algae in low pH environments in the UK and USA revealed low species diversity and many similarities in species composition (Warner, 1971; Hargreaves *et al.*, 1975; Blouin, 1989; Mataloni, 1999; Ceosel & Meesters, 2007). This is due to cell wall damage caused by highly acidic pH (Vymazal, 1995) which limits species diversity.

Algal communities on bogs are typically species-poor especially at low pH (Hooper, 1981; Yung *et al.*, 1986). Locally, algal diversity can be enhanced due to abundance of desmids, the predominant algae in bogs. Among the most frequent algal groups found in peat bogs are Chlorophyta, Cyanophyta and Bacillariophyta. Green algae mainly of Desmidiaceae and Zygnematales are abundant within peat moss. These algae grow between the cells of peat moss or in cavities within plants. At the same time, desmids also occurred as a free-living species. In our study, we focused on

Desmidiaceae that occupy the mucilage on top of mosses. The desmids were retrieved by scraping the mucilage off the moss, thus releasing the algae.

Nowadays, N deposition has altered the vegetation of many bogs, especially in Central Europe. Epiphytic algae have increased in the areas where N deposition is highest (Brakenhielm & Qinghong, 1995; Krupa, 2003). The enhanced nutrient level is thought to reduce the photosynthetic capacity of its substratum, to which the algae are attached (Wear *et al.*, 1999; Limpens *et al.*, 2003) such as *Calluna* or moss. Even after the reduction of substratum (*Sphagnum*) due to high N, desmids continue to grow in that area providing that there is enough moisture.

Uherkovich (1984) reported that desmids in bogs are more sensitive to drying out than other algae and can thus be a suitable indicator for environmental changes. As freshwater algae in the bog ecosystem as elsewhere are closely correlated with chemical variables and changes in the environment, it is important to understand the ecology, occurrence patterns and abundance of algal assemblages (Coleman, 2002; Cerna, 2010). These variations in terms of distribution, abundance and composition of species should enhance our understanding of the underlying mechanisms which regulate biodiversity (Hubbell, 2001).

Patterns of algal distribution and abundance vary according to habitat or time and geography (Bridges *et al.*, 1994; Neustupa *et al.*, 2009). According to Foster *et al.* (1988), algal distribution and abundance show little variation over the small scale and temporal distribution is dominated by seasonal effects (Dethier, 1982; Cubitt, 1984).

5.1.3 Aims and objectives

In place of relying on natural deposition of pollutants, the study described in this chapter uses a facility for introducing measured amounts of the gases under study to a natural environment, in this case an ombrotrophic bog. It investigates the effect on algae of 8 years simulated atmospheric deposition of ammonium (NH_4^+) and nitrate (NO_3^-) applied either alone or in combination with phosphorus and potassium (PK).

The objectives are as follows:

- 1) To investigate whether or not algal species diversity differs between wet and dry N treatments.
- 2) To investigate how algal population density responds to long term exposure to N deposition in field plots.
- 3) To separate the effects of reduced and oxidised N on algal density.
- 4) To investigate whether one N form is more damaging than another.
- 5) To study the relationship between pH and algal density in both wet and dry deposition plots.
- 6) To see if added PK on treated plots has any effect on algal populations.

5.2 Materials and Methods

5.2.1 Site Descriptions

This study was carried out at the spatially heterogeneous Whim Moss (UK grid reference of NT 210 530). It is located approximately 20 km south of Edinburgh in Scotland (Leith *et al.*, 2001; Sheppard *et al.*, 2004). The 1 ha site is part of an ombrotrophic bog and represents a transition between a lowland raised bog and a blanket bog. No management has been employed in this area for approximately 60

years. This study utilized the large-scale automated field N-manipulation system set up in 2002 and managed by the Centre for Ecology and Hydrology (CEH) Edinburgh.

This ombrotrophic bog is classified as M19 under the National Vegetation Classification system. Vegetation in this area is mainly dominated by the heathland dominant species, *Calluna vulgaris*. Other species present in abundance are *Eriophorum vaginatum*, *Erica tetralix*, *Empetrum nigrum*, *Vaccinium myrtillus* and *V. oxycoccus*. Lower plants comprise mosses such as *Sphagnum capillifolium*, *S. papillosum*, *S. fallax* and *Hypnum jutlandicum*, amongst other species. Among algae species found at this site are *Cylindrocystis* sp., *Desmidium* sp., *Oocystis* sp., and *Chlorococcus* sp. Lichen species that can be found here are *Pleurozium schreberi* and *Cladonia portentosa*. Habitats colonised include epiphytic growth on *Sphagnum* and *Calluna*, and a species colonising the surface of the peat soil (**Figure 5.1**).



Figure 5.1: Pictures showing terrestrial algae in the Whim Bog:
a) Green slimy patches of algae on the surface of soil and dying *Sphagnum* in the dry deposition plot. b) Thick green layer of colonies of algae on *Calluna vulgaris* in the wet deposition plot.

Areas at the site are comprised of deep acid peat, 3-6 m thick, with an ambient surface pH of 3.27-3.91. Average mean temperature is 10.7° C with an annual rainfall of approximately 900 – 1000 mm. The site is considered to have a low ambient N

deposition, approximately $8 \text{ kg N ha}^{-1} \text{ year}^{-1}$. Since the site has not been managed for a long time, historical N and S inputs were low and have not been subject to elevated N or S inputs over the last century (Cape *et al.*, 2008; Phuyal *et al.*, 2008).

There is an automated field release system separated into two areas receiving either wet or dry deposition treatments (**Figure 5.2**). The method of N application at the wet deposition site was by tracking the rainfall data. The dry deposition of the NH_3 fumigation system was coupled to wind direction. Thus these plots closely mimic the real environment. The ammonia is released whenever meteorological conditions permit, either night or day.

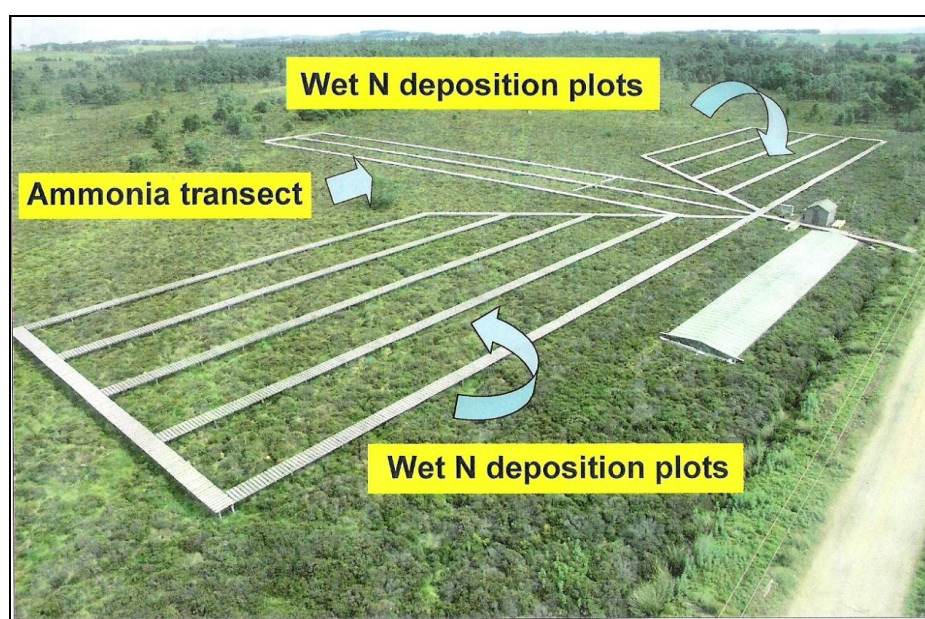


Figure 5.2 : Aerial view of wet and dry N deposition plots in the 1 ha ombrotrophic Whim bog, Scotland, UK. Ammonia transect represent the Dry Deposition Plots and the two extended wings represents the Wet Deposition Plots. Picture credit to Lucy Sheppard from the Centre for Ecology and Hydrology, Edinburgh.

5.2.2 N Treatments in Dry Plots

Dry deposition treatments were applied as an NH_3 release, equivalent to the emissions from 24,000 broiler hens. A 60 m transect utilises automatic release of

NH₃ from two pipes, 5 m wide apart. The NH₃ was released when the wind direction is between 180 and 215° from North and wind speed is 2.5 ms⁻¹ or higher (Sheppard *et al.*, 2008; Cape *et al.*, 2008). A sonic anemometer is used to measure the wind direction every 5 s.

Ammonia concentrations downwind of the source were measured using a combination of passive diffusion tube samplers and 'ALPHA' passive samplers. The ALPHA sampler was developed by CEH (Tang *et al.*, 2001). The samplers were located 0.1 m above the vegetation canopy at 1, 2, 4, 6, 8, 12, 16, 32 and 60m from source. Acidified filter discs held inside the ALPHA samplers were used to measure ammonia in the air filtered by the sampler. The filter was soaked in 5ml of de-ionised water, and NH₃ concentrations in solution were measured using the AMFIA flow injection analysis system (Puchalski *et al.*, 2011).

NH₃ concentration is measured at a range of heights and positions along the transect as monthly mean concentrations, with the timing and duration of exposure logged continuously.

5.2.3 N Treatments in Wet Plots

Wet deposition treatments were applied using an automated spray of rainwater collected at the site. Each 13 m² plot was treated with an equivalent 100mm of rain, to avoid increasing the rainfall volume by more than 10%. No treatment was applied in the absence of rain, nor when temperatures fell below 0°C. There are 44 experimental plots with a diameter of 4 m each. The plots were arranged in four randomized blocks. The treatments consist of controlled additions of ammonium or

nitrate of three different levels of N deposition. Some treatments also contained potassium and phosphorus. Treatments were supplied at a maximum concentration of 4 mM.

The NH₃ release system was controlled through a Campbell 23x logger, linked to a mass flow controller on an NH₃ cylinder. The precipitation was released from the cylinder into a 10 m diameter perforated pipe 0.5 m above the ground. (Leith *et al.*, 2001; Sheppard *et al.*, 2004).

The experimental plots distinguished the effect of oxidised (NO₃⁻ in the form of NaNO₃) and reduced (NH₄⁺ in the form of NH₄Cl) N by different treatments. There were five treatments, comprised of:

- i) Control (rainwater only with no added water or nutrients)
- ii) NH₄Cl
- iii) NH₄Cl + PK
- iv) NaNO₃
- v) NaNO₃ + PK

P and K were added as K₂HPO₄ in a 1:14 P: N ratio. Three different doses of nitrogen were applied at 16, 32 and 64 kg ha⁻¹ year⁻¹ (Sheppard *et al.*, 2004). An ambient deposition at 8 kg ha⁻¹ year⁻¹ was also included in the calculation.

5.2.4 Algal Collection and Quantification

Algal samples from dry-deposition plots were collected at ten distances along the NH₃ transect at 8, 12, 16, 20, 24, 28, 32, 40, 50, 60 m. Since this study focused on

epiphytic terrestrial algae only, collection of algae was only made where algal slime was present on top of soil or vegetation in the plots. No collection has been made from the soil itself. The algal slime samples were collected within 10 x 10 cm quadrats, and placed in labelled plastic bags before being taken to the laboratory. The samples were then diluted into 20 ml deionised water and subjected to cell counts and identification. Quantification of algae was made using the Scepter automatic counter. No control area was selected because algal slimes were only found within the plots treated with NH₃.

Collection of algae in the wet-deposition plots was done by scraping the algae off the twigs of *Calluna vulgaris* using a scalpel. On certain occasions where the twigs were very small, algae were removed from the substratum by placing it in a vial and shaking vigorously to loosen the algae from the twigs. The same process of identification and quantification as in the dry plots was repeated for samples of algae from the wet plots.

5.2.5 Statistical Analysis

All data were tested for normality using Anderson-Darling prior to analysis. Log transformation was done when necessary. Non-parametric tests were used when data were not normally distributed. All errors are displayed as ± 1 standard error of the mean. ANOVA was performed using MINITAB version 15 to see whether there were any significant differences between treatments. Correlations between variables were calculated using Pearson's correlation coefficient (Crawley, 1993, 2005; Shaw, 2003; Tucker, 2003; Wardlaw, 2000).

5.3 Results

5.3.1 Algal Diversity and Density

5.3.1.1 Algal Diversity and Density in the Wet-deposition Plots

The wet deposition plots which received a variety of N treatments were mainly dominated by algae from the division Chlorophyta. There were 3 main genera in this area namely *Cylindrocystis* sp., *Desmidium* sp. and *Chlorococcus* sp (Figure 5.3). These algae formed thick layers of greenish colour on the twigs of *Calluna vulgaris*.

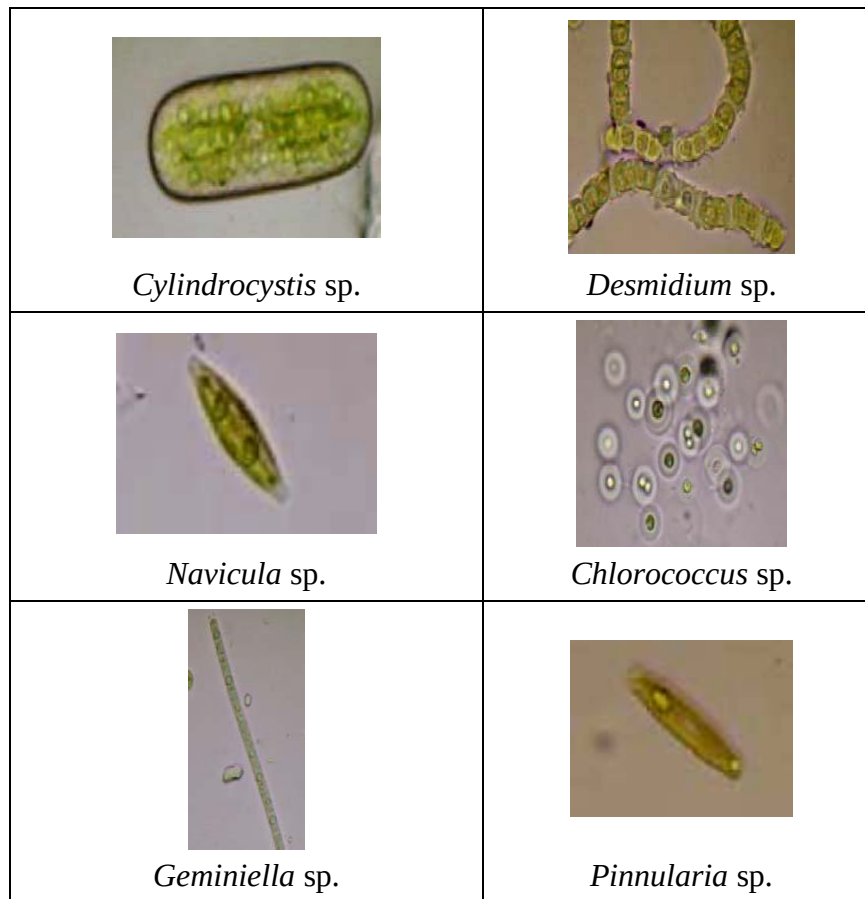


Figure 5.3: Images of three major genera of algae and a selection of diatoms inhabiting wet-deposition plots. All images were taken using Brunel Digital Microscope at 400x magnification.

The highest number of cells was of *Cylindrocystis* sp. treated with NH_4Cl at 64 kg N $\text{ha}^{-1} \text{yr}^{-1}$ + PK (Figure 5.4). There were $29,180 \pm 6,411$ cells / ml, equivalent to

11,672 cells/cm². This species was found in all plots treated with NH₄Cl except the one with NH₄Cl at 16 kg N ha⁻¹ yr⁻¹. On the contrary, *Desmidium* sp. was found on all plots except the one treated with NH₄Cl at 64 kg N ha⁻¹ yr⁻¹ + PK.

Interestingly, no *Cylindrocystis* sp. was found in any plot treated with NaNO₃ except the one treated with NaNO₃ at 64 kg N ha⁻¹ yr⁻¹ + PK. The same situation also occurred in control plots where no *Cylindrocystis* sp. was found. Another major genus, *Chlorococcus* sp. was found in all plots regardless of treatments received. Desmids such as *Cylindrocystis* and *Desmidium*, and diatoms were also abundant, an indicator for N eutrophication. However, there were no *Desmococcus-Pleurococcus* assemblages found in these plots.

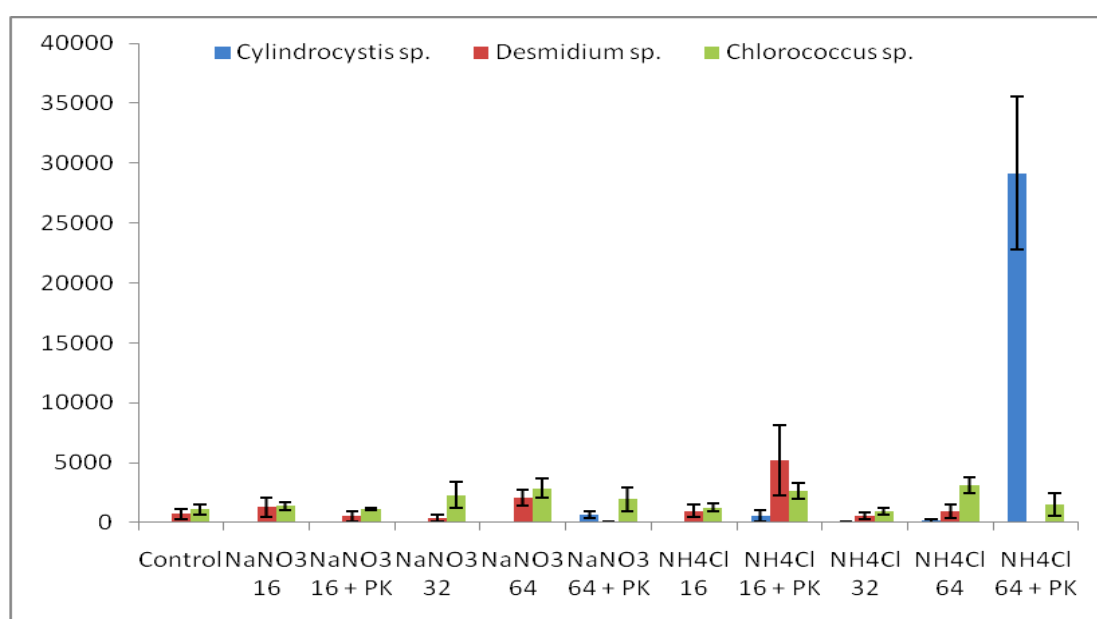


Figure 5.4: Algal composition of three major genera, in plots receiving wet-deposition treatments. Number of algal cells is based on mean cells per treatment in the 10 x 10 cm² quadrats. Each bar represents mean number of algae/ml ± SEM.

5.3.1.2 Algal Diversity and Density in the Dry-deposition Plots

The dry-deposition plot was also dominated by algae from the division Chlorophyta.

There were 6 genera drawn from the Chlorophyta, Bacillariophyta and Euglenophyta

(**Figure 5.5**). These algae formed a thin layer of gelatinous slimy green patches on soil and on moss. Among the dominant genera were *Cylindrocystis* sp., *Desmidium* sp. and *Spirogyra* sp.

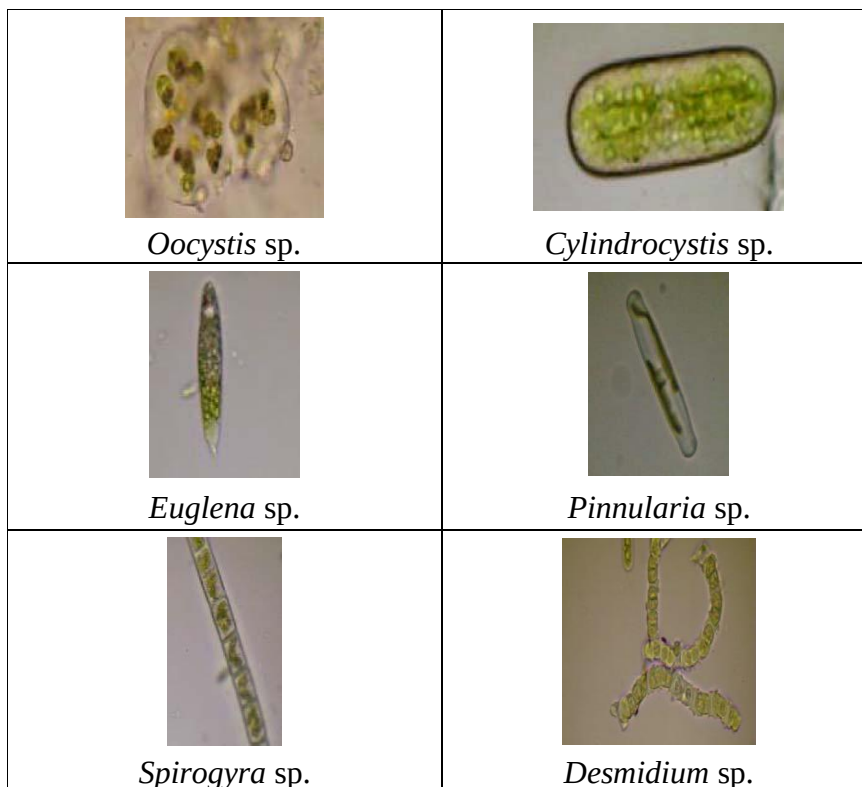


Figure 5.5: Images of algae recorded in the plots which received dry N deposition, along NH_3 gradient transect. Images were taken using a Brunel Digital Microscope under 400 x magnifications.

Algae in dry-deposition plots were dominated by *Cylindrocystis* sp. and almost all plots contained solely this species. A high population of *Cylindrocystis* is common in acidic environments, particularly in *Sphagnum* vegetation. The highest density of *Cylindrocystis* sp. was found in plots 28 m from the NH_3 source (**Figure 5.6**). This species increased in density from 8 m up to 28 m from the source but then declined with increasing distance. *Desmidium* sp. was erratically distributed and showed no obvious pattern in relation to nutrient deposition. As in the case with *Cylindrocystis* sp., the highest density of *Desmidium* sp. was found at 28 m from source. There was no *Desmidium* sp. at 8 m from the NH_3 source. Another alga, *Spirogyra* sp. was

found only within plots receiving high doses of ammonia (8 m and 16 m from the source). Number of cells increased from 783 ± 205 cells / ml (equivalent to 313 cells/cm²) at 8 m to $3,047 \pm 387$ cells / ml at 16 m which equates to 1,218 cells/cm².

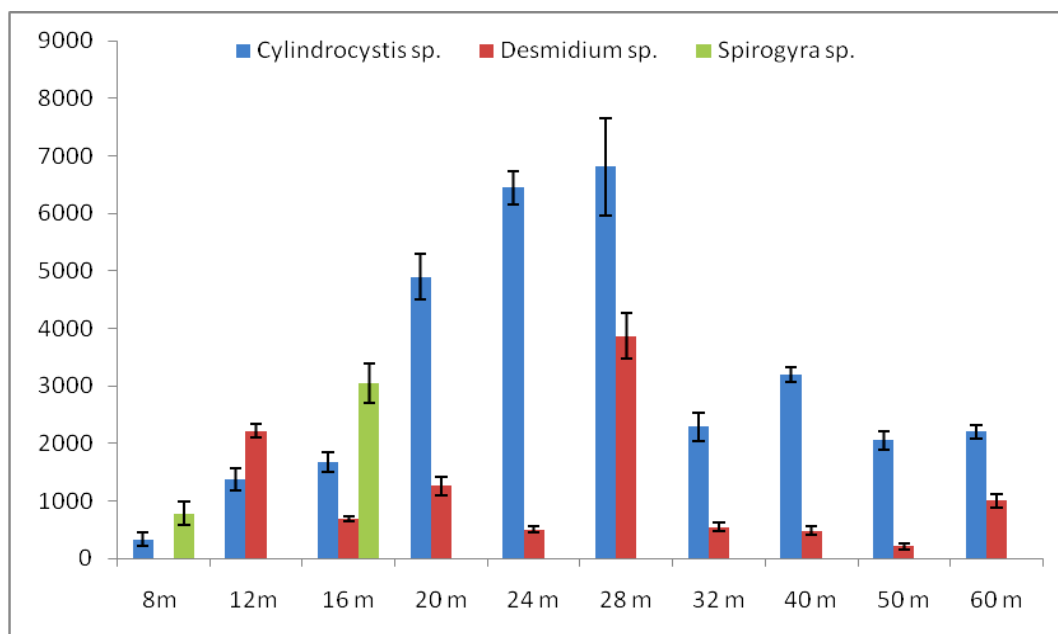


Figure 5.6: Algal composition of the three major genera recorded in the plots receiving dry N deposition, along the NH₃ gradient transect. Each bar represents mean number of algae / ml ± SEM.

5.3.2 Effect of treatments

5.3.2.1 The effect of treatments on total algal density in wet-deposition plots

In general, total algal density in treated plots increased compared to control plots (**Figure 5.7**). The highest number of cells was found in plots treated with NH₄Cl at 64 kg N ha⁻¹ y⁻¹ + PK. The number in this plot (dominated by *Cylandrocystis* sp was significantly greater than in any of the others (1-way ANOVA, $F_{10,33} = 7.28$, $p < 0.001$). Mean number of cells in this plot was $30,680 \pm 6744$ cells / ml (equivalent to 12,272 cells/cm²) compared to only $1,840 \pm 290$ cells / ml at the control plots, which equates to 736 cells/cm². The second highest number of cells was found in plots treated with NH₄Cl at 16 kg N ha⁻¹ y⁻¹ + PK with $8,395 \pm 2,275$ cells / ml or an equivalent to 3,358 cells/cm².

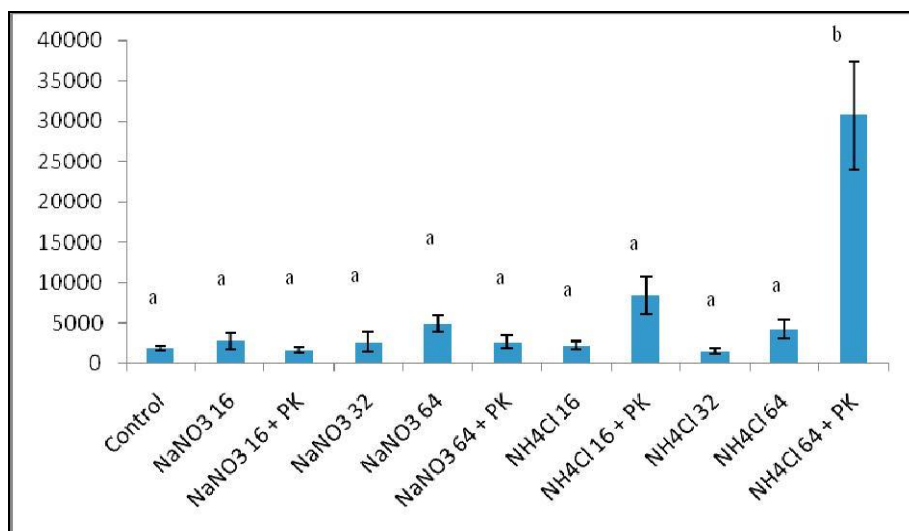


Figure 5.7 : Total numbers of all algae recorded in wet-deposition plots. Each bar represents mean number of algae / ml $\pm$ SEM.

Added PK in treated plots was clearly played a major role in influencing algal density. The number of cells is greater at the higher doses of PK. However, this pattern was true for plots treated with NH₄Cl but not plots treated with NaNO₃. In NaNO₃ plots, added PK appears to reduce algal density. For example, plots treated with NaNO₃ at 16 kg N ha⁻¹ yr⁻¹ contained 2,700 $\pm$ 1019 cells / ml, an equivalent to 1,080 cells/cm² while plots with the same treatment but with added PK showed that the algal density decreased to 1,640 $\pm$ 374 cells / ml which equates 656 cells/cm². The same pattern was observed on plots treated with NaNO₃ at 64 kg N ha⁻¹ y⁻¹.

At the same time, another pattern emerged in plots treated with NaNO₃. Algal density increased as the doses of N increased. At 16 kg N ha⁻¹ y⁻¹, there were 2,700 $\pm$ 1019 cells / ml (an equivalent to 1,080 cells/cm²) but the number increased to 2,640 $\pm$ 1,258 cells / ml (an equivalent to 1,056 cells/cm²) and 4,950 $\pm$ 1,003 cells / ml (an equivalent to 1,980 cells/cm²) at 32 kg N ha⁻¹ y⁻¹ and 64 kg N ha⁻¹ y⁻¹ respectively.

On another note, **Figure 5.8** shows that algal density was negatively correlated with pH of the *Calluna* bark (Pearson correlation, $R^2 = 0.6778$, $r = -0.823$, $p = 0.002$). Regression analysis showed that number of cells of algae was significantly decreased as the bark pH increased.

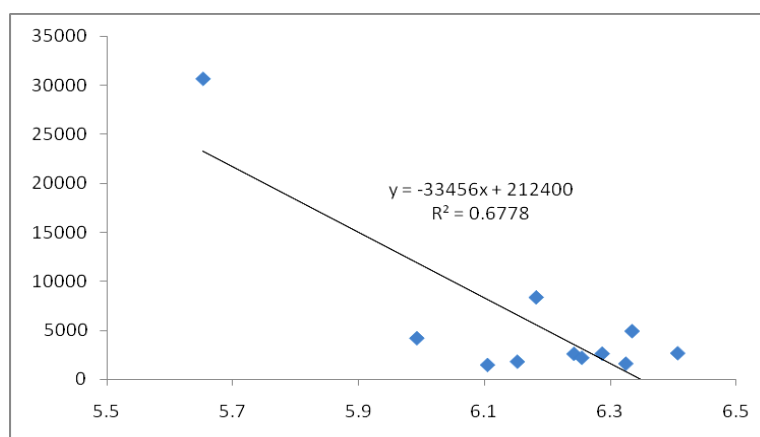


Figure 5.8: Relationship between algal density and *Calluna* bark pH, in the plots receiving wet N deposition. Each datum point (♦) represents mean density of algae / ml.

5.3.2.2 The effect of treatments on total algal density in dry-deposition plots

Algal density at different distances from the N source showed significant differences (1-way ANOVA, $F_{9,20} = 71.03$, $p < 0.001$). The high F value showed that the variance between groups was larger than the variance within groups. **Figure 5.9** shows that at 28 m, the number of algal cells was significantly higher ($10,687 \pm 1230$ cells / ml, equivalent to $4,275$ cells/cm²) than in any other plots. In fact, all plots from 16 to 28 m had a significantly higher density of algae than the rest of the plots.

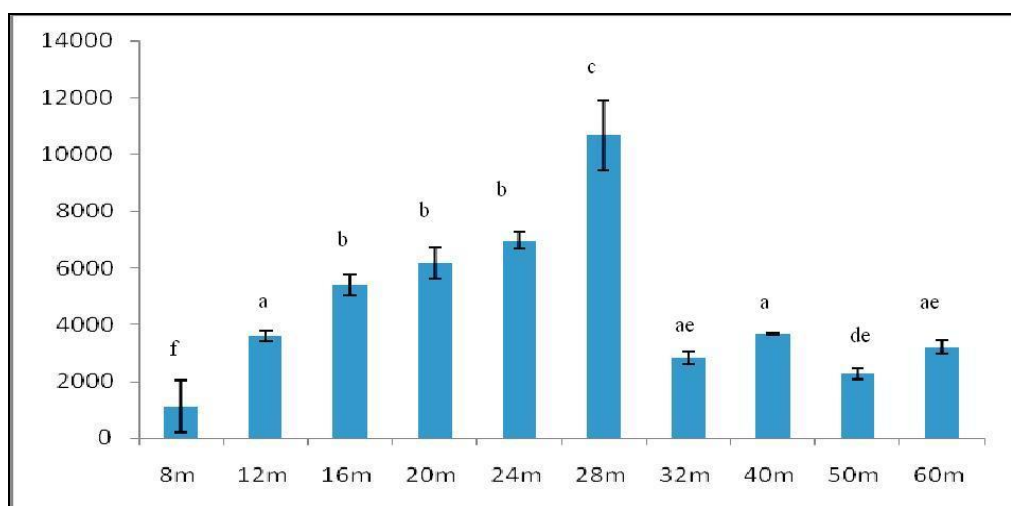


Figure 5.9 : Mean number of algae in each plot along NH_3 gradient of the dry-deposition plots. Each bar represents mean number of algae/ml $\pm$ SEM. Bars denoted by the same letter do not differ significantly ($p=0.001$).

On the contrary, the number of cells 8 m from the NH_3 source was significantly lower (1120 ± 92 cells/ml, an equivalent to 448 cells/cm^2) than the rest of the plots. In addition to that, plots between 32 m to 60 m from the source were not significantly higher than plots at 12 m from the source.

The relationship between algal cells and soil water pH was investigated using regression analysis (**Figure 5.10**) which shows no correlation between algal density and soil water pH ($r = -0.08$, $p = 0.826$), which was not statistically significant.

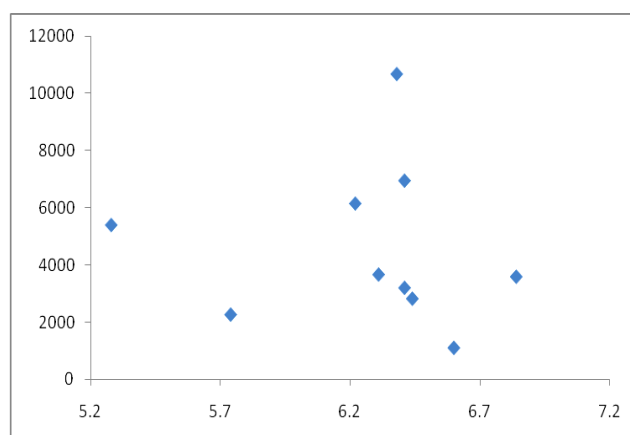


Figure 5.10: Relationship between algal density and soil water pH, in the plots receiving dry N deposition. Each datum point (♦) represents mean density of algae / ml.

5.4 Discussion

5.4.1 Species Composition of Algae in Wet and Dry Deposition Plots

5.4.1.1 Wet-deposition Plots

The acidic environment of this ombrotrophic bog resulted in low algal density compared to other habitats. Low pH is known to be correlated with low diversity of algae (Mataloni, 1999; Coesel & Meesters, 2007) especially of terrestrial epiphytic types. Apart from a variety of diatoms which will not be discussed in this chapter due to their low numbers, there were 3 main genera that dominated the wet plots, namely *Cylindrocystis* sp., *Desmidium* sp. and *Chlorococcus* sp.

It is generally believed that in a habitat of low pH such as this, species diversity is generally low with just a few or even a single dominant species, often of simple, typically cylindrical morphology (Coesel *et al.*, 1978). Here, the dominant species will account for a large proportion of the total biomass with a very high cell number compared with the less dominant species (Neustupa *et al.*, 2009).

However, *Desmococcus* sp. or *Apatococcus* sp. (formerly known as *Pleurococcus*) assemblages were not found in this plot. We speculate that the tiny branches of *Calluna vulgaris* were not a suitable type of substratum for *Desmococcus* sp. and *Apatococcus* sp. due to some unknown reason.

The three main algal genera that dominated the wet plots were among the typical algae found in acidic soils such as typical of bogs (Wayda, 2004; Neustupa *et al.*, 2009). Out of these, *Cylindrocystis* sp. was highest in abundance and this was especially true for plots treated with NH_4Cl at 64 kg ha⁻¹ yr⁻¹ + PK. The number

of *Cylindrocystis* sp. in these plots was more than three times higher than any other genus (**Figure 5.4**). This figure also shows that the number of cells of *Cylindrocystis* sp. increased as the level of NH_4Cl increased, and even more so with an addition of PK. It indicates that growth of *Cylindrocystis* sp. was favoured by higher doses of NH_4Cl and added PK. In particular luxuriant growth of *Cylindrocystis* sp. seems to be favoured by reduced forms of N (NH_4^+) compared to oxidized N (NO_3^-). In support of this hypothesis, there was no *Cylindrocystis* sp. in the plots treated with NaNO_3 . Even with added PK, *Cylindrocystis* sp. showed little response to NaNO_3 . Only at one site where plots were treated with NaNO_3 at $64 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ with added PK, does *Cylindrocystis* sp. emerge in the plots, but at low numbers, approximately one-tenth of that at the same doses of NH_4Cl .

Desmidium. is a unicellular green alga, often forming filaments typically inhabiting acidic peatland environments. It was found on all plots except the one treated with NH_4Cl at $64 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ + PK. Its densest populations were found in plots treated with NH_4Cl at $16 \text{ kg ha}^{-1} \text{ yr}^{-1}$ + PK with $5,185 \pm 2932 \text{ cells/ml}$, an equivalent to $2,074 \text{ cells/cm}^2$. Out of the three dominant species, *Chlorococcus* sp. was found in all plots, but only at low cell numbers from $920 \pm 274 \text{ cells/ml}$ to $3,120 \pm 703 \text{ cells/ml}$ which equates to 368 and 1,248 cells/cm^2 respectively.

Desmids in low pH habitats tend to have lower surface-to-volume ratios than those at higher pH thus increasing the cell fitness (Coesel, 1982). This might be the factor contributing to the large number of *Desmidium* sp. in the plots. Desmids are known to thrive in acidic water, usually in the pH range of 4-7. Only a few taxa are recorded from alkaline environments (Brook, 1981; Gerath, 1993; Wayda, 2004).

5.4.1.2 Dry-deposition Plots

Leith *et al.* (2001) in his study concluded that the majority of NH_3 was deposited within 30-50 m of its source. Within this distance, ammonia concentration was approximately 3-4 times higher than the background which itself was relatively low considering that the site was in a pristine area.

Algal composition in dry-deposition plots was similar to that in the wet plots. There was one difference in terms of species diversity in that *Chlorococcus* sp. was replaced by *Spirogyra* sp. The study showed that at the highest ammonia concentrations algal populations were suppressed, a finding in keeping with that of Abeliovich & Azov (1976) who reported that NH_3 concentrations of over 2.0 mM inhibits the photosynthetic process and growth of many algal species. Total nitrogen also significantly influences species composition (Neustupa *et al.*, 2009). A study on Desmids of Central Europe by these authors reported that total N was negatively correlated with species diversity, a finding in agreement with a previous study from Gilbert *et al.*, (1998).

The most abundant alga, *Cylindrocystis* sp. was found in almost all plots, regardless of distance from the NH_3 source. Population density increased steadily with distance from the source, peaking at 28m but thereafter declined.

Desmidium sp. was also found in high numbers: it was absent from the site closest to the source but was found in all other sites. Its distribution seemed to show no obvious pattern across the plots but from 16 m up to 28 m it increased almost exponentially until it decreased drastically after 28 m. The probability is that *Desmidium* sp. does

not thrive at high doses of NH_4Cl probably due to the difficulty of maintaining a neutral cytoplasmic pH in an acidic environment (Cerna & Neustupa, 2010).

The other species, *Spirogyra* sp. is a green alga belonging to the order Zygnematales. It has a helical or spiral chloroplast and usually appears as a slimy green mat on stagnant waters and on *Sphagnum*. Its outer cell wall is composed of pectin which partly dissolves in water, making the filament slimy to touch. It is commonly found in high nutrient waters (Schindler, 1971). This may explain why *Spirogyra* sp., was only found in plots located nearer to the NH_3 source. As the NH_3 concentration decreased with distance, *Spirogyra* sp., decreased as it 'struggles' in a low N environment.

5.4.2 Response of Algae Towards Pollutants

5.4.2.1 Response of Algae Towards Pollutants in the Form of Precipitation

Results have shown that total algal populations increased with introduced N, either in the form of NaNO_3 or NH_4Cl . Enhanced N levels stimulated the growth of algae, agreeing with the study by Limpens *et al.* (2003) where they found that algal patches expanded over the years and eventually were distributed equally in the plots that received N. They concluded that enhanced N stimulated the growth of algae in the plots and suggested a mechanism that invoked the leaking of N from *Sphagnum* as this bryophyte was no longer able to sustain the saturated N (Limpens *et al.*, 2003). Plots with added PK also showed significant differences from plots which did not received added PK (**Figure 5.7**).

There was an opposite effect when considering the two different forms of nitrogen. Oxidized N supported less growth of algal cells compared with the reduced form of N. The effect was pronounced with *Cylindrocystis* sp. which showed a decrease in the number of cells when treated with oxidized N. The same result has also been reported by Carfrae *et al.* (2007) who studied the effect of N deposition on *Sphagnum capillifolium* at the same field site. Plots treated with NaNO₃ showed that the number of algal cells decreased when PK was added to the treatments.

On the other hand, when treated with NH₄Cl, algal cells increased markedly when PK was added to the treatment. This result is in agreement with a study conducted by Adamsen & King (1993) which reported that nitrate uptake was about half that of ammonium. However, Limpens *et al.* (2003) in their study concluded that adding N stimulated algal growth and the addition of P hampered growth.

In this study, algal density decreased as the plant surface (bark) pH increased, a result which might be expected since algae in ombrotrophic bogs are naturally adapted to low pH (see the next section for a further discussion of this effect). The results showed that algal density was negatively correlated with bark pH (Pearson correlation, $r = -0.823$, $p = 0.002$). See **Figure 5.8**.

5.4.2.2 Response of Algae to Pollutants in the Form of Gaseous NH₃

Distances from the source of NH₃ had a significant effect on the number of algae in the plots (1-way ANOVA, $F_{9,20} = 71.03$, $p < 0.001$). Broadly, ammonia was stimulatory to algal growth but at a distance of less than 28m from source

concentrations were too high and inhibitory. At 12 m population numbers were no higher than at 32 m and beyond.

There was no consistent relationship between algal density and soil water pH ($r = -0.08$, $p = 0.826$). **Figure 5.10.** When pH is increased, the concentration of bicarbonate ions increases rapidly and the majority of soluble inorganic carbon which is a major component for photosynthesis, is in the form of bicarbonate. Not many algal species are capable of using bicarbonate as a substrate for photosynthesis (Brook, 1981), which may explain the failure of algal populations to respond fully to ammonia as a nutrient. For comparison, Wayda (2004) recorded only 7 species of alga compared to 23 species before raising alkalinity via liming. At pH values over 8.0, the growth of many algal species such as *Scenedesmus obliquus*, *Chlorella pyrenoidosa*, *Anacystis nidulans*, and *Plectonema boryanum* was inhibited (Abeliovich & Azov, 1976) while other work conducted by Gardnet et al. (1997) reported that algal growth has a limiting value of pH 9.

From this study, it could be hypothesised that algal diversity was positively correlated with pH while total population size was negatively correlated with pH. This phenomenon might be due to the fact that as the pH increased, the very acidic substratum changed to a milder one and provided better conditions for other algal species which are less tolerant of very low pH. This is supported by the work of Neustupa *et al.* (2009), who in their study regarding morphological disparity in Central European peatlands reported that species diversity of desmids in the benthic assemblages was positively correlated with pH.

5.5 Conclusion

As a conclusion, we can say that :

- Species composition of algae in this study differed slightly between plots treated with gaseous NH_3 (dry plots) and those treated with nitrogen in solution (wet plots).
- Total populations were greater in plots that received wet treatment in the form of NH_4^+ and NO_3^- compared with plots that received dry deposited NH_3 .
- Different algal species responded differently towards a variety of treatments.
- Oxidized N in the form of NaNO_3 was more damaging to algae compared with reduced N in the form of NH_4Cl . Both *wet and dry plots* showed that algal density was negatively correlated with plant surface (bark) pH and also with soil water pH.
- The addition of PK had a damaging effect when it supplemented NaNO_3 treatments but not NH_4Cl treatments.

Chapter 6

Field Experiment to Explore the Effect of Dissolved N Compounds and Bisulphite on *Desmococcus* sp. and *Trentepohlia* sp. on Oak and Beech Trees

6.1 Introduction

Algae are the first level of the trophic chain (primary producer or pioneer organisms). Therefore, any disturbance in their dynamics might affect the ecosystem at higher levels. Algae are very sensitive to changes in their environment (Gupta & Agrawal, 2008; Silva *et al.*, 2009) and many studies have been conducted utilizing algae as bio-indicators for pollution, especially of marine and freshwater ecosystems (Carmichael *et al.*, 2001; Abe *et al.*, 2004). Freystein *et al.* (2008) who studied algal biofilms on tree bark reported that central Leipzig in Germany, the most polluted site in their study, showed the greatest diversity of algae compared to other sites. In a separate study carried out in Sweden, Brakenheilm & Qinghong (1995) reported that colony thickness and colonization rate of algae on spruce needles reacted positively to increased N deposition. Similarly, Bates *et al.* (1990) and Goransson *et al.* (2007) also observed an increase of algal growth upon enhanced N deposition. Haapala *et al.* (1996) who studied the ecology of forests around the eastern part of the Gulf of Finland, regarded *Trentepohlia umbrina* as an indicator of alkaline pollution.

Almost all epiphytic terrestrial algae on tree bark are green algae. Green algae are known as an ancient group of aquatic photosynthetic organisms which gave rise to the land plants. The aquatic character of algae makes it possible to survive whenever there is water either in a marine environment, freshwater bodies or in the terrestrial habitat. Terrestrial algae is the term used for algae which inhabit (sometimes only briefly) aquatic environments on land, while soil algae are those that survive in a film of soil water. Algae which live in symbioses with fungi are known as lichen algae.

The classification of green algae in the past was mostly based on morphological characters (Smith, 1950; Ettl & Gärtner, 1995) but increasingly includes molecular data of late (Lopez-Bautista, 2006; Rindi *et al.*, 2009). Subaerial green microalgae are among the most widespread and evolutionarily diverse organisms that inhabit many terrestrial environments (Rindi & Cinelli, 1995). These algae represent a polyphyletic complex of organisms in which their genetic diversity is much higher and more complex than their simple morphologies suggests (Rindi *et al.*, 2009).

The systematics of green epiphytic algae especially in the order Trentepohliales has been traditionally problematic and surrounded by great controversy (Rindi *et al.*, 2009). This is due to the fact that algae in this order are characterized by great plasticity, in particular the morphology of members of this order is greatly influenced by environmental conditions (Roberts, 1985; Rindi & Guiry, 2002). Thus, it is common for specimens of *Trentepohlia* to be identified only at the genus level or sometimes only at family or order level (Rindi *et al.*, 2009).

6.1.1. General overview of *Desmococcus* sp. and *Trentepohlia* sp.

6.1.1.1 *Trentepohlia* sp.

Trentepohlia sp. is a member of the order Trentepohliales. This order contains a single family, the Trentepohliaceae, and four genera namely *Trentepohlia*, *Phycopeltis*, *Printzina* and *Cephaleuros*. Algae from this genus are found throughout Britain and survive as free-living algae in terrestrial habitats or as lichen photobionts. *Trentepohlia* spp. are cosmopolitan with a world-wide distribution, especially in the tropics. To date, there are about forty species of *Trentepohlia* worldwide but most of them are found only in tropical and subtropical climates (Van den Hoek *et al.*, 1995; John *et al.*, 2002). Five species have been recognized as widely distributed in Britain and Ireland (Rindi & Guiry, 2002).

The most familiar species are *Trentepohlia aurea* and *Trentepohlia abietina* which are common on oak trees. They can be found in scattered localities in Ireland and wetter areas of the British Isles such as Scotland. Another common species is *Trentepohlia umbrina* (Rindi & Guiry, 2002; Neustupa & Škaloud, 2008).

One distinctive character of *Trentepohlia* spp. is the absence of pyrenoids in their chloroplasts, while other common green algal such as *Desmococcus*, have pyrenoid (Chapman & Henk, 1983; Roberts, 1984). The filamentous species are often found forming turf or crust-like structures in a wide range of habitats such as tree bark, leaves, wooden poles, stones and plastic (López-Bautista *et al.*, 2002; An *et al.*, 2003). These algae usually form layers of red, orange or yellow colour on tree bark. This colour results mainly from their pigments called haematochrome (carotenoids). These

pigments consist of varying additions of α -carotene produced in the algal cells (Lopez-Bautista, 2006; Aptroot & Van Herk, 2007).

6.1.1.2 *Desmococcus* sp.

Algae of this genus are common members of the Order Chaetophorales and Family Chaetophoraceae. This alga has globular cells, which are uninucleate with a single, parietal chloroplast and pyrenoid. *Desmococcus* spp. are cosmopolitan on soil-free subaerial surfaces such as walls and the bark of trees especially in shaded and polluted habitats. According to the Alga Database (www.algaebase.org), so far only three species have been accepted as valid taxonomically, namely *Desmococcus endolithus*, *D. olivaceus* and *D. spinocystis*.

Desmococcus and the apparently similar *Apatococcus* are not easy to distinguish using light microscopy only (Picket-Heaps, 1975). These species look very similar, although their 18S rDNA is phylogenetically distinct. *Desmococcus* are free-living algae and can be found almost everywhere, including high latitudes (Antarctica), high altitudes and soils worldwide (Broady, 1981, 1989; Broady & Ingerfeld, 1993). Algae of this genus can also live as lichen phycobionts (Broady & Ingerfield, 1993; Brakenheilm & Qinghong, 1995). One species of this genus, *Desmococcus olivaceus* has been described as the commonest green alga in the world (Laundon, 1985).

6.1.2. Effect of atmospheric pollutants on green algae

Bates *et al.* (2001) in their study of lichen and other epiphytes in South East England, reported that green algae, probably *Desmococcus viridis* (currently known as *D. olivaceus*), had increased in parallel with the decline of SO₂. They also reported that

Desmococcus sp. was the most tolerant epiphyte compared with others studied such as *Lecanora conizaeoides* and *Lepraria incana*. This is in agreement with the work of Hawksworth & Rose (1970, 1976), also conducted in the South East of England which reported that *Desmococcus viridis* was the most tolerant taxon on acid tree bark, compared with lichens and other epiphytes.

All of these reports showed that green algae increased with the decline of SO₂ concentration which followed on from the various clean air acts. However, after SO₂ concentrations drastically decreased the problem was later replaced by that of high N concentrations (after 1990), and a more noticeable increase of terrestrial algae has been observed at many sites especially in cities. The algal cover at this time was about 70 % as compared to 20 – 30 % in the mid-late 1980s. By 1990, algal cover continued to increase at many sites along the transect studied by Bates *et al.* (2001) and the most prominent difference was seen at Kensington Gardens which was located in the city centre. Algae on this site were absent before 1986 but reappeared after 1990.

This phenomenon was linked to decreased SO₂ and increased N deposition in the city centre. The same situation has been observed at several other places across Europe. Brakenhielm & Qinhong (1995) in Sweden reported that aerial algae on spruce needles responded positively to increased nitrogen deposition. In Finland, Poikolainen *et al.* (1998) who studied the abundance of epiphytic green algae in relation to nitrogen status observed that they were most abundant where nitrogen deposition was highest. Such algae have become considerably more abundant in Finland in the years 1985 - 1995. Even though these authors stated that the increase

may have been caused by any of several concurrent changes such as a slight rise in mean annual temperature their data certainly do not rule out the effect of decreased sulphur and increased N. In France, Gombert *et al.* (2005) reported that *Pleurococcus viridis* (*Desmococcus*) was present in 96% of the sampling area and populations could benefit from increased N. A study conducted by Sochting (1997) has also reported that algae on spruce needles have increased in Denmark and neighbouring countries in recent years.

To the best of our knowledge, there is no specific study to date on the effect of N deposition on *Trentepohlia* sp. A study by Aptroot & van Herk (2007) described increasing levels of *Trentepohlia* sp. as a lichen phycobiont in heavily polluted areas, but they did not correlate this finding directly with atmospheric pollution but attributed it to global warming. Haapala *et al.* (1996) whose study on the ecological condition of forests around the eastern part of the Gulf of Finland stated that areas of high sulphur deposition give rise to enhanced growth of *Trentepohlia umbrina*. This algal species was abundant on pine trunks and the emergence of large numbers of *Trentepohlia umbrina* was regarded as an indicator for pollution. This area was located within close proximity of a limestone quarry which was characterized by high calcium content, nearly 50-fold above that of the background area (Jokela *et al.*, 1990). Therefore, it seems one prominent effect of atmospheric pollutants on algae and other epiphytes is an elimination of diversity and a reduction of the species list (Liska & Herben, 2008), where pollutant-tolerant species will survive while other less pollutant-tolerant species will disappear.

6.1.3 Nitrate and ammonium in precipitation

N in precipitation is present mainly as ammonium and nitrate (Colbeck & MacKenzie, 1994). Xie *et al.* (2008) stated that the concentration of nitrate in precipitation was always higher than that of ammonium. In the UK, the majority of the N deposition received in the west of the UK falls as wet deposition, while in the east of the UK it is mainly deposited as dry in the form of ammonia (Sutton *et al.*, 2001). This is in agreement with a report from NEG-TAP (2001) which stated that N concentrations in precipitation in the west of the UK are low compared to the east of UK. Similarly, the more polluted areas in Sweden were reported to have higher concentrations of ammonium in precipitation, up to ten times that in the more pristine area in central Sweden (Hallingback, 1991). In general air pollution has been correlated with the disappearance of cyanobacterial lichens and green algal photobionts (Hallingback, 1991).

Dortch (1995) reported that algal growth was significantly affected by levels of nitrate and phosphate. In contrast with other researchers, Tubea *et al.* (1981) and Pietilainen & Niinioja (2001) found that levels of phosphate had no significant effect on algal growth. Apart from these, not many studies have been conducted in this area. Jonsson & Aoyama (2007) who studied the in vitro effect of pollutants from agriculture on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) observed that heavy metals such as Hg^{2+} , Al^{3+} and Cu^{2+} and the surfactant alkyl benzenesulphonate (LAS) altered more than 50% of the algal enzyme activity (phosphatase). No specific work has been carried out to monitor the effect of nitrate or phosphate on *P. subcapitata*. Many in-vitro studies have been conducted to investigate the potential of algae in removing nutrients from wastewater and sewage.

Among the algae involved were *Chlorella*, *Scenedesmus*, *Spirulina* and *Botryococcus braunii* (Martinez *et al.*, 2000; Lee & Lee, 2001; An *et al.*, 2003). Another separate study carried out by Aslan & Kapdan (2006) who investigated the nutrient removal performance of *Chlorella vulgaris* at different nitrogen and phosphorus concentrations in batch operation, reported that *C. vulgaris* was more tolerant towards ammonia-nitrogen concentrations compared to phosphate, and was able to remove higher amount of the former from solution.

In addition to causing algal blooms, nitrogen in wet deposition also contributes to water bodies becoming more acidic due to acid rain. Among the important components in acid rain are sulphur dioxide and nitrogen dioxides. Besides causing direct impact to aquatic ecosystem, acid rain can also affect the growth of certain terrestrial epiphytic algae (Krupa, 2003). This situation will sooner or later lead to community changes.

6.1.4 Type of tree bark as a factor influencing algal presence

Algal growth is influenced by many factors, one of which is type of substratum. The bark of trees constitutes one of the commonest types of substratum and different tree species typically carry their own flora since different bark types constitute different ecophysiological niches (Fogg, 1969). Tree bark or wooden poles usually provide a suitable place for coccoid or filamentous green algae such as *Desmococcus* sp., *Trentepohlia* sp. and *Printzina* sp. (Hoffmann 1989, Ettl & Gärtner, 1995; Rindi *et al.*, 2006). Most of these species are a common sight on oak, beech, alder and ash trees. The bark of orchard trees such as *Malus* is most frequently covered in layers of

reddish to purple-brown algae, while the bark of forest trees such as *Fagus sylvatica* has layers of bright green algae.

Neustupa & Skaloud (2010) who studied the diversity of subaerial algae and cyanobacteria growing on bark and wood in the lowland tropical forests of Singapore reported that there was a strong correlation between substratum type and species composition of algae. However, they also concluded that substratum type alone did not determine algal population size or structure: other factors such as light intensity were believed also to influence the algal flora. There was almost twice the number of species in open space habitats compared to shaded areas in the forest (Neustupa & Skaloud, 2008).

Different tree species are each characterised by different types of epiphyte community (Bates *et al.*, 1992). This might due to the fact that each tree species has a specific range of bark pH over which the tree could support the algae. However, very low pH (due to N deposition) has a negative effect on epiphytes since the hydrogen ion is toxic to algal photobiont (Tarhanen, 1998). On the contrary, high bark pH is known to be good for epiphytes. The high pH of tree bark is reported to facilitate spore germination of many epiphyte species especially of bryophytes (Wiklund & Rydin, 2004).

Besides pH the water holding capacity of bark also plays a role in affecting the algal community. Hauck *et al.* (2000) reported that water holding capacity coupled with atmospheric humidity, evaporation and precipitation all determined the water supply to epiphytic species. Epiphytes which have higher tolerance to drought will survive

where other species will not. Since many epiphytes are poikilohydric (Barkman, 1958), algae and lichens are found on many tree species.

To the best of our knowledge, there are no field studies on the relationship between types of tree bark and algal growth. However, studies on lichens such as that conducted by Gauslaa (1985) concluded that there are correlations between bark chemistry and the epiphyte community. He studied the ecology of *Lobaria pulmonariae* and *Parmelia caperata* in *Quercus*-dominated forests in Norway. One mechanism by which air pollution is known to negatively affect epiphytes is by changing the bark pH to a more acidic value (Farmer *et al.* 1991).

Tree bark covered with epiphytic terrestrial algae also contains green photosynthetically active cells (Wittmann *et al.*, 2001; Berweiller & Damesin 2008; Pilarski *et al.*, 2008). Epiphytic terrestrial algae that form biofilms on tree bark are usually homoiochlorophyllous, which means they retain their chlorophyll during desiccation (e.g. Tuba *et al.*, 1998; Benko *et al.*, 2002). Almost all desiccation-tolerant or poikilohydrous algae are homoiochlorophyllous (Tuba *et al.*, 1998; Tuba, 2008).

6.1.5 Hypotheses:

A twin approach to studying the influence of atmospheric pollutants on populations of algal epiphytes is taken in this thesis. In other chapters we study species richness and numbers and attempt to correlate the data with monitored levels of pollutants. In this chapter a more direct approach is taken by applying ‘model’ pollutants to habitats and looking for changes in epiphyte populations. Thus the sampling approach with all its

difficulties of habitat variability is complemented by the controlled replicated trial approach. This study tests the hypothesis that *Desmococcus* sp. and *Trentepohlia* sp. are tolerant of both oxidized and reduced N compounds and also that the latter species is relatively intolerant of SO₂ pollution which is here simulated by application of a dilute bisulphite solution.

6.1.6 Aims and Objectives

The aim of this experimental study is as follows:

- i) To determine which of the two algae tested (*Desmococcus* sp. and *Trentepohlia* sp.) is more tolerant of atmospheric pollutants.
- ii) To provide evidence that sulphur in the form of sodium bisulphite is harmful to epiphytic terrestrial algae.
- iii) To investigate whether oxidized N (NO₃⁻ in the form of sodium nitrate) or reduced N (NH₄⁺ in the form of ammonium chloride) has the greater effect on population numbers of epiphytic terrestrial algae.
- iv) To investigate whether the effect of pollutants on the algae is influenced by the type of substratum.

6.2 Materials and Methods

6.2.1 Site Description

This experimental study was conducted at the Silwood Park estate of Imperial College, Berkshire, about 35 km west of the centre of London. Mean annual temperature is in the range 7 to 11°C. This area has relatively hot dry summers and cool winters. It is typically warmer and dryer than most of the UK and receives less

than 650 mm of rain per year. The habitat of Silwood Park is mixed, largely regenerated woodland dominated by birch, beech, ash and oak.

6.2.2 Treatment Solutions Application

This experiment was set up on more than 40 trees of oak (*Quercus robur*) and beech (*Fagus sylvatica*), in the woodland areas of Silwood Park. A total of at least 10 trees for each category were carefully selected to ensure that each quadrat comprised more or less the same coverage of algae on its bark and of the same algal species. The categories were as follows:

- i) *Desmococcus* sp. on oak trees, *Desmococcus* sp. on beech trees.
- ii) *Trentepohlia* sp. on oak trees, *Trentepohlia* sp. on beech trees.

Due to limited space and to ensure homogeneity of algal coverage, sometimes more than one tree was required to accommodate a complete set of treatments. Each treatment was applied to a 15 x 15 cm² quadrat, using a hook to hang the quadrat outline on the tree. Ten replicates were made for each treatment. Care has been taken to ensure that no quadrat was put directly beneath another so as to prevent run-off from liquid treatments flowing down the tree and contaminating another quadrat. The quadrat itself was constructed with four metal walls that prevent the treatment solutions from contaminating the other quadrats during the spraying process. The treatment solutions were Sodium nitrate (0.2 mM), Sodium nitrate (2 mM), Ammonium chloride (0.2 mM), Ammonium chloride (2 mM), Sodium bisulphite (1.2 mM) and deionized water as control.

Treatment solutions were sprayed weekly onto the quadrat using a hand sprayer. The volume of the sprayed solution for each quadrat was approximately 4 ml, enough to

moisten the algae in the quadrat. Treatment solutions were prepared using stock solutions with the exception of sodium bisulphite which had to be freshly prepared every week due to its fast reaction with oxygen, converting the harmful bisulphite (HSO_3^{2-}) to the less damaging sulphite (SO_3^{2-}).

This spraying process was done weekly, for more than 18 months, but was done more frequently when there were heavy rains within the week. This experimental work was started in April 2009 and ended in November 2010. The weekly spraying was put on hold during the period November 2009 – February 2010 due to prolonged sub-zero temperatures, when England had its heaviest snowfall for 18 years. The spraying was resumed in March 2010 until the end of the experiment.

6.2.3 Algal sampling and quantification

Algal sampling and quantification followed general method (See Chapter 2 for details).

6.2.4 Data Analysis and Statistics

Data were analysed using MINITAB version 15 and R statistical software. Samples were tested for normality using the Anderson-Darling or Kolmogorov-Smirnov normality test. Non-normally distributed data were transformed to $\log_{10}$ prior to analyses. ANOVA 1-way and 2-way were used in many cases and significant results were later explored using Tukey's Pairwise Comparison. Interactions between variables were explored using a General Linear Model in R.

6.3 Results

6.3.1 The effect of solute sprays on *Desmococcus* sp. and *Trentepohlia* sp. (general trends)

Epiphytic terrestrial algae were found to respond differently towards the range of pollutants (**Table 6.1**). In addition to that, different pollutant concentrations also triggered different responses in the algae. In this experimental study, the number of algal cells of *Trentepohlia* sp. on both oak and beech was significantly affected by treatments. *Trentepohlia* sp. on oak was highly significantly affected by the simulated atmospheric pollutants (1-way ANOVA, $F_{5,54}=11.57$, $p < 0.001$). The same result was observed for *Trentepohlia* sp. on beech (1-way ANOVA, $F_{5,54}= 12.05$, $p < 0.001$). On the other hand, *Desmococcus* sp. was not significantly affected by treatments, neither on oak nor on beech.

Table 6.1: Number of algal cells of *Desmococcus* sp. and *Trentepohlia* sp., treated with solutes designed to simulate the effects of atmospheric pollutants. Data are recorded $\pm$ standard error.

Treatments	<i>Desmococcus</i> sp. (on oak) $\times 10^4$ cells/ml	<i>Desmococcus</i> sp. (on beech) $\times 10^4$ cells/ml	<i>Trentepohlia</i> sp. (on oak) $\times 10^4$ cells/ml	<i>Trentepohlia</i> sp. (on beech) $\times 10^4$ cells/ml
Sodium nitrate (0.2 mM)	143 $\pm$ 14	141 $\pm$ 20	30 $\pm$ 11	62 $\pm$ 7
Sodium nitrate (2 mM)	119 $\pm$ 19	180 $\pm$ 13	58 $\pm$ 14	152 $\pm$ 11
Ammonium chloride (0.2 mM)	127 $\pm$ 21	134 $\pm$ 22	82 $\pm$ 12	103 $\pm$ 10
Ammonium chloride (2 mM)	155 $\pm$ 15	124 $\pm$ 13	58 $\pm$ 8	88 $\pm$ 10
Sodium bisulphite (1.2 mM)	96 $\pm$ 23	111 $\pm$ 21	23 $\pm$ 5	81 $\pm$ 11
Deionized Water (Control)	134 $\pm$ 15	124 $\pm$ 20	115 $\pm$ 12	128 $\pm$ 5

i) Sodium nitrate (0.2 mM)

Quadrats treated with a low concentration of sodium nitrate (0.2 mM) had marginally more *Desmococcus* sp., both on oak and beech trees, $143 \pm 15 \times 10^4$ cells / ml and $141 \pm 20 \times 10^4$ / ml compared with $134 \pm 15 \times 10^4$ cells / ml and $124 \pm 20 \times 10$ /ml in the control. These differences however were not significant.

On the other hand, the opposite effect was recorded for *Trentepohlia* sp. This algal species declined in the low nitrate quadrat compared to the control quadrat. *Trentepohlia* on oak was three times more prolific in the control quadrat ($115 \pm 12 \times 10^4$ cells / ml) compared with only $30 \pm 11 \times 10^4$ cells / ml in the low nitrate quadrat. The same situation was reported for *Trentepohlia* on beech: the control quadrat contained more than twice as many algal cells at $128 \pm 5 \times 10^4$ cells / ml while the low nitrate quadrat contained only $62 \pm 7 \times 10^4$ cells / ml.

ii) Sodium nitrate (2 mM)

The quadrats treated with sodium nitrate at higher concentrations (2 mM) showed a specific pattern in which the number of algal cells on oak was found to be lower but counts on beech were higher than the control quadrats. This is true for both *Desmococcus* sp. and *Trentepohlia* sp. Cell counts on oak were $119 \pm 19 \times 10^4$ cells / ml for *Desmococcus* sp. and $58 \pm 14 \times 10^4$ cells / ml for *Trentepohlia* sp compared with $134 \pm 15 \times 10^4$ cells / ml for *Desmococcus* sp. and $115 \pm 12 \times 10^4$ cells / ml for *Trentepohlia* sp in the untreated controls. On beech the situation was reversed with mean number of *Desmococcus* cells on beech being $180 \pm 13 \times 10^4$ cells / ml, slightly higher than the control quadrat at $124 \pm 20 \times 10^4$ cells / ml. The same situation was reported for *Trentepohlia* sp. with a mean number of cells in the treated sites ($152 \pm$

11 x 10⁴ cells / ml) being slightly higher than the control quadrats (128 ± 5 x 10⁴ cells / ml).

iii) Ammonium chloride (0.2 mM)

Low concentrations of ammonium chloride had a negative effect on *Trentepohlia* sp. populations both on oak and beech trees. *Trentepohlia* cells in the control quadrat on oak and beech trees were 115 ± 12 x 10⁴ cells / ml and 128 ± 5 x 10⁴ cells / ml respectively. These numbers were reduced to 82 ± 12 x 10⁴ cells / ml on oak and 103 ± 10 x 10⁴ cells / ml on beech, when treated with low concentrations of ammonium solution. In contrast low concentrations of ammonium chloride did not seem to have any significant effect on *Desmococcus* populations on either tree. Quadrats treated with low concentrations of ammonium contained a mean of 127 ± 21 x 10⁴ cells / ml compared to 134 ± 15 x 10⁴ cells / ml in the control quadrat. The same alga on beech showed a slightly lower number of cells, at 124 ± 20 x 10⁴ cells / ml compared to 134 ± 22 x 10⁴ cells / ml in the control quadrat.

iv) Ammonium chloride (2 mM)

Similar to the effect of low concentration of ammonium, a higher concentration (2 mM) had a more markedly negative effect on *Trentepohlia* sp. numbers. The populations of *Trentepohlia* on both oak and beech trees were less than half those of the control quadrats. In the case of *Desmococcus*, as at the lower concentration there was little evidence of an effect of this nitrogen source.

v) Sodium bisulphite (1.2 mM)

The most interesting result was shown in quadrats treated with sodium bisulphite at 1.2 mM. All 40 quadrats treated with this pollutant showed fewer algal cells than the control quadrats, although in the case of *Desmococcus* on beech this was non-significant. The most prominent effect of bisulphite was on *Trentepohlia* sp. on oak. There were $115 \pm 12 \times 10^4$ cells / ml in the control plot but this number was five-fold lower in quadrats treated with bisulphite ($23 \pm 5 \times 10^4$ cells / ml). *Trentepohlia* on beech also showed a reduction in the number of cells, from $128 \pm 5 \times 10^4$ cells / ml in the control quadrat to $81 \pm 11 \times 10^4$ cells / ml in the treated with bisulphite.

6.3.2 Effect of sulphur on epiphytic terrestrial algae

Although *Desmococcus* sp. and *Trentepohlia* sp. both showed a response towards sulphur (in the form of sodium bisulphite), only *Trentepohlia* sp. on oak showed a decline that was highly significant (1-way ANOVA, $F_{5,54} = 11.57$, $p < 0.001$) from the control. **Figure 6.1** shows that mean number of *Trentepohlia* cells was significantly lower when treated with sodium bisulphite at 1.2 mM compared to other pollutants. Cell numbers were five times lower when treated with sodium bisulphite ($23 \pm 5 \times 10^4$ cells / ml) compared to the control ($115 \pm 12 \times 10^4$ cells / ml). Sodium nitrate at both low (0.2 mM) and high (2 mM) concentration was nearly as damaging to this alga.

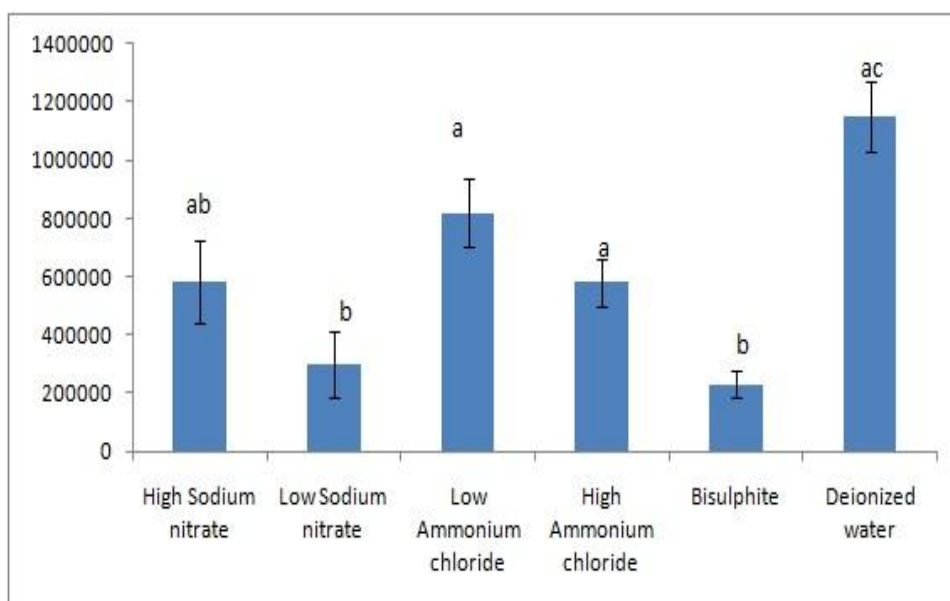


Figure 6.1: Graph showing mean number of algal cells of *Trentepohlia* sp. on oak trees. Treatments marked with the same letter do not differ significantly at $p = 0.001$.

6.3.3 Effect of oxidized and reduced N on epiphytic terrestrial algae.

Mean number of *Trentepohlia* cells on oak trees showed no significant difference between the effect of oxidized N (nitrate) and reduced N (ammonium). Similarly for *Desmococcus* sp. on both oak and beech there was no difference between the effects of reduced and oxidised forms of N, nor were either significantly different from the control.. Only with *Trentepohlia* sp. on beech was there a significant effect of reduced N on algal cell populations. At the lower concentration, oxidized N was more damaging than reduced N (1 way ANOVA, $F_{1,18} = 10.60$, $p < 0.005$). *Trentepohlia* populations in quadrats treated with 0.2 mM nitrate at $62 \pm 7 \times 10^4$ cells / ml were almost half those at sites treated with 0.2 mM ammonium at $103 \pm 10 \times 10^4$ cells / ml. However, at higher concentration (2 mM), this situation was reversed. Ammonium chloride was more damaging to algal cells (1 way ANOVA, $F_{1,18} = 18.91$, $p < 0.001$) than the same concentration of sodium nitrate. Cells counts of *Trentepohlia* sp. were significantly lower when treated with 2 mM ammonium chloride, at $88 \pm 10 \times 10^4$ cells / ml compared to 152 ± 11 cells / ml when treated with 2 mM sodium nitrate.

6.3.4 Concentration dependence of effects of pollutants on algae

There was no significant effect of concentration on the numbers of *Desmococcus* cells on oak or beech treated with different pollutants. Indeed this species seemed very resilient in the face of any of the chemicals. The same result was observed for *Trentepohlia* sp. on oak, the apparent differences being statistically insignificant at $P=0.001$. However, the same species when growing on beech did show concentration-dependent differences (1 way ANOVA, $F_{1,18} = 46.24$, $p < 0.001$; **(Table 6.1)**. *Trentepohlia* populations were significantly higher when treated with 2 mM of sodium nitrate, at $152 \pm 11 \times 10^4$ cells / ml compared to only $62 \pm 7 \times 10^4$ cells / ml when treated with 0.2 mM. With ammonium chloride there was no significant effect of concentration, although if anything the trend was reversed.

6.3.5 Interaction between treatments and substratum in affecting number of algal cells

Desmococcus sp. populations on oak and beech were not statistically influenced by substratum as a variable. However, *Trentepohlia* sp. clearly favoured beech over oak as a substratum, the mean number of *Trentepohlia* cells being almost twice as high on the former. The overall mean number on beech was $102 \pm 5 \times 10^4$ cells / ml compared to oak at $62 \pm 6 \times 10^4$ cells / ml (1-way ANOVA, $F_{1,118}=26.99$, $p < 0.001$). The statistical analysis also showed that algal number was significantly affected by both chemical treatment and substratum (2-way ANOVA, $F=4.28$, $p = 0.001$).

6.4 Discussion

6.4.1 Effect of nitrogen treatments on epiphytic terrestrial algae

The purpose of these treatments was to simulate the effect of wet deposition of atmospheric nitrogenous compounds on the tree bark habitat of terrestrial green algae. The treatments have to be considered as being in addition to the background rate of nitrogen deposition (both wet and dry) typical of south-east England. Although the raw figures suggest that both algal species were affected by the treatments the changes in *Desmococcus* sp. populations were not found to be significant. On the contrary, *Trentepohlia* sp. was significantly affected by treatments both on oak and beech trees (**Table 6.1**). Thus, we can conclude that *Desmococcus* sp. was more tolerant to the effect of atmospheric pollutants than was *Trentepohlia* sp. Similarly, Hawksworth & Rose (1970, 1976) and Bates *et al.* (2001) also concluded that *Desmococcus* sp. was a pollutant tolerant species. There might be some possible reasons why *Desmococcus* is more tolerant than *Trentepohlia*. It could be that the former has the ability to excrete nitrate, ammonium and sulphur from its cells or is able to metabolise the pollutants into other substances that are beneficial to the alga. Since most atmospheric pollutants arrive dissolved in precipitation, algae on tree bark which are sensitive to a change in water chemistry (Singh & Agrawal, 2008) are more affected than those with the ability to utilise, exclude or excrete exogenous chemicals.

To the best of our knowledge, no specific study has been conducted to investigate the ability of *Desmococcus* sp. to remove these chemical substances. However, many studies with the same objectives have been carried out using other algae that are morphologically similar to *Desmococcus* such as *Chlorella*. Shi *et al.* (2007) reported that *Chlorella vulgaris* and *Scenedesmus rubescens* removed up to 90 % of initial

phosphate, ammonium and nitrate from their environment in only nine days. Other similar research has been conducted by Tam & Wong (1996); Perez *et al.* (2007) and Khan & Yoshida (2008) on *Chlorella vulgaris*. Abe *et al.* (2008) are among researchers who investigated the ability of algae to remove nutrients from wastewater and sewage. We think that pollutants absorbed by the algae could possibly be utilized by the cell, thus it has no negative effect on the alga itself, although research is needed to investigate this claim. It was clear that *Desmococcus* sp. could survive increased N deposition. Bates *et al.* (2001) observed that *Desmococcus viridis* (currently known as *Desmococcus olivaceus*) was abundant at the urban stations in their study which were characterised by having higher N concentrations. So a likely explanation is that *D. viridis* actually benefits from high levels of N, a conclusion in agreement with study carried out by Leith *et al.* (2008). They reported that there are strong relationships between tissue N concentration of bryophytes and total N deposition in rainfall that is dominated by ammonium deposition. Central to any consideration of the effect of nitrogenous pollutants is knowledge about what is the optimal form (and its concentration) for growth of the two algae. Unfortunately such data are not readily available, but see chapter 7. A study carried out by Abe *et al.* (2002) using NH_4Cl as N source, reported that biomass density of *Trentepohlia aurea* decreased with increased nitrate concentration. However, after a long period, the growth rate stabilised and reverted to that without N addition. The same study also reported that algal growth rate increased markedly with the addition of ammonium, which inhibited nitrate assimilation. In 2003, Abe *et al.* reported that algal growth was increased when cultured with nitrate and phosphate ions. This response however varied between species.

Another possible reason why *Desmococcus* sp. is more tolerant than *Trentepohlia* sp. is its carbohydrate structure. Shnyukova *et al.* (2005) proposed that the carbohydrate composition in *Desmococcus* sp. helps it survive in unfavourable environments, probably by acting as a protective layer of cell wall against desiccation. The same material also helps to maintain a mucilaginous sheath around cells and allow the storing of reserve water during dry periods. The comparative performance of *Desmococcus*, and *Trentepohlia* in this study can possibly be explained in part by their relative susceptibilities to desiccation. Desiccation is an act of drying and rewetting cycles caused by changes in the weather. It has been reported that carotenoid pigments in *Trentepohlia* play a role in maintaining its survival. Based on work carried out by Rindi & Guiry (2002), *Trentepohlia* sp. was found to secrete some of their red pigment (b-carotene and phenolics) during wetting and drying cycles. Reduced amount of pigment correlated with poor survival although there was no evidence that the connection was causal. *Trentepohlia* also showed a poor performance upon recovery after longer periods of desiccation. Laundon (1985) also concluded that *Desmococcus* sp. was tolerant to desiccation.

6.4.2 Toxicity of sulphur to epiphytic terrestrial algae

Bisulphite was shown to reduce greatly the number of algal cells of both *Desmococcus* sp. and *Trentepohlia* sp. (**Table 6.1**). But as with nitrogen, the effect was more marked with *Trentepohlia* than with *Desmococcus*. This result is in agreement with many similar studies conducted on various epiphytes. Sheridan (1978) who studied the toxicity of bisulphite to *Chlorococcum* sp, showed that both photosynthesis and respiration were reduced and observed that the degree of inhibition decreased with time following the removal from bisulphite. Silva *et al*

(2009) who studied the ability of *Chlorella vulgaris* to remove pollutants from wastewater reported that the addition of sodium bisulphite increased the effluent toxicity. While Wodzinski *et al.* (1978) who studied the effects of low concentrations of bisulphite-sulphite and nitrite reported that photosynthesis of algae was decreased as pH increased.

Other studies have been conducted using bryophytes. Ferguson & Lee (1979) concluded that bisulphite at 0.1 mM caused an instantaneous reduction in photosynthesis of *Sphagnum*. Baxter *et al.* (1989) in his work on *Sphagnum* reported that application of bisulphite to *Sphagnum cuspidatum* sampled from polluted sites produced less than maximum growth of the moss. This effect was far greater with *Sphagnum* from unpolluted sites. They also observed that the photosynthetic rate of *Sphagnum* from an unpolluted site decreased steadily over time and ceased after 21 days of exposure. However, *Sphagnum* from a polluted site was stimulated by initial bisulphite exposure, but then decreased over time as well. Hallingback & Kellner (1992) who studied the effect of simulated nitrogen-rich and acid rain on the nitrogen-fixing lichen *Peltigera aphthosa* reported that sulphuric acid had a negative effect on nitrogen fixation rate, more so when in combination with ammonium. Bates *et al.* (2001) who studied the effects of bisulphite on respiration and photosynthesis of *Lecanora conizaeoides* and *Pertusaria pertusa* reported that photosynthesis was enhanced by bisulphite at a concentration range of 0.2-2.0 mM but was inhibited completely above 2.0 mM. This lichen species did not recover photosynthetic nor respiratory activity after exposure to high concentrations of bisulphite.

6.5 Conclusions

Based on the results presented on this study, *Desmococcus* sp. was found to be tolerant of both oxidized and reduced forms of N. On the other hand *Trentepohlia* sp. was significantly affected by the same treatments. Among the six solutions sprayed onto the algae, sulphur in the form of bisulphite was the most damaging in reducing algal density. *Trentepohlia* also responded to the nature of the substratum, populations on oak declining more than those on beech. Nitrogen in both reduced and oxidised forms was also detrimental to *Trentepohlia* populations, again the effect on oak being significantly greater. A difference in bark properties might contribute to this result and investigating the physical and chemical differences in habitat between the two trees is a logical future extension of the work. Since *Desmococcus* sp. was shown to be little affected by atmospheric pollutants, further studies are recommended to investigate the physiology and mechanism of this algal species to understand what makes it tolerant to increased N. This might help us in conservation efforts for other less tolerant species. The present work has provided a broad picture of the effect of simulated N and S pollutants, but many fine details remain to be elucidated. Choosing a lengthy exposure and only a single harvest meant that no seasonal effects could be detected, but it was far from clear at the outset that briefer exposure would have revealed any changes at all. Interpretation of the effects seen awaits information on the nutritional requirements of the algae under study, as well as information on the background level of N and S deposition. Further, although the effects reported here are real, other pollutants not included in the study may have modified the response shown by the algae.

Chapter 7

Effects of Short-Term Exposure of liquid-cultured *Desmococcus* sp. and *Trentepohlia* sp. to chemicals simulating atmospheric pollutants

7.1 Introduction

This in-vitro work has been carried out to complement the in-situ experiment presented in Chapter 6 of this thesis. The work described here examines the effects of raised N and S levels, provided as inorganic salts, on the growth of selected algae under defined environmental conditions. The study focuses on the same genera, *Desmococcus* and *Trentepohlia* as used in the fieldwork.

7.1.1 Algae and their ability to overcome stress

It is generally believed that some species of algae inhabit diverse habitats and often occur in extremely hostile environments, achieving this by their remarkable ability to tolerate various kinds of stress. Some of the stresses are naturally occurring such as various concentrations of chemical elements in the atmosphere, different availability of light, temperature, pressure, pH and many more. In addition, further sources of stress result from human activities such as agricultural activities, sewage treatment and transportation systems. These activities lead to an increase in atmospheric pollutants and subsequent exposure of epiphytic terrestrial algae.

Stress is defined as an external constraint that limits the rate of resource acquisition, growth or reproduction of organisms (Grime, 1989). The short and long-term effects of stress do not necessarily have the same results; indeed sometimes the opposite is observed (Rees & Lawton, 1993). Stress can be divided into two groups, limitation stress and disruptive stress (Davison & Pearson, 1996). Limitation stress is caused by an inadequate supply of resources while disruptive stress results from damage caused by adverse conditions which can include an excess of resources. In this chapter we examine pollutants as a stress factor potentially limiting growth, the surmise being that either algal cells are able to tolerate the stress or if not this will contribute to lower productivity. Nitrate, ammonium and bisulphite dissolved in rain water are among the stress factors influencing algal growth in the sub-aerial environment, which are considered here.

Among the many eukaryotic algal groups, the Chlorophyceae are among the most resilient which explains their success colonizing extreme terrestrial habitats (Fogg, 1969). They possess a variety of adaptations enabling them to survive factors such as desiccation, low temperatures and high light intensity (Rai & Gaur, 2001). The green alga *Chlorella* is known to be tolerant to all sorts of physical and chemical stress which has made it a chosen experimental model for many studies regarding cell metabolism. Other species such as *Chlorococcum* are very resistant to heat and have an ability to overcome extreme low pH (Rai & Gaur, 2001). These algae produce pigments to protect them from excess photosynthetically active radiation and UV radiation. Another species, *Dunaliella* has the ability to adjust its cytoplasmic pH according to environment. To the best of our knowledge, no specific study has been

conducted to see the response of *Desmococcus* or *Trentepohlia* towards any stress factor.

7.1.2 The effect of pollutants as a stress factor to algae

Almost all recent studies relating to the effect of stress on algae focus on their contribution for bioremediation and as a source of biofuels (Aslan & Kapdan, 2006; Shi *et al.*, 2007; Doshi *et al.*, 2008); one such example being the effect of nitrogen and phosphate starvation on the accumulation of lipid in algae (Khozin-Goldberg & Cohen, 2006; Wu, 2009). Other studies have examined the factors limiting algal growth. Some have shown that nitrogen is the most nutrient-limiting factor for algal growth while others have reported that it is phosphorus (Hecky & Kilham, 1988; Xu *et al.*, 2001). Basically, this depends on the ecosystem. In temperate zones phosphorus is usually the limiting factor for algal productivity (White *et al.*, 1982), but when phosphorus is freely available nitrogen becomes the limiting factor. The N:P ratio is believed to influence the outcome of inter-species competition and can influence species diversity (Nandini & Rao, 2000). An N:P ratio of less than 10 will result in N limitation and if the N:P ratio is greater than 17, this will result in phosphorus limitation (Forsberg *et al.*, 1978). Lynn *et al.* (2000) reported that N and P concentrations in the cytoplasm of algae vary in response to changes in the environment and their cellular conditions. Other researchers distinguish between marine habitats where nitrogen is considered limiting and freshwater where phosphorus is seen as limiting (Schindler, 1971; Nalewajko & Prepas, 1996; Martinez *et al.*, 2000). In the case of epiphytic terrestrial algae phosphorus is more likely as the limiting factor because inputs of this element are substantially lower than those of nitrogen from in the atmosphere.

Many studies examined the uptake of nitrogen and phosphorus from water, especially by *Scenedesmus*, *Chlorella* and *Spirulina* (Olguin *et al.*, 2003; Hernandez *et al.*, 2006; Shi *et al.*, 2007). In one study a total of 53 % of the uptake of dissolved N in a freshwater ecosystem came from ammonia while only 19 % was from nitrate (Berman & Bronk, 2003). Yeesang & Cheirsilp (2011) who worked on the effect of nitrogen, salt and iron content in the growth medium of freshwater microalgae in Thailand, reported that all strains tested in their experiment showed an increase in biomass under nitrogen-rich condition. The algal cells appeared bleached and no growth was observed in the absence of nitrogen. This correlated with a decrease in chlorophyll and protein content, and increased carbohydrate and lipid content (Richardson *et al.*, 1969). When nitrogen depletion occurs, algal cells respond by accumulating a surplus of carbon metabolites such as lipids and fats (Ahlgren & Hyenstrand, 2003; Dayananda *et al.*, 2005) thus providing longevity but less growth in terms of number of cells.

Xin *et al.* (2010) who also studied the effect of nitrogen supply to the green alga *Scenedesmus*, reported that nitrogen limitation caused a decrease in the cellular content of thylakoid membranes, activation of acyl hydrolase and stimulation of phospholipid hydrolysis. They also reported that after only 13 days growth, *Scenedesmus* sp. removed almost 100 % of the phosphorus from the medium.

In the simulation study carried out by Kuwata & Miyazaki (2000) the blue-green bacterium, *Microcystis* was found not to be significantly affected by ammonium concentrations of 20 – 100 mM, but higher amounts in the medium contributed to

larger final biomass. In a separate study, Maberley *et al.* (2002) reported that single additions of phosphorus, nitrate or ammonium had no effect on microalgae but combinations of nitrogen and phosphorus stimulated growth and yield. They also reported that ammonium was a significantly better source of N than nitrate, in terms of algal yield. This is in agreement with work conducted by Dortch (1990) where algae treated with ammonium recorded higher growth than on nitrate.

Normally, high N concentration is linked to high biomass productivity (Hsieh & Wu, 2009). However, the effect of reduced and oxidized N varies according to algal species. Ammonium stimulated higher biomass and lipid content than nitrate for *Ellisoidion* sp. (Xu *et al.*, 2001). On the other hand, nitrate produced higher biomass of *Chlorella* sp. and *Neochloris oleoabundans* (Pruvost *et al.*, 2011). Lipid content was also higher when exposed to nitrate than ammonia. Lin & Lin (2011) also reported that algae treated with ammonia showed higher growth than with nitrate over the first 5 days of the experiment. However after 17 days, this trend was reversed.

For those epiphytic terrestrial algae that inhabit the tree trunk as their substratum, nitrogen depletion is faster than for soil or freshwater algae. Due to the nature of the habitat there is no reservoir of this or other nutrients. Rainwater or leachate from bark must satisfy the mineral requirements of bark surface residents and availability is characteristically erratic. Epiphytic algae have adapted to survive in a dilute nutrient solution. Whether they respond positively or negatively to enhanced inputs is the subject of the current study.

7.1.3 Aims and objectives

The aim of this study is to investigate the effects of sulphur dioxide, ammonia and nitrogen dioxide on the growth rate of *Desmococcus* sp. and *Trentepohlia* sp. cultured from field populations growing on oak and beech trees. The specific objectives are as follows:

- i) To investigate which of the dissolved pollutant treatments caused the most damage to algal survival and growth.
- ii) To separate the effect of reduced and oxidized forms of nitrogen on algal survival and growth.
- iii) To examine the concentration dependence of any effects seen in (i) and (ii) above.
- iv) To determine which of the two algae, *Desmococcus* or *Trentepohlia*, is more tolerant of short-term application of dissolved atmospheric pollutants.
- v) To provide information on whether short-term exposure of dissolved sulphur affected algal survival and growth.
- vi) To test whether time of exposure played an important role in any effects seen above.

7.2 Materials and Methods

7.2.1 Algal preparations and culture medium

This short-term physiological experiment was conducted in a controlled temperature room at Imperial College, Silwood Park campus. *Desmococcus* and *Trentepohlia* were collected from the trunks of oak and beech trees at the same site as the in-situ experiment (Chapter 6). The isolates were named DO, DB, TO and TB to represent

Desmococcus on oak, *Desmococcus* on beech, *Trentepohlia* on oak and *Trentepohlia* on beech respectively.

These isolates were cultured on Bold Basal Medium (BBM) with 3 fold nitrogen and vitamins (Tompkins *et al.*, 1995). Stock solutions were obtained from the UK Culture Collection of Algae and Protozoa (CCAP). The final medium contained the following salts in the amounts shown made up to a final volume of 1 litre:

- (1) 25.0 g NaNO_3 30 ml
- (2) 2.5 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 10 ml
- (3) 7.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 10 ml
- (4) 7.5 g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ 10 ml
- (5) 17.5 g KH_2PO_4 10 ml
- (6) 2.5 g NaCl 10 ml
- (7) trace element solution 6 ml
- (8) vitamin B1 1 ml
- (9) vitamin B12 1 ml

1 litre stock medium was made up by adding distilled water. The stock medium was autoclaved at 15 psi for 15 minutes. Trace element solution (7) was prepared by adding 1000 ml of distilled water to 0.75 g Na_2EDTA and then adding minerals in the following sequence and amounts:

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 97 mg, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 41 mg, ZnCl_2 5 mg, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 2 mg, and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 4 mg.

Vitamin B1 (8) was obtained by adding 0.12 g thiamine hydrochloride to 100 ml distilled water. Vitamin B12 (9) was prepared by adding 0.1 g cyanocobalamin to 100 ml distilled water. 1 ml of this solution was added to 99 ml distilled water and filter sterilised. 20 ml of freshly made culture medium was dispensed into 40 ml polycarbonate culture flasks with membrane layer tops, using an automatic dispenser.

The caps of the flasks were loosened to allow an exchange of air through the membrane layer of the cap. After inoculation, contents were mixed by shaking the flasks. During incubation cultures were shaken regularly to prevent cells from clumping.

7.2.2 Controlled temperature room and algal treatments

Culture flasks were maintained in a controlled temperature room at 18°C with a photon flux density of 25-30 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and a day length of 14 hr obtained using white fluorescent tubes supplemented by tungsten bulbs.

A total of 36 culture flasks was inoculated with each algal species at an initial cell concentration of 67,490 cells/ml for DO, 43,120 cells/ml for DB, 45,3800 cells/ml for TO and 27,4070 cells/ml for TB. Flasks were subjected to one of the following six treatments in triplicate: 0.2 mM or 2mM sodium nitrate (to represent an oxidised form of N), 0.2mM or 2mM ammonium chloride (to represent a reduced form of N), 2mM bisulphite (to simulate sulphur pollution) and deionized water (as a control). Culture flasks were treated weekly (top up treatments) by adding a further 1ml of the stock solutions described below once a week for 4 weeks (**Figure 7.1**). Algal in

culture flasks was checked regularly under light microscope to ensure that no contamination has occurred.

To avoid confusion, the chemical additives were labelled using synchronyms as follows:

LoSo = 0.2 mM sodium nitrate, HiSo = 2 mM sodium nitrate, LoAm = 0.2 mM ammonium chloride, HiAm = 2 mM ammonium chloride, Bis = 1.2 mM sodium bisulphite, Con = Control, using deionized water only.



Figure 7.1: Arrangement of culture flasks containing algae randomised within the growth chamber.

7.2.3 Determination of algal cells

At the end of each week, 1 ml of algal culture was pipetted from the culture flask into a 1.5 ml centrifuge tube. The samples were then mixed with 0.5 ml phosphate buffer solution, shaken and ready for count. An automatic handheld counter (Scepter, Millipore UK) was used to count the algal cells. The readings were made by dipping the Scepter tip into the centrifuge tube for a few seconds until an audible indication that a valid reading had been taken. Data were then displayed on the LCD monitor and transferred to the computer using Scepter Application software. Readings were

taken weekly apart from a two week interval before the final counts. The mean of three replicates was calculated: data were recorded as cells per ml.

7.3 Results

7.3.1 General pattern of changes in number of algal cells

In this controlled short-term experiment, the number of algal cells of *Desmococcus* and *Trentepohlia* varied according to treatment and also fluctuated for the duration of the study (**Figure 7.2**). *Desmococcus* populations, regardless of source, remained stable for the first two weeks of incubation and then, with one exception showed a period of growth over the final two weeks.

Trentepohlia, on the other hand showed a fluctuating pattern with a significant decline in cell counts between days 7 and 14, followed by a recovery which was more marked in the case of the population from beech trees. Overall, most changes were minor and statistical analysis showed that the main effect of treatment was not significant at $P=0.001$. However, for both algae the period of exposure was highly significant (2-way ANOVA, $F_{5,3} = 142.76$, $p < 0.0001$).

The decline in *Trentepohlia* showed that this species reacted negatively towards the liquid pollutants. The Scepter automatic cell counter measures live cells in the flasks so the decline in counts over the first 14 days must represent mortality suggesting that this alga reacts adversely to the transfer to liquid medium.

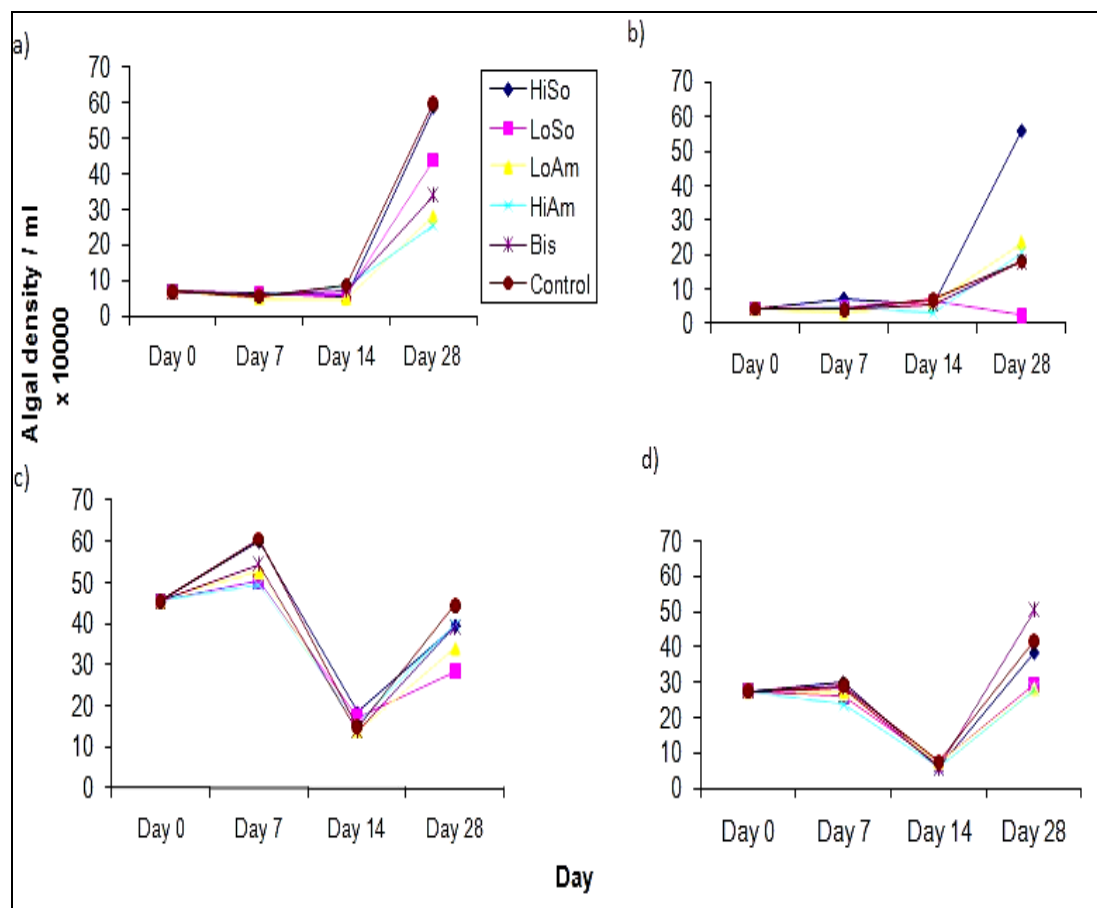


Figure 7.2: Changes in number of algal cells in culture flasks in relation to time over a period of 28 days. Points in line graph representing the mean number of cells in the flasks for:

- a) *Desmococcus* isolated from oak
- b) *Desmococcus* isolated from beech
- c) *Trentepohlia* isolated from oak
- d) *Trentepohlia* isolated from beech

One interesting finding was that after 14 days of given treatments, algal cell density increased substantially in all samples of both *Desmococcus* and *Trentepohlia*, regardless of the origin of the isolation (either from oak or beech). A reasonable interpretation could be that after a period of adaptation to the new environment the algae show a recovery phase where the number of cells begin to increase rapidly after slow growth for the early period after inoculation.

i) *Desmococcus* from oak (DO)

No obvious changes in terms of number of algal cells was recorded for DO until after 14 days, whereafter cell number proliferated rapidly showing a six-fold increase in both controls and the high nitrate treatments (**Figure 7.2a**). The same pattern was true to a lesser extent for all treatments.

ii) *Desmococcus* from beech (DB)

Number of algal cells of DB exhibited the same pattern as DO where treatments have no prominent effect until after 14 days (**Figure 7.2b**). By day 28 most cultures had made considerable growth with the exception of the 0.2 mM sodium nitrate (labelled as LoSo on graph) treatment where there was growth inhibition to the extent of 45 %.

iii) *Trentepohlia* from oak (TO) and from beech (TB)

Trentepohlia populations seemed to be more easily affected by the simulated atmospheric pollutants than *Desmococcus*. Regardless of source tree, populations showed a fluctuating pattern typically with a slight initial increase followed by a steep decline and a final recovery phase which was most marked in the case of TB (**Figure 7.2c,d**). In all cases cultures with additions of simulated pollutants behaved in a similar fashion to the controls in respect of changes with time.

7.3.2 Effects of simulated atmospheric pollutants on number of algal cells

Algal cells of *Desmococcus* and *Trentepohlia* responded to the various dissolved pollutants by increasing or declining in number in the culture flasks, but for the most part changes were minor and statistical analysis showed that the main effect of treatment was not significant at $P=0.001$. However, for both algae the period of

exposure was highly significant (2-way ANOVA, $F_{5,3} = 142.76$, $p < 0.0001$) confirming that the population fluctuations seen (**Fig 7.2**) were real. Based on these results, it is impossible to pinpoint main effects of pollutants, there being no clear cut trend. Clearly at these concentrations none of the chemical additives are toxic. There is some suggestion of nitrate N being stimulatory and ammonium N being inhibitory but it is not possible to eliminate this being an effect of the different counter ions used.

7.4 Discussion

7.4.1 Toxicity of short-term exposure to dissolved atmospheric pollutants

Short-term application of N and S sources as simulated atmospheric pollutants had no significant effect on populations of *Desmococcus* and *Trentepohlia* growing in liquid media. Populations fluctuated over time, and this effect was shown to be statistically significant. Even though there are some nutrients already present in the growth medium such as sodium nitrate and sulphur, these nutrients are very small in concentration thus did not affect the overall concentration when added with liquid pollutants.

We conclude that short-term exposures of atmospheric pollutants at these concentrations were not toxic. Since epiphytic algae are known to be very robust and tolerant, this factor might explain the apparent lack of ill-effects in this experiment. To the best of our knowledge, there have been no physiological studies on either *Desmococcus* or *Trentepohlia* so far. However, other green algae such as *Chlorella vulgaris* and *Scenedesmus quadricauda* are known to have the ability to eliminate toxins (Mohamed, 2008). Furthermore, green algae have been reported to have

variable responses to a variety of toxicants (Laundon, 1985). This supports the initial thought that different algal species respond differently towards external disturbances.

Statistical analyses has proven that time of exposure was highly significant in influencing the algal cell numbers. This is in agreement with our long-term field experiment (Chapter 6) where the dissolved atmospheric pollutants were applied to the algae for over 18 months, and again time had a significant effect towards the outcome. If the current experiment had been continued for longer treatment effects might have become apparent at a later stage, but equally conducting the experiment with a wider range of concentrations could help differentiate between nutritional and toxicity effects of the additives, especially given the known ability of algae especially green algae to eliminate toxins such as ammonia and excess nitrogen from their cells (Martinez *et al.*, 2000). Following the result, it is quite difficult to determine which of the treatments caused the most or least damage to the algal cells. Since there is no significant effect of treatments on the cultures, we can only describe the response of the algae towards treatments in terms of their general trends. As an example, a low concentration of sodium nitrate (0.2 mM) was found to be most damaging to *Desmococcus* isolated from beech. On day 28 of the experiment, *Desmococcus* numbers had declined by 45% compared with both controls and other treatments (**Figure 7.2b**). On the other hand, a higher concentration of sodium nitrate (2 mM) was beneficial to *Desmococcus* numbers, presumably for nutritional reasons, but it is unclear why there is a reversal of the effect with concentration (**Figure 7.2**).

This data also provide information on the effect of sulphur on epiphytic terrestrial algae. In the field experiment (Chapter 6), the number of algal cells was found to be

significantly affected by sulphur (in the form of dissolved bisulphite). The results in this chapter however reveal no significant effect of bisulphite on the growth of either alga. It is unclear at present whether this difference is due to the shorter period of exposure or differences in concentration of the potential toxicant.

When comparing the effect of reduced (ammonium) and oxidized (nitrate) N on the algae, it is clear that the oxidized form of N had a greater growth-promoting effect compared to the reduced form. However, since there seems to be a recovery phase at the end of the experiment it is possible that this trend may change in the long run. Recycling of nitrogen in old cultures is an established phenomenon in all microorganisms as cells die and proteins are broken down. In such a closed environment the form in which nitrogen is found during autolysis will bear little resemblance to the form in which it was originally supplied

7.4.2 Which algal species is most affected by atmospheric pollutants?

Desmococcus appears to be more tolerant than *Trentepohlia* because it shows no notable response in terms of number of algal cells until after day 14 of the experiment. On the contrary, *Trentepohlia* responded quite quickly to treatments, with some effects appearing within days of application (**Figure 7.2**). This trend is in agreement with our field experiment where *Trentepohlia* was found to be significantly affected by treatments while *Desmococcus* was more resilient to change (Chapter 6).

Even though both species under study here are green algae, *Desmococcus* is more robust and may have specific mechanisms to remove or utilize atmospheric pollutants for its own benefit. As mentioned in an earlier chapter, *Desmococcus* has a reputation

as pollutant-tolerant because at the time when no other epiphytes could survive due to high concentrations of SO₂, *Desmococcus* was the only epiphyte that could thrive in this environment (Hawksworth & Rose, 1970; Bates *et al.*, 2001). There has been no conclusive work on the mechanisms of *Desmococcus* that makes the cells pollutant-tolerant. However, we hypothesize that the protective outer layer of the cells in *Desmococcus* helps to prevent the cytoplasm from the toxic effect of pollutants. This cell wall stores reserve water in a mucilaginous sheath, for use when the environment becomes extreme (Shnyukova *et al.*, 2005).

Trentepohlia, on the other hand is more pollutant-sensitive. Any changes in water chemistry will be absorbed by their surface cells and will directly influence the growth of *Trentepohlia* cells. This algal species was reported to decrease in abundance with enhanced nitrate concentrations but increase when treated with ammonium (Abe *et al.*, 2001). Carotenoid pigments called haematochromes in *Trentepohlia*, that give them the reddish-orange colour and functions to protect the cell against external influences such as high light and UV rays, seems not to be enough to shield the cell from all stresses. This might be due to the fact that these pigments work at an optimum level when there is long day/short night with high light intensity. Arguably these conditions are not met in either the controlled temperature room used here, nor on the north side of trees in our study.

One further tentative conclusion from this in-vitro experiment also points to the relative resilience of *Desmococcus* over *Trentepohlia*. Over the 28 day period of culture *Desmococcus* did in almost all treatments increase its population size well above starting level. On the other hand, *Trentepohlia* populations decreased rapidly

during the first 14 days of culture and even after the recovery phase, were in most cases still lower (*Trentepohlia* on oak) or only slightly higher than the initial number of cells (*Trentepohlia* on beech).

7.5 Conclusions

Based on data provided in this chapter, we conclude that short-term exposure of dissolved atmospheric pollutants has neither toxic nor beneficial effects on populations of either of *Desmococcus* or *Trentepohlia* isolated from oak and beech trees. Compared to our long-term field experiment, we draw a conclusion that persistent exposure to atmospheric pollutants over longer periods and probably at higher concentrations is needed to influence the growth of these algae in terms of number of cells. A short-period exposure to these pollutants as used here does not seem to provide useful data for extrapolation to the field. We further conclude that although care was taken to source cells from the bark of two contrasting trees (as used in the field trial), there is no evidence that the populations from oak and beech differed materially.

Even though there are no significant effects of the treatments on the algae, in general, oxidized N was more beneficial to algal growth than the reduced form. In contrast with the long-term study, bisulphite did not show any toxicity to the algae under the conditions of use. The same bisulphite concentration, when applied for a long period directly to tree bark (Chapter 6) is damaging to these algae.

Chapter 8

General Discussion

8.1 Summary of the current work

This work was undertaken mainly to provide some pioneer information of the effect of atmospheric pollutants on epiphytic terrestrial algae. Compared with other lower plants such as lichens and bryophytes, terrestrial algae in general and epiphytic terrestrial algae in particular have been given little attention by biologists. This situation seems to be due to the lack of information about the ecology of these organisms and the complexity of their taxonomy. Data and references available to the present date mainly focus on the taxonomy of terrestrial algae that inhabit walls and stones but almost none (except for *Trentepohlia* and *Printzina* in tropical forests) relate to epiphytic terrestrial algae that are commonly seen in temperate surroundings.

This work started out of curiosity about the effect of atmospheric pollutants on algae in the urban area of London compared with the relatively pristine area of rural South East England. As the work progressed, we also investigated the localized effect of some specific pollutants such as ammonia emanating from an intensive husbandry unit. To understand further the effect of enhanced nitrogen on algal growth we utilized an existing field experiment at Whim bog in Scotland to examine the effects of pollutants, artificially introduced at known levels as both wet and dry deposition, on algal abundance. Based on this field experiment, the effect of reduced and

oxidized forms of nitrogen was identified and separated. In parallel a long-term field experiment conducted in Silwood Park provided an understanding of the effect of atmospheric pollutants in precipitation on epiphytic terrestrial algae. The same pollutants were added at known concentrations to cultured algae in liquid medium to assess what effect they would have on growth under defined conditions.

To the best of our knowledge, no previous studies have monitored in detail the effect of atmospheric pollutants on epiphytic terrestrial algae. Some incidental information has emerged during studies on lichens, usually with no more than a brief mention of ‘green algae most probably *Desmococcus-Pleurococcus*’ (currently accepted taxonomically as *Desmococcus olivaceus*) in their study. However, no further work has been carried out to give us more understanding on the impact that atmospheric pollutants have on epiphytic terrestrial algae. It was our intention to provide as accurate species identification as possible. However, due to time and finance limitations, the original aim to support our morphologically-based species identification through molecular work did not materialize. Thus, although a high level of confidence can be ascribed to genus-level identification, naming to species level in this work has to be regarded as presumptive or tentative and awaiting further confirmation.

In this final chapter, the results produced from the preceding chapters are summarized briefly so as to recapture and give an idea of the relevance of one study to another. The overall conclusions drawn are based on the entirety of the experiments that have been carried out for the whole duration of this research work. At the end of this chapter, we describe supplemental studies that are needed to explore further and

enhance our understanding of the effect played by atmospheric pollutants on epiphytic terrestrial algae.

8.1.1 The relationship between individual studies carried out in this investigation

To understand the effect of atmospheric pollutants on epiphytic terrestrial algae, we have looked in terms of localized effects of specific pollutants (**Chapter 3**) and the wider effect of various pollutants (**Chapter 4**). Our results are in agreement with many studies conducted regarding the diffusion of ammonia from poultry farms where the gas is known to diffuse quickly and concentrations fall rapidly from a point source. The area within close proximity of the ammonia source was much more heavily colonized than the same substratum (tree trunks) only a short distance away. Thus we concluded that increased ammonia was a contributing factor in the proliferation of algal growth on a scale that, if it were in an aqueous habitat, would be described as a bloom. The existence of only one dominant species of epiphytic alga in this zone suggests that the species concerned, *Desmococcus olivaceus*, is a true nitrophyte that favours such a high N environment and can outcompete other species that normally inhabit the same substratum.

After looking at the localized effect of N on epiphytic terrestrial algae, we then extended this to consider the pattern of algae along a transect that encompasses both urban and rural environments. In the urban area oxides of nitrogen are the dominant pollutants, mostly being emitted by motor vehicles and other components of the transportation system. In a rural area, especially where there is significant agricultural

activity, the major contributing pollutant is ammonia. Even though the urban and rural areas were dominated by different pollutants, one thing in common is that increased N (either ammonia or oxides of nitrogen) triggered an increase in the number of algal cells. This study thus supports those of Thomsen (1992), Brakenhielm & Qinghong (1995) and Poikolainen *et al.* (1998) which concluded that sub-aerial algae are most abundant where nitrogen deposition is greatest.

Since we have established that enhanced N increases the number of algal cells on a tree bark substrate, we then investigated which forms of N exert the greatest influence on algal numbers (**Chapter 5**). To achieve this, we utilized the on-going long-term field experiment where rain simulation treatments are applied to a terrestrial environment dominated by bog habitat plants with both epiphytic and ground surface algae present. We found that number of algal cells was increased when enhanced N was in the form of precipitation, rather than dry deposition, particularly of reduced N (ammonium) and coupled with the addition of phosphorus and potassium (PK). On the other hand, fewer algae were seen following exposure to oxidised forms of nitrogen, an effect exaggerated when coupled with PK.

Since algae are known to be robust and quite tolerant of atmospheric pollutants, we investigated the time period over which exposure to pollutants might exert an effect on algal growth. In the long-term study (**Chapter 6**), we found out that *Desmococcus* was tolerant of both oxidized and reduced forms of N. Using the same methodology but conducted under controlled environments in the laboratory, we observed that *Desmococcus* was not significantly affected by short-term exposure to dissolved atmospheric pollutants (**Chapter 7**). Sulphur in the form of bisulphite was also

detrimental to algal density in the field but had little effect *in vitro* over a short period of exposure. Atmospheric pollutants are believed to alter bark pH and thus provide a better substratum for some algal species and at the same time suppress others. This probably explains why *Desmococcus* was not affected by pollutants both in the short and long term experiment but another alga, *Trentepohlia* was greatly affected. Even though the short-time exposure of the *in vitro* experiment did not affect the growth of *Trentepohlia*, in the long-term experiment this algal species was significantly suppressed by some treatments.

Overall, this study found that epiphytic terrestrial algae are reliable as an indicator for air pollution. The response of algae by increasing (for some species such as *Desmococcus*) or decreasing (in others such as *Trentepohlia*) in population density showed that algae are responsive to atmospheric pollutants under prolonged exposure. Taking *Desmococcus* into consideration as an air pollution indicator, this species is proven to increase under high N deposition, regardless of types of pollutants (reduced or oxidized N) but particularly when exposed to reduced N (NH_3 and NH_4). *Desmococcus* which is able to grow well in a high N environment eventually suppresses and outcompetes other algal species which can not tolerate high concentration of N in the atmosphere. Thus the dominance of *Desmococcus* (with a very few other species or none at all) in a given area could be the first visual indicator for the level of atmospheric pollution.

Further evidence of high nitrogen aiding algal growth can be seen in Chapter 4 where areas of high N such as in the city centre (main N source from motor transport – NO , NO_2 , NO_x) and rural areas (main N source from large poultry farms – NH_3) exhibited

a higher algal density than suburban or rural periphery areas. However, the response of epiphytic terrestrial algae to atmospheric pollutants is also influenced by time of exposure. Based on the current study, results from the short and long-term exposures contradict each other. Long-term exposure to atmospheric pollutants showed that reduced N in the forms of NH_4Cl was more favourable to algal growth as compared to oxidized N. In contrast short-term exposure showed that oxidized N was more favourable to algal growth. In a study to investigate the effect of local N on epiphytic terrestrial algae (Chapter 3), long-term exposure of algae to localized NH_3 was most probably the main factor leading to the dominance of *Desmococcus*. In this case, the role of reduced N has proven to favour algal growth. While the short-term study (Chapter 7) showed that oxidized N was more favourable for algal growth, it is possible that the trend would be reversed if the study had been continued. Either way, epiphytic terrestrial algae were responsive to both short and long-term exposure to atmospheric pollutants. Thus using epiphytic terrestrial algae, in particular *Desmococcus*, as indicators of air pollution seems to be a reliable choice as an indicator of air pollution.

8.1.2 Recommendations for future work

In general, some aspects of this study would benefit from further investigation. When exposed to atmospheric pollutants for only weeks, neither *Desmococcus* nor *Trentepohlia* were significantly affected by treatments (**Chapter 7**). However, when exposed to a longer period of the order of months, *Trentepohlia* was significantly affected by atmospheric pollutants while *Desmococcus* did not show any significant responses (**Chapter 6**). Over an even longer timescale of years, *Desmococcus* was

also found to be significantly affected by atmospheric pollutants (**Chapter 5**). Ideally the laboratory and field approaches should be integrated so that the benefits of the controlled application trial can be extended in both time and space. The liquid phase trials should be run for longer and incorporate a wider range of concentrations of the key chemicals replenished and measured on a regular basis. Experiments could be mirrored by parallel trials of the same algae growing on solid substrates designed as bark substitutes. These could be chosen to give a range of hydrophobicities and intrinsic pH values. Given suitable facilities factors such as humidity and light intensity could be controlled to optimise growth of the algae. All such trials should be conducted with pure cultures of confirmed identity to determine the physiological parameters of individual species; but could be followed by mixed culture trials to study competition between species as influenced by the chemical environment.

Also, since *Desmococcus* was shown not to be easily affected by atmospheric pollutants, further studies are recommended to investigate the physiology and mechanism of resilience of this algal species, to understand what makes it tolerant to increased N. This might provide useful information and give better understanding of this pollutant-tolerant species, and could also be extended to include other toxicants or to run the experiments using pure mono-algal culture.

Finally it is desirable to confirm the identity of the algae used in the current study by molecular methods, and to extend the identification to species level. The inability to identify routinely to species level could have biased some of the trends seen in these studies and obscured others. The morphology-based identification used here is believed to be accurate and to have been applied consistently across time. Accurate

species identification is crucial to avoid misunderstanding in the future and for enabling comparison of data between laboratories. We trust that with the passage of time, more interest will be shown on this subject matter and that the current work will provide a base-line for future study.

REFERENCES

- ABE, K., IMAMAKI, A. & HIRANO, M. 2002. Removal of nitrate, nitrite, ammonium and phosphate ions from water by the aerial microalga *Trentepohlia aurea*. *Journal of Applied Phycology*, 14, 129-134.
- ABE, K., MATSUMURA, I., IMAMAKI, A. & HIRANO, M. 2003. Removal of inorganic nitrogen sources from water by the algal biofilm of the aerial microalga *Trentepohlia aurea*. *World Journal of Microbiology & Biotechnology*, 19, 325-328.
- ABE, K., TAKIZAWA, H., KIMURA, S. & HIRANO, M. 2004. Characteristics of chlorophyll formation of the aerial microalga *Coelastrella striolata* var. *multistriata* and its application for environmental biomonitoring. *Journal of Bioscience and Bioengineering*, 98, 34-39.
- ABE, K., TAKAHASHI, E. & HIRANO, M. 2008. Development of laboratory-scale photobioreactor for water purification by use of a biofilter composed of the aerial microalga *Trentepohlia aurea* (Chlorophyta). *Journal of Applied Phycology*, 20, 283-288.
- ABELIOVICH, A. & AZOV, Y. 1976. Toxicity Of Ammonia To Algae In Sewage Oxidation Ponds. *Applied and Environmental Microbiology*, 31, 801-806.
- ADAMSEN, A. P. S. & KING, G. M. 1993. Methane Consumption In Temperate And Sub-Arctic Forest Soils - Rates, Vertical Zonation, And Responses To Water And Nitrogen. *Applied and Environmental Microbiology*, 59, 485-490.
- AERTS, R. 1990. Aboveground biomass and nutrient dynamics of *Calluna vulgaris* and *Molinia caerulea* in a dry heathland. *Oikos*, 56, 31-38.
- AHLGREN, G. & HYENSTRAND, P. 2003. Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms, *Scenedesmus quadricauda* (Chlorophyceae) and *Synechococcus* sp (Cyanophyceae). *Journal of Phycology*, 39, 906-917.
- ALDOUS, A. R. 2002. Nitrogen translocation in *Sphagnum* mosses: effects of atmospheric nitrogen deposition. *New Phytologist*, 156, 241-253.
- ALEXOPOULOS, C.J. & BOLD, H.C. 1967. *Algae and Fungi*. MacMillan Publishing. New York. 135 pp.
- ALLEN, A. G., HARRISON, R. M. & WAKE, M. T. 1988. A Meso-Scale Study Of The Behavior Of Atmospheric Ammonia And Ammonium. *Atmospheric Environment*, 22, 1347-1353.
- ALLUVIONE, F., BERTORA, C., ZAVATTARO, L. & GRIGNANI, C. 2010. Nitrous Oxide and Carbon Dioxide Emissions Following Green Manure and Compost Fertilization in Corn. *Soil Science Society of America Journal*, 74, 384-395.
- AMSLER, D.A. 2009. *Algal Chemical Ecology*. Springer. Berlin. 313 pp.
- AN, J. Y., SIM, S. J., LEE, J. S. & KIM, B. W. 2003. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *Journal of Applied Phycology*, 15, 185-191.
- ANDERSEN, R.A. 2005. *Algal Culturing Techniques*. Elsevier Academic Press. Burlington, MA. 578pp.
- ANDERSEN, R., FRANCEZ, A. J. & ROCHEFORT, L. 2006. The physicochemical and microbiological status of a restored bog in Quebec: Identification of relevant criteria to monitor success. *Soil Biology & Biochemistry*, 38, 1375-1387.

- ANDERSON, R.A. 1992. Diversity of Eukaryotic Algae. *Biodiversity and Conservation* 1:267-292.
- ANEJA, V. P., NELSON, D. R., ROELLE, P. A., WALKER, J. T. & BATTYE, W. 2003. Agricultural ammonia emissions and ammonium concentrations associated with aerosols and precipitation in the southeast United States. *Journal of Geophysical Research-Atmospheres*, 108.
- ANGUS, A. J., HODGE, I. D. & SUTTON, M. A. 2006. Ammonia abatement strategies in livestock production: A case study of a poultry installation. *Agricultural Systems*, 89, 204-222.
- APSIMON, H. M., KRUSE, M. & BELL, J. N. B. 1987. Ammonia Emissions And Their Role In Acid Deposition. *Atmospheric Environment*, 21, 1939-1946.
- APTROOT, A. & VAN HERK, C. M. 2007. Further evidence of the effects of global warming on lichens, particularly those with *Trentepohlia* phycobionts. *Environmental Pollution*, 146, 293-298.
- ASHMORE, M. R., DALPRA, C. & TICKLE, A. K. 1988. Effects of ozone and calcium nutrition on native plant species. *Air pollution and ecosystems. Proceedings of an international symposium held in Grenoble, France, 18-22 May 1987.*, 647-652.
- ASLAN, S. & KAPDAN, I. K. 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, 28, 64-70.
- ASMAN, W. A. H., SUTTON, M. A. & SCHJORRING, J. K. 1998. Ammonia: emission, atmospheric transport and deposition. *New Phytologist*, 139, 27-48.
- AYRES, E., VAN DER WAL, R., SOMMERKORN, M. & BARDGETT, R. D. 2006. Direct uptake of soil nitrogen by mosses. *Biology Letters*, 2, 286-288.
- BAILEY, N.T.J. 1995. *Statistical Methods in Biology*. Cambridge University Press. Cambridge. 255pp.
- BARINOVA, S. S., NEVO, E. & BRAGINA, T. M. 2011. Ecological assessment of wetland ecosystems of northern Kazakhstan on the basis of hydrochemistry and algal biodiversity. *Acta Botanica Croatica*, 70, 215-244.
- BARKMAN, J. J. 1988. Some Reflections On Plant Architecture And Its Ecological Implications A Personal View Demonstrated On Two Species Of *Quercus*. *Werger, M. J. a., Et Al. (Ed.). Plant Form and Vegetation Structure: Adaptation, Plasticity and Relation to Herbivory. Academic Publishing the Hague, Netherlands*. 1-8.
- BARKMAN, J. J. 1990. Ecological Differences Between *Calluna*-Dominated And *Empetrum*-Dominated Dry Heath Communities In Drenthe, The Netherlands. *Acta Botanica Neerlandica*, 39, 75-92.
- BATES, J. W. & BROWN, D. H. 1981. Epiphyte Differentiation Between *Quercus petraea* And *Fraxinus excelsior* Trees In A Maritime Area Of South West England. *Vegetatio*, 48, 61-70.
- BATES, J. W., BELL, J. N. B. & FARMER, A. M. 1990. Epiphyte Recolonization Of Oaks Along A Gradient Of Air-Pollution In South-East England, 1979-1990. *Environmental Pollution*, 68, 81-99.
- BATES, J. W. 1992. Influence Of Chemical And Physical Factors On *Quercus* And *Fraxinus* Epiphytes At Loch Sunart, Western Scotland - A Multivariate-Analysis. *Journal of Ecology*, 80, 163-179.
- BATES, J. W., MCNEE, P. J. & MCLEOD, A. R. 1996. Effects of sulphur dioxide and ozone on lichen colonization of conifers in the Liphook forest fumigation project. *New Phytologist*, 132, 653-660.

- BATES, J. W., BELL, J. N. B. & MASSARA, A. C. 2001. Loss of *Lecanora conizaeoides* and other fluctuations of epiphytes on oak in SE England over 21 years with declining SO₂ concentrations. *Atmospheric Environment*, 35, 2557-2568.
- BATTY, K., BATES, J. W. & BELL, J. N. B. 2003. A transplant experiment on the factors preventing lichen colonization of oak bark in southeast England under declining SO₂ pollution. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 81, 439-451.
- BAXTER, R., EMES, M. J. & LEE, J. A. 1989. Effects Of The Bisulphite Ion On Growth And Photosynthesis In *Sphagnum-Cuspidatum* Hoffm. *New Phytologist*, 111, 457-462.
- BELL, S. & ASHENDEN, T. W. 1997. Spatial and temporal variation in nitrogen dioxide pollution adjacent to rural roads. *Water Air and Soil Pollution*, 95, 87-98.
- BELL, J.N.B. & TRESHOW, M. 2002. Air Pollution and Plant Life. John Wiley. Chicester. 465 pp.
- BELTMAN, B., VANDENBROEK, T. & BLOEMEN, S. 1995. Restoration of acidified rich-fen ecosystems in the Vechtplassen area: Successes and failures. *Restoration of Temperate Wetlands*, 273-286.
- BENKO, Z., JUHASZ, A., POCS, T. & TUBA, Z. 2002. Desiccation survival times in different desiccation-tolerant plants. *Acta Biologica Szegediensis*, 46, 231-233.
- BERMAN, T. & BRONK, D. A. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquatic Microbial Ecology*, 31, 279-305.
- BERVEILLER, D. & DAMESIN, C. 2008. Carbon assimilation by tree stems: potential involvement of phosphoenolpyruvate carboxylase. *Trees-Structure and Function*, 22, 149-157.
- BILGRAMI, K.S & SAHA, L.C. 2004. A Textbook of Algae. CBS Publishers & Distributors. New Delhi. 260pp.
- BIODIVERSITY. 1995. Biodiversity : The UK Steering Group Report. Volume 1: Meeting the Rio Challenge. HMSO. London. 103pp.
- BLOUIN, A. C. 1989. Patterns Of Plankton Species, pH And Associated Water Chemistry In Nova-Scotia Lakes. *Water Air and Soil Pollution*, 46, 343-358.
- BOLD, H.C. & WYNNE, M.J. 1978. Introduction to the Algae: Structure and Reproduction. Prentice Hall. New Jersey. 706 pp.
- BOLIER, G. 1985. Algal Growth-Potential Test - An Indicative Method To Estimate The Influence Of Water-Quality Changes On The Algal Biomass. *Water Science and Technology*, 17, 1403-1405.
- BOUBEL, R. W., FOX, D. L., TURNER, D. B. & STERN, A. C. 1994. Fundamentals of air pollution, Third edition. *Fundamentals of air pollution, Third edition*.
- BOUWMAN, A. F., LEE, D. S., ASMAN, W. A. H., DENTENER, F. J., VANDERHOEK, K. W. & OLIVIER, J. G. J. 1997. A global high-resolution emission inventory for ammonia. *Global Biogeochemical Cycles*, 11, 561-587.
- BOWER, J. S., LAMPERT, J. E., STEVENSON, K. J., ATKINS, D. H. F. & LAW, D. V. 1991. A Diffusion Tube Survey Of Nitrogen Dioxide Levels In Urban Areas Of The Uk. *Atmospheric Environment Part B Urban Atmosphere*, 25, 255-266.
- BRAKENHIELM, S. & LIU, Q. H. 1995. Spatial And Temporal Variability Of Algal And Lichen Epiphytes On Trees In Relation To Pollutant Deposition In Sweden. *Water Air and Soil Pollution*, 79, 61-74.

- BRASSEUR, G.P., SCHULTZ, M.G & DIEHL T. 2003. Air Pollution and Climate-Forcing Impacts of a Global Hydrogen Economy. *Science*. Volume 302 Issue 5645 : 624-627.
- BRIDGES, T. S., LEVIN, L. A., CABRERA, D. & PLAIA, G. 1994. Effects Of Sediment Amended With Sewage, Algae, Or Hydrocarbons On Growth And Reproduction In 2 Opportunistic Polychaetes. *Journal of Experimental Marine Biology and Ecology*, 177, 99-119.
- BROADY, P. A. 1981. Ecological And Taxonomic Observations On Subaerial Epilithic Algae From Princess-Elizabeth Land And MacRobertson Land, Antarctica. *British Phycological Journal*, 16, 257-266.
- BROADY, P. A. 1989. Broadscale Patterns In The Distribution Of Aquatic And Terrestrial Vegetation At 3 Ice-Free Regions On Ross Island, Antarctica. *Hydrobiologia*, 172, 77-95.
- BROADY, P. A. & INGERFELD, M. 1993. 3 New Species And A New Record Of Chaetophoracean (Chlorophyta) Algae From Terrestrial Habitats In Antarctica. *European Journal of Phycology*, 28, 25-31.
- BROADY, P. A. 1996. Diversity, distribution and dispersal of Antarctic terrestrial algae. *Biodiversity and Conservation*, 5, 1307-1335.
- BRODO, I. M. 1973. The Lichen Genus *Coccotrema* In North America. *Bryologist*, 76, 260-270.
- BROOK, A. J. 1981. The Accumulation Of Barium-Sulfate By Desmids. *Phycologia*, 20, 101-101.
- BRUNIALTI, G., GIORDANI, P., ISOCRONO, D. & LOPPI, S. 2002. Evaluation of data quality in lichen biomonitoring studies: The Italian experience. *Environmental Monitoring and Assessment*, 75, 271-280.
- BUIJSMAN, E., ABEN, J. M. M., VAN ELZAKKER, B. G. & MENNEN, M. G. 1998. An automatic atmospheric ammonia network in the Netherlands set-up and results. *Atmospheric Environment*, 32, 317-324.
- CAIRNS, J. JR. 1974. The Sequential Comparison Index – a Simplified Method of Non-biologists to Estimate Relative Difference in Biological Diversity in Stream Pollution Studies. *J. Wat. Pollut. Control Fed.* 40: 1607-1613.
- CAMPBELL, G. W. 1988. Measurements Of Nitrogen-Dioxide Concentrations At Rural Sites In The United-Kingdom Using Diffusion Tubes. *Environmental Pollution*, 55, 251-270.
- CAMPBELL, G. W., STEDMAN, J. R. & STEVENSON, K. 1994. A Survey Of Nitrogen-Dioxide Concentrations In The United-Kingdom Using Diffusion Tubes, July-December 1991. *Atmospheric Environment*, 28, 477-486.
- CAPE, J. N., JONES, M. R., LEITH, I. D., SHEPPARD, L. J., VAN DIJK, N., SUTTON, M. A. & FOWLER, D. 2008. Estimate of annual NH₃ dry deposition to a fumigated ombrotrophic bog using concentration-dependent deposition velocities. *Atmospheric Environment*, 42, 6637-6646.
- CARFRAE, J. A., SHEPPARD, L. J., RAVEN, J. A., LEITH, I. D. & CROSSLEY, A. 2007. Potassium and phosphorus additions modify the response of *Sphagnum capillifolium* growing on a Scottish ombrotrophic bog to enhanced nitrogen deposition. *Applied Geochemistry*, 22, 1111-1121.
- CARMICHAEL, W. W., SIRENKO, L. A., KLOCHENKO, P. D. & SHEVCHENKO, T. F. 2001. A comparative assessment of the toxicity of algae and cyanobacteria in water bodies of Ukraine. *Phycologia*, 40, 15.
- CERNA, K. 2010. Small-scale spatial variation of benthic algal assemblages in a peat bog. *Limnologia*, 40, 315-321.

- CERNA, K. & NEUSTUPA, J. 2010. The pH-related morphological variations of two acidophilic species of Desmiales (Viridiplantae) isolated from a lowland peat bog, Czech Republic. *Aquatic Ecology*, 44, 409-419.
- CHADWICK, D. R., SNEATH, R. W., PHILLIPS, V. R. & PAIN, B. F. 1999. A UK inventory of nitrous oxide emissions from farmed livestock. *Atmospheric Environment*, 33, 3345-3354.
- CHAPMAN, R. L. & HENK, M. C. 1983. Ultrastructure Of *Cephaleuros virescens* (Chroolepidaceae, Chlorophyta) . Absolute-Configuration Analysis Of The Cruciate Flagellar Apparatus And Multilayered Structures In The Pre-Release And Post-Release Gametes. *American Journal of Botany*, 70, 1340-1355.
- CHARMAN, D. 2002. Peatlands and environmental change. *Peatlands and environmental change*.
- COESEL, P. F. M. 1978. Environmental Changes In The Oisterwijk Netherlands Moorland Pool Area Evident From The Composition Of The Desmid Flora. *Hydrobiological Bulletin*, 12, 52-53.
- COESEL, P. F. M. 1982. Structural Characteristics And Adaptations Of Desmid Communities. *Journal of Ecology*, 70, 163-177.
- COESEL, P. F. M. 2007. Taxonomic notes on Dutch desmids IV: new species, new names, new combinations. *Systematics and Geography of Plants*, 77, 5-14.
- COLBECK, I. & MACKENZIE, A.R. 1994. Air Pollution by Photochemical Oxidants. Elsevier. London. 376 pp.
- COLEMAN, M. A. 2002. Small-scale spatial variability in intertidal and subtidal turfing algal assemblages and the temporal generality of these patterns. *Journal of Experimental Marine Biology and Ecology*, 267, 53-74.
- CRAWLEY, M.J. 1993. GLIM for Ecologists. Blackwell Scientific. Oxford.
- CRAWLEY, M.J. 2005. An Introduction Using R. John Wiley, Chichester. 327pp.
- CREESE, C., LEE, A. & SACK, L. 2011. Drivers Of Morphological Diversity And Distribution In The Hawaiian Fern Flora: Trait Associations With Size, Growth Form, And Environment. *American Journal of Botany*, 98, 956-966.
- CUBIT, J. D. 1984. Herbivory And The Seasonal Abundance Of Algae On A High Intertidal Rocky Shore. *Ecology*, 65, 1904-1917.
- DARLEY, W. 1982. Algal Biology: A Physiological Approach. Blackwell Scientific. Oxford.
- DAVIES, L., BATES, J. W., BELL, J. N. B., JAMES, P. W. & PURVIS, O. W. 2007. Diversity and sensitivity of epiphytes to oxides of nitrogen in London. *Environmental Pollution*, 146, 299-310.
- DAVISON, I. R. & PEARSON, G. A. 1996. Stress tolerance in intertidal seaweeds. *Journal of Phycology*, 32, 197-211.
- DAYANANDA, C., SARADA, R., BHATTACHARYA, S. & RAVISHANKAR, G. A. 2005. Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. *Process Biochemistry*, 40, 3125-3131.
- DEFRA. Air Quality Expert Group. Great Britain. Great Britain. Department for Environment, Food & Rural Affairs. 2001. Transboundary air pollution (NEGTA). DEFRA, London. 314pp.
- DEFRA. Air Quality Expert Group. Great Britain. Department for Environment, Food & Rural Affairs. 2004. Nitrogen dioxide in the United Kingdom. DEFRA, London. 337pp.
- DEFRA. Air Quality Expert Group. Great Britain. Department for Environment, Food & Rural Affairs. 2011. Air Pollution in the UK 2010. DEFRA, London. 78pp.
- DETHIER, M. N. 1982. Pattern And Process In Tidepool Algae -

- Factors Influencing Seasonality And Distribution. *Botanica Marina*, 25, 55-66.
- DoE. The United Kingdom Air Quality Strategy. 1997. Department of the Environment. Stationery Office. London. 189pp.
- DOERING, P. H., OVIATT, C. A., NOWICKI, B. L., KLOS, E. G. & REED, L. W. 1995. Phosphorus And Nitrogen Limitation Of Primary Production In A Simulated Estuarine Gradient. *Marine Ecology-Progress Series*, 124, 271-287.
- DORTCH, Q. 1990. The Interaction Between Ammonium And Nitrate Uptake In Phytoplankton. *Marine Ecology-Progress Series*, 61, 183-201.
- DOSHI, H., RAY, A. & KOTHARI, I. L. 2008. Bioremediation potential of Chlorella: Spectroscopic, kinetics, and sem studies. *International Journal of Phytoremediation*, 10, 264-277.
- DOWNING, J. A. & RIGLER, F. H. 1984. IBP International Biological Program Handbook No. 17. A Manual On Methods For The Assessment Of Secondary Productivity In Fresh Waters. Blackwell Scientific Publications: Palo Alto, Calif., USA.
- DUPRE, C., STEVENS, C. J., RANKE, T., BLEEKER, A., PEPPLER-LISBACH, C., GOWING, D. J. G., DISE, N. B., DORLAND, E., BOBBINK, R. & DIEKMANN, M. 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344-357.
- DYTHAM, C. 2003. Choosing And Using Statistics : A Biologist's Guide. Blackwell Pub. Oxford. 248pp.
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). 1994. Ammonia Emissions to Air in Western Europe. ECETOC. Brussels.
- EGERTON-WARBURTON, L.M & ALLEN, E.B. 2000. Shifts in *Arbuscular mycorrhizal* Communities Along an Anthropogenic Nitrogen Deposition Gradient. *Ecological Applications*. 10: 484-496.
- ERISMAN, J. W., VERMETTEN, A. W. M., ASMAN, W. A. H., WAIJERSIJPELAAN, A. & SLANINA, J. 1988. Vertical-Distribution Of Gases And Aerosols - The Behaviour Of Ammonia And Related Components In The Lower Atmosphere. *Atmospheric Environment*, 22, 1153-1160.
- ERISMAN, J. W., GRENNFELT, P. & SUTTON, M. 2003. The European Perspective On Nitrogen Emission And Deposition. *Environment International*, 29, 311-325.
- ETTL, H. & GARTNER, G. 1995. Syllabus der Boden-, Luft-und Flechtenalgen, Gustav Fischer, Stuttgart. 721pp.
- FANGMEIER, A., GRUTERS, U., VERMEHREN, B. & JAGER, H. J. 1996. Responses of some cereal cultivars to CO₂ enrichment and tropospheric ozone at different levels of nitrogen supply. *Journal of Applied Botany-Angewandte Botanik*, 70, 12-18.
- FARMER, A. M., BATES, J. W. & BELL, J. N. B. 1991. Seasonal-Variations In Acidic Pollutant Inputs And Their Effects On The Chemistry Of Stemflow, Bark And Epiphyte Tissues In 3 Oak (*Quercus Petraea* (Mattuschka) Liebl Woodlands In Nw Britain. *New Phytologist*, 118, 441-451.
- HEMOND, H.F. & FECHNER-LEVY, E.J. 2000. Chemical Fate and Transport in the Environment. Academic Press. London.
- HESTER, R.E. & HARRISON, R.M. 1996. Agricultural Chemicals and the Environment. Royal Society of Chemistry. Cambridge.

- HOBBS, P.V. 2000. Introduction To Atmospheric Chemistry : A Companion Text To 'Basic Physical Chemistry For The Atmospheric Sciences. Cambridge University Press. Cambridge. 262pp.
- FERGUSON, P. & LEE, J. A. 1979. Effects Of Bisulphite And Sulfate Upon Photosynthesis In *Sphagnum*. *New Phytologist*, 82, 703-712.
- FERRIS-KAAN, R. 1995. Managing forests for biodiversity. *Technical Paper - Forestry Commission*.
- FERRIS, R. & CARTER, C. 2000. Managing rides, roadsides and edge habitats in lowland forests. *Forestry Commission Bulletin*.
- FERRY, B.W., BADDELEY, M.S. & HAWKSWORTH, D.L. 1973. Air Pollutions and Lichens. Athlone Press. London.
- FLAIG, H. & MOHR, H. 1996. Nova Acta Leopoldina; Critical loads of the nitrogen cycle: Management strategies. 70, 168p.
- FOGG, G. E. 1969. Survival of algae under adverse conditions. *Symposia of the Society for Experimental Biology*, 23, 123-42.
- FORSBERG, C., RYDING, S. O., FORSBERG, A. & CLAESSION, A. 1978. Research On Recovery Of Polluted Lakes Part 1 Improved Water Quality In Lake Boren And Lake Ekoln After Nutrient Reduction. *International Association of Theoretical and Applied Limnology, Proceedings, Vol. 20. Part 1 and 2*. 825-832.
- FOSTER, D. R., WRIGHT, H. E., THELAUS, M. & KING, G. A. 1988. Bog Development And Landform Dynamics In Central Sweden And Southeastern Labrador, Canada. *Journal of Ecology*, 76, 1164-1185.
- FOWLER, D., PITCAIRN, C. E. R., SUTTON, M. A., FLECHARD, C., LOUBET, B., COYLE, M. & MUNRO, R. C. 1998. The mass budget of atmospheric ammonia in woodland within 1 km of livestock buildings. *Environmental Pollution*, 102, 343-348.
- FOWLER, D., SMITH, R. I., MULLER, J. B. A., HAYMAN, G. & VINCENT, K. J. 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, 137, 15-25.
- FRATI, L., BRUNIALTI, G. & LOPPI, S. 2008. Effects of reduced nitrogen compounds on epiphytic lichen communities in Mediterranean Italy. *Science of the Total Environment*, 407, 630-637.
- FRANCESCO, F., MANUELA, D.S & MICHELOZZI, P. 2010. The Italian Contribution to EU Research Programs on Health Effects of Air Pollution and Climate in a Changing Environment. *Epidemiologia & Prevenzione*. 34: 39-46.
- FRENEY, J. R., SIMPSON, J. R. & DENMEAD, O. T. 1983. Volatilization of ammonia. *Gaseous loss of nitrogen from plant-soil systems*, 1-32.
- FREYSTEIN, K., SALISCH, M. & REISSER, W. 2008. Algal biofilms on tree bark to monitor airborne pollutants. *Biologia*, 63, 866-872.
- FRITZ, O., BRUNET, J. & CALDIZ, M. 2009. Interacting effects of tree characteristics on the occurrence of rare epiphytes in a Swedish beech forest area. *Bryologist*, 112, 488-505.
- GALLOWAY, D.J. 1991. Tropical Lichens : Their Systematics, Conservation and Ecology. Clarendon Press. London. 302 pp.
- GALLOWAY, J. N., ABER, J. D., ERISMAN, J. W., SEITZINGER, S. P., HOWARTH, R. W., COWLING, E. B. & COSBY, B. J. 2003. The nitrogen cascade. *Bioscience*, 53, 341-356.

- GAUSLAA, Y. 1985. The Ecology Of *Lobarion pulmonariae* And *Parmelion caperatae* In *Quercus* Dominated Forests In Southwest Norway. *Lichenologist*, 17, 117-140.
- GAUSLAA, Y. & SOLHAUG, K. A. 1996. Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. *Functional Ecology*, 10, 344-354.
- GERATH, M., SILVA, T., BOWEN, J. & SIGDA, J. 1993. Prediction Of Water Availability In River Headwaters And Control Of Facility Water Demand In Response To River Flows. *Engineering Hydrology - Proceedings of the Symposium*, 1066-1071.
- GILBERT, O.L. 2004. Lichens. Scottish Natural Heritage. Perth. 39 pp.
- GILBERT, D., AMBLARD, C., BOURDIER, G. & FRANCEZ, A. J. 1998. The microbial loop at the surface of a peatland: Structure, function, and impact of nutrient input. *Microbial Ecology*, 35, 83-93.
- GODISH, T. 2004. Air Quality. Boca Raton, Fla.: London. 460pp.
- GOMBERT, S., ASTA, J. & SEAWARD, M. R. D. 2005. The use of autecological and environmental parameters for establishing the status of lichen vegetation in a baseline study for a long-term monitoring survey. *Environmental Pollution*, 135, 501-514.
- GORANSSON, H., FRANSSON, A.-M. & JONSSON-BELYAZID, U. 2007. Do oaks have different strategies for uptake of N, K and P depending on soil depth? *Plant and Soil*, 297, 119-125.
- GOWARD, T. & ARSENAULT, A. 2000. Cyanolichen distribution in young unmanaged forests: A dripzone effect? *Bryologist*, 103, 28-37.
- GRAFEN, A. 2002. Modern Statistics for the Life Sciences. Oxford University Press. Oxford. 351pp.
- GRAHAM, L., GRAHAM, J.M & WILCOX L.W. 2009. Algae. Benjamin Cummings. San Francisco California. 640pp.
- GRANLI, T. & BOCKMAN, O. C. 1994. Ammonia and nitrous oxide emissions from agriculture. *Nitric Acid-Based Fertilizers and the Environment*, 21, 123-131.
- GREGOR, D. J. & MUNAWAR, M. 1989. Assessing Toxicity Of Lake Diefenbaker (Saskatchewan, Canada) Sediments Using Algal And Nematode Bioassays. *Hydrobiologia*, 188, 291-300.
- GRIME, J. P. 1979. Succession and competitive exclusion. *Ecology and design in amenity land management. Proceedings of conference, Wye College, April 8th-11th, 1979 in association with Recreation Ecology Research Group.*, 57-70.
- GRIME, J. P. 1989. Whole-Plant Responses To Stress In Natural And Agricultural Systems. *Plants under Stress: Biochemistry, Physiology and Ecology and Their Application to Plant Improvement*, 39, 31-46.
- GROFFMAN, P. M. & TIEDJE, J. M. 1991. Relationships Between Denitrification, CO₂ Production And Air-Filled Porosity In Soils Of Different Texture And Drainage. *Soil Biology & Biochemistry*, 23, 299-302.
- GUNNARSSON, U. & RYDIN, H. 2000. Nitrogen fertilization reduces *Sphagnum* production in bog communities. *New Phytologist*, 147, 527-537.
- GUNNARSSON, U. 2004. Populations of *Sphagnum angermanicum* in Sweden: distribution, habitat requirements and threats. *Lindbergia*, 29, 129-135.
- GUPTA, S. & AGRAWAL, S. C. 2008. Vegetative survival of some wall and soil blue-green algae under stress conditions. *Folia Microbiologica*, 53, 343-350.

- HAAPALA, H., GOLTSOVA, N., PITULKO, V. & LODENIUS, M. 1996. The effects of simultaneous large acidic and alkaline airborne pollutants on forest soil. *Environmental Pollution*, 94, 159-168.
- HALLINGBACK, T. 1991. Blue-Green Algae And Cyanophilic Lichens Threatened By Air Pollution And Fertilization. *Svensk Botanisk Tidskrift*, 85, 87-104.
- HALLINGBACK, T. & KELLNER, O. 1992. Effects Of Simulated Nitrogen Rich And Acid-Rain On The Nitrogen-Fixing Lichen *Peltigera aphthosa* (L.) Willd. *New Phytologist*, 120, 99-103.
- HANDY, R. D. & POXTON, M. G. 1993. Nitrogen Pollution In Mariculture - Toxicity And Excretion Of Nitrogenous Compounds By Marine Fish. *Reviews in Fish Biology and Fisheries*, 3, 205-241.
- HANNINEN, O., RUUSKANEN, J. & OKSANEN, J. 1993. A Method For Facilitating The Use Of Algae Growing On Tree Trunks As Bioindicators Of Air-Quality. *Environmental Monitoring and Assessment*, 28, 215-220.
- HARGREAVES, J. W., LLOYD, E. J. H. & WHITTON, B. A. 1975. Chemistry And Vegetation Of Highly Acidic Streams. *Freshwater Biology*, 5, 563-&.
- HARRISON, R. M. 2001. Pollution: causes, effects and control. *Pollution: causes, effects and control*.
- HAUCK, M., JUNG, R. & RUNGE, M. 2000. Does water-holding capacity of bark have an influence on lichen performance in dieback-affected spruce forests? *Lichenologist*, 32, 407-409.
- HAWKESFORD, M.J. & DE KOK, L.J. 2007. Sulphur in Plants : An Ecological Perspective. Springer. The Netherlands. 264 pp.
- HAWKINS, D. 2005. Biomeasurement: Understanding, Analysing and Communicating Data in the Biosciences. Oxford University Press. Oxford. 284pp.
- HAWKSWORTH, D.L. & ROSE, F. 1970. Qualitative Scale For Estimating Sulphur Dioxide Air Pollution In England And Wales Using Epiphytic Lichens. *Nature*, 227, 145-&.
- HAWKSWORTH, D. L. & ROSE, F. 1976. Lichens as Pollution Monitors. The Camelot Press Ltd. Southampton. 59pp.
- HAWKSWORTH, D. L. & McMANUS, P. M. 1989. Lichen Recolonization In London Under Conditions Of Rapidly Falling Sulfur-Dioxide Levels, And The Concept Of Zone Skipping. *Botanical Journal of the Linnean Society*, 100, 99-109.
- HECKY, R. E. & KILHAM, P. 1988. Nutrient Limitation Of Phytoplankton In Fresh-Water And Marine Environments - A Review Of Recent-Evidence On The Effects Of Enrichment. *Limnology and Oceanography*, 33, 796-822.
- HEMOND, H.F. & FECHNER-LEVY, E.J. 2000. Chemical Fate and Transport in the Environment. Academic Press. London.
- HERNANDEZ, J. P., DE-BASHAN, L. E. & BASHAN, Y. 2006. Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*. *Enzyme and Microbial Technology*, 38, 190-198.
- HOOVER, C. A. 1981. Microcommunities Of Algae On A *Sphagnum* Mat. *Holarctic Ecology*, 4, 201-207.
- HSIEH, C.-H. & WU, W.-T. 2009. A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. *Biochemical Engineering Journal*, 46, 300-305.

- HUBBELL, S. P. 2001. Monographs in Population Biology. The unified neutral theory of biodiversity and biogeography. *Monographs in Population Biology. The unified neutral theory of biodiversity and biogeography*.
- IRVINE, D.E.G. & JOHN. D.M. 1984. Systematics of the Green Algae. Academic Press. London.
- JACOBSON, M.Z. 2002. Atmospheric Pollution: History, Science and Regulation. Cambridge University Press. Cambridge. 399pp.
- JIN, X., NALEWAJKO, C. & KUSHNER, D. J. 1996. Comparative study of nickel toxicity to growth and photosynthesis in nickel-resistant and -sensitive strains of *Scenedesmus acutus* f *alternans* (Chlorophyceae). *Microbial Ecology*, 31, 103-114.
- JOHN, D.M, WHITTON, B.A & BROOK, A.J. 2002. The Freshwater Algal Flora of the British Isles. Cambridge University Press. London. 702pp.
- JOKELA, A. & HUTTUNEN, S. 1990. The Effect Of Nitrogen Fertilization On The Inner Structure Of Pine Needles. *Physiologia Plantarum*, 79, A118.
- JONSSON, C. M. & AOYAMA, H. 2007. In vitro effect of agriculture pollutants and their joint action on *Pseudokirchneriella subcapitata* acid phosphatase. *Chemosphere*, 69, 849-855.
- KARNOSKY, D. 2003. Air Pollution, Global Change and Forests in the New Millenium. Elsevier. London. 469 pp.
- KAUPPI, M. 1980. Fluorescence Microscopy And Micro-Fluorometry For The Examination Of Pollution Damage In Lichens. *Annales Botanici Fennici*, 17, 163-173.
- KEOGH, S. M., ALDAHAN, A., POSSNERT, G., FINEGAN, P., VINTRO, L. L. & MITCHELL, P. I. 2007. Trends in the spatial and temporal distribution of I-129 and Tc-99 in coastal waters surrounding Ireland using *Fucus vesiculosus* as a bio-indicator. *Journal of Environmental Radioactivity*, 95, 23-38.
- KHAN, M. & YOSHIDA, N. 2008. Effect of L-glutamic acid on the growth and ammonium removal from ammonium solution and natural wastewater by *Chlorella vulgaris* NTM06. *Bioresource Technology*, 99, 575-582.
- KHOZIN-GOLDBERG, I. & COHEN, Z. 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. *Phytochemistry*, 67, 696-701.
- KIEU, N. D., MICHELS, E. & DE MEESTER, L. 2001. Phototactic behavior of *Daphnia* and the continuous monitoring of water quality: Interference of fish kairomones and food quality. *Environmental Toxicology and Chemistry*, 20, 1098-1103.
- KOECHY, M. & WILSON, S. D. 1997. Nitrogen deposition and vegetation change in six Canadian national parks. *Bulletin of the Ecological Society of America*, 78, 271.
- KOERKAMP, P. 1994. Review On Emissions Of Ammonia From Housing Systems For Laying Hens In Relation To Sources, Processes, Building Design And Manure Handling. *Journal of Agricultural Engineering Research*, 59, 73-87.
- KOERKAMP, P., SPEELMAN, L. & METZ, J. H. M. 1998. Litter composition and ammonia emission in aviary houses for laying hens. Part 1: Performance of a litter drying system. *Journal of Agricultural Engineering Research*, 70, 375-382.
- KOOIJMAN, A. M. & BAKKER, C. 1995. Species Replacement In The Bryophyte Layer In Mires - The Role Of Water Type, Nutrient Supply And Interspecific Interactions. *Journal of Ecology*, 83, 1-8.

- KRICKE, R. 2002. Measuring bark pH. *Monitoring with Lichens - Monitoring Lichens*, 7, 333-336.
- KRUPA, S. V. 2003. Effects of atmospheric ammonia (NH₃) on terrestrial vegetation: a review. *Environmental Pollution*, 124, 179-221.
- KUWATA, A. & MIYAZAKI, T. 2000. Effects of ammonium supply rates on competition between *Microcystis novacekii* (Cyanobacteria) and *Scenedesmus quadricauda* (Chlorophyta): simulation study. *Ecological Modelling*, 135, 81-87.
- LANGAN, S.J. 1999. The Impact of Nitrogen Deposition on Natural and Semi-Natural Ecosystems. Kluwer. London. 251 pp.
- LARSEN, R. S., BELL, J. N. B., JAMES, P. W., CHIMONIDES, P. J., RUMSEY, F. J., TREMPER, A. & PURVIS, O. W. 2007. Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. *Environmental Pollution*, 146, 332-340.
- LAUNDON, J. R. 1985. *Desmococcus-Olivaceus* - The Name Of The Common Subaerial Green-Alga. *Taxon*, 34, 671-672.
- LEITH, I. D., SHEPPARD, L. J., PITCAIRN, C. E. R., CAPE, J. N., HILL, P. W., KENNEDY, V. H., TANG, Y. S., SMITH, R. I. & FOWLER, D. 2001. Comparison of the effects of wet N deposition (NH₄Cl) and dry N deposition (NH₃) on UK moorland species. *Water Air and Soil Pollution*, 130, 1043-1048.
- LEITH, I. D., PITCAIRN, C. E. R., SHEPPARD, L. J., HILL, P. W., CAPE, J. N., FOWLER, D., TANG, S., SMITH, R. I. & PARRINGTON, J. A. 2002. A comparison of impacts of N deposition applied as NH₃ or as NH₄Cl on ombrotrophic mire vegetation. *Phyton-Annales Rei Botanicae*, 42, 83-88.
- LEITH, I. D., MITCHELL, R. J., TRUSCOTT, A. M., CAPE, J. N., VAN DIJK, N., SMITH, R. I., FOWLER, D. & SUTTON, M. A. 2008. The influence of nitrogen in stemflow and precipitation on epiphytic bryophytes, *Isoetecium myosuroides* Brid., *Dicranum scoparium* Hewd. and *Thuidium tamariscinum* (Hewd.) Schimp of Atlantic oakwoods. *Environmental Pollution*, 155, 237-246.
- LEWIN, R.A. 1962. Physiology and Biochemistry of Algae. Academic Press. London. 929 pp.
- LIMPENS, J., RAYMAKERS, J., BAAR, J., BERENDSE, F. & ZIJLSTRA, J. D. 2003. The interaction between epiphytic algae, a parasitic fungus and *Sphagnum* as affected by N and P. *Oikos*, 103, 59-68.
- LIN, Q. & LIN, J. 2011. Effects of nitrogen source and concentration on biomass and oil production of a *Scenedesmus rubescens* like microalga. *Bioresource Technology*, 102, 1615-1621.
- LINDSAY, R. A. 1993. Peatland Conservation - From Cinders To Cinderella. *Biodiversity and Conservation*, 2, 528-540.
- LISKA, J. & HERBEN, T. 2008. Long-term changes of epiphytic lichen species composition over landscape gradients: an 18 year time series. *Lichenologist*, 40, 437-448.
- LOFLUND, M., KASPER-GIEBL, A., STOPPER, S., URBAN, H., BIEBL, P., KIRCHNER, M., BRAEUTIGAM, S. & PUXBAUM, H. 2002. Monitoring ammonia in urban, inner alpine and pre-alpine ambient air. *Journal of Environmental Monitoring*, 4, 205-209.
- LOPEZ-BAUTISTA, J. M., RINDI, F. & GUIRY, M. D. 2006. Molecular systematics of the subaerial green algal order Trentepohliales: an assessment based on

- morphological and molecular data. *International Journal of Systematic and Evolutionary Microbiology*, 56, 1709-1715.
- LOPPI, S. & De DOMINICIS, V. 1996. Effects of agriculture on epiphytic lichen vegetation in Central Italy. *Israel Journal of Plant Sciences*, 44, 297-307.
- LOPPI, S. & FRATI, L. 2004. Influence of tree substrate on the diversity of epiphytic lichens: Comparison between *Tilia platyphyllos* and *Quercus ilex* (Central Italy). *Bryologist*, 107, 340-344.
- LUDWIG, G. & SCHITTLER, M. 1996. Rote Liste Gefährdeter Pflanzen Deutschlands. Schriftenreihe für Vegetationskunde. 28: 1-744.
- LUND, J.W.G. 1962. Classical and Modern Criteria Used in Algal Taxonomy with Special Reference to Genera of Microbial Size. Symposia of the Society for General Microbiology. 12: 68-110.
- LYNN, S. G., KILHAM, S. S., KREEGER, D. A. & INTERLANDI, S. J. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *Journal of Phycology*, 36, 510-522.
- MANN, D.G. & DROOP, S.J.M. 1996. Biodiversity, biogeography and conservations of diatoms. *Hydrobiologia* 336: 19-32.
- MANNING, W.J. & FEDER, W.A. 1980. Biomonitoring Air Pollutants with Plants. Applied Science. London.
- MARTINEZ, M. E., SANCHEZ, S., JIMENEZ, J. M., EL YOUSFI, F. & MUNOZ, L. 2000. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 73, 263-272.
- MATALONI, G. Ecological studies on algal communities from Tierra del Fuego peat bogs. *Hydrobiologia*, 391, 157-171.
- MCCORMICK, P. V. & CAIRNS, J. 1994. Algae As Indicators Of Environmental-Change. *Journal of Applied Phycology*, 6, 509-526.
- MCCRORY, D. F. & HOBBS, P. J. 2001. Additives to reduce ammonia and odor emissions from livestock wastes: A review. *Journal of Environmental Quality*, 30, 345-355.
- MELVILLE, F. & PULKOWNIK, A. 2006. Investigation Of Mangrove Macroalgae As Bioindicators Of Estuarine Contamination. *Marine Pollution Bulletin*, 52, 1260-1269.
- MILOW, P AND SALLEH, A. 2006. Epiphytic Terrestrial/Subaerial Algae Of The Genera *Trentepohlia* And *Printzina*. University of Malaya Press.
- MISSELBROOK, T. H., PAIN, B. F. & HEADON, D. M. 1998. Estimates of ammonia emission from dairy cow collecting yards. *Journal of Agricultural Engineering Research*, 71, 127-135.
- MOFIDPOOR, M., KRZIC, M. & PRINCIPE, L. 2009. Effects of peat source and length of storage time in bales on selected properties of *Sphagnum* peat. *Canadian Journal of Soil Science*, 89, 635-644.
- MOHAMED, Z. A. 2008. Polysaccharides as a protective response against microcystin-induced oxidative stress in *Chlorella vulgaris* and *Scenedesmus quadricauda* and their possible significance in the aquatic ecosystem. *Ecotoxicology*, 17, 504-516.
- MOSS, B. 1973. Influence Of Environmental Factors On Distribution Of Freshwater Algae - Experimental Study .2. Role Of pH And Carbon Dioxide Bicarbonate System. *Journal of Ecology*, 61, 157-177.
- MUNAWAR, M. & MUNAWAR, I. F. 1987. Phytoplankton Bioassays For Evaluating Toxicity Of In-situ Sediment Contaminants. *Hydrobiologia*, 149, 87-105.

- NALEWAJKO, C. & PREPAS, E. E. 1996. Responses of phytoplankton photosynthesis and phosphorus kinetics to resuspended sediments in copper sulphate-treated ponds. *Journal of Environmental Quality*, 25, 80-86.
- NANDINI, S. & RAO, T. R. 2000. Microcosm experiments on the effect of nutrient enrichment and a soil layer on the development of freshwater plankton. *Limnologia*, 30, 9-19.
- NEGTA (National Expert Group on Transboundary Air Pollution). 2001. Transboundary Air Pollution : Acidification, Eutrophication And Ground-Level Ozone In The UK. Department for Environment, Food & Rural Affairs. Great Britain. DEFRA. London. 314 pp.
- NEUSTUPA, J. & SKALOUD, P. 2008. Diversity of subaerial algae and cyanobacteria on tree bark in tropical mountain habitats. *Biologia*, 63, 806-812.
- NEUSTUPA, J., CERNA, K. & ST'ASTNY, J. 2009. Diversity and morphological disparity of desmid assemblages in Central European peatlands. *Hydrobiologia*, 630, 243-256.
- NEUSTUPA, J. & SKALOUD, P. 2010. Diversity of subaerial algae and cyanobacteria growing on bark and wood in the lowland tropical forests of Singapore. *Plant Ecology and Evolution*, 143, 51-62.
- NEWSHAM, K. K. 2003. Response of saprotrophic fungal communities to declining SO₂ pollution in the natural environment. *Pedobiologia*, 47, 77-84.
- NIELSEN, G. D., HANSEN, L. F. & WOLKOFF, P. 1997. Chemical and biological evaluation of building material emissions .2. Approaches for setting indoor air standards or guidelines for chemicals. *Indoor Air-International Journal of Indoor Air Quality and Climate*, 7, 17-32.
- NIMIS, P. L., CASTELLO, M. & PEROTTI, M. 1990. Lichens As Biomonitors Of Sulphur Dioxide Pollution In La-Spezia (Northern Italy). *Lichenologist*, 22, 333-344.
- NIMIS, P.L., SCHEIDEGGER, C. & WOLSELEY, P.A. 2002. Monitoring With Lichens – Monitoring Lichens. Kluwer. Dordrecht. 408 pp.
- OLGUIN, H. F., SALIBIAN, A. & PUIG, A. 2000. Comparative sensitivity of *Scenedesmus acutus* and *Chlorella pyrenoidosa* as sentinel organisms for aquatic ecotoxicity assessment: Studies on a highly polluted urban river. *Environmental Toxicology*, 15, 14-22.
- OLGUIN, E. J., RODRIGUEZ, D., SANCHEZ, G., HERNANDEZ, E. & RAMIREZ, M. E. 2003. Productivity, protein content and nutrient removal from anaerobic effluents of coffee wastewater in *Salvinia minima* ponds, under subtropical conditions. *Acta Biotechnologica*, 23, 259-270.
- OLGUIN, H. F., PUIG, A., LOEZ, C. R., SALIBIAN, A., TOPALIAN, M. L., CASTANE, P. M. & ROVEDATTI, M. G. 2004. An integration of water physicochemistry, algal bioassays, phytoplankton, and zooplankton for ecotoxicological assessment in a highly polluted lowland river. *Water Air and Soil Pollution*, 155, 355-381.
- PAIN, B. F., VAN DER WEERDEN, T. J., CHAMBERS, B. J., PHILLIPS, V. R. & JARVIS, S. C. 1998. A new inventory for ammonia emissions from UK agriculture. *Atmospheric Environment*, 32, 309-313.
- PALMER, C.M. 1969. A Composite Rating of Algae Tolerating Organic Pollution. *J. Phycol.* 5: 78-82.

- PALMER, C.M. 1977. Algae and Water Pollution: An Illustrated Manual on the Identification, Significance and Control of Algae in Water Supplies and Polluted Water. US Environmental Protection Agency. USA.
- PALMES, E. D., GUNNISON, A. F., DIMATTIO, J. & TOMCZYK, C. 1976. Personal Sampler For Nitrogen-Dioxide. *American Industrial Hygiene Association Journal*, 37, 570-577.
- PATRICK, R. 1971. Effects Of Increasing Light And Temperature On Structure Of Diatom Communities. *Limnology and Oceanography*, 16, 405-&.
- PATRICK, R. 1972. Aquatic Communities As Indices Of Pollution. *Thomas, Williams a. (Ed.). Indicators of Environmental Quality. Proceedings of a Symposium. Philadelphia, Pa., Dec. 26-31, 1971. X+275p. Illus. Map. Plenum Press: New York, N.Y., U.S.a.; London, England*, 93-100.
- PATTERSON, P. H. & ADRIZAL 2005. Management strategies to reduce air emissions: Emphasis - Dust and ammonia. *Journal of Applied Poultry Research*, 14, 638-650.
- PAULISSEN, M., VAN DER VEN, P. J. M., DEES, A. J. & BOBBINK, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. *New Phytologist*, 164, 451-458.
- PEARCE, I. S. K., WOODIN, S. J. & VAN DER WAL, R. 2003. Physiological and growth responses of the montane bryophyte *Racomitrium lanuginosum* to atmospheric nitrogen deposition. *New Phytologist*, 160, 145-155.
- PEPPERS, R.A. & HARVEY, R.D. 1997. Distribution of Boghead Algae in Illinois Basin Coal Beds. Illinois Geological Survey. Champaign.
- PEREZ, G., QUEIMALINOS, C., BALSEIRO, E. & MODENUTTI, B. 2007. Phytoplankton absorption spectra along the water column in deep North Patagonian Andean lakes (Argentina). *Limnologica*, 37, 3-16.
- PETITTE, J. M. & ORMROD, D. P. 1992. Sulphur Dioxide And Nitrogen-Dioxide Affect Growth, Gas-Exchange, And Water Relations Of Potato Plants. *Journal of the American Society for Horticultural Science*, 117, 146-153.
- PHUYAL, M., ARTZ, R. R. E., SHEPPARD, L., LEITH, I. D. & JOHNSON, D. 2008. Long-term nitrogen deposition increases phosphorus limitation of bryophytes in an ombrotrophic bog. *Plant Ecology*, 196, 111-121.
- PICKETT-HEAPS, J.D. 1975. Green Algae: Structure, Reproduction and Evolution in Selected Genera. Sinauer Associates. Sunderland, MA. 606 pp.
- PIETILAINEN, O. P. & NIINIOJA, R. Nitrogen and phosphorus as algal growth limiting factors in a boreal lake. 27th Congress of the International-Association-of-Theoretical-and-Applied-Limnology, 2001 Dublin, Ireland. 2944-2947.
- PILARSKI, J., TOKARZ, K. & KOCUREK, M. 2008. Optical Properties of the Cork of Stems and Trunks of Beech (*Fagus Sylvatica* L.). *Polish Journal of Environmental Studies*, 17, 773-779.
- PITCAIRN, C. E. R., FOWLER, D. & GRACE, J. 1995. Deposition Of Fixed Atmospheric Nitrogen And Foliar Nitrogen-Content Of Bryophytes And *Calluna-Vulgaris* (L) Hull. *Environmental Pollution*, 88, 193-205.
- PITCAIRN, C. E. R., LEITH, I. D., SHEPPARD, L. J., SUTTON, M. A., FOWLER, D., MUNRO, R. C., TANG, S. & WILSON, D. 1998. The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environmental Pollution*, 102, 41-48.

- PITCAIRN, C.E.R., LEITH, I.D. & SHEPPARD, L. 2001. A Comparison of Impacts of N Deposition Applied as NH_3 or as NH_4Cl on Ombrotrophic Mire Vegetation. *Phyton-Annales Rei Botanicae* 42: 83-88.
- POIKOLAINEN, J., LIPPO, H., HONGISTO, M., KUBIN, E., MIKKOLA, K. & LINDGREN, M. 1998. On the abundance of epiphytic green algae in relation to the nitrogen concentrations of biomonitors and nitrogen deposition in Finland. *Environmental Pollution*, 102, 85-92.
- POSTHUMUS, A. C. 1982. Biological indicators of air pollution. *Effects of gaseous air pollution in agriculture and horticulture*, 27-42.
- PRENDERGAST-MILLER, M., COLE, L., STANDEN, V., REES, R., PARKER, J., LEITH, I. & SHEPPARD, L. 2008. Are enchytraeid worms (Oligochaeta) sensitive indicators of ammonia-N impacts on an ombrotrophic bog? *European Journal of Soil Biology*, 44, 101-108.
- PRENDERGAST-MILLER, M., STANDEN, V., LEITH, I. D. & SHEPPARD, L. J. 2009. Response of enchytraeid worm populations to different forms of nitrogen (ammonia, ammonium, and nitrate) deposition. *Soil Organisms*, 81, 225-236.
- PRESCOTT, G.W. 1982. Algae of the Western Great Lakes Area. Otto Koelz Science Publisher. West Germany. 977 pp.
- PRESS, M. C., WOODIN, S. J. & LEE, J. A. 1986. The Potential Importance Of An Increased Atmospheric Nitrogen Supply To The Growth Of Ombrotrophic *Sphagnum* Species. *New Phytologist*, 103, 45-55.
- PRINS, A. H., BERDOWSKI, J. J. M. & LATUHIHIN, M. J. 1991. Effect Of NH_4 -Fertilization On The Maintenance Of A *Calluna-Vulgaris* Vegetation. *Acta Botanica Neerlandica*, 40, 269-279.
- PRUVOST, J., VAN VOOREN, G., LE GOUIC, B., COUZINET-MOSSION, A. & LEGRAND, J. 2011. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. *Bioresource Technology*, 102, 150-158.
- PUCHALSKI, M. A., SATHER, M. E., WALKER, J. T., LELUNANN, C. M. B., GAY, D. A., MATHEW, J. & ROBARGE, W. P. 2011. Passive ammonia monitoring in the United States: Comparing three different sampling devices. *Journal of Environmental Monitoring*, 13, 3156-3167.
- PURVIS, O. W., CHIMONIDES, J., DIN, V., EROTOKRITOU, L., JEFFRIES, T., JONES, G. C., LOUWHOFF, S., READ, H. & SPIRO, B. 2003. Which factors are responsible for the changing lichen floras of London? *Science of the Total Environment*, 310, 179-189.
- PURVIS, O. W., SEAWARD, M. R. D. & LOPPI, S. 2007. Lichens in a changing pollution environment: An introduction. *Environmental Pollution*, 146, 291-292.
- QUINN, G.P. 2002. Experimental Design And Data Analysis For Biologists. Cambridge University Press. Cambridge. 520pp.
- RAI, L. C. & GAUR, J. P. 2001. Algal adaptation to environmental stresses: Physiological, biochemical and molecular mechanisms. *Algal adaptation to environmental stresses: Physiological, biochemical and molecular mechanisms*, i.
- REES, M. & LAWTON, J. H. 1993. What can models tell us? *Plant adaptation to environmental stress*, 65-83.
- REINERS, W. A. & OLSON, R. K. 1984. Effects Of Canopy Components On Throughfall Chemistry - An Experimental-Analysis. *Oecologia*, 63, 320-330.

- RICHARDSON, B., ORCUTT, D. M., SCHWERTNER, H. A., MARTINEZ, C. L. & WICKLINE, H. E. 1969. Effects Of Nitrogen Limitation On The Growth And Composition Of Uni Cellular Algae In Continuous Culture. *Applied Microbiology*, 18, 245-250.
- RINDI, F. & CINELLI, F. 1995. Contribution To The Knowledge Of The Benthic Algal Flora Of The Isle Of Alboran, With Notes On Some Little-Known Species In The Mediterranean. *Cryptogamie Algologie*, 16, 103-114.
- RINDI, F., GUIRY, M. D., BARBIERO, R. P. & CINELLI, F. 1999. The marine and terrestrial prasiolales (chlorophyta) of Galway City, Ireland: A morphological and ecological study. *Journal of Phycology*, 35, 469-482.
- RINDI, F. & GUIRY, M. D. 2002. Diversity, life history, and ecology of *Trentepohlia* and *Printzina* (trentepohliales, chlorophyta) in urban habitats in Western Ireland. *Journal of Phycology*, 38, 39-54.
- RINDI, F., GUIRY, M. D. & LOPEZ-BAUTISTA, J. M. 2006. New records of Trentepohliales (Ulvophyceae, Chlorophyta) from Africa. *Nova Hedwigia*, 83, 431-449.
- RINDI, F., LAM, D. W. & LOPEZ-BAUTISTA, J. M. 2009. Phylogenetic relationships and species circumscription in *Trentepohlia* and *Printzina* (Trentepohliales, Chlorophyta). *Molecular Phylogenetics and Evolution*, 52, 329-339.
- ROBERTS, K. R. 1985. The Flagellar Apparatus In Batophora And Trentepohlia And Its Phylogenetic Significance. Irvine, D. E. G. and D. M. John (Ed.). *Systematics Association Special Volume No. 27. Systematics of the Green Algae; Meeting, London, England, Mar. 29-31, 1983. Xi+449p. Academic Press Inc., Publishers: Orlando, Fla., USA; London, England. Illus*, 331-342.
- ROELOFS, J. G. M., BOBBINK, R., BROUWER, E. & DEGRAAF, M. C. C. 1996. Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in The Netherlands. *Acta Botanica Neerlandica*, 45, 517-541.
- ROSE, C. I. & HAWKSWORTH, D. L. 1981. Lichen recolonization in London's cleaner air. *Nature*. 289, 289-292.
- ROUND, F.E. & CHAPMAN, D.J. 1982. Progress in Phycological Research. Volume 1. Elsevier Biomedical Press. Oxford. 383 pp.
- RUBIANO, L. J. 1988. Lichens As Indicators Of Contamination In The *Betania colombia* Industrial Complex And The Zipaquira Thermoelectric Plant *Cundinamarca colombia*. *Acta Biologica Colombiana*, 1, 95-126.
- RUISI, S., ZUCCONI, L., FORNASIER, F., PAOLI, L., FRATI, L. & LOPPI, S. 2005. Mapping environmental effects of agriculture with epiphytic lichens. *Israel Journal of Plant Sciences*, 53, 115-124
- RUOSS, E. 1999. How agriculture affects lichen vegetation in Central Switzerland. *Lichenologist*, 31, 63-73.
- RYDIN, H. & KLUM, J.K. 2006. The Biology of Peatlands. Oxford University Press. Oxford. 343 pp.
- RYTHER, J. H. & DUNSTAN, W. M. 1971. Nitrogen, Phosphorus, And Eutrophication In Coastal Marine Environment. *Science*, 171, 1008-&.
- SAGGAR, S., BOLAN, N. S., BHANDRAL, R., HEDLEY, C. B. & LUO, J. 2004. A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, 47, 513-544.

- SCHIEFERSTEIN, B. & LORIS, K. 1992. Ecological Investigations On Lichen Fields Of The Central Namib .1. Distribution Patterns And Habitat Conditions. *Vegetatio*, 98, 113-128.
- SCHINDLER, D.W. 1971. Carbon, Nitrogen, And Phosphorus And Eutrophication Of Freshwater Lakes. *Journal of Phycology*, 7, 321-&.
- SCHLESINGER, W. H. 1997. Biogeochemistry: an analysis of global change. *Biogeochemistry: an analysis of global change*.
- SCHLICHTING, H.E. 1975. Some Subaerial Algae from Ireland. British Phycological Society. 10:257-261.
- SCHNEIDER, T. & GRANT, L. 1982. Air Pollution by Nitrogen Oxides. Elsevier Scientific Company. New York. 1100 pp.
- SCHULTZ, M. G., DIEHL, T., BRASSEUR, G. P. & ZITTEL, W. 2003. Air pollution and climate-forcing impacts of a global hydrogen economy. *Science*, 302, 624-627.
- SHAW, P. 2003. Multivariate Statistics for the Environmental Sciences. Hodder & Stoughton. London. 233pp.
- SHEPPARD, L. J., CROSSLEY, A., HARVEY, F. J., SKIBA, U., COWARD, P. & INGLEBY, K. 2004. Effects of five years of frequent N additions, with or without acidity, on the growth and below-ground dynamics of a young Sitka spruce stand growing on an acid peat: implications for sustainability. *Hydrology and Earth System Sciences*, 8, 377-391.
- SHEPPARD, L. J., LEITH, I. D., CROSSLEY, A., VAN DIJK, N., FOWLER, D., SUTTON, M. A. & WOODS, C. 2008. Stress responses of *Calluna vulgaris* to reduced and oxidised N applied under 'real world conditions'. *Environmental Pollution*, 154, 404-413.
- SHERIDAN, R. P. 1978. Toxicity Of Bisulphite To Photosynthesis And Respiration. *Journal of Phycology*, 14, 279-281.
- SHI, J., PODOLA, B. & MELKONIAN, M. 2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *Journal of Applied Phycology*, 19, 417-423.
- SHNYUKOVA, E. I., MIKHAILYUK, T. I., DARIENKO, T. M. & KONDRATYUK, S. Y. 2005. Contribution to the study of carbohydrates in terrestrial algae. *International Journal on Algae*, 7, 201-212.
- SHUBERT, L.E. 1984. Algae as Ecological Indicators. Academic Press. London.
- SILVA, A., FIGUEIREDO, S. A., SALES, M. G. & DELERUE-MATOS, C. 2009a. Ecotoxicity tests using the green algae *Chlorella vulgaris*-A useful tool in hazardous effluents management. *Journal of Hazardous Materials*, 167, 179-185.
- SKIBA, U., DICK, J., STORETON-WEST, R., LOPEZ-FERNANDEZ, S., WOODS, C., TANG, S. & VANDIJK, N. 2006. The relationship between NH₃ emissions from a poultry farm and soil NO and N₂O fluxes from a downwind forest. *Biogeosciences*, 3, 375-382.
- SKINNER, R. A., INESON, P., JONES, H., SLEEP, D., LEITH, I. D. & SHEPPARD, L. J. 2006. Heathland vegetation as a bio-monitor for nitrogen deposition and source attribution using delta N-15 values. *Atmospheric Environment*, 40, 498-507.
- SMITH, G.M. 1950. The Freshwater Algae of the United States. McGraw Hill Book Company. New York. 719p.
- SOCHTING, U. 1995. Lichens as monitors of nitrogen deposition. *Cryptogamic Botany*, 5, 264-269.

- SOCHTING, U. 1997. Epiphyllic cover on spruce needles in Denmark. *Annales Botanici Fennici*, 34, 157-164.
- SOMMER, S. G., HUTCHINGS, N. J. & CARTON, O. T. 2001. Ammonia losses from field applied animal manure. *DIAS Report, Plant Production*.
- SOMMER, S. G., OSTERGARD, H. S., LOFSTROM, P., ANDERSEN, H. V. & JENSEN, L. S. 2009. Validation of model calculation of ammonia deposition in the neighbourhood of a poultry farm using measured NH₃ concentrations and N deposition. *Atmospheric Environment*, 43, 915-920.
- STAPPER, N.J., FRAHM, J. & FRANZEN-REUTER, I. 2006. Bioindication with epiphytic lichens - a comparison of different methods on the basis of a mapping of North Rhine-Westphalia (NRW, Germany) with special regard to the new guideline 6: 267-271.
- STAPPER, N. J., FRANZEN-REUTER, I. & FRAHM, J. P. 2011. Epiphytic lichens as indicators of climate changes in the Dusseldorf area. *Gefahrstoffe Reinhaltung Der Luft*, 71, 173-178.
- STARKS, T. L., SHUBERT, L. E. & TRAINOR, F. R. 1981. Ecology Of Soil Algae - A Review. *Phycologia*, 20, 65-80.
- STEIN, J.R. 1973. Handbook of Phycological Methods : Culture Methods and Growth Measurements. Phycological Society of America. Cambridge.
- STEVENS, C. J., DISE, N. B., MOUNTFORD, J. O. & GOWING, D. J. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876-1879.
- STEWART, W.D.P. 1974. Algal Physiology and Biochemistry. Blackwell Scientific. Oxford. 989 pp.
- STEWART, N.F & CHURCH, J.M. 1992. Red Data Book of Britain and Ireland: Stoneworts. JNCC Peterborough. 144pp.
- STUBBS, C. S. 1989. Patterns Of Distribution And Abundance Of Corticolous Lichens And Their Invertebrate Associates On *Quercus rubra* In Maine. *Bryologist*, 92, 453-460.
- STUDLAR, S. M. 1982. Host Specificity Of Epiphytic Bryophytes Near Mountain-Lake, Virginia. *Bryologist*, 85, 37-50.
- SUTTON, M. A., PITCAIRN, C. E. R. & FOWLER, D. 1993a. The Exchange Of Ammonia Between The Atmosphere And Plant-Communities. *Advances in Ecological Research*, Vol 24, 24, 301-393.
- SUTTON, M. A., LEE, D. S., DOLLARD, G. J. & FOWLER, D. 1998. Atmospheric ammonia: Emission, deposition and environmental impacts - Introduction. *Atmospheric Environment*, 32, 269-271.
- SUTTON, M. A., TANG, Y. S., DRAGOSITS, U., FOURNIER, N., DORE, A. J., SMITH, R. I., WESTON, K. J. & FOWLER, D. 2001. A spatial analysis of atmospheric ammonia and ammonium in the U.K. *The Scientific World JOURNAL*, 1, 275-286.
- SUTTON, M. A., ASMAN, W. A. H., ELLERMANN, T., VAN JAARSVELD, J. A., ACKER, K., ANEJA, V., DUYZER, J., HORVATH, L., PARAMONOV, S., MITOSINKOVA, M., TANG, Y. S., ACHERMANN, B., GAUGER, T., BARTNIKI, J., NEFTEL, A. & ERISMAN, J. W. 2003. Establishing the link between ammonia emission control and measurements of reduced nitrogen concentrations and deposition. *Environmental Monitoring and Assessment*, 82, 149-185.
- SUTTON, M.A., REIS, S. & BAKER, S.M.H. 2009. Atmospheric Ammonia : Detecting Emission Changes and Environmental Impacts. Results of an Expert

- Workshop Under the Convention on Long-Range Transboundary Air Pollution. Dordrecht. Springer. London. 464 pp.
- TAM, N. F. Y. & WONG, Y. S. 1996. Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media. *Bioresource Technology*, 57, 45-50.
- TANG, Y. S., CAPE, J. N. & SUTTON, M. A. 2001. Development and types of passive samplers for monitoring atmospheric NO₂ and NH₃ concentrations. *TheScientificWorldJournal*, 1, 513-29.
- TARHANEN, S. 1998. Ultrastructural responses of the lichen *Bryoria fuscescens* to simulated acid rain and heavy metal deposition. *Annals of Botany*, 82, 735-746.
- TAYLOR, D. I., NIXON, S. W., GRANGER, S. L. & BUCKLEY, B. A. 1999. Responses of coastal lagoon plant communities to levels of nutrient enrichment: A mesocosm study. *Estuaries*, 22, 1041-1056.
- THOMSEN, M. G. 1992. Epiphytic growth on spruce needles in Norway in relation to nitrogen deposition and climate. *Rapport fra Skogforsk*.
- THONI, L., BRANG, P., BRAUN, S., SEITLER, E. & RIHM, B. 2004. Ammonia monitoring in Switzerland with passive samplers: Patterns, determinants and comparison with modelled concentrations. *Environmental Monitoring and Assessment*, 98, 93-107.
- THORNBURGH, C. S., DIMILLA, P., NIXON, S. W. & MCKINNEY, R. A. 2008. Natural and anthropogenic nitrogen uptake by bloom-forming macroalgae. *Marine Pollution Bulletin*, 56, 261-269.
- TILMAN, D. & LEHMAN, C. L. 1997. Habitat destruction and species extinctions. *Monographs in Population Biology; Spatial ecology: The role of space in population dynamics and interspecific interactions*, 30, 233-249.
- TINGEY, D. T., HECK, W. W., DUNNING, J. A. & REINERT, R. A. 1971. Vegetation Injury From Interaction Of Nitrogen-Dioxide And Sulfur-Dioxide. *Phytopathology*, 61, 1506-&.
- TUBA, Z., PROCTOR, M. C. F. & CSINTALAN, Z. 1998. Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: a comparison and an ecological perspective (vol 24, pg 211, 1998). *Plant Growth Regulation*, 26, 71-71.
- TUBA, Z. 2008. Notes on the poikilochlorophyllous desiccation-tolerant plants. *Acta Biologica Szegediensis*, 52, 111-113.
- TUBEA, B., HAWXBY, K. & MEHTA, R. 1981. The Effects Of Nutrient, pH And Herbicide Levels On Algal Growth. *Hydrobiologia*, 79, 221-227.
- TUCKER, L.A. 2003. Simplistic Statistics : A Basic Guide To The Statistical Analysis Of Biological Data. Chalcombe Publications. Lincoln. 68pp.
- UHERKOVICH, G. 1993. A Contribution To The Knowledge Of Algae Of Some Waters In C-Slovakia. *Biologia*, 48, 3-6.
- VAN DEN HOEK, C., MANN, D. G. & JAHNS, H. M. 1995. Algae: An introduction to phycology. *Algae: An introduction to phycology*.
- VAN DER SWALUW, E., ASMAN, W. A. H., VAN JAARSVELD, H. & HOOGERBRUGGE, R. 2011. Wet deposition of ammonium, nitrate and sulphate in the Netherlands over the period 1992-2008. *Atmospheric Environment*, 45, 3819-3826.
- VAN DOBBEN, H. F. & TER BRAAK, C. J. F. 1998. Effects of atmospheric NH₃ on epiphytic lichens in the Netherlands: The pitfalls of biological monitoring. *Atmospheric Environment*, 32, 551-557.

- VAN HERK, C. M. 1999. Mapping of ammonia pollution with epiphytic lichens in the Netherlands. *Lichenologist*, 31, 9-20.
- VAN HERK, C. M. 2001. Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time. *Lichenologist*, 33, 419-441.
- VAN HERK, C. M. 2003. Long distance nitrogen air pollution effects on lichens in Europe. vol 35, pg 347..
- VITOUSEK, P. M. & FARRINGTON, H. 1997. Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry*, 37, 63-75.
- VYMAZAL, J. 1995. Algae and element cycling in wetlands. *Algae and element cycling in wetlands*.
- WARDLAW, A.C. 2000. Practical Statistics For Experimental Biologists. John Wiley. Chichester. 249pp.
- WARNER, R. W. 1971. Distribution Of Biota In A Stream Polluted By Acid Mine Drainage. *Ohio Journal of Science*, 71, 202-216.
- WATHES, C. M. 1998. Aerial emissions from poultry production. *Worlds Poultry Science Journal*, 54, 241-251.
- WATSON, C. A., RITZ, K., YOUNIE, D. & FRANKLIN, M. 1996. Nitrogen and soil biomass dynamics in ley/arable crop rotations. *Aspects of Applied Biology*, 43-50.
- WAYDA, M. 2004. Changes in species composition of desmids in the "Bloto" peat bog (the Niepolomice Forest) from 1954 to 2001. *Acta Societatis Botanicorum Poloniae*, 73, 239-246.
- WEAR, D. J., SULLIVAN, M. J., MOORE, A. D. & MILLIE, D. F. 1999. Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. *Marine Ecology Progress Series*, 201-213.
- WEHR, J. D. & SHEATH, R. G. 2003. *Freshwater Algae Of North America: Ecology And Classification*. Academic Press.
- WELLBURN, A. 1994. Air Pollution and Climate Change: The Biological Impact. Longman Scientific. Harlow.
- WEST, G.S & FRITSCH, F.E. 1927. A Treatise on the British Freshwater Algae. Cambridge University Press, Cambridge. 534 pp.
- WHITE, E., PAYNE, G., PICKMERE, S. & PICK, F. R. 1982. Factors Influencing Ortho-Phosphate Turnover Times - A Comparison Of Canadian And New-Zealand Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 39, 469-474.
- WHITTON, B. A. & KELLY, M. G. 1995. Use Of Algae And Other Plants For Monitoring Rivers. *Australian Journal of Ecology*, 20, 45-56.
- WHITTON, B.A., JOHN D.M, JOHNSON L.R, BOULTON, P.N.G, KELLY M.G, HAWORTH E.Y. 1998. A Coded List of Freshwater Algae of the British Isles. NERC, LOIS Publication Number 222. Institute of Hydrology, Wallingford. 274pp.
- WIEDERMANN, M. M., NORDIN, A., GUNNARSSON, U., NILSSON, M. B. & ERICSON, L. 2007. Global change shifts vegetation and plant-parasite interactions in a boreal mire. *Ecology*, 88, 454-464.
- WIKLUND, K. & RYDIN, H. 2004. Ecophysiological constraints on spore establishment in bryophytes. *Functional Ecology*, 18, 907-913.

- WILLIAMS, T. 1972. Aquatic Communities as Indices of Pollution. Indicators of Environmental Quality. Proceedings of A Symposium. Philadelphia, PA. Illus. Map. Plenum Press: New York. 93-100.
- WILLIAMS, E. J., GUENTHER, A. & FEHSENFELD, F. C. 1992. An Inventory Of Nitric-Oxide Emissions From Soils In The United-States. *Journal of Geophysical Research-Atmospheres*, 97, 7511-7519.
- WITTMANN, C., ASCHAN, G. & PFANZ, H. 2001. Leaf and twig photosynthesis of young beech (*Fagus sylvatica*) and aspen (*Populus tremula*) trees grown under different light regime. *Basic and Applied Ecology*, 2, 145-154.
- WODZINSKI, R. S., LABEDA, D. P. & ALEXANDER, M. 1978. Effects Of Low Concentrations Of Bisulphite-Sulphite And Nitrite On Microorganisms. *Applied and Environmental Microbiology*, 35, 718-723.
- WOLSELEY, P. A. & JAMES, P. W. 2002. Assessing changes in density and condition of lichens for species recovery programmes. *Monitoring with Lichens - Monitoring Lichens*, 7, 391-393.
- WOLSELEY, P. A., JAMES, P. W., THEOBALD, M. R. & SUTTON, M. A. 2006. Detecting changes in epiphytic lichen communities at sites affected by atmospheric ammonia from agricultural sources. *Lichenologist*, 38, 161-176.
- WOODIN, S. J. & FARMER, A. M. 1993. Impacts Of Sulphur And Nitrogen Deposition On Sites And Species Of Nature Conservation Importance In Great-Britain. *Biological Conservation*, 63, 23-30.
- WU, X., LI, H., WEI, P., WU, X. Y., LI, H. & WEI, P. 2009. Optimization of the conditions for using sewage to cultivate *Navicula tenera*. *Agricultural Science & Technology - Hunan*, 10, 68-73.
- XIE, Y.-X., ZHANG, S.-L., ZHAO, X., XIONG, Z.-Q. & XING, G.-X. 2008. Seasonal variation patterns of $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$ ratio and $\delta^{15}\text{N}$ value in rainwater in Yangtze River Delta. *The journal of applied ecology* 19, 2035-41.
- XIN, L., HU, H.-Y., KE, G. & SUN, Y.-X. 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresource technology*, 101, 5494-500.
- XU, N. J., ZHANG, X. C., FAN, X., HAN, L. J., ZENG, C. K. & TSENG, C. K. 2001a. Effects of nitrogen source and concentration on growth rate and fatty acid composition of *Ellipsoidion* sp (Eustigmatophyta). *Journal of Applied Phycology*, 13, 463-469.
- YEESANG, C. & CHEIRSILP, B. 2011. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresource Technology*, 102, 3034-3040.
- YUNG, Y. K., STOKES, P. & GORHAM, E. 1986. Algae Of Selected Continental And Maritime Bogs In North-America. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 64, 1825-1833.
- ZEDDA, L. & RAMBOLD, G. 2004. Diversity change of soil-growing lichens along a climate gradient in Southern Africa. *Bibliotheca Lichenologica*, 88, 701-714.
- ZHAI, S., YANG, L. & HU, W. 2009. Observations of Atmospheric Nitrogen and Phosphorus Deposition During the Period of Algal Bloom Formation in Northern Lake Taihu, China.

APPENDIX

PRELIMINARY STUDY

A preliminary study was conducted to find the best possible method for algal collection of epiphytic terrestrial algae. Collections were made of algae on oak, beech, birch and ash. Birch and ash was later removed from this study due to insufficient number of trees. This study was conducted in the natural habitats of the Imperial College estate at Silwood Park, Ascot, Berkshire (51° 24'N, 0° 39'W). Silwood Park is about 35 km west of the centre of London and includes a remnant of the former Windsor Forest which lies at an altitude of about 65 m. The soil overlies sand and gravel, with clay locally, but it is nearly always very light and therefore dries out very rapidly. The study area comprises 93 ha of acid grassland, scrubland and woodland mostly dominated by oak, beech and birch. This area has relatively hot dry summers and cool winters. The annual mean temperature from January to July ranges from 7 to 11°C. This area receives less than 650 mm of rain per year. In this preliminary study, algae were sampled on 30 trees, 15 trees each of pedunculate Oak (*Quercus robur*) and Beech (*Fagus sylvatica*).

To permit comparisons of the algal density data with older studies using percentage cover determinations, an estimate of total algal cover was made in the preliminary study (individual species could not usually be distinguished). Cover of the total terrestrial algae in each quadrat was estimated visually within 5% classes. Samples with less than 5% cover, were estimated to the nearest 1% interval. In an attempt to explain variation in the distribution of algae among the samples, some simple environmental measurements were made at the quadrats. These included slope angle which is a pointer to the microclimatic conditions and bark pH. A flat glass electrode

was used to measure bark pH in-situ but since oak bark is generally coarse and fissured, resulting in inaccurate pH measurement, a conventional pH electrode was on bark samples taken to the laboratory. See chapter 2 and Kricke (2002). Average inclination or slope of the bark surface was measured in the field with a clinometer. No attempt was made to estimate the density of the tree canopy above each quadrat although this may be an important factor in determining the total exposure to sunlight at each point sampled.

Results

A total of ten algal taxa from nine genera were recorded from the 90 samples examined in this survey. Most of them (70%) were members of the Division Chlorophyta. This division was represented by seven out of ten species. The Division Bacillariophyta (diatoms) was represented by two species (20%) while the Division Cyanophyta was represented by only one species (10%) (**Table A1**).

On beech, *Desmococcus olivaceus* had the highest mean number of cells with $2,875 \pm 65.6 \times 10^2$ cells per ml, i.e 51,118 cells/cm², while on oak, *Trentepohlia abietina* had the highest mean number of cells with $4,074 \pm 105 \times 10^2$ cells per ml, equivalent to 72,435 cells/cm² (**Table A1**). The mean percentage covers of algae of all species on beech and oak trees were 62.8% (± 6.0) and 63.7% (± 4.6) respectively. Percentage cover on beech ranged from 21.7 to 88.3% and on oak from 31.7 to 86.7%. A Kolmogorov-Smirnof test showed no significant departure from normality in cover values for either species ($P = 0.096$ for beech and $P > 0.15$ for oak). An F-test also showed that there is no significant difference between the variances for the two tree species.

A t-test confirmed that mean covers of total algae on beech and oak did not differ significantly ($t=0.82$, $p=0.427$ (two-tailed), $d.f = 15$). Even though there was no difference in total cover of algae between beech and oak, the algal density on oak trunks ($8,714 \times 10^2$ cells per ml, equivalent to $154,934$ cells/cm²) was significantly different from and twice as great as on beech ($4,438 \times 10^2$ cells per ml, equivalent to $78,907$ cells/cm²). This was confirmed by a t-test ($t=2.76$, $p=0.012$ (two-tailed), $d.f = 21$).

Table A1: The epiphytic algae recorded on beech and oak trees at Silwood Park, their mean densities and the results of a t-test comparing their mean densities on the two tree species. The t-tests were performed using data transformed to logarithms (base 10). Legend ^a: Algae belonging to division Bacillariophyta (diatoms). Legend ^b : Algae belonging to Cyanophyta (currently known as Cyanobacteria). Results are of mean algal densities $\pm$ SE.

No	Species	Mean number of cells per ml on beech ($\times 10^2$)	Mean number of cells per ml on oak ($\times 10^2$)	<i>p</i> -value
1	<i>Apatococcus lobatus</i>	461 ± 37.6	0	-
2	<i>Trentepohlia abietina</i>	788 ± 39.2	4074 ± 105	0.236
3	<i>Trentepohlia umbrina</i>	36 ± 1.11	12 ± 0.8	0.301
4	<i>Chlorella</i> sp.	28 ± 2.8	0	-
5	<i>Trebouxia</i> sp.	0	2061 ± 50.1	-
6	<i>Desmococcus olivaceus</i>	2875 ± 65.6	1989 ± 40	0.056
7	<i>Chlorococcus</i> sp.	236 ± 13.6	578 ± 22	0.818
8	<i>Gloeotrichia</i> sp. ^a	2 ± 0.2	0	-
9	<i>Pinnularia</i> sp. ^b	6 ± 0.6	0	-
10	<i>Tabellaria</i> sp. ^b	6 ± 0.6	0	-

There was no correlation between algal % cover and density on either beech or oak ($r = 0.239$, $p = 0.391$ and $r = 0.442$, $p = 0.099$ respectively), using Pearson's correlation coefficient (**Figure A1**). All data were transformed to log 10 prior to analysis. None of the algal species was found to be significantly different in terms of mean density, between the two tree species.

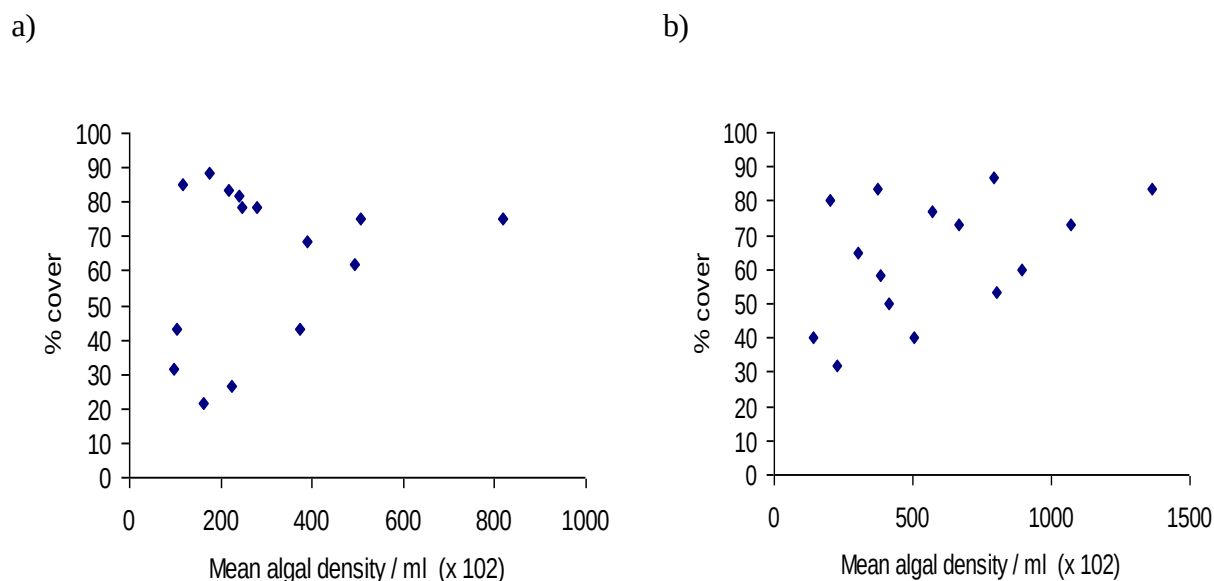


Figure A1. Variations of total algal % cover as a function of total algal density. No correlations were found for % cover and density for either beech or oak.

a) Beech

b) oak

In terms of bark pH, beech is significantly less acidic than oak. The range for beech was slightly higher (4.9-5.9) than that for oak (4.1-5.0) but there is some overlap. Differences in the angle of leaning between the two trees were not statistically significant (**Table A2**).

Table A2: A comparison of mean levels of environmental variables between beech and oak samples employing 2-tailed t-tests. *, $p \leq 0.05$.

Environmental Variable	Mean Value in Beech	Mean Value in Oak	<i>t</i>	<i>P</i> -value
Bark pH	4.9 ± 0.1	4.7 ± 0.07	2.00	0.045*
Slope Angle	83.33 ± 1.07	84.40 ± 1.27	-0.64	0.526

In this study, there was no correlation between algal density and degree of leaning (slope angle) on both beech and oak. *Desmococcus olivaceus* is the only species which showed a strong positive correlation ($r=0.649$, $p=0.009$) with bark pH (**Figure**

A2). No other species showed any correlation in regards to bark pH and algal density either on beech or oak.

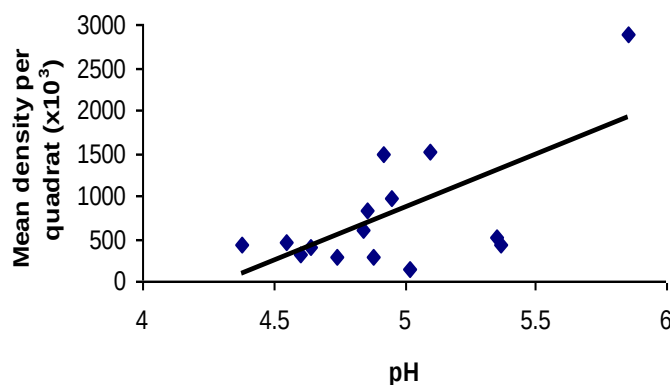


Figure A2 Variation of mean density of *Desmococcus olivaceus* on beech as a function of bark pH. ($r=0.649$, $p=0.009$)

Discussion

The epiphytic algal flora at Silwood Park was dominated by most of the common genera associated with temperate regions, namely *Desmococcus*, *Trentepohlia*, *Trebouxia* and *Apatococcus*. Algal density at Silwood Park was appreciable, totalling almost 500,000 cells per ml, equating to a population of 89k cells per cm² of quadrat (**Table A1**).

There were some marked differences in abundance and distribution of algae between beech and oak. Thus the total density of algal cells on oak was more than twice that on beech and yet the commonest alga, *Desmococcus olivaceus*, was significantly more abundant on beech than on oak. Five species were recorded only on beech whereas one was recorded only on oak. Substantial differences occurred in the total densities of some of the other species between the two trees, but the mean numbers were not significantly different. These differences might be due to the differing properties of the host trees' bark which can fundamentally influence the type of

epiphytic vegetation present (Hawksworth & Rose, 1970; Bates & Brown, 1981; Barkman, 1988; Loppi, 2004).

Bark pH can be one of the most influential factors determining the occurrence of lichen and bryophyte epiphytes on trees (Brodo, 1973; Studlar, 1982; Bates, 1992; Larsen *et al.*, 2007; Fritz, 2009). In the Silwood study, oak bark was slightly but significantly more acid than beech bark (**Table A2**). Whether or not this can explain the differences in algal density is uncertain. The most striking difference, the prevalence of *D. olivaceus* on beech which is the less acidic substratum, is supported by the additional observation that on beech the density of this alga is positively correlated with bark pH (**Figure A2**). The many literature references suggesting that low pH is detrimental to terrestrial algae include those of Tarhanen (1998) working with lichen photobionts. Gauslaa *et al.*, (1996) also reported that low pH reduced photosynthesis in the lichen *Lobaria pulmonaria*, while Goward & Arsenaud (2000) suggested that nitrogenase activity in cyanobacterial photobionts was also limited by low pH. Moss (1973) found that the growth of green algae increased with pH, and that most of the chlorophytes would not establish at pH lower than 4.48. This is in agreement with Nalewajko *et al.* (1996) where they concluded that no growth of the green alga, *Scenedesmus acutus* occurred at pH 4.8 or less.

The texture of tree bark may also influence algal density. The coarsely fissured bark of oak traps more water than the relatively smooth bark of beech, and thus remains moister for longer following precipitation. High moisture content of the substratum can facilitate the development and germination of autospores, aplanospores and zoospores (depending on algal species) and thus assist reproduction. However, the

most abundant algae in the Silwood study were mostly found on beech rather than on oak. It is possible that bark pH is a more relevant factor in determining *D. olivaceus* density than the texture of bark.

The degree of leaning of a tree is known to affect epiphyte growth (Schieferstein & Loris; 1992; Zedda & Rambold, 2004). If a tree leans more strongly water drains away more slowly thus favouring epiphyte growth (Barkman, 1988). No significant differences between the lean of oak and beech were found in this study.

Finally, no correlation was found between algal percentage cover and algal density in the Silwood Park survey (**Figure A2**). This probably means that the algae may grow in either few or in many layers on tree bark, something that is not obvious when making visual assessments of percentage cover. This finding is important because some earlier studies of epiphytic algae in relation to atmospheric pollutants (e.g. Bates *et al.*, 1990; Bates, *et al.*, 2001) were based on macroscopic cover determinations rather than on microscopic assessments of density.