AIR POLLUTION IMPACTS TO AGRICULTURAL CROPS

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PhD

The University of York
Environment

September, 2010

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ABSTRACT

Although air pollution levels are increasing, there is no knowledge of air pollution effects on agricultural crops in the Peshawar region of Pakistan. The aim of this study was to assess the impact of ozone (O_3) and hydrogen fluoride (HF) on agricultural crops in Peshawar through a combination of field work and experiments.

The mean monthly O_3 concentration in Peshawar, measured using passive samplers, was between 25-30ppb in February and March, but increased to 35-55ppb in April and May; it fell to 15-20ppb in November/December. O_3 injury was found on potato (black flecking), onion (leaf tipburn) and cotton (white stipples) in a survey in April/May, but was absent from many other crops. No O_3 injury was found on any crops during a winter survey. O_3 fumigation experiments on spinach (*Beta vulgaris*) and onion (*Allium cepa*) in open-top chambers in UK showed that high O_3 concentrations can affect both species in terms of visible injury and growth. However, onion is at greater risk in the field as it is a summer crop and is likely to be exposed to high O_3 concentrations, unlike spinach, which is grown in the winter season. An EDU experiment on spinach under field conditions in Peshawar showed no effect on growth during winter season. However, elemental contents of spinach were significantly reduced in EDU treated plants.

The HF concentrations in Peshawar, measured using passive samplers were higher in summer than in winter in areas close to brick kiln fields. The mean summer concentration was 0.2μg HF m^{-3}, with maximum of 0.3μg HF m^{-3} in May. HF was below detection limits of <0.1μg HF m^{-3} in November-December. Severe HF injuries to mango, apricot and plum leaves, in form of necrotic leaf margins and tipburn, were found near the brick kiln fields. Tomato, maize, wheat and sugarcane were found to be less sensitive, but also showed some HF injury. The fluoride content of fruit leaves, wheat grains and spinach was significantly higher in the brick kiln area than at control sites. There was no significant difference between the soil fluoride content of wheat fields in the brick kiln area and at control sites. Wheat grown at different NaF levels in alkaline soils similar to those in Peshawar, in a greenhouse experiment in the UK showed no effect of fluoride on growth. The degree of powdery mildew infestation increased with increased fluoride concentrations in the soil and ear emergence was also delayed in all treatments except the control.

It was concluded that O_3 and HF are significant pollutants in Peshawar, especially for summer crops. More detailed studies should be conducted to determine the magnitude of damage caused by these pollutants in the Peshawar region.

Acknowledgments

First and the foremost, thanks to Almighty Allah for His countless blessings and providing me with peace of mind and courage to successfully complete this dissertation.

I would like to thank Higher Education of Pakistan for financial support to pursue my PhD at York University and make my dream come true of higher education from a well-reputed foreign institution.

My sincerest thanks to my supervisor Professor Mike Ashmore for his continued, untiring and generous help throughout my research work over the last 3 years. He helped me thinking innovatively and creatively with his amazingly optimistic approach. With his help and affectionate advice, research experimentation, analysis and data interpretation went smoothly and delightfully. I would also like take this opportunity to thank my research group members, Patrick Bücker and Leon Van Den Berg, for their productive comments and suggestions during my research work.

Special thanks to my colleague Ishaq A. Mian, Muhammad Riaz and Afia Zia for their always being available to help in my lab work and analysis. I am also very thankful to my colleagues in Pakistan, Dr. Abdul Wahid, Prof. Hamid Ullah Shah, Tariq Masood, Muhammad Suleman, Waqar Ali and all the local farmers for helping me in my field work; with out them it would have been very difficult to complete this thesis.

My gratitude to Rebecca Sutton for advice on the Elementar Vario, Dionex and Atomic Absorption Spectrometry and thanks to Dr. Pierre Delmelle for providing the Fluoride Electrode for fluoride analysis.

Last, but not least, my heartiest thanks to my family for their patience, love and support over the entire period of my study. My sincere thanks to many friends in the UK, especially in Environment Department, who made my stay really pleasant and enjoyable through uncountable cheerful moments and events.

Author's declaration

This thesis is submitted in fulfilment of the requirements for the degree of PhD in Environmental Science at the Environment Department, The University of York, UK. The work described in this thesis was conducted between January 2008 and October 2010. I declare that work included in this thesis is my own original research work except where clearly acknowledged in the text and are other contributors are referred to.

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CHAPTER 1

INTRODUCTION

1.1. AIR POLLUTION AND ITS IMPACTS

The atmosphere of the earth has been distorted since the start of the last millennium, due to the rise of the industrial age causing air pollution (Finlayson and Pitts, 1999). Air pollution is a physical, chemical or biological substance that is present in high enough concentrations in air to harm humans, animals and vegetation, and usually results from human activity. There are many substances in the air that may affect the condition of plants and animals. Concentrations of air pollutants such as nitric oxides (NOx) and ozone (O_3) are continuing to increase in the atmosphere due to the rise in urbanisation in developing countries (Chameides *et al*., 1994). Because of the increase in the concentrations of these pollutants, there is much unease about future changes in our environment (IPCC, 2000). Major air pollutants that are harmful to vegetation, in terms of physical injury, yield loss, and altered nutritional quality, include sulphur dioxide (SO_2), nitrogen oxides (NOx), ozone (O_3), fluorides (F), heavy metals and acid deposition (UQM, 2007).

Local and regional air pollution affects the lowest layer of the atmosphere called the troposphere, which, at its widest, extends from the Earth's surface to about 16 km. If the load of pollutants added to the troposphere were equally distributed, the pollutants would be spread over vast areas and air pollution might almost escape our notice. Pollution sources tend to be concentrated in the troposphere, however, especially in and around cities or close to individual sources (Wahid, 1995a & b) and can have greater effects on the immediate

surroundings. There are two types of air pollutants, primary and secondary pollutants. Primary pollutants are emitted directly from sources e.g. nitric oxide (NO) and SO_2, while secondary pollutants are derived from the primary pollutants e.g. ozone formation from NO_x and volatile organic compounds (VOCs) emitted into the atmosphere. However, secondary pollutants are among the most significant pollutants, especially ozone, which can occur at high concentrations a long way from sources and can damage human health and vegetation over large areas (Emberson *et al*., 2003).

Most of the work related to air pollution impacts on plants has been carried out in western countries, where effects of air pollutants like ozone and SO_2 have been the focus (UQM, 2007). In contrast, their effects in the regions such as South Asia have been much less studied (Emberson *et al*., 2009).

1.1.1 Air pollution and its impacts on South Asia

South Asia is one of the populous regions on earth. 1.3 billion People live here, with 27% of them in urban areas (Kojima *et al*., 2000). Most of the countries are in their developmental stage such as India and Pakistan (Kojima *et al*., 2000). The biggest emission sources in South Asia are from automobiles, industries and domestic activities (Emberson *et al*., 2003). South Asians are facing negative effects of air pollution on human health as well as on vegetation, due to the rapid rise in air pollution (Wahid 2006a & b). It has been estimated that 800,000 deaths are caused by air pollution annually, and almost two third of these are in the developing countries of Asia (BAQ, 2004).

In South Asia, several studies have been carried out on the effects of air pollution on different crops. For the last 30 years, potato, wheat, rice, legumes, barley and spinach have been subjected to ozone, SO_2 and NO_x in both field and controlled conditions, which has revealed visible injuries, and growth and yield reduction in these crops (Bell and Treshow, 2002). Most of the recent studies are related to O_3 effects on different crops, as O_3 is a regional pollutant. Bambawale (1986), Agrawal *et al*. (2005), Tiwari *et al*. (2005), and Tiwari and Agrawal (2009) in India, and Wahid *et al*. (1995, 2001) and Wahid (2006a & b) in Pakistan have revealed significant ozone damage to crops. According to Emberson *et al*. (2009), Asian varieties of staples such as wheat and rice may be more sensitive to O_3 than North American varieties. The annual yield loss of wheat, rice, maize and soybean grown in China, Japan and South Korea due to O_3 is estimated to be about US $5 billon (Wang and Mauzerall 2004). In Pakistan, the economic cost of air pollution effects is estimated at US$998 million/year for urban air pollution (PCAN, 2008), due to effects on human health, but economic losses due to effects on agriculture are unknown.

In this chapter, some of the important air pollutants and their impacts on vegetation are discussed. The air pollution situation in Pakistan, and previous studies carried out on air pollution effects on crops are described. Finally the current pollution situation in Peshawar and the reasons it was selected for a study of pollution and its potential effects on vegetation are discussed.

1.1.2. Sulphur dioxide (SO_2)

Sulphur dioxide is one of the main products from the combustion of sulphur compounds in most energy fuels and is of significant environmental

concern. SO_2 is a primary pollutant, which is released directly to the atmosphere from domestic and industrial processes, especially those using petroleum and coal combustion (Emberson *et al.*, 2001). Industry and power generation are the main regional sources, but vehicular emissions may be important within major cities (Emberson *et al*, 2003). SO_2 can be oxidised in the atmosphere to form sulphate aerosols that contribute to acid deposition (Holleman and Wiberg, 2001).

1.1.2.1. SO_2 Impacts

SO_2 is at the core of many pressing air pollution problems in developing countries, where it contributes both to urban pollution and to regional acid deposition (Cofala *et al*., 2004). Acid deposition can damage forests and crops by acidification of soil; it also causes lakes and stream acidification (USEPA, 2007). Gaseous SO_2 can cause direct injury to crops and forests by entering the leaves through the stomata and deposition to external surfaces, leading to negative effects on the growth and yield of the plant. Acute visible injury (Fig 1.1) to plants is caused by absorption of high concentrations of SO_2 over a relatively short time. The foliar symptoms are usually interveinal chlorosis (whitened areas), which run through to the edges of the leaves. The fully expanded leaves are more sensitive to acute SO_2 injury, as compared to the very youngest and oldest leaves (Heather, 2003).

Fig 1.1: Acute SO_2 injury to raspberry (Rubus idaeus) (Linzon, 1978)

In the leaf cells, SO_2 dissolves to give bisulphite (HSO_3^-) and sulphite ions (SO_3^{2-}). Sulphite is toxic, but at low concentrations it is metabolized in the chloroplasts to sulphate (SO_4^{2-}) that is not toxic. At low concentrations, bisulfite and sulphite are effectively detoxified by plants, and SO_2 then provides a sulphur source for the plants (Zeiger and Taiz, 2006). The crop species that are generally considered susceptible to sulphur dioxide are alfalfa, barley, wheat, clover, oats, pumpkin, radish, spinach, squash and tobacco. Resistant crop species include asparagus, cabbage, corn, onion and potato (Kondo and Sugahara, 1978).

1.1.3. Nitrogen Oxides (NO_x)

Nitric oxide (NO) and nitrogen dioxide (NO_2) are the two forms of NO_x that are known to have direct impacts on vegetation. NO_2 is mainly a secondary pollutant formed by the reaction of NO and O_3 in the atmosphere. NO_x is produced in high temperature combustion; the main emission sources are power generation and motor vehicles (Emberson *et al.*, 2001).

1.1.3.1. NO_x Impacts

The main route of entry of NO_x into plant leaves is through the stomata (CLAG, 1996). NO_x then dissolves in leaf cells and gives rise to nitrite ions (NO_2^-), which are toxic at high concentrations, and nitrate ions (NO_3^-) that enter into nitrogen metabolism as if they had been absorbed through the roots (Zeiger and Taiz, 2006). NO_x at high concentrations in the atmosphere can significantly reduce the growth of the plant especially under high soil nitrogen conditions. However, NO_x can increase the nitrogen content of the plants and stimulate growth, when soil nitrogen is limiting and the concentration in the air is moderate. Exposure to very high concentrations of NO_x in a relatively short time will cause

abnormal symptoms (Zeiger and Taiz, 2006), and visible foliar injury to the plant, in the form of patches of chlorosis and necrosis on the leaves. Exposing the plant to lower concentrations of NO_x for a longer duration rarely causes visible injury but can affect growth by suppressing the rate of photosynthesis. NO_x in combination with other pollutants, especially SO_2, can cause more damage to vegetation than would be expected from the effects of the individual pollutants depending upon the environmental conditions (Emberson *et al*., 2001).

1.1.4. Ozone (O_3)

Tropospheric ozone (O_3) is an important phytotoxic air pollutant. Although ozone has an important role in protecting the biosphere by absorbing harmful ultraviolet radiation in the upper stratosphere, in the troposphere it is a phytotoxic air pollutant (Khan and Soja, 2003). The detrimental effects of photochemical oxidant mixtures on plants were first recognised in the late 1940s (Middleton *et al*., 1950). Northern hemisphere background surface concentration of ozone concentrations have been rising steadily, from 10-20 ppb to 20-40 ppb, over the last century (Emberson *et al*., 2001). The concentration of O_3 increases mainly due to two different processes, O_3 transfer from stratosphere to troposphere and photochemical reactions. The second process is the formation of O_3 from the recombination of atomic and molecular oxygen, through the reactions involving NO_x and hydrocarbons emitted in to the atmosphere (Fig 1.2). The tropospheric ozone formation consists of a series of reactions that first produce an H atom from the reaction of hydroxyl radical (OH) with CO or VOCs, which then quickly reacts with O_2 to give a peroxy radical (HO_2).

$$OH + CO \rightarrow H + CO_2$$
$$H + O_2 \rightarrow HO_2$$

The peroxy radicals then react with nitric oxide (NO) to form nitrogen dioxide (NO_2), and NO_2 in the presence of light (hν) can split into the NO and O molecules, which ultimately react with O_2 to form ozone (O_3) (USEPA, 2007).

$$HO_2 + NO \rightarrow OH + NO_2$$
$$NO_2 + h\nu \rightarrow NO + O$$
$$O + O_2 \rightarrow O_3$$

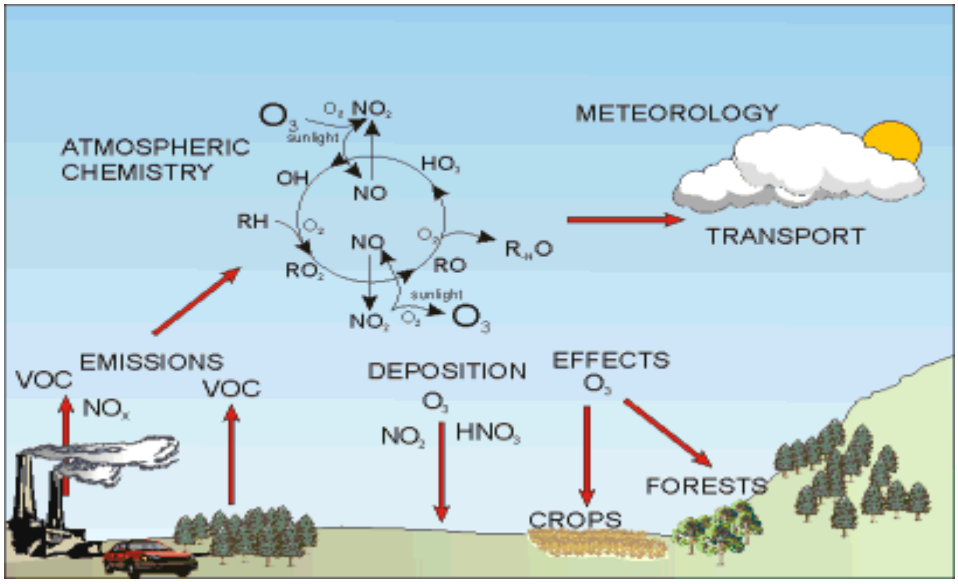


Fig 1.2: Schematic diagram showing the emission of air pollutants (NO_x and VOCs) from different sources and its conversion process to ozone with the help of light and then transported to different places where it can damage vegetation (Source: www.ars.usda.gov)

1.1.4.1. O_3 Impacts

O_3 is an important part of the air pollution climate in urban-industrialized areas of the world. Its effects are not localized, because O_3 precursor's travels long distances in the atmosphere depending upon the wind speed and direction, with higher concentrations often found in rural areas. O_3 pollution is known to have substantial effects on agricultural production in North America, Western Europe and many other countries of the world (Zeiger and Taiz, 2006; Wang *et al,*. 2005). Northern hemisphere O_3 concentrations, and especially those in developing countries, are expected to rise from mean 10ppm to 20ppm O_3 concentrations in the atmosphere by the end of 21st century due to the increase in its precursor emissions (oxides of nitrogen and volatile hydrocarbons), which is

linked to the increase in the number of motor vehicles and industrialization worldwide (Wahid *et al*. 2006a). O_3 is a highly reactive substance and, before entering the stomata, can damage the receptors of the guard cells near the stomata, which are then unable to respond to environmental signals (Calatayud *et al*., 2002). Inside the leaf apoplast, it reacts with water to form free redicals like hydroperoxide and superoxides, which reacts with intercellular fluid in the cell wall and alters the mesophyll cells just inside the epidermis, which are the main route of CO_2 from the stomata to the cells responsible for photosynthesis. It also affects the ion balance near the chloroplast that is linked to the synthesis of energy molecule adenosine diphosphate (ATP) (Calatayud *et al*., 2002; Zeiger and Taiz, 2006).

Fig 1.3: Ozone injury to soybean foliage (Koiwai et al., 1974)

O_3 toxicity causes different foliar injury symptoms, such as flecking, stipples, bronzing (Fig 1.3) and reddening. Usually the symptoms appear when the plant is exposed to a high concentration of O_3 for a short time period (Kley *et al*., 1999). O_3 symptoms typically occur between the veins on the upper leaf surface of older and middle-aged leaves, but may also involve both leaf surfaces (bifacial) for some species. The type and severity of injury is dependent on several factors including the duration and concentration of O_3 exposure, weather conditions, and plant genetics. Longer exposure to O_3 can often result in major crop yield losses

and reductions in annual biomass increments for forest trees (Fuhrer *et al*., 1997). Other studies have shown that O_3 can alter the nutritional value of crops such as wheat and soya bean (Agrawal *et al*., 2006).

1.1.5. Suspended Particulate Matter (SPM)

SPMs are finely divided solids or liquids that are dispersed through the air and are formed from combustion processes, and domestic and industrial activities, and from natural sources such as volcanoes, dust and forest fires (Emberson *et al.*, 2001). SPM in the atmosphere includes aerosols and fine particles and is commonly defined as two size classes, PM_{10} (particles with aerodynamic diameter <10μm) and $PM_{2.5}$ (Particle with aerodynamic diameter < 2.5μm). SPM can be a local problem close to large sources, but under certain conditions, it can be a regional scale pollution issue e.g. in South Asia (Fig 1.4).

Figure 1.4: Aerosol pollution over Northern India and Bangladesh (source NASA, 2006)

The particle size and composition depends upon the source e.g. mineral dusts originate from mineral oxides and the material from the earth's crust (UNEP, 2007). SPM is generally divided into two types; primary particles, which

originate directly from sources, and secondary particles, formed by the combination with other compounds e.g. the photo-oxidation of NO_x to form nitrates.

1.1.5.1. SPM Impacts

SPM can affect plants in a wide variety of ways, depending upon the composition of the particle, and is known to have direct or indirect effects on agricultural plants. Dust particles are of localized importance near roads, quarries, cement works, and other industrial areas. Apart from screening out sunlight in the atmosphere, the direct impact of the dust on leaves reduces radiation to chloroplasts, and stomatal conductance, and can affect control of water loss by physically preventing stomata closure (Zeiger and Taiz, 2006). SPM can also affect the photosynthetically active radiation (PAR) reaching the ground during pollution episodes (Siegfried *et al*. 1982). The direct impact of particles containing contaminants such as heavy metals can also cause phytotoxicity (Erickson, 1979). Accumulation of particulates on the surface of the plants can indirectly alter plant susceptibility to pathogens and pests (Emberson *et al.*, 2001).

1.1.6. Fluorides

Fluorine (F) does not occur in its elemental state in nature, because it is the most electronegative element in nature. It reacts with all elements, except oxygen and the noble gases, to form fluorides (WHO, 1984). Fluoride air contamination is mostly produced from industrial processes. The most important fluoride emitting industrial sources are aluminium smelters, phosphate fertilizer factories, and brick kiln factories (Xie *et al*., 2003), which mainly produce hydrogen fluoride (HF)

and sodium fluorides. Coal combustion, steel and glass works may also significantly contribute to fluoride emissions (Emberson *et al*., 2001).

1.1.6.1. Fluoride Impacts

Fluorides have wide range of impacts on the environment, not only on humans and animals, but also on vegetation. Almost all soils and waters contain inorganic and organic fluorides, while fluoride accumulates in humans by consumption of plants and animals for food, and through water (WHO, 1984). Fluoride in plants contributes to human and animal dietary fluoride (Weinstein, 1977). Exposure of plants to airborne fluorides leads to deposition on the outer surface and uptake into plant tissues. Long-term exposure of plants to fluoride concentrations of >0.2μg m^{-3} may cause injury to plants (WHO, 1984). Fluorides, usually in the form of HF gas, diffuse through stomata and enter the intercellular spaces. Fluoride is then transported through the transpiration stream to the margins and tips of the leaf (Fig 1.5), where it accumulates and causes injury as a grey or light-green water-soaked lesion, which turns tan to reddish-brown (Weinstein and Davison, 2003). Less than 10 mg kg^{-1} F is usually found in the foliage of plants grown in uncontaminated soils (Davison *et al*., 1985). Close to fluoride sources, the fluoride concentration in the foliage of the plant can increase to several hundred to 1000 mg kg^{-1}, depending upon the concentration of fluoride in the air, soil and type of plant species (Weinstein and Davison, 2003).

Figure 1.5: Fluoride injury to plum foliage (Taylor, 1973)

The most susceptible plant species to fluorides are apricot, barley, blueberry, peach, grape, plum, sweet corn and tulip. Resistant plants include alfalfa, asparagus, bean, cabbage, carrot, cauliflower, cucumber, eggplant, pea, pear, pepper, potato, squash, tobacco and wheat (Weinstein and Davison, 2003).

1.2. AIR POLLUTION IN PAKISTAN

Pakistan is a country located in South Asia. It has a 1,046 km coastline along the Arabian Sea and Gulf of Oman in the south, and is bordered by Afghanistan and Iran in the west, the Republic of India in the east and the People's Republic of China in the far northeast. Pakistan has total land area of about 80 million ha, of which 22 million ha is cultivated for crop production. About 80% (18 million ha) is cultivated by irrigation, while the remaining area is rain fed. Agriculture contributes to the growth of supplies of raw materials to industry as well as providing a market for industrial products. It also contributes 60% of Pakistan's export earnings. 67% of the population is directly involved in activities related to agriculture. The crop sector contributes about 60% of the agricultural GDP, compared to 40% from livestock, forestry and fisheries (Pakistan Economic Survey, 2006).

Air pollution is a fast growing problem in many developing countries including Pakistan, because of the increase in the population. Pakistan is the 6th largest country in the world in terms of population (160.9 million, Pakistan Economic Survey, 2006). Urbanization is on the rise as people are moving from rural areas to the cities in search of jobs, education and better standard of living. In the near future, half of the world's population will be living in cities (UNFPA, 2007). Other important factors contributing to the rise in air pollution include industrialization and unchecked vehicular emissions, brick kilns and industries, while some commercial activities, hotels, restaurants, households and solid waste disposal also cause air pollution (EPA, 2007). As a result, concentrations of toxic gases, such as CO, NO_x and SO_x, as well as smoke and dust, have increased in the air over recent decades (Wahid, 2006a). Of the total emissions in the city of

Lahore in the late 1990's, automobiles contributed 32% of suspended particulate matter (SPM), 92% of carbon monoxide (CO), 89% of hydrocarbons (HC's), 75% of nitrogen oxides (NOx), 45% of sulphur dioxide (SO_2) and 100% of aldehydes (Wahid and Marshall, 2000).

Much attention has been given to the impacts of air pollution on human health in large cities and suburban areas, but less attention has been given to its effects on agricultural production and quality. However, in many developing countries, particularly in parts of Asia, crop yield and forest productivity are being severely affected by ambient air pollution concentrations (Emberson *et al*, 2001). The average wheat yield in Pakistan was stagnant over the period from 2000-2007, while the population has increased significantly, widening the gap between demand and supply of basic staple food (Wahid, 2006a). Due to deteriorating air quality and the potential impacts of these pollutants on crop yield, there is an urgent need for air pollution impact assessment studies on the yield and nutritional value of crops in Pakistan, as the agricultural sector is sensitive to pollution effects and is of prime significance in feeding the fast growing human population (Bell and Treshow, 2002; Wahid *et al.*, 2006a).

However, little research work has been done in Pakistan on crop sensitivity to ambient levels of air pollution compared to other Asian countries, like India and China, and to Europe and North America. In Pakistan, experiments have been carried out mainly at one location in a suburban area of Lahore, on wheat, rice, soybean and barley, from the 1990's to 2006. These reveal the potential for Pakistan to suffer significant crop yield loss due to air pollution, mainly from tropospheric ozone (O_3). In these studies (described in detail in

Chapter 2 & 3), open top chambers and EDU experiments were carried out to check the effect of current levels of air pollution in Pakistan and to test the sensitivities of different varieties of wheat and other crops to ambient air pollution. These studies showed that air pollution can cause considerable damage to crop yield and growth; the annual yield loss in a suburban area of Lahore was about 40% in wheat and rice, and 57% in soybean, due to the effect of ambient air pollution (IIED, 2008). Wheat is the main staple food in Pakistan. Its yield is much lower than in the USA and many other countries including India; Pakistan is the 7th largest wheat growing country in the world, but still imports wheat to fulfil the demand of the increasing population (Wahid *et al.*, 2006a).

It is therefore essential to examine the current and future importance of the air pollution as a constraint to agricultural productivity in developing countries like Pakistan (Wahid *et al*., 2006a). Few steps have been taken to investigate air pollution impacts on the nutritive quality of crops and vegetables, and no steps have been taken to test the impact of air pollution on medicinal plants and on the forest trees that are abundant in northern Pakistan. The research studies in Lahore should be extended to the north and south of Pakistan to get a bigger and clearer picture of air pollution effects on crops and other sensitive plants, as population growth rates and emission levels are both rapidly increasing in the country (Wahid *et al.*, 2006a).

1.3. AIR POLLUTION SOURCES AND EFFECT ON PESHAWAR CITY

Peshawar is the capital of North West Frontier Province (NWFP). Its elevation is about 510 m and area is 2257 km² (Govt. NWFP, 2006). The population of Peshawar was about 2.9 million in 2005 (Pakistan Economic Survey, 2006). The city has a very strategic location as it is bounded by Afghanistan to the west and north, the territory of Jammu and Kashmir to the northeast, Punjab province to the southeast, and Baluchistan province to the southwest (Fig 1.6). It is also a cultural capital and is about 4000 year old. In the past, Peshawar was governed by the Gadara's, Persians, Mughals, Sikhs and the British kingdoms (Govt. NWFP, 2006).

Summers in Peshawar are hot, with a maximum temperature of 49°C, and a minimum of 25°C, with a mean temperature of 30°C (EPA, 2007). The winter season is also very distinct, with daily maximum daytime air temperatures of 16°C or less, and cold nights, with minimum air temperatures below 2°C. The average annual rainfall is 400 mm, and most rain falls during the summer monsoon from July to September. The cultivable area is 74,287 hectares (Economic Survey of Pakistan, 2006). The major cash crops are maize, wheat, rice and sugarcane and popular fruits are apricot, plum, pear and peach. Most of the land is irrigated by the Warsak dam, which is situated to the north west of the city (Govt. NWFP, 2006).

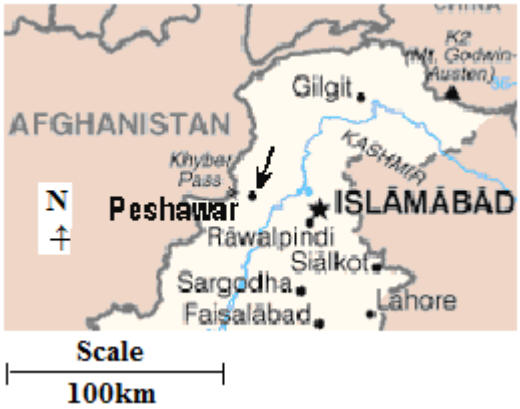


Fig 1.6: Peshawar located in the north west of Pakistan near the border of Afghanistan and 165 km away from Islamabad, the Capital of Pakistan (www.peshawar.png)

The large influx of Afghan refugees during the past 25 years has also led to a demand for more food, more shelter, more transportation and more manufacturing activities, which has ultimately resulted in a faster depletion of natural resources, deforestation, water pollution, air pollution and land pollution, particularly in Peshawar (EPA, 2007). Dense smoke emissions have also been reported from foundries, brick kilns and rolling mills in Peshawar (EPA, 2007). The major pollution sources in Peshawar are described below.

1.3.1. Vehicular emissions

Peshawar, like other major cities of Pakistan, is facing a serious air pollution problem from rapidly increasing traffic density. According to the Police Department in Peshawar, 42948 auto vehicles were registered in 2000, with an average annual increase of about 10 percent; these include 28,000 tri-wheeler 'rickshaws' which cause chaos on roads (Daily Times, 2007). Old vehicles which are not properly maintained were a particular problem. Lead and NO_x emissions from automobile exhausts are reportedly high in some parts of the city during traffic peak hours (EPA, 2007). This results in an increase of SO_2 and NO_2 concentrations in the city. A large amount of suspended dust is generated due to vehicles driving on unpaved road shoulders, and poorly maintained and

overcrowded roads. The 8h daily average concentrations of NO_x, SO_2 and PM in the air are about 80, 72 and 834 μg m^{-3} respectively, in central Peshawar (EPA, 2007). This situation is alarming from the point of view of both human health and effects on vegetation.

1.3.2 Industrial emissions

Regulations and zoning policies have not been clearly defined for setting up industries in Pakistan. In NWFP province, there are about 1500 factories most of which are located in and around Peshawar (Matthew, 2001). Because of the urbanization, cities have grown up around industrial areas that were originally located well outside the city limits. In Peshawar city, most of the stone crushing, marble, match and plastic goods factories are located within the walls of the city and thus there is a mix of residential, commercial, and small to medium-scale industrial establishments (EPA, 2007). Most of the industries discharge their waste, without any primary treatment, into the environment, contaminating air, soil, and water (Matthew, 2001). Untreated waste not only pollutes soil and surface water, but also threatens groundwater. Discharge of the untreated industrial wastes is contributing to water quality deterioration of the Kabul River system, which is the main water reservoir for irrigation. Besides raising the biological and chemical oxygen demand of the water, the effluents are adding a variety of toxic substances to the rivers that may bio-accumulate in fish and may be taken up by plants, and will hence have unknown implications and effects on the food chain (EPA, 2007).

Similarly, stack emissions from most of the industries are unregulated and uncontrolled except for a few industries which have installed treatment facilities.

These untreated gaseous emissions from small-scale industries are playing a major role in rising air pollution. Indiscriminate burning of municipal solid wastes also contributes to air pollution (Matthew, 2001).

1.3.3 Emission from brick kiln

Approximately 400 brick kilns were situated in and around Peshawar in 2000 (EPA, 2007). On average, a brick kiln producing 800,000 bricks uses large amounts of rubber to start fires and burns a total of eight tons of low-quality coal or 20 drums of used vehicle oil (EPA, 2007). The brick kiln emissions include many toxic pollutants such as nitrogen oxides (NOx), carbon monoxide (CO) and dioxins (EPA, 2007). The emission of smoke from brick kilns on the peripheries of cities and towns in Pakistan often causes a thick black smoke cover, especially in the evening. The rising number of brick kilns situated in NWFP province, particularly in Peshawar, have almost doubled the level of air pollution (NO_x and VOCs) in the city, mainly due to the use of large amounts of rubber, and the low quality of coal and used oil for burning purposes (EPA, 2007).

1.3.4. Brick kiln factory pollution

In the course of field research for this thesis (see Chapter 4), large visible black clouds of thick smoke were observed in the brick kiln area. The thick smoke was primarily emitted by brick kilns operating in the area. It was also noted that the brick kiln factories were constructed in the middle of agricultural farms and close to the houses of farmers' families (Fig 1.7). These brick kilns operate around the year nonstop and the operation is only halted during wet weather. Large trucks full of used rubber tyres (Fig 1.8) that were used as a fuel

were seen going into the brick kiln area. Brick kilns can produce toxic gases like HF, SO_x and NO_x (Pandey *et al*., 1985)

Figure 1.7: One of the hundreds of brick kilns working in the middle of agricultural areas in the outskirts of Peshawar

Figure 1.8: Transportation of large numbers of used rubber tyres to the brick kiln area for fuelling

1.4. AIM OF THE STUDY AND THESIS STRUCTURE

Keeping in mind the deteriorating air quality, and the air pollution levels that have been recorded in Peshawar, there are potential effects of air pollution on local crops. However, no studies haves been carried out to date. Therefore the principle aim of this study was to assess the potential effects of two major air pollutants on crops in Peshawar.

The work described in this thesis focused on two major phytotoxic pollutants, ozone (O_3) and hydrogen fluoride (HF). O_3 was selected for this study, as it is a regional pollutant that can reduce the yield of important cash crops, such as wheat and rice that can affect livelihood and sustainability. Previous studies in Lahore (Wahid et al., 2006a & b) have found negative effects of O_3 on different crops. HF is a local rather than a regional pollutant, and was selected mainly due to its phytotoxic nature and the fact that it is thought to be emitted by the large number of brick kilns operating in and around Peshawar. The main objectives of the study were:

- To assess the O_3 and HF concentrations during the summer and winter seasons
- To identify the visible injuries to different local crops possibly caused by O_3 and HF
- To identify the sensitive and resistant local crop species to O_3 and HF
- To assess the response of individual crop species to a range of O_3 and soil fluoride concentrations under controlled conditions

These objectives were achieved by a combination of field surveys and experiments in Peshawar, and controlled experiments in the UK. Concentrations of O_3 and HF were measured using passive samplers (Chapter 2 & 4) at different times of the year in Peshawar. Field surveys aimed to identify and assess visible injuries due to air pollution to different cash crops, fruits and vegetables in Peshawar (Chapter 2 & 4). During the surveys, plant and soil samples were also collected from different polluted and control sites for fluoride analysis (Chapter 4).

Apart from the field surveys, an EDU application experiment was carried out in Peshawar on winter spinach (Chapter 3), and O_3 fumigation experiments in open top chambers (OTCs) were conducted on onion and winter spinach at Imperial College London (Chapter 2), to asses the impact of O_3 on growth, yield and visible injuries. A local spinach variety was selected for both the EDU and OTC fumigation experiments because of the lack of previous experimental data and also its high nutritional value. Onion was chosen for OTC O_3 fumigation experiment because of its sensitivity to O_3 and also because suspected O_3 visible injury was identified on onion leaves during the summer research survey in Peshawar (Chapter 2). Finally, a Pakistani wheat variety, collected from an area of Peshawar polluted with HF, was grown in alkaline soil at different levels of NaF in greenhouse conditions to assess the effect of soil fluoride on growth and visible injury of wheat (Chapter 5). This experiment was conducted based on the evidence of visible leaf injury damage to wheat in HF polluted area of Peshawar (Chapter 4) and to get a clearer picture of whether the injury is caused by soil fluoride or HF in the air emitted by nearby brick kiln factories.

The research described in this thesis is a step forward in providing an assessment of the risk posed by these two pollutants to different cash crops, fruits and vegetables in the Peshawar area. This study also aims to help with the broader issue of saving Pakistan from future food security crises, when the economy is already in turmoil.

1.5. SELECTED RESEARCH FIELD SITES IN AND AROUND PESHAWAR

Five sites were selected in and around Peshawar city to investigate the effect of ambient air pollution on crops and vegetables. The sites were at the Agricultural University (AUP), in agricultural fields near brick kiln factories (BKF), at the Agricultural Research Institute (ARI), in Hayatabad township and at Charsadda, which served as a control site. The locations are shown in Figure 1.9. These sites were selected on the basis of their distance from the main roads and population, the presence of a range of agricultural crops, and their proximity to pollution sources.

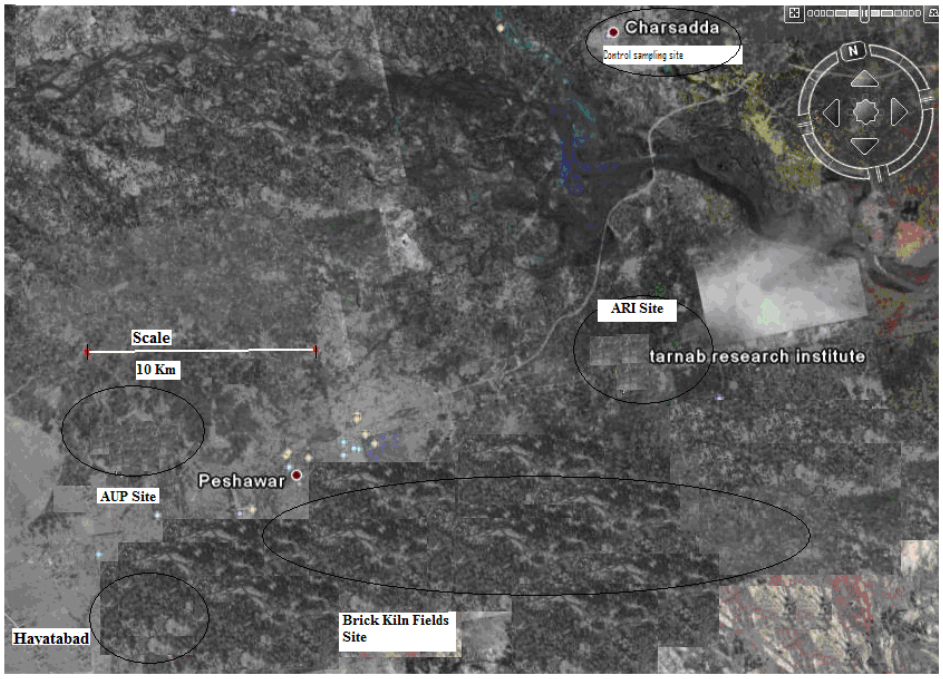


Fig 1.9: Selected sampling sites of AUP, ARI, brick kiln field, Hayatabad and Charsadda (Control) in Peshawar city (Source: Google earth, 2008)

1.5.1. The Agricultural University Peshawar (AUP)

The Agricultural University is situated on the western side of Peshawar, about 2 km from the city. It has a research farm of 700 acres, where most of the graduates carry out research work (Fig 1.10). All most all cash crops and fruits are grown here. The entire farm is irrigated by a canal which originates from Warsak dam. This site was chosen because it is located on the opposite side of the city to the large brick works area, and it is also situated near the main city road.

Fig 1.10: Agricultural University Peshawar (AUP) Site (Source: Google Earth, 2008)

1.5.2. Brick Kiln Field (BKF) site

Most of the brick kiln factories are located on the south eastern side of the city, starting about 2 km from Peshawar, and spreading well an over area of about 40km^2 (Figure 1.11). There are about 400-450 brick kiln factories actively operating in the area (EPA, 2007). These factories have rapidly increased in the last 10 years or so, because it is believed that the soil of this area is considered best for brick making. The bricks from here are exported all over the country and

are also exported to Afghanistan. This area is also one of the most fertile areas in the suburban regions of Peshawar for growing crops.

Fig 1.11: Brick kiln factories (BKF) are clearly visible and can be seen in oval shape in between the agricultural land, situated to the east of Peshawar (Source: Google Earth, 2008)

1.5.3 Agricultural Research Institute Tarnab Site (ARI)

ARI is one of the oldest agricultural institutes in the province, and is spread over 100 acres of land. It is situated to the north east, and about 12km from Peshawar city. Scientists grow different varieties of cash crops and vegetables for research purposes, and several varieties of wheat have been developed here. It is also situated near the busy main Great Trunk (G.T) road, which links Peshawar to Islamabad (Fig 1.12).

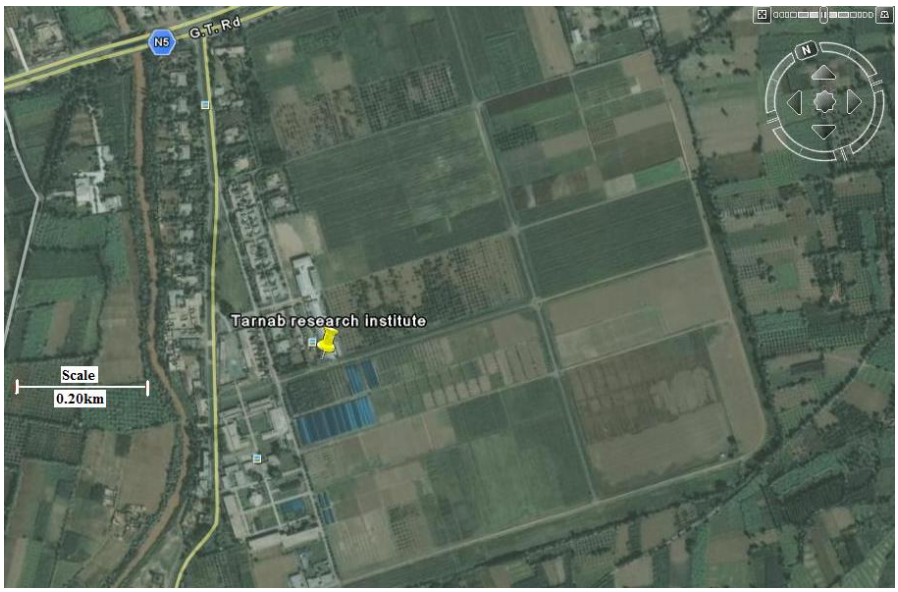


Fig 1.12: ARI Peshawar (Source: Google Earth, 2008)

1.5.4. Hayatabad site

Hayatabad town is located south west of the city of Peshawar (Fig 1.9); about 4km from the brick works fields. It is a modern town with all the basic facilities. It was built in 1975 and currently half of the city population lives here, most of them are the Afghan refugees.

1.5.5 Charsadda (Control site)

This site is located about 30 km north of Peshawar city (Fig 1.9). Charsadda is one of the oldest towns of the subcontinent dating back to the era of the Gandharas', when it was the capital. It was chosen because there are no brick works or major industrial activity in this area, and hence this area was used as a control site.

CHAPTER 2

O3 IMPACT ON CROPS IN PESHAWAR

2.1. INTRODUCTION

Air pollution is one of the main problems faced by the modern world. Among the main pollutants is O_3, which is harmful to humans and animals, as well as plants (Emberson *et al.*, 2001). A rise in urbanization increases NO_x and VOC emissions from transport and industry, which are mainly responsible for the gradual rise of ground level O_3 concentrations that is affecting crop growth and yield around the world (Emberson *et al.*, 2001; Ashmore, 2005). In Pakistan, ambient O_3 concentrations have been found to cause significant yield reductions in different varieties of wheat, rice and soybean in a suburban area of Lahore (Wahid *et al.*, 1995a & b; Maggs and Ashmore, 1998; Wahid *et al*., 2001 and Wahid, 2006a & b). The yield loss at this site was estimated to be about 40% in wheat and 57% in both soybean and rice (IIED, 2008).

There is a need to assess the impact of ambient O_3 on crops and vegetables in other regions of Pakistan, including Peshawar, because no such studies have been carried out previously in these regions. For this purpose, a visible injury symptom survey was carried out during the summer and winter at different sites around Peshawar on a variety of crops. An EDU experiment on winter spinach was also performed from October to December, 2008, at the Agricultural Research Institute (ARI), for the first time in this region (see Chapter 3). Open top chamber O_3 fumigation experiments on winter spinach and onion varieties that are grown in the Peshawar region were conducted in the summer of 2009, at the

Silwood Park campus, Imperial College London, to assess their sensitivity to a range of O_3 concentrations.

Spinach was chosen for the OTC experiment as it is sensitive to O_3 and a very important vegetable in the Peshawar region. Spinach has been cited as a very sensitive crop having shown visible injuries and yield reduction to O_3 by Sakaki *et al.* (1983), David (1998), Agrawal *et al*. (2003), Calatayud *et al.* (2003), Singh *et al*. (2005), and Tiwari and Agrawal (2009). Sakaki *et al.* (1983), David (1988), Takahama (1992) and Calatayud *et al.* (2003) used *Spinacia oleracea* varieties in their study, while Singh *et al*., (2005), and Tiwari and Agrawal (2009) worked on the effects of O_3 on the summer spinach variety *Beta vulgaris*, All Green.

Spinach is one of the most important vegetables in Pakistan and is an excellent source of iron and calcium, as well as beta carotene, vitamin C and E, potassium, sulphur, sodium, folic acid and oxalic acid (Singh *et al.,* 2005). It has more protein than most vegetables and it is one of the vegetables with the highest amount of chlorophyll. Spinach, which is locally known as 'palak', is an annual dioecious plant belonging to the family Chenopodiacea. There are two main varieties of spinach grown in Pakistan. One, called winter spinach (*Beta vulgaris* L), has thin leaves, is grown in October and harvested in February. The other needs a somewhat warmer climate and is called summer spinach (*Beta vulgaris cicla*); it has thick leaves, is grown in early spring and harvested in summer. Winter spinach is more commonly consumed because of its flavour and good taste. Spinach also provides a cheap source of nutrients for the lower and middle classes of Pakistan (Waseem *et al*., 2001); therefore it is important to study the

effects of air pollution, especially ambient O_3, on the growth and yield of winter spinach.

Winter spinach is grown in Pakistan on a large scale (Waseem *et al*., 2001; Hanif *et al*., 2006). Spinach is generally grown in broadcasting form (scattering of seeds by hand, or mechanically on flat beds) in Pakistan (Waseem *et al*., 2001). In the 2002-03 season, the total spinach cultivated area in NWFP was 15,798 hectares, with a total production of 172,542 tonnes (Agricultural statistics of NWFP, 2002-2003). This production is for all varieties grown in the region.

Onion was selected for the OTC experiment because it is termed very sensitive to O_3 (e.g. Temple *et al*., 1990; McCool *et al*., 1987; and Engle *et al*., 1965). As there was no previous study on the response of local varieties of onion to O_3, it was important to carry out an OTC O_3 fumigation experiment in controlled conditions in order to assess the sensitivity of the local onion variety, Swat-1, to O_3.

Onion (*Allium cepa* L.) is one of the most important crops grown around the world in different seasons. It is used in green form (salads) as well as in mature form (bulb). In Pakistan, because of its flavour, it is an important ingredient of recipes such as soups, sauces and a number of seasonal foods (Khan *et al*., 2005). In recent years, onion production has increased gradually. According to the Agricultural Statistics, Government of Pakistan, the total cultivated area of onion in Pakistan in 2001 was 105.6 thousand hectares, with a total production of 1563.3 thousand tonnes of onion bulbs. In NWFP, the total cultivated area was 6,900 hectares, with a total production of 120,500 tonnes, in 2001. Calcium,

carbohydrates and phosphorus are the main nutrients of onion bulbs, along with lower amounts of protein, sodium and potassium (Nayerabi, 2001). In Pakistan, it is sown in January/February and harvested in June/July (Jilani and Ghaffoor, 2003). It is first sown in a form of nursery by sowing seedlings in seed beds for 4-6 weeks, and then transferred into the field with 10cm distance between plants and 20-25cm between the rows (PARC, 2009). After sowing and transplantation, water is given immediately and then after every 7-10 days, depending upon the environmental conditions. Irrigation is stopped before neck fall and plants are harvested after 75% of the plants show neck fall (PARC, 2009). There are different varieties grown in different provinces of Pakistan as shown in Table 2.1.

Table 2.1: Different varieties of onion grown in four provinces of Pakistan (Source: PARC, 2009)

Provinces	**Varieties**
Punjab	Desi red
NWFP	Swat-1
Sindh	Phulkara
Baluchistan	Sariab Red

2.1.1. Aims and Objectives

Overall, this study aimed to identify the extent of O_3 injury to summer and winter crops, and also to provide information regarding the potential for O_3 damage to crops, in Peshawar.

The specific objectives were:-

1. To assess the extent of O_3 visible injuries in the Peshawar region, characterised by chlorosis, necrosis and white stippling to different crops.

2. To assess the ambient O_3 concentrations at ground level in the Peshawar region, using passive samplers.
3. To assess the effect of different O_3 concentrations on spinach and onion growth and biomass in open top chambers (OTC).
4. To assess the effect of different O_3 concentrations on visible injuries to spinach and onion leaves in OTC conditions.
5. To compare the results of the OTC studies with O_3 exposures in Peshawar and other regions of South Asia.

2.2. SURVEY FOR O3 VISIBLE INJURY

2.2.1. Materials and methods

Two sites were selected in and around Peshawar city to investigate the effects of ambient O_3 pollution on crops and vegetables. The sites were at the Agricultural University (AUP) and Tarnab Research Farm (ARI). Locations and details of the sites and why these sites were selected for the O_3 survey can be found in Chapter 1.

Pollution and climate data were closely monitored at the experimental sites in order to relate any foliar injury symptoms to the pollution climate and to provide information on the risk resulting from O_3. The O_3 concentration was measured using a passive sampler delivered by IVL in Gothenburg, Sweden. The passive sampler contains a reactive substance (nitrite) protected by a net. O_3 in the air diffuses into the filter and reacts with nitrite to form nitrate. The O_3 concentration was calculated from the exposure time (4 weeks), and the initial and final nitrate concentration (www.cma.gva.es). Passive samplers were installed at AUP and ARI from February, 2008 to June, 2008 and from November to December 2008, only at the ARI site. The samplers were kept under metal plates to avoid direct sunlight and rain. The plates were then attached to wooden poles at 2m height from the ground. Air temperature and humidity was monitored by the local weather station at ARI and AUP by taking readings at 8:00am and 5:00pm on a daily basis.

2.2.1.1. Identification of O_3 visible injuries

The foliar injury survey was carried out from February to June 2008, and in November and December 2008. Observations on different crop leaves were

carried out at the AUP and ARI sites at weekly intervals. The mature leaves of lady finger (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), sugarcane (*Saccharum officinarum*), maize (*Zea mays*), onion (*Allium cepa* L.), summer spinach (*Beta vulgaris*), peach (*Prunus persica*) and grapes (*Vitis vinifera*) were investigated during the summer survey, whereas winter spinach (*Beta vulgaris*), wheat (*Triticum aestivum*) and clover (*Trifolium repens*) were examined during the winter survey. The crop species were selected for the survey because of their importance in the agricultural economy of the region. The injuries were identified as typical tip burn and leaf interveinal necrosis. The injuries were compared to published pictures of O_3 injury to crops in Europe from the ICP Vegetation (www.icpvegetation.ceh.ac.uk). The damage by O_3 was assessed on the basis of % injury to mature fully expanded leaves as follows:

No damage = 0% injury

Little damage = <30% injury

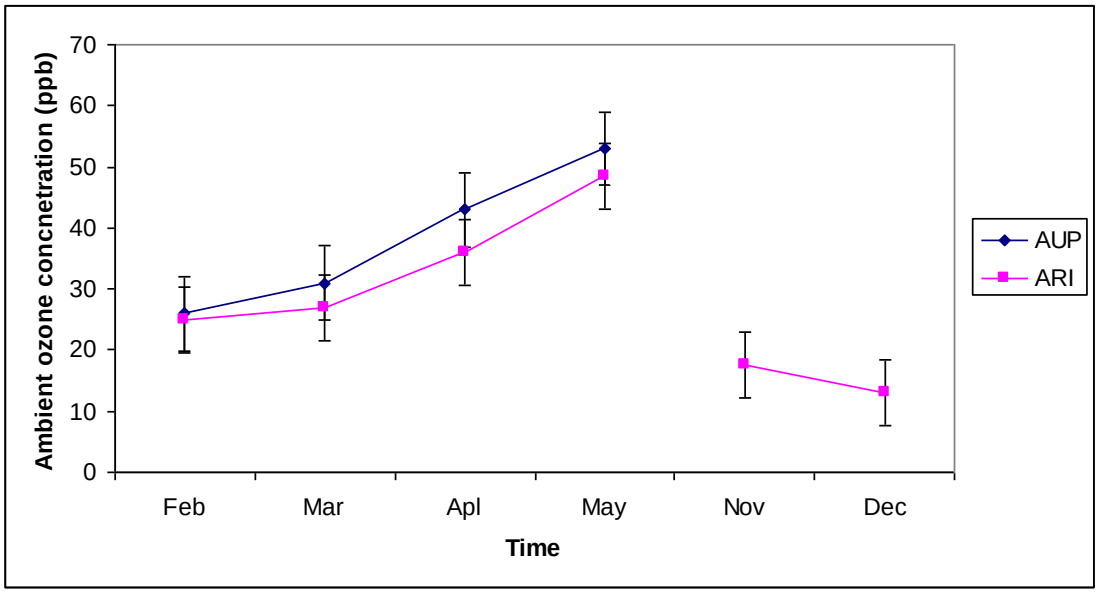
Significant damage= >30-70% injury

Severe damage = >70% injury

2.2.2. Results

The passive sampler data (Figure 2.1) show the O_3 concentrations at both AUP and ARI sites. The concentrations measured by the passive samplers, expressed as 4 week mean values, ranged from 26 to 53ppb at AUP, and 25 to 48.5ppb at ARI, from mid February to June. The O_3 concentration increased significantly from spring to summer and was higher at AUP compared to ARI (Fig 2.1). The mean monthly O_3 concentration was between 25-30ppb in February and March at both sites, but increased to 35-55ppb at both sites in April and May (Fig 2.1). The overall mean O_3 concentration was 38.2ppb and 34ppb for AUP and ARI, respectively. The O_3 mean monthly winter concentrations were low compared to summer O_3 concentration values, and ranged from 12-22ppb in November and December, 2008 (Fig 2.1). Winter O_3 measurements were only made at ARI.

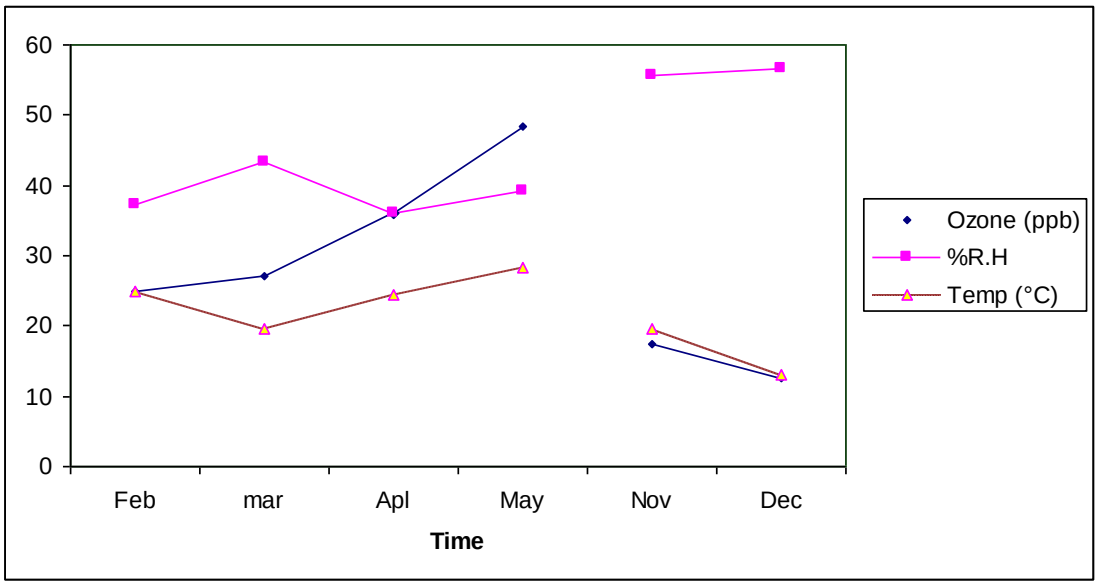
Figure 2.1: O3 concentrations (ppb) at AUP and ARI. Error bars indicate standard error of the values



The O_3 concentrations trended to increase with an increase in air temperature. Monthly mean O_3 concentrations of 35-40ppb and above only

occurred with a temperature of 25°C or above, although February had a high mean temperature but relatively low mean O_3 values (Fig 2.2). However, there was a negative influence of relative humidity on monthly O_3 concentration during the two surveys, as shown in Figure 2.2. The mean relative humidity was higher in winter and spring when the O_3 concentrations were lower and was lower in summer when the O_3 concentrations were at their peak (Fig 2.2).

Figure 2.2: Mean monthly O3 concentrations (ppb), temperature (°C) and % Relative humidity at Peshawar, from February to June and from December to November, 2008.



No O_3 injuries were recorded during the spring season (February-March). However, suspected O_3 foliar injuries were observed on potato and onion at the ARI site in the month of May, when the mean O_3 concentration was 48.5 ppb (Figure 2.3 & 2.4). Typical black flecking was observed on mature leaves of potato grown at ARI, when the plants were three months old. It was noted that suspected O_3 injuries appeared when the plants were about three months old, during the month of May 2008. Suspected severe injury in form of white stippling and necrotic tip burn was also observed on leaves of most onion plants at the ARI. Suspected O_3 injury (white stippling) was also found on cotton leaves at AUP site in mid May when the mean O_3 concentration was 53ppb (Figure 2.5). There was

no O_3 injury found on wheat, peach, cucumber, tomato, summer spinach, lady finger, maize, sugarcane and grapes during the summer survey period (February to June) as it was very difficult to distinguish O_3 injury from insect injury (Figure 2.6). Plants that had insect infestation on their leaves were not considered for O_3 injury assessments, e.g. for clover. No O_3 injuries were seen on crops during winter surveys at the AUP and ARI sites.

Fig 2.3: Suspected O_3 injury to potato leaf at ARI Date 19/05/2008

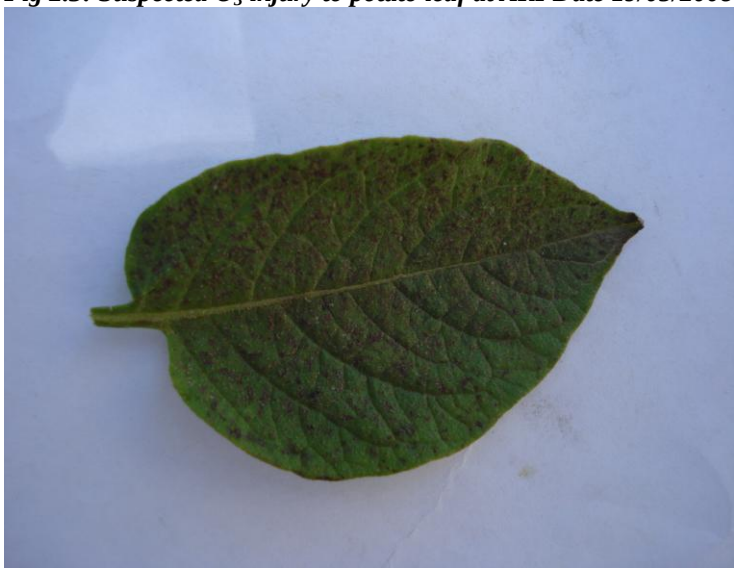


Fig 2.4: Suspected O_3 injury to onion leaf at ARI Date 19/05/2008

Fig 2.5: Suspected white stippling injury to cotton leaf at AUP Date 19/05/2008

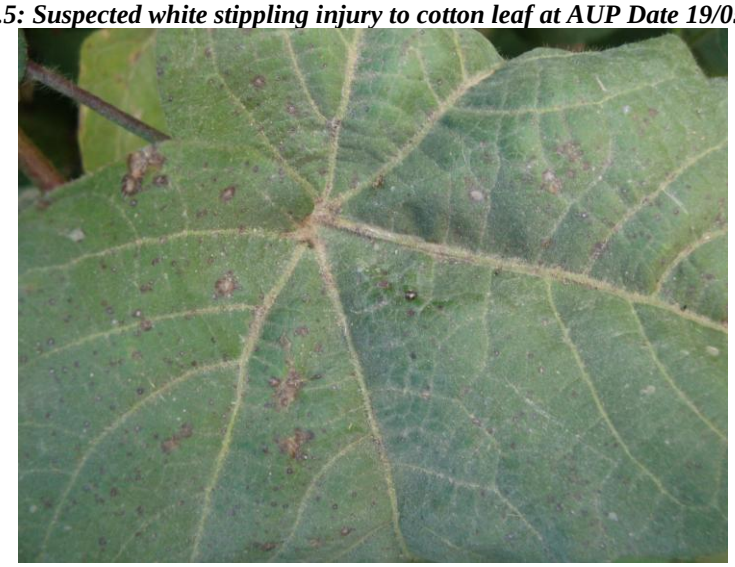
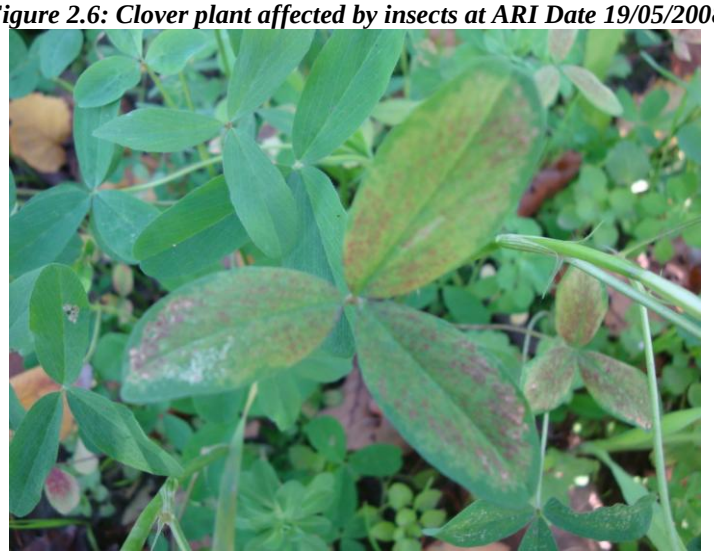


Figure 2.6: Clover plant affected by insects at ARI Date 19/05/2008



The results from the O_3 injury surveys are summarised in Table 2.2. The survey showed that some summer crops are at risk to visible injury due to high O_3 concentrations. Most of the O_3 injury on crops was found at ARI, although the O3 concentration was slightly higher at AUP than at ARI (Fig 3.1). The most O_3 injured crops were potato and onion at the ARI site, while typical white stipples were found on cotton leaves at AUP. Injury to clover was not very clear as it was under infestation of insects at AUP, because of which it was very difficult to assess it for O_3 visible injury. No O_3 injury was found on other crops at both sites.

Table 2.2: The extent of damage and suspected O_3 injury symptoms of summer (S) and winter (W) crop species pollution at AUP and ARI sites. The injury to leaves was categorised into no injury, little injury, significant injury and severe injury.

Sites	Plant species	No injury	Little injury	Significant injury	Severe injury	Leaf injury symptoms
ARI	Tomato (S)	X				No Injury
	Potato (S)			X		Black flecking
	Onion (S)			X		White stipples & leaf tip burn
	Spinach (S, W)	X				No Injury
	Cucumber (S)	X				No Injury
	Wheat (W)	X				No Injury
	Lady finger (S)	X				No Injury
	Maize (S)	X				No Injury
	Clover (W)	X				Interveinal necrosis
	Sugarcane (S)	X				No injury
	Peach (S)	X				No Injury
	Grapes (S)	X				No Injury
AUP	Tomato (S)	X				No Injury
	Potato (S, W)	X				No Injury
	Onion (S)					Tip burn
	Spinach (S, W)	X				No Injury
	Cucumber (S)					Necrotic margins
	Wheat (W)	X				No Injury
	Lady finger (S)	X				No injury
	Sugarcane (S)	X				No injury
	Maize (S)	X				No Injury
	Cotton (S)			X		Necrosis and white stipples
	Clover (W)	X				No Injury

2.3. EFFECT OF O_3 FUMIGATION ON SPINACH

2.3.1. Materials and methods

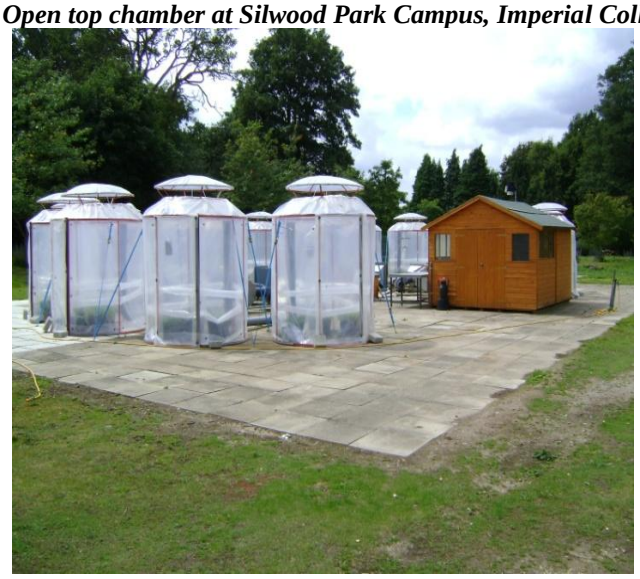
2.3.1.1. Plant growth and O_3 exposure

In the summer of 2009, plants of a Pakistani spinach '*Nare palak'* variety (*Beta vulgaris*) were exposed to four different target concentrations (FA, 30, 60 and 90ppb) of O_3 in open top chambers (OTC) at Silwood Park, Imperial College London for 4 weeks. The same variety was used earlier for the EDU field study at Peshawar (see Chapter 3). Spinach seeds were first sown in nursery trays using Westland John Innes No. 2 compost soil inside the greenhouse at Imperial College on 1st June 2009. After germination, one week old plants were transferred to 30x30 pots, each pot carrying five plants. Before transplantation, pots were filled with Westland John Innes No. 2 compost and were irrigated with tap water. After transplantation, a total of 32 pots were transferred carefully to 16 chambers on 6th June 2009, with each chamber containing two pots.

The 16 chambers were divided into four O_3 treatments (Fig 2.7). The chambers were 2m high and 2m in diameter. The distance between each chamber was 0.5m. Each group of four chambers received FA (charcoal filtered air) daily from 5pm to 9am, with O_3 added at 0ppb, 30ppb (low), 60ppb (medium) and 90ppb (high) between 9am to 5pm. Pots were watered with tap water as required, depending upon environmental conditions. Pots within the treatments were moved between replicate chambers on a weekly basis. No pesticide applications were made during the experiment. The plant height and leaf number were recorded on Day 1 of the O_3 fumigation and then at weekly intervals. Air temperature and relative humidity was monitored by the local weather station at Silwood Park throughout the experiment.

The actual O_3 concentrations were monitored by O_3 monitor. O_3 exposures were summarised as an AOT40 value for each individual chamber over the course of the experiment. AOT40 is the accumulated O_3 exposure over a threshold level of 40ppb (Mills *et al*., 2007).

Figure 2.7: Open top chamber at Silwood Park Campus, Imperial College London



2.3.1.2. Injury assessment

Injury assessments were made on a weekly basis. The injury assessments were carried out based on the number of damaged fully expanded leaves. Each plant was graded as either healthy (H) or abnormal, with the cause of the abnormality graded as 1 (slight), 2 (moderate), or 3 (severe) using the key in Table 2.3.

Table 2.3: Abbreviations for each injury caused by different factors

Abbreviations	Injury names
S1, S2, S3	Stunted
D1, D2, D3	Diseased
I1, I2,I3	Insect damaged
SL1,SL2,SL3	Slug Damaged
A1,A2,A3	Animals (rabbits, goats, birds etc.)
V1,V2,V3	Virus

The injury assessments for O_3, based on the fraction of fully developed/expanded leaves exhibiting injury, were also made on a weekly basis, so that the date of first occurrence of O_3 injury could be noted (Table 2.4).

Table 2.4: The injury assessments for O_3, based on the fraction of fully developed/expanded leaves

I.D	***Injury scale***
0	No injury
1	Very slight injury, < 5 % of fully expanded leaves with slight injury
2	Slight injury, 5 -15 % of fully expanded leaves with slight injury
3	Moderate injury, 15-30 % of fully expanded leaves with injury
4	Heavy injury, 30-50 % of fully expanded leaves injured
5	Very heavy injury, 50-90 % of fully expanded leaves injured
6	Total injury, 90 -100 % of fully expanded leaves are injured

2.3.1.3 Plant harvest

The spinach plants were harvested after 4 weeks of fumigation, on 2nd July 2009. The experiment was halted ahead of the planned time as the spinach plants flowered prematurely, and also there was increased insect attack by green aphids due to the high temperature and humidity. The entire above-ground biomass of each plant was put into a labelled paper bag (i.e. one bag per plant). The labelled bags containing spinach plants were brought to the laboratory of the University of York within 24 hours. Upon arrival, each plant was separated into stem, live leaves and dead leaves. The plants were weighed using a high precision electronic balance (AND, 202) and then dried at about 70°C for about 48 hours in an electric oven. After drying, the samples were weighed again to obtain dry weight data.

2.3.1.4. Statistical summary

The data summary (means and standard errors) was carried out using Microsoft Excel (2003) and the statistical analysis was carried out using SPSS 17.0. The dataset for all parameters was explored for skewness, kurtosis and normality,

using the Shapiro-Wilk-test. The insect injury parameter, showing major deviations from a normal distribution, was normalised by using log_{10} transformation. One-way ANOVA was then used for each of the parameters to determine whether the effect of O_3 was significant. Tukey's HSD post hoc test was used for multiple comparisons between treatments at $p<0.05$.

2.3.2. Results

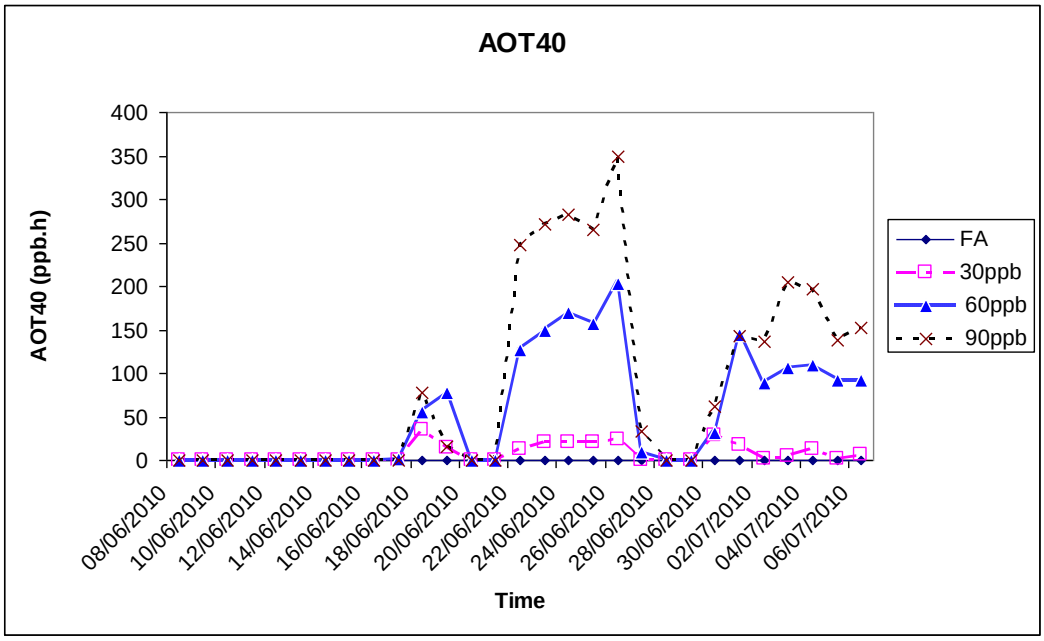
2.3.2.1. O3 exposure

The mean air temperature and relative humidity, from the local weather station near the OTCs, for the whole experimental period was 21.4°C and 61.6%, respectively. There was little O_3 exposure, and no difference between the AOT40 values in the chamber treatments, during the first 2 weeks of O_3 fumigation, mainly due to malfunctioning of the O_3 generators (Fig 2.8). There was significant O_3 exposure in the final 2 weeks, although there was a gap for 3 days from 28-30th June.

Table 2.5: ANOVA results (probability values) showing the difference between the AOT40 values among the treatments for the entire spinach experiment. Post-hoc differences were tested for significance at P=0.05.

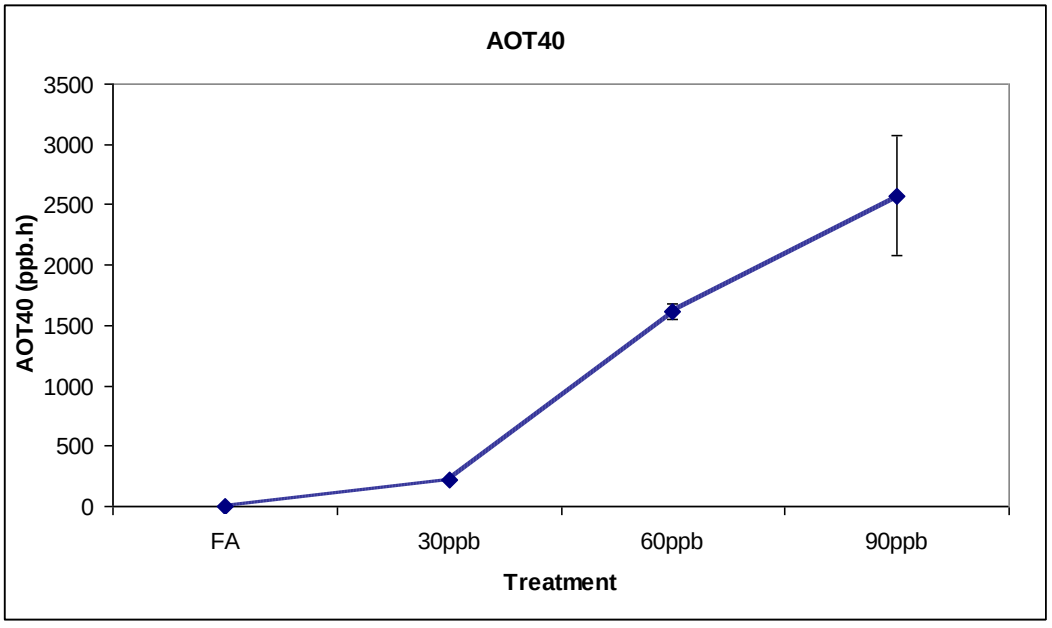
Chambers	Effects of chambers	Post-hoc
O3 concentration (ppb.h)	0.000	90ppb,60ppb>FA,30ppb

Fig 2.8: Mean AOT40 (ppb.h) values for each day for the entire spinach experimental period



According to the ANOVA test (Table 2.5) AOT40 values in the 60ppb and 90ppb chambers were significantly higher than those of FA and 30ppb. However, AOT40 in the 90ppb chambers was not significantly different from 60ppb chambers (Table 2.5; Fig 2.9). The mean AOT40 values for the whole experiment for the 60 and 90ppb chambers were 1615ppb.h and 2574ppb.h, respectively (Fig 2.9).

Fig 2.9: Mean AOT40 (ppb.h) values for FA, 30, 60 and 90ppb chambers for the entire spinach experimental period. Error bars show the standard errors of the values in individual chambers



2.3.2.2. Statistical summary

The ANOVA results of the effects of four weeks O_3 fumigation in OTCs on different parameters (plant height, number of leaves, visible O_3 injury and plant biomass) are given in Tables 2.6, 2.7, 2.8 and 2.9. The first growth assessment was carried out on day 1 of the O_3 fumigation and showed no significant difference between treatments on plant height and number of leaves (data not shown).

Table 2.6: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height, and number of leaves/plant, after 2 weeks. Post-hoc differences were tested for significance at P=0.05 (ns = non significant). No O_3 or insect injury was found during this week.

Parameters	Effects of O_3	Post-hoc
Plant height	0.858	*ns*
Leaf number	0.416	*ns*

Table 2.7: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height, number of leaves/plant, O_3 injury and insect injury to spinach after 3 weeks. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	Effects of O_3	Post-hoc
Plant height	0.580	*ns*
Leaf number	0.254	*ns*
O3 injury	**0.002**	90ppb>60ppb>30ppb, FA
Insect injury	**0.000**	30ppb>FA,60ppb,90ppb

Table 2.8: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height, number of leaves/plant, O_3 injury and insect injury to spinach at final harvest in Week 4. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	Effects of O_3	Post-hoc
Plant height	**0.010**	FA,30ppb,60ppb>90ppb
Leaf number	0.206	*ns*
O3 injury	**0.000**	90ppb>60ppb>30ppb>FA
Insect injury	**0.000**	FA>90ppb

Table 2.9: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the above ground biomass of spinach at final harvest in Week 4. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	Effects of O_3	Post-hoc
Live leaf biomass	0.077	*ns*
Dead leaf biomass	**0.002**	FA, 30ppb,60ppb<90ppb
Stem biomass	0.383	*ns*
Total biomass	0.090	*ns*

2.3.2.3. Plant Height

No significant difference was observed between the mean plant height of the four treatments for the first three weeks of O_3 fumigation (Tables 2.6, 2.7). However, the mean plant height on the day of harvest (Week 4) was significantly reduced, by 24%, in 90ppb compared to filtered air (FA) as shown in Figure 2.10. The mean plant height did not differ significantly between FA, 30 and 60ppb treatments at the final harvest (Fig 2.10d; Table 2.9).

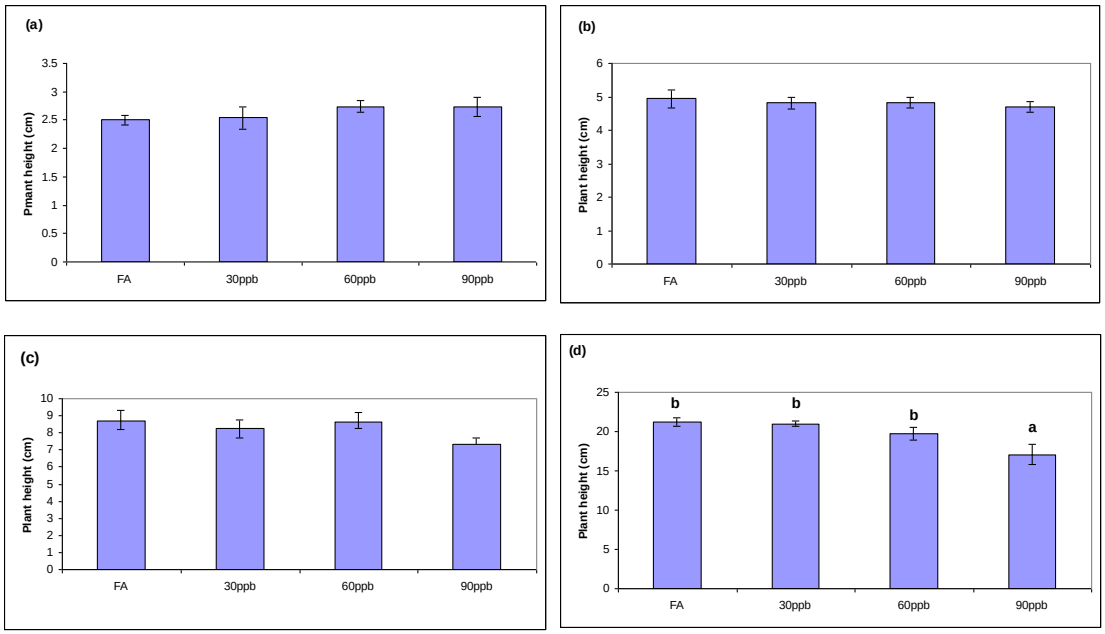


Figure 2.10: The effect of O_3 fumigation on plant height on (a) day 1, (b) week 2, (c) week 3 and (d) week 4 in OTCs for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the means for each chamber. Bars sharing different letters differ significantly from each other at P=0.05

2.3.2.4. Leaf Numbers/plant

The average numbers of leaves per plant were not significantly different throughout the experiment, as shown in Table 2.6, 2.7 and 2.8. However, there was an apparent 19%, 20% and 29% reduction of leaf number per plant in Week 2, 3 and 4, respectively, in 90ppb treated plants compared to FA (Figure 2.11).

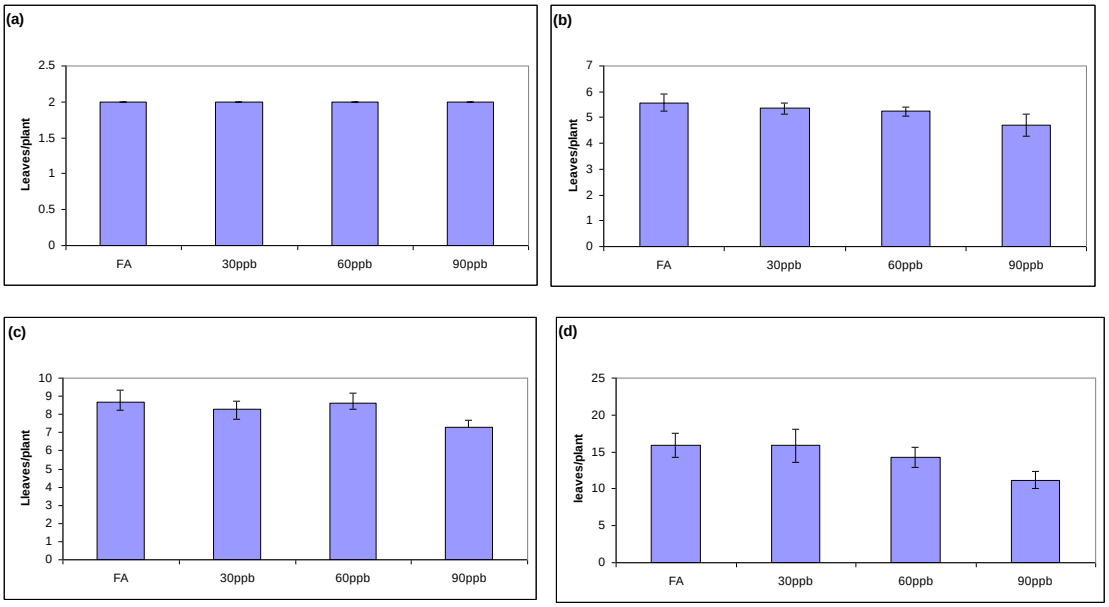


Figure 2.11: The effect of O_3 fumigation on leaf numbers/spinach plant on (a) day 1, (b) week 2, (c) weeks 3 and (d) week 4 in OTC for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means.

2.3.2.5. Insect Injury

Insects, mostly pea aphids *(Acyrthosiphon pisum)* appeared in the 3rd week of O_3 fumigation, when there was hot weather and high humidity. The insect attack was found only in the FA and 30ppb chambers (Fig 2.4.5), with injury in the 30ppb treatment being significantly greater than in the rest of the treatments (Table 2.7). However, in week 4, the insect damage increased in FA chambers, and was significantly higher than in 90ppb chambers. The FA treatment also had 57% and 73% more insect damage than the 30 and 60ppb treated plants, but these differences were not significant (Fig 2.12; Table 2.8). There was very little, or no, insect damage found on spinach plants in 90ppb chambers throughout the experiment, as shown in Figure 2.12.

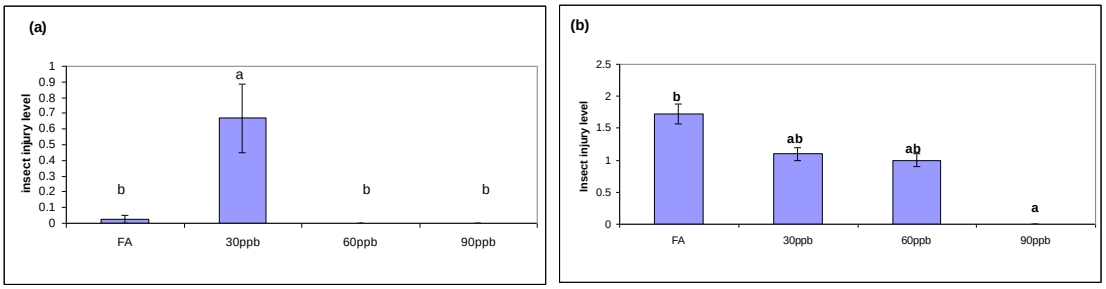


Figure 2.12: (a) Insect damage to spinach plants in Week 3 of O_3 fumigation. (b) Insect damage to spinach plants in Week 4 of O_3 fumigation. All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each other at P=0.05

2.3.2.6. O_3 Injury

No O_3 injury symptoms were observed in the first two weeks of fumigation in all chambers (Table 2.6). However, O_3 injury appeared in the 3rd week of the fumigation, in a form of interveinal necrosis and senescence of mature leaves at the bottom of the plants in the 60 and 90ppb chambers (Fig 2.16). There was no O_3 injury in FA and 30ppb treated plants (Fig 2.15), and the 90ppb treated plants had significantly higher O_3 injury (83%) than 60ppb treated plants (Fig 2.13a). In Week 4, O_3 injury symptoms also appeared on 30ppb plants. The final Week 4 measurements showed that injury in all treatments was significantly different from each other (Table 2.8) in ascending order from FA to 90ppb, as shown in Figure 2.13b. O_3 injury in 30ppb treated plants was 31% and 65% lower than that in the 60 and 90ppb treated plants respectively, while injury to 60ppb treated spinach was 39% significantly lower than that in 90ppb treated plants (Fig 2.13b). Senescence was recorded mostly in 90ppb treated plants, along with interveinal necrosis and tip burn (Fig 2.14), while 60ppb treated plants had only O_3 damage in the form of leaf necrosis and tip burn.

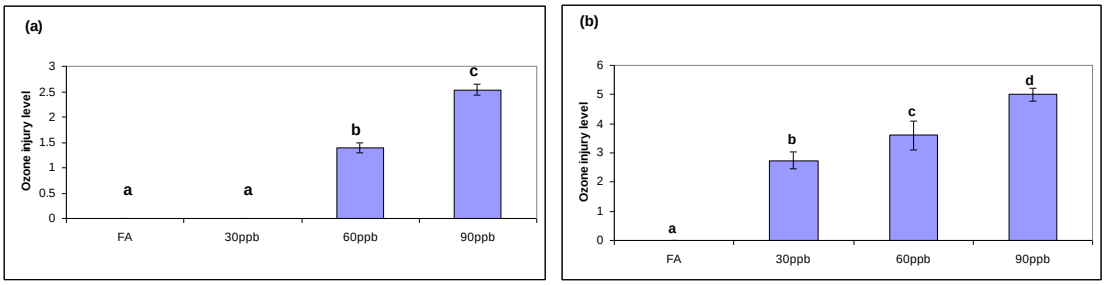


Figure 2.13: (a) O_3 damage to spinach plants in Week 3 of O_3 fumigation. (b) O_3 damage to spinach plants in Week 4 of O_3 fumigation. All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each others at P=0.05

Figure 2.14: Comparison between 90ppb and FA fumigated spinach after 4 weeks of O_3 fumigation.

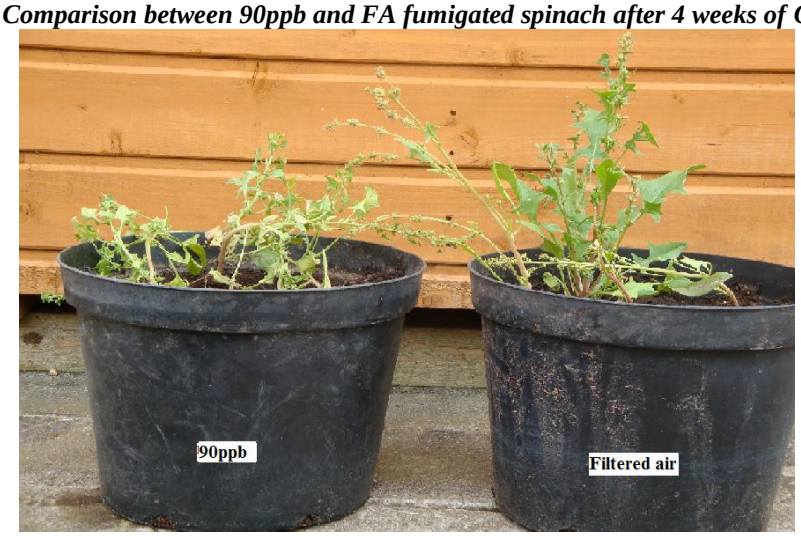


Figure 2.15: FA fumigated spinach after 4 weeks of O_3 fumigation

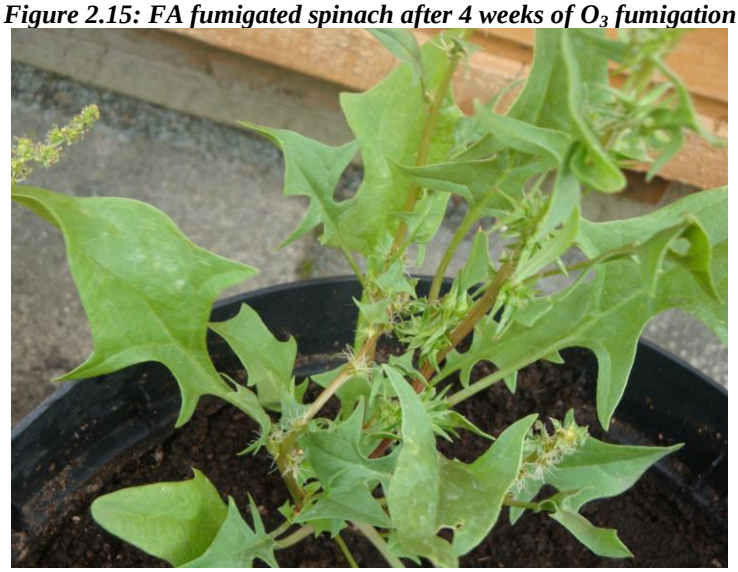


Figure 2.16: Interveinal necrosis on spinach leaves fumigated at 90ppb for four weeks.

2.3.2.7. Biomass

The biomass of each plant was divided into live leaves, dead leaves, stem and leaf + stem biomass. The biomass of the flowers that appeared, due to unfavourable conditions during the experiment, was included with the stem biomass as it was very difficult to separate them. The biomass of live leaves did not differ significantly between the treatments (Table 2.9), although there was an apparent reduction in the biomass of 90ppb treated plants (Fig 2.17a). Dead leaves were only found in 90ppb chambers (Fig 2.17b). There was no significant difference between the stem biomass among the treatments (Fig 2.17c). The leaf + stem biomass was also not significantly different between the four treatments (Table 2.9; Fig 2.17d).

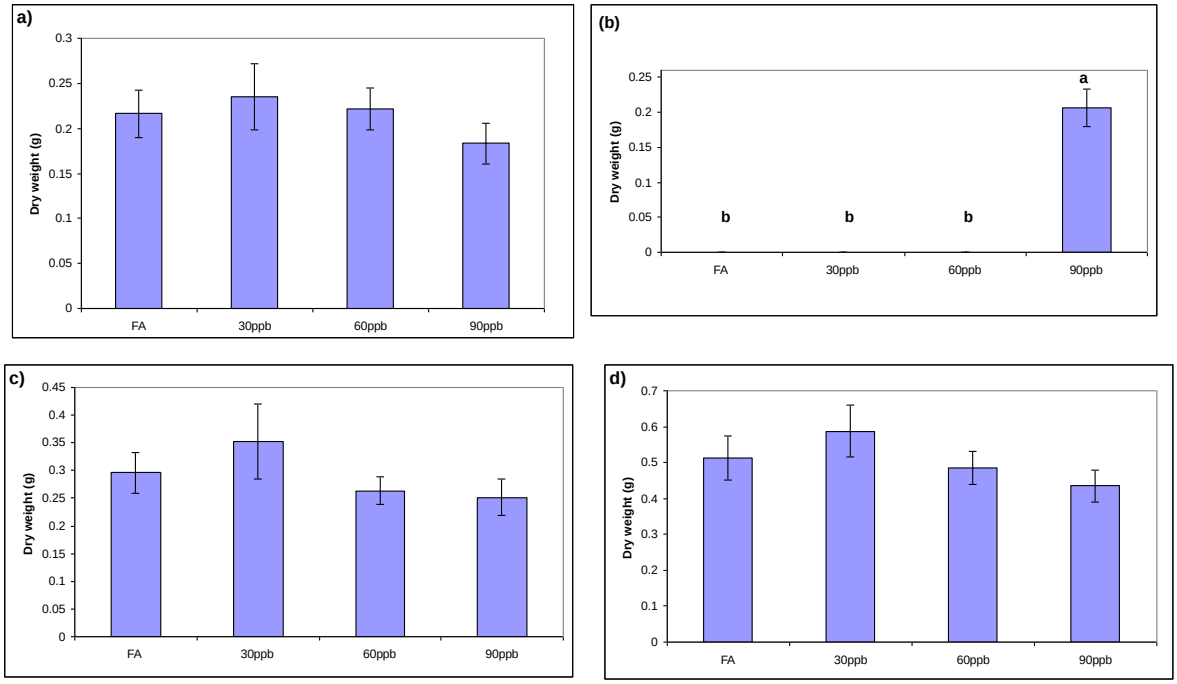
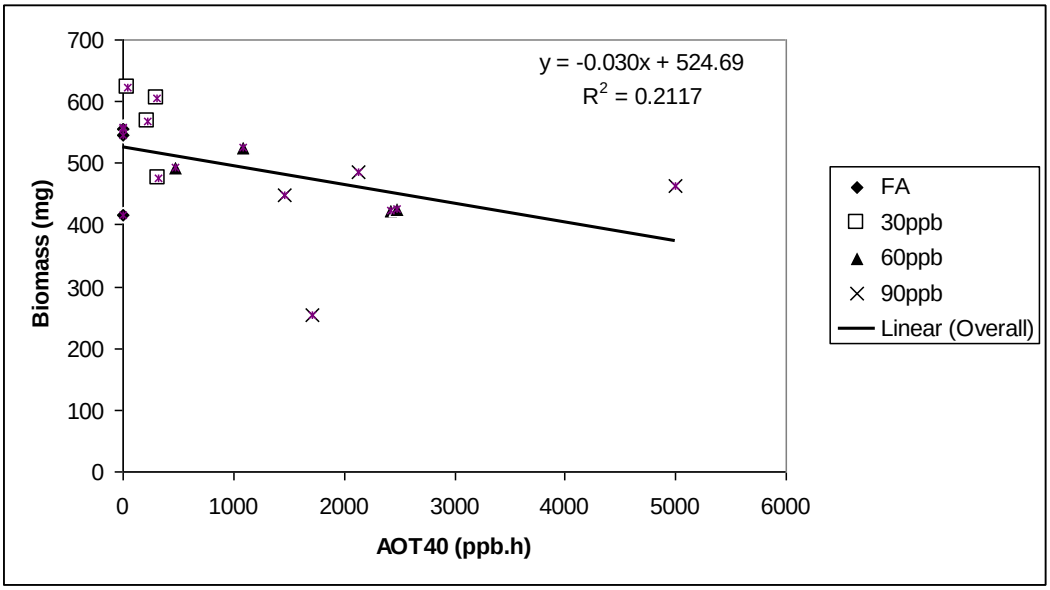


Figure 2.17: Biomass of (a) live leaf (b) dead leaf (c) stem and (d) leaf + stem for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each other at P=0.05

A linear regression between AOT40 (ppb.h) and biomass for individual chambers showed a significant reduction ($p < 0.001$) in biomass with an increase in O_3 exposure (Fig 2.18). Two of the 60ppb chambers had higher AOT40 values than the other two (Fig 2.18), and also had a lower biomass; this strengthens the case that O_3 had significant effects on the total biomass, even if this was not shown by the ANOVA, which was based on treatment means.

Fig 2.18: Relationship between O_3 exposure (AOT40 ppb.h) and total biomass of spinach after four weeks of O_3 fumigation



2.4. EFFECT OF O_3 FUMIGATION ON ONION

2.4.1. Materials and methods

2.4.1.1. Plant growth and O_3 exposure

During the summer survey of 2008, onion (*Allium cepa* L.) plants of the Swat-1 variety grown at the ARI site showed suspected O3 injury symptoms. Therefore the variety was exposed to four different concentrations (FA, 30, 60 and 90ppb) of O3 in open top chambers (OTC) at Silwood Park, Imperial College London for 4 weeks in August, 2009. Onion seeds were first sown in nursery trays using Westland John Innes No. 2 compost inside the greenhouse at Imperial College on 28th July 2009. After germination, one week old plants were transferred to 30x30 pots, each pot carrying five plants. Before transplantation, pots were filled with Westland John Innes No. 2 compost and were irrigated with tap water. After transplantation, a total of 32 pots were transferred carefully to 16 chambers on 6th August 2009, with each chamber containing two pots.

The 16 chambers were divided into four O3 treatments. Each group of four chambers received FA (charcoal filtered air) daily from 5pm to 9am, with O_3 added at 0ppb, 30ppb (low), 60ppb (medium) and 90ppb (high) between 9am to 5pm. Pots were watered with tap water as required, depending upon environmental conditions. Pots within the treatments were moved between replicate chambers on a weekly basis. No pesticide applications were made during the experiment. The plant height and leaf number were recorded on Day 1 of the O_3 fumigation and then at weekly intervals. The visible injury was recorded using the same scale as for spinach, as summarised in Table 3.4. Air temperature and relative humidity was monitored by the local weather station at Silwood Park throughout the experiment. The actual O3 concentrations were

monitored by O_3 monitor. O_3 exposures were summarised as an AOT40 value for each individual chamber.

2.4.1.2. Plant harvest

The plants were harvested after 4 weeks, on day 27 (1st September). The entire above-ground biomass of each plant was put into a labelled paper bag (i.e. one bag per plant). The labelled bags containing onion plants were brought to the laboratory of the University of York, UK within 24 hours. Upon arrival, each plant was separated into stem, live leaves and dead leaves. The plants were weighed using a high precision electronic balance (AND, 202) and then dried at approx. 70°C for approx. 48 hours in an electric oven. After drying, the samples were weighed again to obtain dry weight data.

2.4.1.3. Statistical summary

The data summary (means and standard errors) was carried out using Microsoft Excel (2003) and the statistical analysis was carried out using SPSS 17.0. The dataset for all parameters was explored for skewness, kurtosis and normality, using the Shapiro-Wilk-test. The O_3 injury, showing major deviations from a normal distribution, was normalised by using $\log_{10}$ transformation. One-way-ANOVA was then used for each of the parameters to determine whether the effect of O3 was significant. Tukey's HSD post hoc test was used for multiple comparisons between treatments at $p<0.05$.

2.4.2. Results

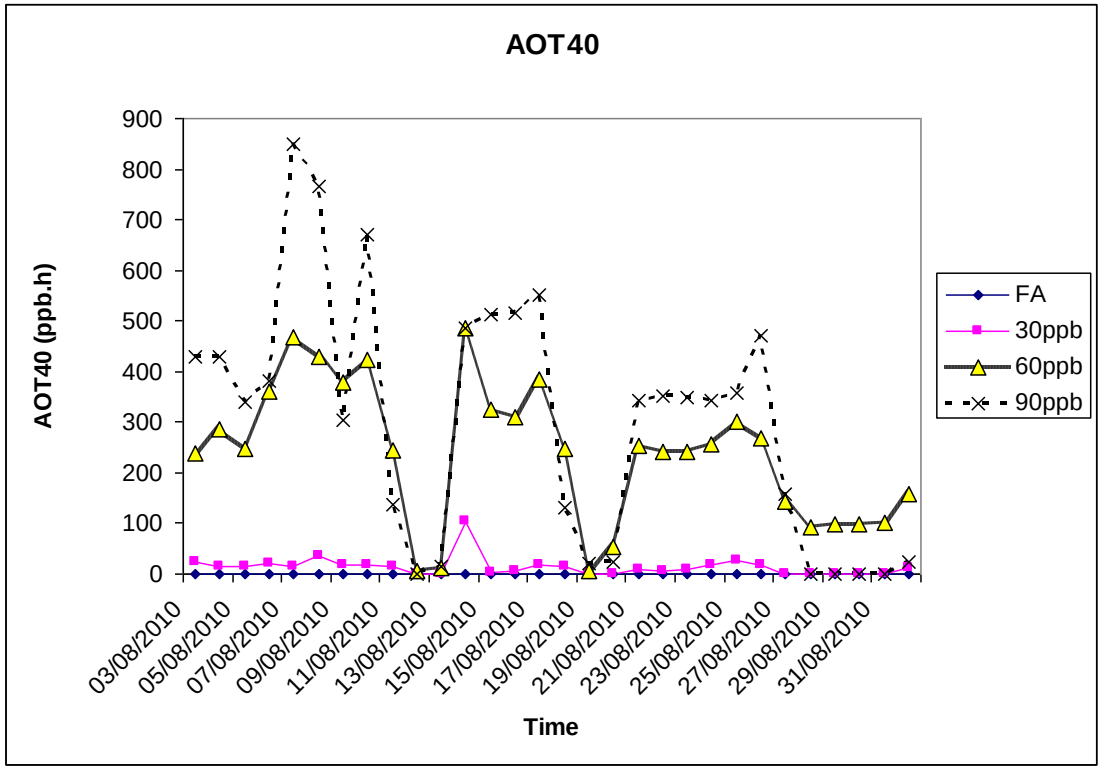
2.4.2.1. O_3 exposure

The mean temperature and relative humidity, measured at the local weather station near the OTCs, for the whole experimental period was 20.5°C and 72.7%, respectively. The O_3 concentration was high for the first 3 weeks, with occasional gaps of 1-2 days, but there was low fumigation, especially in the 90ppb chambers, during the last week of O_3 fumigation, which was mainly due to the malfunctioning of the O_3 generators (Fig 2.19).

Table 2.10: ANOVA results (probability values) showing the difference between the AOT40 values between the treatments for the entire onion experiment. Post-hoc differences were tested for significance at P=0.05.

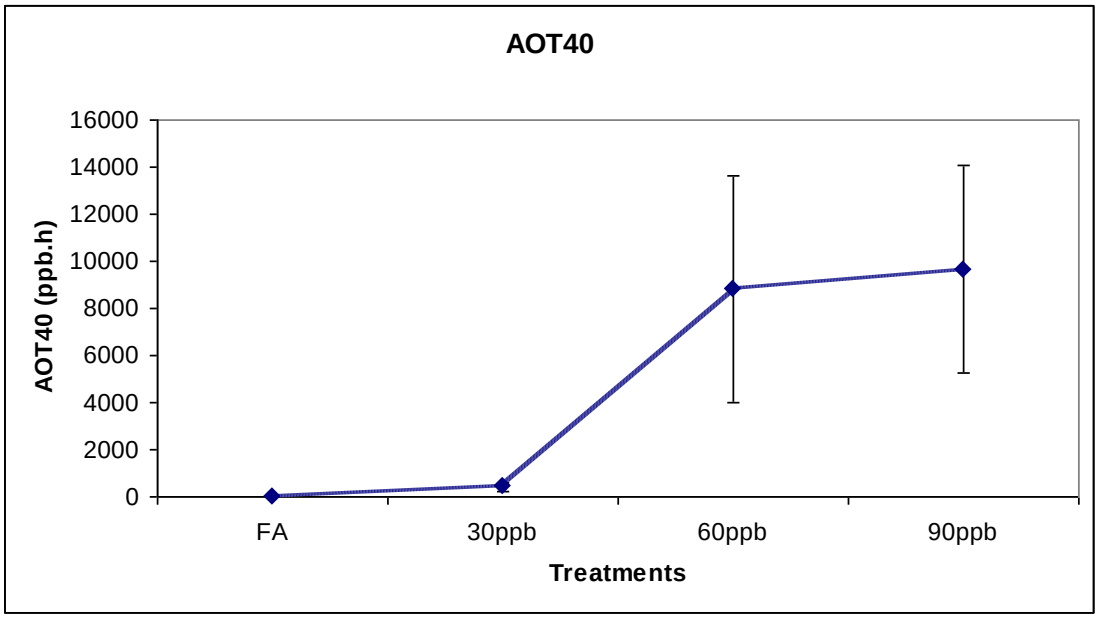
Chambers	Effects of chambers	Post-hoc
O3 concentration (ppb.h)	0.000	90ppb,60ppb>FA,30ppb

Figure 2.19: Mean AOT40 (ppb.h) values for each day (treatment means) for the entire onion experimental period



According to the ANOVA test (Table 2.10), the AOT40 values in the 60ppb and 90ppb chambers were significantly higher than those of FA and 30ppb (Fig 2.20). However, the AOT40 values in the 90ppb chambers were not significantly different from the 60ppb chambers (Table 2.10; Fig 2.20). The mean AOT40 values (ppb.h) for the whole experiment for the 60ppb and 90ppb chambers was 88.2ppb.h and 9654ppb.h, respectively (Fig 2.20).

Figure 2.20: Mean AOT40 (ppb.h) values for FA, 30, 60 and 90ppb chambers for the entire onion experimental period. Error bars represent standard errors of the individual chamber mean values.



2.4.2.2. Statistical summary

The ANOVA results for the effects of four weeks O_3 fumigation on plant height, number of leaves, visible O_3 injury and plant biomass are given in Tables 2.11, 2.12, 2.13 and 2.14. The first growth assessment was carried out on Day 1 of the O_3 fumigation, and showed that there was no significant difference between plant height and number of leaves, and there was no visible O_3 injury on leaves (Table 2.11). No insect injury was recorded during the entire experiment.

Table 2.11: ANOVA results (probability value, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height and number of leaves/plant on day 1. Post-hoc differences were tested for significance at P=0.05 (ns = non significant).

Parameters	Effects of O_3	Post-hoc
Plant height	0.644	*ns*
Leaf number	0.410	*ns*

Table 2.12: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height, number of leaves/plant and O3 injury to onion after 2 weeks. Post-hoc differences were tested for significance at P=0.05.

Parameters	Effects of O_3	Post-hoc
Plant height	**0.000**	FA, 30ppb>60ppb,90ppb
Leaf number	**0.000**	FA> 30ppb>90ppb
O_3 injury	**0.000**	FA, 30ppb>60ppb> 90ppb

Table 2.13: ANOVA results (probability values, those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the plant height, number of leaves/plant and O3 injury to onion after final harvest (week 4). Post-hoc differences were tested for significance at P=0.05.

Parameters	Effects of O_3	Post-hoc
Plant height	**0.001**	FA, 30ppb>60ppb,90ppb
Leaf number	**0.015**	FA, 30ppb>90ppb
O3 injury	**0.000**	FA,30ppb>60ppb>90ppb

Table 2.14: ANOVA test results (probability values: those significant at p = 0.05 are shown in bold) showing the effects of O_3 on the above ground biomass of onion. Post-hoc differences were tested for significance at P=0.05.

Parameters	Effects of O_3	Post-hoc
Live leaves	**0.000**	FA, 30ppb>60ppb, 90ppb
Dead leaves	**0.000**	90ppb, 60ppb>FA, 30ppb
Total biomass	**0.000**	FA, 30ppb>60ppb, 90ppb

2.4.2.3. Plant Height

There was no significant difference between the plant height in the FA and 30ppb treatments, and that in the 60ppb and 90ppb treatments, in Week 2 and Week 4. However, the plant height in the 60ppb and 90ppb treatments was significantly lower than in FA and 30ppb treatments in both weeks. The height of the 60ppb treated plants was 55% & 42% lower than that of the FA plants in weeks 2 and 4, respectively (Fig 2.21b), whereas the height in 90ppb chambers was 47% & 71% lower than that in FA in weeks 2 and 4, respectively (Fig 2.21c).

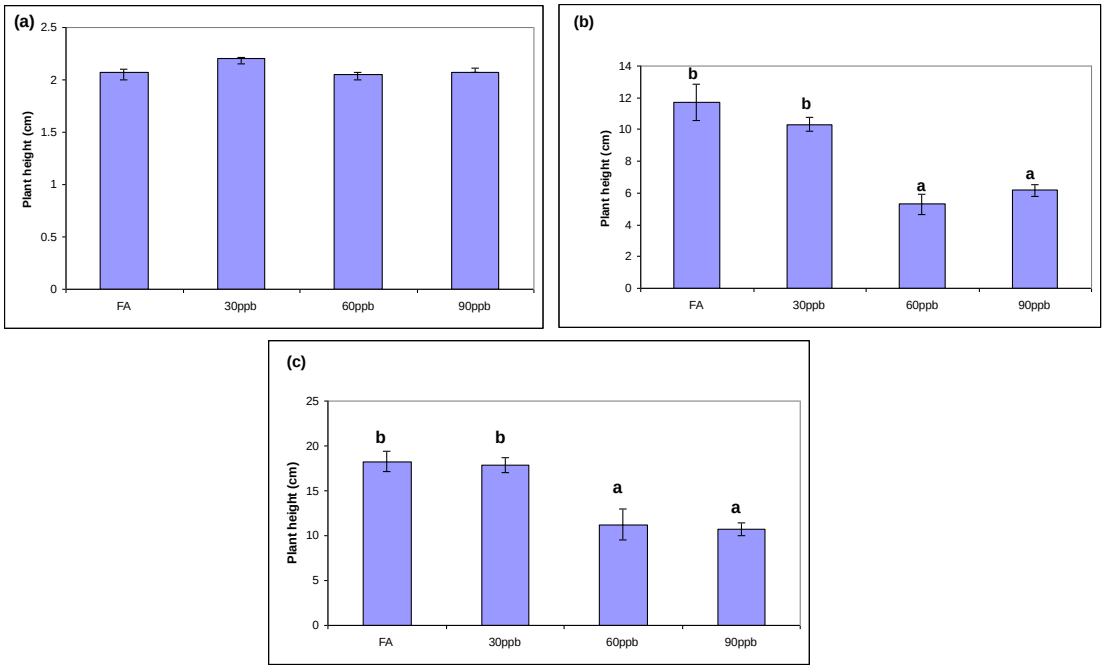


Figure 2.21: The effect of O_3 fumigation on onion plant height on (a) day 1, (b) week 2 and (c) week 4 in OTC for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each others at P=0.05

2.4.2.4. Leaf number

Leaf number per plant was significantly different among the treatments in Week 2 (Fig 2.22b; Table 2.12). The leaf number of 90ppb treated plants was significantly reduced, by 34%, compared with FA treated plants. The leaf number of 30ppb treated plants was also significantly lowered, by 15%, compared with FA treated plants. Leaf numbers of 30ppb treated plants were not significantly different from those in 60ppb, but were significantly higher than those in 90ppb in Week 2 (Fig 2.22b; Table 2.12). The number of leaves per plant in 90ppb was reduced by 47% compared to that in FA and 30ppb in Week 4 (Fig 2.22c). However, there was no significant difference in leaf number between FA, 30 and 60ppb treated plants in Week 4 (Table 2.12).

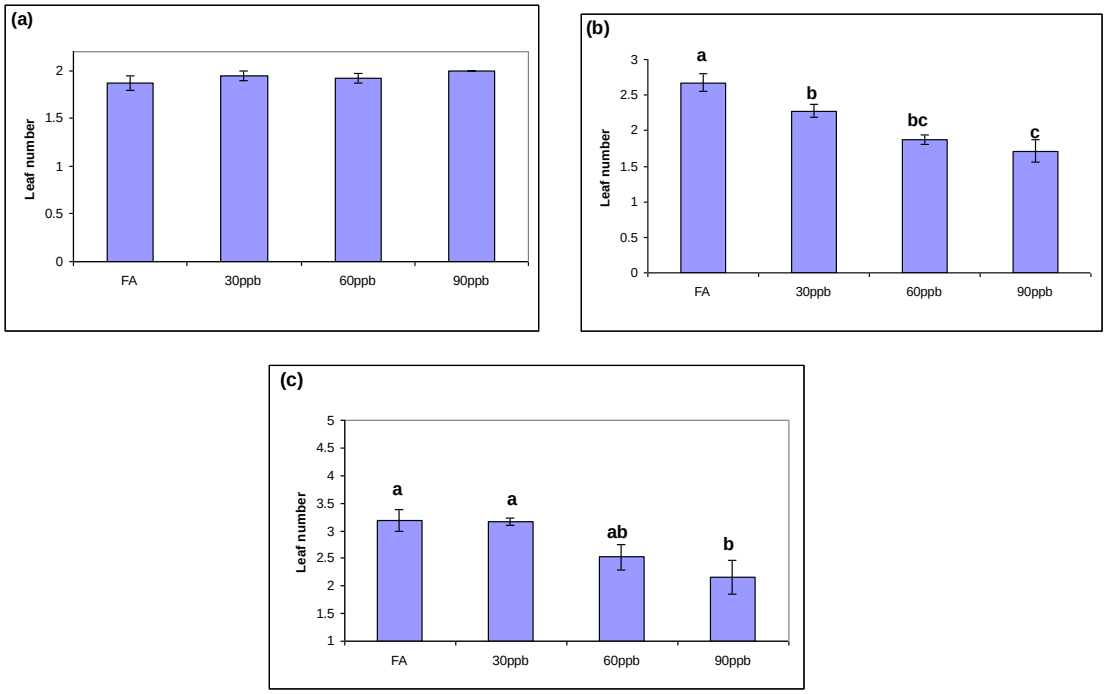


Figure 2.22: The effect of O_3 fumigation on onion leaf number on (a) day 1, (b) week 2 and (c) week 4 in OTC for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each other at P=0.05

2.4.2.5. O_3 Injury

Typical O_3 injury (leaf tip burn) appeared in the second week of O_3 fumigation, when the plants were 3 weeks old. O_3 injury varied significantly between the treatments (Fig 2.23b). There was little or no O_3 injury recorded in FA and 30ppb chambers. The O_3 injury in 90ppb treatments was significantly higher than in 60ppb treated plants, by 27% and 49%, in weeks 2 and 4, respectively (Fig 2.23; Table 2.13).

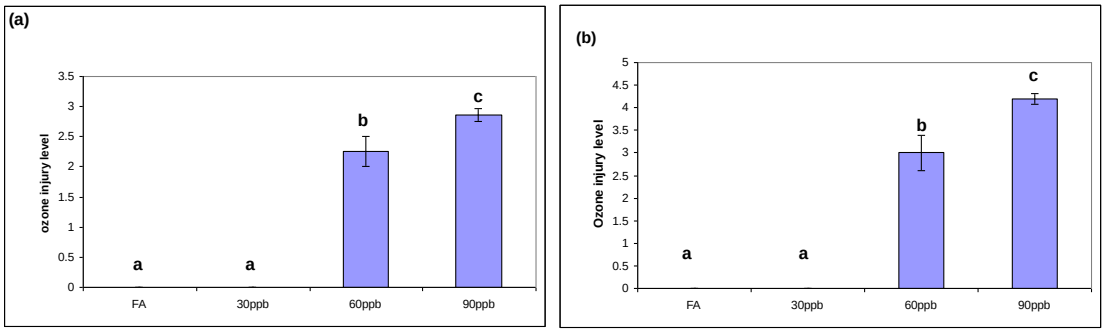


Figure 2.23: O_3 visible injury to onion during O_3 fumigation in (a) week 2 and (b) week 4 in OTC for four treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05

2.4.2.4. Biomass

The biomass of each plant was divided into live leaves and dead leaves. The live and dead leaf biomass in the FA and 30ppb, and in the 60ppb and 90ppb treatments, did not significantly differ from each other. The leaf biomass of FA and 30ppb plants was significantly higher than 60 and 90ppb treated plants (Table 2.14). The live leaf biomass of 90ppb treated plants was reduced by 78% compared to FA plants (Fig 2.24a). The dead leaf biomass of onion plants in 60ppb was not significantly different than that in 90ppb, although there was an apparent reduction of 24% in 60ppb compared to 90ppb. There were no dead leaves in 30ppb and FA treated plants. Total biomass of 90ppb treated plants was 75% lower than FA treated plants, and the biomass of the 60ppb treatment was reduced significantly from FA treated plants by 66%.

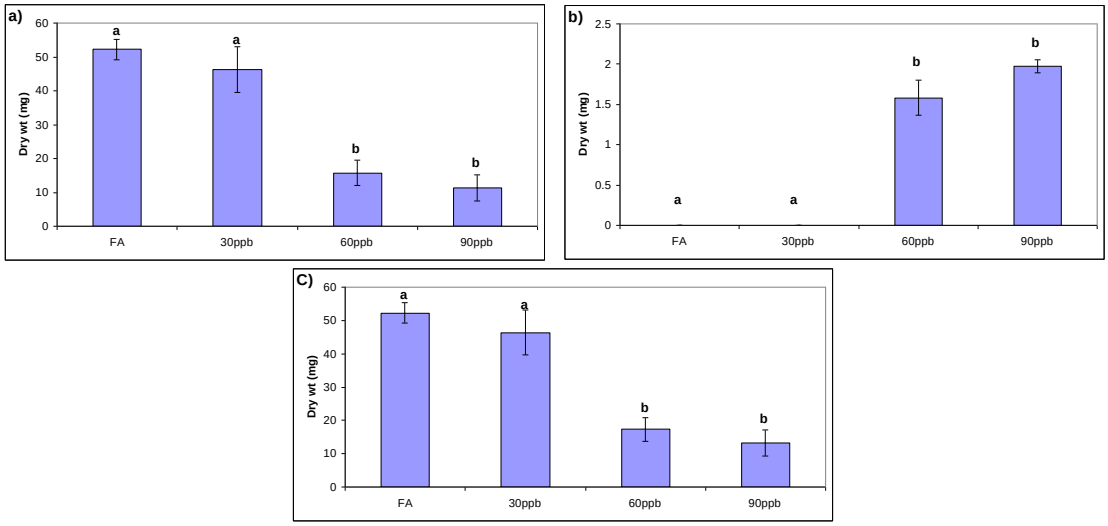
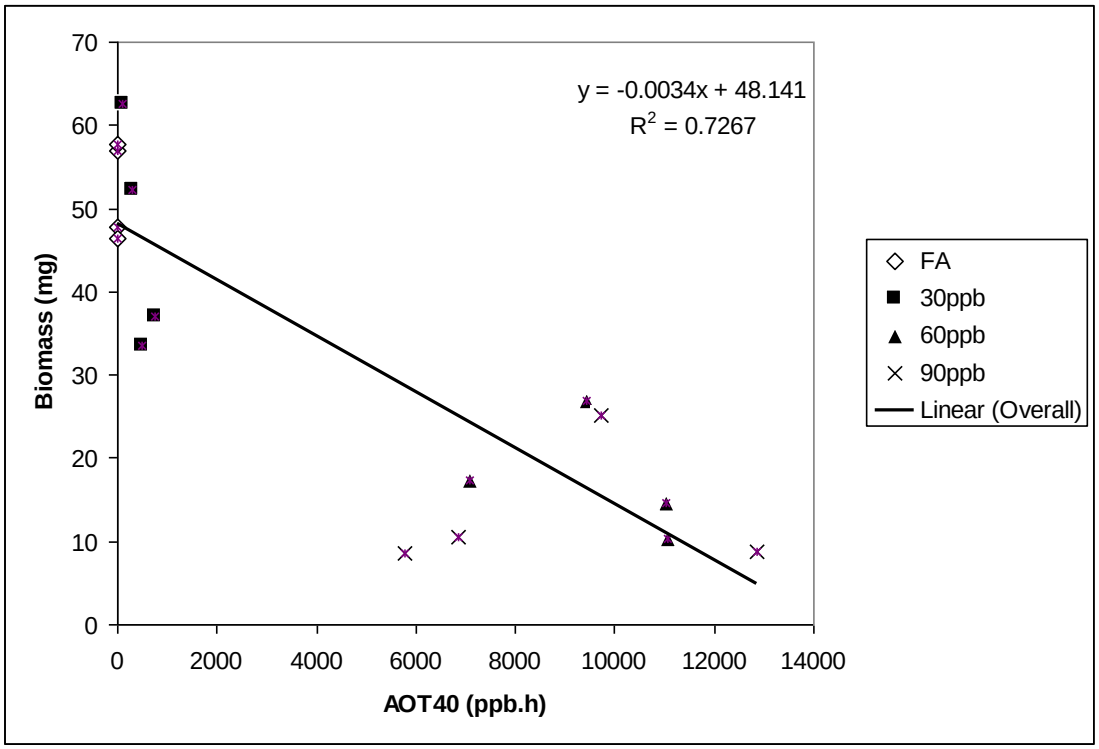


Figure 2.24: Biomass of (a) live leaves, (b) dead leaves and (c) total onion plant for four O_3 treatments (FA, 30, 60 and 90ppb). All values are the means of values for 4 replicate chambers. Error bars indicate standard errors of the chamber means. Bars sharing different letters differ significantly from each others at P=0.05

A linear regression model also showed that the biomass decreased significantly (P = 0.001) with an increase in O_3 exposure (Fig 2.25). However, the biomass values of 60ppb and 90ppb were widely scattered against their AOT40 values, but were not significantly different from each other.

Figure 2.25: Relationship between AOT40 (ppb.h) and total biomass of onion in individual chambers after four weeks of O_3 fumigation



2.5. DISCUSSION

2.5.1. Field survey

This was the first ground level O_3 pollution survey in this part of Pakistan. There was no previous record of ground level O_3 concentrations, and its potential impact on vegetation, in Peshawar. O_3 concentrations were positively correlated with temperature at both monitoring sites. There was gradual increase in O_3 concentration with the increase in mean temperature and the duration of daylight from February to May. Based on the average of the values at the AUP and ARI sites, the mean O_3 concentration in February was 26ppb at 22°C, which increased to 42.2ppb in May, when the mean temperature was 28°C. The mean O_3 concentration at the AUP site was higher (38.2ppb) than at the ARI site (34.1ppb) based on the mean concentration for the 4 months. This might be due to AUP being a shorter distance from the main city centre, where most of the heavy traffic exists. In contrast to the spring and summer values, the mean monthly O_3 concentration at ARI in winter was low, ranging from 22ppb in November to 13ppb in December, with an overall mean of 15ppb and mean temperature of 16°C.

These findings were in line with previous studies. Pandey and Agrawal (1992) also found significant positive correlations between temperature and O_3 concentrations in urban areas of Varanasi, India. The low temperatures and reduced sunlight in winter slow down the reactions between the O_3 precursors (VOCs and NO_x), which are primarily produced by vehicular and industrial emissions. O_3 is often formed during the transport of these primary pollutants on the prevailing wind in suburban areas during periods of high temperatures (Singh *et al.*, 2005; Ren *et al.*,

2007). Beaney and Gough (2002), Agrawal *et al*., (2003) and Tiwari *et al*., (2005) have reported higher O_3 concentrations during summer than winter months in peri-urban areas. Farag *et al.* (1993) also reported that monthly O_3 concentrations were higher during summers (65 ppb) than winter (24 ppb) in the urban areas of Cairo, Egypt.

Passive samplers for O_3 measurement were used for this survey, as the study was conducted at field sites where it is very difficult to install continuous O_3 monitors, which are very expensive and required continuous electricity. Passive samplers are considered an alternative, cheap method that does not require electricity, to measure O_3 concentration over a longer duration (Sanz *et al*., 2001). Several studies have been carried out to assess ambient O_3 concentrations, and their effects on different crops, using passive samplers in recent years (e.g. Manning *et al*., 1996; Blum *et al*., 1997; Brace and Peterson, 1998; Krupa and Legge, 2000, and Sanz *et al*., 2001).

The use of passive samplers to measure O_3 can give values that are comparable to active O_3 samplers. Delgado-Saborit and Esteve-Cano (2008) used passive samplers for the assessment of O_3 effects on citrus crops in a western Mediterranean area. Their passive samplers showed a precision of 6.1%, while the passive samplers from the current study showed on average a difference of 9.3% between the duplicate samplers, a value that is comparable to the 9% reported by Wedlich *et al.* (in press). However, passive samplers may provide measurements that are consistently lower or higher than active samplers, as documented by Delgado-Saborit and Esteve-Cano (2008); for example Wedlich *et al*. (in press) reported that

passive samplers gave a positive bias of 30% when compared with continuous O_3 analysers. Lurmann *et al*. (1994) compared the relative performance of active and passive samplers and concluded that active samplers have better (lower) detection limits than passive samplers. The measurements of two active O_3 monitors over the same period also showed greater agreement than passive sampler replicates, suggesting that O_3 concentrations measured with passive samplers are not as precise as those measured using active O_3 monitors. In addition, the passive sampler O3 concentrations are based on monthly mean values; they do not record daytime O3 peak values, which is when effects of O_3 primarily occur. Nevertheless, passive samplers offer a cheap and effective method for ambient O_3 assessment in the absence of active O_3 monitors.

The results of the summer and winter surveys for O_3 concentrations were also consistent with the results of the two surveys of for visible injuries, which found injury during the summer, when O_3 concentrations were high, but not during winter or spring, when O_3 concentrations measured by passive samplers were low. Suspected O_3 visible injuries were observed on four crop species at the AUP and the ARI sites. Most of the visible injuries were seen in the month of May, when the daytime temperature reached 40°C and the mean O_3 concentration exceeded 45ppb. Suspected O_3 injury, of white stippling and tip burn on onion leaves and black flecking on potato leaves, was found, but only at the ARI site. Both crops were about 3 months old and the injuries were only found on mature leaves. The black spots on potato leaves were only on the upper epidermis. White stipples were also found on mature leaves of cotton plants in later stages of plant growth in a research plot at AUP. These results suggests that these three crop species may be sensitive to O_3 during vegetative

growth, or that they might be vulnerable to mean monthly O_3 concentration above 40ppb, which is why these crops started to show O_3 injury symptoms in the month of May, with the increase in O_3 concentrations. Suspected visible injury to clover was also found at AUP, but there was insect activity on clover, which made it very difficult to distinguish between O_3 and insect injury.

The visible foliar injuries to onion and potato at ARI, and to cotton at AUP, were similar to O_3 injuries reported in the literature. Engle and Gabelman (1965), Wukasch and Hofstra (1977a & b) and Temple (1990) reported white flecking and tip burn injury on onion leaves. The leaf spot visible injury on potato at ARI was similar to the injuries reported by Bambawale (1986) in India and Hooker *et al*. (1973) in USA. However, the extent of these injuries varies from cultivar to cultivar due to genetic resistance and O_3 exposure (Engle and Gabelman, 1966). Moreover, due to the lack of previous O_3 foliar injury data in the region, it was hard to say definitely that the injuries were caused by O_3, as no experimental and survey studies on O_3 effects on these crops in this area have been reported to date. Symptoms observed on these three crops were similar to the pictures of O_3 injury in Europe that have been collected by ICP Vegetation (www.icpvegetation.ceh.ac.uk). O_3 damage to leaves cannot be analyzed chemically, so it is suggested that local crops should be subjected to EDU or O_3 exposure experiments, in order to confirm that the symptoms are due to O_3. For this reason, the onion variety that showed sensitivity to O_3 at ARI was subjected to different O_3 concentrations under controlled conditions in OTCs at Imperial College London, to help in diagnosis of the O_3 foliar injury on onion. However, potato and cotton were not used for OTC experiments, as the necessary space was not available to grow them inside the OTCs at Imperial College.

Most of the crops did not show any O_3 foliar injury, but some of these crop species (e.g. tomato and spinach) have been reported to be sensitive to O_3 in the literature. Iriti *et al*. (2006) observed red necrotic symptoms on a currant tomato variety. Deveau *et al*. (1987) and Oguntimehin *et al*. (2010) also found interveinal chlorosis and necrosis on tomato caused by O_3 fumigation. Spinach (*Spinacia oleracea*) and clover (*Trifolium resupinatum*) had been reported to be sensitive to O_3 (www.icpvegetation.ceh.ac.uk), but the different species in Peshawar (*Beta vulgaris* and *Trifolium resupinatum*) may show different response to O_3. The local varieties are also different from those reported in the literature and O_3 effects differ from one region to another depending upon the environmental conditions and variety (Emberson *et al*., 2001). The surveys were also carried out in a limited area because of the prevailing security situation in Peshawar. The author was also inexperienced, and was carrying out this type of field survey for the first time, which made it difficult to locate O_3 foliar injury and to differentiate it from other types of injury in the field. The lack of O_3 visible injury on several species does not mean that these crops will not be affected by high O_3 concentrations in Peshawar, as O_3 can affect the growth and yield of crops in the absence of visible symptoms (Agrawal *et al*., 2005). For example, maize and sugarcane are reported to be less sensitive to O_3 (e.g. Leitao *et al*., 2007 for maize; Grantz and Vu, 2009 for sugarcane). In the current study, maize and sugarcane did not show any visible foliar O_3 injury, but it is still possible that the crops are affected by O_3 in terms of growth and yield.

The results suggested that there were significant differences in ambient O_3 levels between the early summer and winter cropping seasons. However, the samplers were only installed for four months in summer and two months in winter. The short

duration of the surveys was because of limited funding, time and the fragile security in the region. In order to get a bigger and clearer picture of O_3 concentrations and potential negative impacts to agricultural crops, it is imperative to install diffusive passive samplers at multiple sites and monitor O_3 concentrations round the year. It is also suggested that the diffusive samplers should be exposed during daytime only (8am-5pm) in order to assess O_3 peak levels and to compare them with other studies carried out in Lahore (Wahid *et al*., 2001 and Wahid, 2006b), where O_3 was monitored using a continuous O_3 analyzer. The visible injury study was also limited to only two sites, which gave limited data on the extent of O_3 injury to crops around the city of Peshawar.

2.5.2 O3 fumigation of spinach

The O_3 fumigation for the first two weeks did not have any significant effects on plant height, leaf number and visible injury. This was mainly due to a fault in the O_3 fumigation system. However, in the later weeks of O_3 fumigation, plant height was reduced by up to 24% in the 60ppb and 90ppb treatments.

O_3 visible injury in the form of interveinal chlorosis, and necrosis on leaf tips and margins, also appeared in the 60 and 90ppb chambers in the last two weeks due to the greater O_3 exposure compared to the first two weeks. The injury that appeared in Week 3 of O_3 fumigation in the 60 and 90ppb chambers on spinach leaves increased by Week 4, which suggested that this variety is more susceptible if the duration of O_3 fumigation is prolonged. However, it is not clear from this experiment whether this variety is susceptible to O_3 injury at an early growth stage, although previous studies suggest that some crops are susceptible to O_3 at this stage. For example, Wukasch and

Hofstra (1977) worked on O_3 and botrytis effects on onion (*Allium cepa* L. 'Autumn Spice') inside open top chambers in Ontario, Canada and concluded that leaf necrosis and tip burn appeared in the second week of fumigation. A similar study on wheat plants by Soja and Soja (1995) reported more O_3 damage in the early growth stage. Due to the lack of O_3 fumigation in the first two weeks, it is suggested that this spinach variety should be further subjected to same O_3 dose at different growth stages in order to confirm its sensitivity to O_3 at specific growth stages.

The apparent reduction in leaf number in the 60 and 90ppb chambers may have been significant if the spinach had been fumigated for a longer duration. Due to the longer day length during the experiment compared with winter growth conditions in Peshawar, flowers appeared prematurely, because of which it was difficult to estimate the actual reduction in plant height and leaf number. The study was in line with Abdel-Latif (2001) who carried out an O_3 dose response study on Egyptian Jew's mallow (*Corchorus olitorius* cv. Balady) in two separate experiments and concluded that longer O_3 fumigation with same exposure system can decrease plant growth and increase visible leaf injury. Similar results were also obtained by Pleijel and Danielsson *et al*. (1997), who worked on *Phleum alpinum*.

The dead leaves were only found in 90ppb chambers. This may be due to O_3 induced stomatal closure, because high O_3 concentrations can affect photosynthetic processes and increase internal CO_2 concentrations. Several studies [e.g. Darrall, (1989), Heath (1994) and Kleier *et al*. (2001)] report that high O_3 concentrations reduce the rate of photosynthesis, and hence affect the growth and yield of the plant, depending upon the duration and concentration of the exposure.

The stem biomass was significantly reduced, by 45%, in 90ppb compared with the FA treatment. Stem biomass also included the biomass of the flowers; assimilates used for flower growth otherwise could have been allocated to the leaves, if the environmental conditions were more favourable. The live leaf and total above ground biomass of spinach did not vary significantly, although there was an apparent reduction in 90ppb compared to FA. This suggests that the overall biomass of the spinach might have been significantly reduced in better conditions for spinach growth and with a longer exposure. Tiwari and Agrawal (2009) conducted an EDU experiment on an Indian summer variety (*Beta vulgaris* all green) at O_3 contractions ranging from 53ppb to 74ppb (daytime 8h mean) and concluded that the biomass was reduced by up to 26% in non EDU treated plants.

The significantly higher attack of green aphids on FA and 30ppb treated plants, compared to 60 and 90ppb, suggested that O_3 affected the insect-plant relationship. No insect injury was found in the 90ppb treatment, suggesting that aphid growth was negatively affected by high O_3 concentrations. The leaf symptoms of the aphid infestation were clearly different from those of O_3 injury. O_3 has a major influence on insect-plant relationships that in turn can affect plant growth. O_3 can increase or decrease insect attack on plants by bringing about biochemical changes and changing the internal pH of plant cells (Braun and Flickiger, 1989). Menendez *et al*. (2010) worked on the aphid and episodic O_3 injury in arugula plants (*Eruca sativa* Mill) grown in open-top field chambers, and concluded that O_3 visible injury was lower in aphid affected plants, and that the aphid growth rate of the new offspring was low in high O_3 concentrations. Holopainen *et al*. (1995) studied the performance of aphids on Scots pine and Norway spruce seedlings by fumigation with air pollutants

(O_3, SO_2 and NO_2) in growth chambers and suggested that SO_2 increases the performance of aphids, but that O_3 and NO_2 did not have any positive or negative effect on aphid growth. However, Warrington (1989) worked on O_3 effects on the growth rate of cereal aphids and concluded that O_3 enhances the growth rate of aphids by remobilising nutrients in prematurely senesced leaves, which increases the amount of nitrogen and the quality of phloem sap for aphids. This suggests that O_3 does not directly affect aphids but changes biochemical composition inside the plant in ways that influence aphid growth rates.

Aphids are not likely to play any role in the field conditions under which this crop is grown in the Peshawar region, as it is grown in winter, when the temperature ranges from 0°C during night to 15°C during day. However, the summer spinach is also grown in Pakistan in temperatures ranging from 30-40°C. It is therefore important to assess the insect-plant relationships under high O_3 concentrations. No such study has been carried out in Pakistan to date.

Spinach plants did not show any significant effect of O_3 in the 30ppb chambers. However, plants exposed to 60ppb had visible leaf injuries but no significant effect on total plant biomass, whereas 90ppb treated plants had O_3 visible injuries and significant biomass reduction. However, the 24h mean O3 concentrations were much lower than 60 and 90ppb during the winter season at Peshawar. An important limitation of the monitoring of ambient O_3 concentrations by passive samplers was that it was based on four weeks mean concentrations and did not record the peak O_3 concentration during day time, when the stomata are open and O_3 can enter the plant and can alter the photosynthetic process. The 24h mean O_3

concentration was 15ppb during the winter months in Peshawar; based on the OTC experiment, this is unlikely to cause any significant damage to plants as no injury or reduction in growth was found in 30ppb chambers.

It should be noted that O_3 fumigation was disrupted on several occasions, especially at the start of the experiment, due to a fault in the O_3 generators; because of this, AOT40 values were quite low and did not exceed the critical level of 3000 ppb.h for effects on crop yield. The aphid infestation of the leaves may have also masked the visible O_3 foliar injury and could have reduced the growth of the plants in FA and 30ppb treated plants, thus reducing the size of any O_3 effect on spinach. However, in spite of these limitations, significant adverse effects were observed in 60ppb and 90ppb treatments. In order to remove these limitations, it is suggested that this variety should be subjected to EDU treatment under field conditions in order to get a clearer picture of the sensitivity of this variety to O_3 under field conditions in Peshawar.

2.5.3. O_3 fumigation of onion

Four weeks of O_3 fumigation in OTCs had a significant effect on the plant height, leaf number, visible injury and biomass of onion, suggesting that this variety is very sensitive to O_3. The experiment was terminated before the planned end date because of the cooler temperature inside the OTCs, due to which the plants did not grow as well as expected. The mean temperature during the experimental period (August-September) was cold (20°C) for this variety, as it is grown in spring and early summer in Peshawar, where normally the temperature ranges from 30-40°C; because of this, the plants did not grow as well as expected.

The AOT40 values in the onion experiment were much higher than those in the spinach experiment. The AOT40 values in the onion experiment were 8802ppb.h and 9654ppb.h for the 60ppb and 90ppb treatments, respectively, whereas the AOT40 values during the spinach experiment were 1614ppb.h for the 60ppb treatment and 2574ppb.h for the 90ppb treatment. There was more O_3 fumigation in the first two weeks for onion compared to the later weeks. Plant height and leaf number of FA treated plants were significantly higher than those in 60ppb and 90ppb in the 2nd and 4th week of O_3 fumigation, suggesting that this variety is sensitive to O_3. The plant height and leaf number decreased further in 60ppb and 90ppb treatments with the passage of time. It is not clear whether this variety is more sensitive with prolonged exposure to O_3 fumigation, because the O_3 concentrations were higher in the first two weeks due to problems with the O_3 generators. If the O_3 concentrations had been uniform during the entire experiment and the target concentrations had been met, then a clearer picture of O_3 effects would have been achieved. The study was in line with Wukasch and Hofstra (1977), who worked on O_3 (150ppb for 4h/day) and botrytis effects on onion *(Allium cepa* L. 'Autumn Spice') inside open top chambers in Ontario, Canada, and concluded that plant height was affected in the second week of O_3 fumigation at 150ppb for 4h/day.

Dead leaves at the bottom of the plants in the 60 and 90ppb chambers were also observed in the 2nd week, which indicates increased leaf senescence. There were no dead leaves in the FA and 30ppb chambers. O_3-induced injury on leaves of the 60 and 90ppb treated plants was in the form of typical tip burn injury. Tip burn injury to onion has been reported by several researchers in OTC experiments (e.g. Engle and Gabelman, 1965; Wukasch and Hofstra, 1977, and Temple *et al*., 1990). Symptoms

found during the OTC study were also similar to the ones observed on this same onion variety grown at the ARI site, Peshawar in the summer of 2008. Suspected O_3-induced white stipples were also associated with tip burn injury in leaves at the ARI site; the symptoms were found on mature leaves. However, no white stipples were found during the OTC experiment and also have not been documented in the literature, except by Temple *et al*. (1990), who reported light ivory and tan flecking on mature onion leaves in an OTC O_3 filtration experiment. This provides strong evidence that the tip burn injury to onion leaves observed in the field was due to O_3, but it is not clear that the white stipples found on onion at ARI were due to high O_3 concentrations. There might be several factors involved (e.g. O_3 exposure time, temperature difference and plant age) that caused onion to develop white stipple symptoms. The ARI plants with white stipples were at least three months old, very large in size, the temperature reached were 40°C, and the monthly O_3 concentration during May was 48.5ppb. In contrast, the OTC experiment was run for four weeks with temperatures between 15-20°C and the plants were small compared to the plants at ARI. White stipples could have appeared, in addition to leaf tip burn, if the onions in the OTC experiment were exposed to high O_3 concentrations for a longer time, and at high temperatures.

The findings are supported by most of the earlier studies conducted on different varieties of onion, which show that onion leaves are sensitive to O_3 injury and have increased leaf senescence. The first injury symptom was reported by Engle and Gabelman (1965), who stated that there was a close relationship between flecking and leaf tipburn symptoms on onion in the field and high O_3 concentrations. Temple *et al*. (1990) worked on the effect of O_3 (50ppb, 8h mean) on different winter cultivars

of onions in open top chambers, and observed that all cultivars had increased leaf senescence and tip burn injury on leaves. However, the extent of injury symptoms varied from cultivar to cultivar due to genetic resistance to O_3 exposure (Engle and Gabelman, 1966).

The O_3 concentrations of 60 and 90ppb significantly reduced the live and total biomass of onion, compared with FA treated plants, by 66% and 78% respectively. Wukasch and Hofstra (1977) examined the effect of O_3 on field grown onions exposed for three months to 150ppb 4h/day mean O_3 concentrations in an OTC experiment and documented that the biomass of onion treated plants was reduced by 28% compared to charcoal filtered air treated plants. McCool *et al*. (1987) also demonstrated a significant yield loss for green bunching onions exposed to various 12-hour seasonal mean O_3 concentrations. The linear regression response model predicted yield losses of 14.9% and 24.8% for seasonal means of 40ppb and 50ppb O_3 using the 12-hour seasonal mean statistic. Temple *et al*. (1990) worked on the effect of four different onion varieties and concluded that O_3 (150ppb, 8h mean for four weeks after germination) induced visible injuries to all varieties but there was no significance difference between the total biomass of O_3 fumigated and non fumigated plants, except for one variety that showed a 10% reduction. The O_3 effects on onion growth in these three studies is low compared to the current study, which suggests that the onion variety (Swat-1) used in the current study is more sensitive to O_3 than the varieties used by Wukasch and Hofstra (1977), McCool *et al*. (1987) and Temple *et al*. (1990).

It is concluded from the survey in Peshawar and the OTC experiment that onion variety Swat-1 grown in Peshawar and surrounding areas is at risk from high ambient O_3 concentrations. The mean O_3 concentration for May, 2008 was 48.5ppb, suggesting that the peak O_3 concentrations during day time might have been around 90-100ppb. This scenario puts onion at risk of negative effects as the plants showed a large and significant reduction in biomass at 60ppb inside the OTCs, but not at 30ppb. The gap between 30ppb and 60ppb is large and it is difficult to identify a threshold level for this variety. Furthermore, the OTC conditions at Imperial College were very different from the local conditions in Peshawar and hence there might be a large difference in sensitivity under field conditions.

This variety is a first choice variety for growing locally in Pakistan due to its high yield (Khan *et al*., 2005; Jilani & Ghuffor, 2003) but high local O_3 concentrations may still restrict this variety from reaching its full potential. It is suggested that this variety, along with other onion varieties and cultivars, should be subjected to further EDU and OTC experiments in local conditions in order to confirm whether O_3 does reduce yield under local conditions and to select the variety most resistant to O_3 that will boost the local agricultural economy.

2.6. CONCLUSIONS

The O_3 pollution surveys in Peshawar and OTC experiments on spinach and onion found that ambient O_3 concentrations were higher during summer than winter; visible injuries were also found on summer crops and no injury was observed in winter. The findings of the OTC experiments on winter spinach (B*eta vulgaris*) and a summer onion variety (Swat-1) suggested that both crops are sensitive to high O_3 concentrations. The percentage yield reduction per 1000ppb.h of AOT40 was 5.7% for spinach (Fig 2.18), and 7.1% for onion (Fig 2.25), which means that the two crops are similar in sensitivity. The difference is that the spinach receives lower O_3 concentrations during winter growth in Peshawar, which makes it at less risk from the negative effects of O_3 compared to onion that is exposed to high O_3 concentrations in the summer.

It is suggested that O_3 concentrations should be monitored round the year at multiple locations in order to assess its distribution in the Peshawar region. Since OTCs are an expensive method to determine O_3 effects on crops, and the unreliable electricity supply in the region makes them impractical, EDU could be used as a research tool for use in the areas where there are high O_3 concentrations. There should be more EDU experiments performed on summer crops, especially those crops which showed suspected visible O_3 injury symptoms, to estimate the extent of damage and to identify crop varieties that are resistant to O_3, after which the seeds should be certified by the government before handing them to the farmers. However, it is also important to evaluate if there are effects of O_3 on the growth and yield in the winter seasons. For this reason winter spinach (*Beta vulgaris*) was subjected to an EDU field experiment

during the winter season of 2008 at ARI to assess the impact of O_3 on the growth and yield of spinach (see Chapter 3 for details).

CHAPTER 3

EDU EXPERIMENT

3.1. INTRODUCTION

EDU was used instead of open top chambers (OTCs) in Peshawar. The installation of such chambers was not possible in the region, as it requires reliable power and a supply of significant funds. EDU is an anti-ozonant compound, ethylenediurea (N-2-[2-{oxo-1-imidizolidimyl} ethyl]-N1-phenylurea) abbreviated as EDU (Carnahan *et al.,* 1978). It has been used to assess acute and chronic effects of O_3 on a variety of plant species under ambient conditions (Manning, 2000). Control studies can be used to identify EDU concentrations which prevent O_3 effects without any direct adverse effects from EDU itself. EDU studies have been conducted on range of crop species including mung bean (Agrawal *et al.,* 2005), tobacco (Nakajima *et al.,* 2002), beans (Tonneijck and Van-Dijk, 2002a), soybean (Wahid *et al*., 2001), and radish & turnip (Hassan *et al*., 1995). Numerous studies have also been carried out on the mode of action of EDU, but the exact mechanism of action is still a controversial matter (Gatta *et al.,* 1997). EDU is applied as soil drenches, stem injections or foliar spray. It is reported to protect plants from premature senescence due to O_3 and allow successful growth leading to optimum productivity (Tiwari *et al.,* 2005).

Spinach was chosen for this EDU application experiment in pots at Peshawar, as it has been cited as a very sensitive crop to O_3 by Sakaki *et al.* (1983), David (1988), Luwe *et al*. (1993), Agrawal *et al*. (2003), Calatayud *et al.* (2003), Singh

(2005) and Tiwari and Agrawal (2009) and also based on the OTC experiment discussed in Chapter 3. In most American and European studies, *Spinacia oleracea* was subjected to EDU, and used in open top chamber experiments (Manning, 1972; Benton *et al*., 2000 and Calatayud *et al*., 2003), whereas experiments carried out in India on O_3 damage assessment in different cities used EDU on summer varieties of *Beta vulgaris* (Singh *et al*., 2005, Tiwari and Agrawal 2009). While O_3 levels are highest in summer, the results of Wahid *et al*. (1995) in Lahore suggest that adverse effects of O_3 could also occur under winter conditions in Peshawar. A majority of research studies conducted on effects of ambient O_3 concentrations on both types of spinach revealed foliar injury, loss of yield and also alteration in the nutritional quality.

3.1.1. EDU mechanism inside plant

It is not exactly clear how EDU alters the effects of O_3 entering the leaf (Lee and Chen, 1982; Lee *et al.*, 1997; and Manning, 2000). Gatta *et al*. (1997) studied the translocation and persistence of EDU in bean plants; they suggested that EDU accumulates in leaves and has a slow rate of degradation, showing persistence for more than 10 days. O_3 damage was lower in those leaves that contained higher EDU contents. EDU does not seem to function directly as an antioxidant, but it helps to maintain cellular antioxidants and to retain chlorophyll during O_3 stress (Lee *et al*., 1997).

It is generally thought that EDU confers tolerance to O_3 through induction of enzyme systems involved in elimination of activated oxygen species and free radicals. In particular, there is evidence that EDU induces changes in the antioxidant status of

leaves by increasing the levels of activity of the antioxidant enzyme superoxide dismutase (SOD) (Lee and Bennett, 1982). Brunschon-Harti *et al.* (1995b) reported effects of EDU on the antioxidant status of bean plants, and found a large increase in particular in levels of ascorbic acid. The mechanism of protection by EDU, however, may not be the same in all species.

Tomlinson and Rich (1973) and Brunschon-Harti *et al.* (1995a) concluded that EDU prevents the accelerated senescence that is caused by O_3. It prevents early leaf senescence in the presence of high O_3 concentrations, thus extending vegetative growth and increasing carbohydrate allocation from leaves to roots and developing fruit (Brunschon-Harti *et al.*, 1995b and Wahid *et al*., 2001). Membrane lipids as well as chlorophyll were also shown to be protected from O_3 in EDU-treated snap bean plants. It is theorized that EDU could stimulate cytokinin production, or have cytokinin like characteristics, in protecting chlorophyll from breakdown, increasing protein and RNA synthesis, and encouraging cell growth (Lee and Chen, 1982).

EDU applied systemically, through root application or as a foliar spray, converted O_3-susceptible plants into highly tolerant ones within 24h of treatment. In addition, both O_3 injury and senescence can be retarded by pre-treating the plants with EDU (Lee *et al*., 1981). Several reports have attributed "anti-senescent" properties to EDU (Wahid *et al*., 2001). Lee *et al*. (1981) showed that the senescence of red clover leaf discs was delayed when the discs were floated on an EDU solution in the dark or under low light. Kostka-Rick and Manning (1993b) also noted delayed foliar senescence of radish *(Raphanus sativus* L.) treated with EDU. It is well known that O_3 induces accelerated foliar senescence (Wahid *et al*., 1995a & b).

3.1.2. Use of EDU in field experiments

Many experiments on crop species have shown that EDU is useful in field conditions, but needs to be applied regularly (Brennan *et al.*, 1990; Miller *et al.*, 1994). EDU has been widely used in biomonitoring studies to determine the extent of O_3 damage because of its anti-ozonant action (Agrawal *et al*., 2001; Sanders *et al.*, 1992; Tonneijck and Van Dijk, 1997b). EDU has been shown to protect plants from O_3 injury (Carnahan, *et al.*, 1978) and has often been used in field studies to estimate crop losses caused by O_3 (Heagle, l989). Higher yields in EDU-treated plants in comparison to untreated plants have been attributed to the specific protective effect of EDU against O_3 damage (Saettler, 1981; Smith *et al.*, 1987). However, several factors are important to consider in evaluating the use of EDU to assess the impacts of O_3 under field conditions.

Firstly, EDU can have significant positive effects on bean plant (*Phaseolus vulgaris*) growth, increasing leaf, root and shoot dry weights in the absence of O_3 (Brunschon-Harti *et al*., 1995a & b). Results from this study showed that the EDU action on plant biomass could be interpreted as a delay in senescence, since EDU-treated plants showed a significant increase in biomass even in the charcoal filtered air (CF) treatment (Brunschon-Harti *et al*., 1995b). EDU and O_3 also have similar effects on carbon partitioning in radish (Kostka-Rick and Manning, 1993b), which constitutes a problem when EDU is used to study effects of O_3 on plant growth. However, in a related study, Kostka-Rick and Manning (1992) found no influence on growth dynamics or biomass partitioning in radish that could be attributed to EDU. Secondly, the level of protection provided by EDU depends on the application, methods, frequency and location. Several studies in Europe have shown that the EDU

protective effect varies between different locations characterized by different meteorological conditions and O_3 concentrations (Ribas and Penuelas, 2000). Therefore, it is important to establish the use of EDU under local Asian conditions. The first Asian EDU study was carried out by Bambawale (1986) in India, who studied the effect of O_3 on crop plants. He used tobacco (var. Bel-W3) and potato (var. Cherokee) as bio-indicators, and showed that the leaf spot symptoms on potato were similar to O_3 stipples reported in USA. He used EDU as a foliar spray and found that it reduced leaf spot disease by about 25-30 %. Tiwari *et al.* (2005) studied the effect of different EDU applications to field-grown two cultivars of wheat (*Triticum aestivum* L.) and suggested that EDU was very useful tool to investigate the effects of O_3 on the growth and yield of these varieties.

3.1.3. EDU Toxicity

A third reason that the use of EDU has been surrounded by controversy is that the chemical can be toxic in higher amounts (Heagle, 1989; Krupa and Nosal, 1989; Eckardt and Pell, 1996; Wahid, 2001). The toxic effects of EDU include marginal necrosis and leaf curling (Kostka-Rick and Manning, 1992). The potential effects of EDU on the growth and yield of radish (*Rapanus sativus* L.) cv. 'Cherry Belly' were studied by Kostka-Rick and Manning (1993c), who were able to show that low EDU concentrations (<400ppm) provided sufficient protection from O_3 injury without becoming phytotoxic. In further studies with O_3-sensitive bush beans *(Phaseolus vulgaris* L.) cv. "BBL 290", Kostka-Rick and Manning (1993c) demonstrated that the timing of EDU application was important and affected reproductive plant part development and yield. The toxicity of EDU depends upon the duration of its applications and fluctuations in ambient O_3 (Elagoz and Manning, 2005). No EDU

studies in South Asia have reported toxic effects of EDU on crops. However, in all these studies, O_3 concentrations were high, and may have disguised any toxic effects of the EDU on crops. It is thus important to assess whether EDU will have any negative effects on the growth, yield and nutritional quality of crops in South Asia, in the presence of low O_3 concentrations in the atmosphere, and also to assess if a dose of 300ppm, based on Tiwari and Agrawal (2009), will not cause any toxic effects on local spinach plants.

3.1.4. EDU effect on nutritional quality of spinach

Spinach is known for its nutritional quality and is an excellent source of iron and calcium, as well as beta carotene, vitamin C and E, potassium, sulphur, sodium, folic acid and oxalic acid (Singh *et al.*, 2005). Spinach also provides a cheap source of nutrients for the lower and middle classes of Pakistan. Tiwari and Agrawal (2009) conducted an experiment on the EDU effect on Indian summer spinach (*Beta vulgaris* L.) all green (Palak) and concluded that O_3 significantly reduces macro elements concentrations (Ca, Na, K, Mg and Fe) in non EDU treated plants compared to EDU treatments. Therefore it is important to study the effects of ambient O_3 on the growth and nutritive value of winter spinach.

3.1.5. EDU studies in different regions of the world

3.1.5.1. North American and European EDU studies

EDU has been used in the last two decades in Europe and North America to assess the quantitative and qualitative effects of O_3 on various crops. For example, EDU has been widely used with beans by researchers in America and Europe and has been reported to reduce foliar injuries and yield reductions (e.g. Brunschön-Harti *et al*., 1995a; Astorino *et al*., 1995; Tonneijck and Van Dijck, 1997a; Elagöz and Manning, 2005). Eckardt & Pell (1996) and Godzik & Manning, (1998) studied the effect of O_3 on potato and tobacco using EDU and concluded that EDU can reduce foliar senescence. Examples of studies on O_3 effects on crops in North America and Europe using EDU have been summarised in Table 3.1 and 3.2, respectively.

Table 3.1: Examples of research conducted on EDU application to different crops in North America

Location	Crop	Method of application and concentration	Average O_3 (ppb) exposure	Response	Reference
USA	Bean (*Phaseolus vulgaris*)	Foliar spray, 500ppm	850ppb for 150 min	Prevention of O_3 leaf injury	Carnahan *et al.* (1978)
Canada	Cucumber (*Cucumis sativus*)	Soil drench, 250ppm	80ppb, 7h mean for10 days	Increase in growth and yield	Proctor *et al.* (1978)
Canada	Potato (*Solanum tuberosum*)	Foliar spray, 1kg ha^{-1}	800ppb, 6h mean for3 months	Reduced early blight infection on potato	Bisessar and Palmer (1982)
Canada	Beans (*Phaseolus vulgaris*)	Soil drench, 400ppm, 600ppm	80ppb, 6h mean for 4 days	No effect on oxyradical scavenger, superoxide dismutase (SOD)	Chanway and Runeckles (1984)
USA	Beans (*Phaseolus vulgaris*)	Soil drench, 500ppm	120ppb, 7h seasonal mean	Increase in yield	Brennan *et al.* (1990)
USA	Potato (*Solanum tuberosum*)	Soil drench, 500ppm	110ppb/hr for three months	Less visible foliar injury and increase in total tuber yield	Clarke *et al.* (1990)
USA	Field pea	Soil drench, 150ppm	250ppb, 4h mean for one day	Visible foliage symptoms were completely prevented	Zilinskas *et al.* (1990)
USA	Black cherry (*Prunus serotina* Ehrh.)	Foliar spray, 1000ppm	80ppb, 13h seasonal mean	Significant increase in biomass, height and growth diameter	Long and Davis (1991)
USA	Radish (*Raphanus sativus*)	Soil drench, 200ppm	58ppb, 7h mean for 6 days	Increase in relative growth rate and carbohydrates for sink and shoot.	Kostka-Rick *et al.* (1992)
USA	Bean (*Phaseolus vulgaris*) and soybean (*Glycine max*)	Soil drench, 500ppm	350ppb for 2 hr	Did not alter stomatal behavior and foliar membrane lipid composition	Lee *et al.* (1992)
USA	Bean (*Phaseolus vulgaris*)	Soil drench, 800ppm	124ppb, 7h mean for 4 weeks	Delayed maturation and senescence	Kostka-Rick and Manning (1993a)
USA	Radish (*Raphanus*	Soil drench,	75ppb, 7h mean for 4 week	Complete protection from visible	Kostka-Rick and

	sativus)	400ppm		injury	Manning (1993b)
USA	Potato (*Solanum tuberosum*)	Soil drench, 150ppm	100ppb, 5h mean for 4 days	Reduced symptoms of toxicity and delayed senescence	Eckardt and pell (1996)
USA	Beans (*Phaseolus vulgaris*)	Soil drench, 500ppm	300ppb for 3 hrs	No effect on superoxide dismutase (SOD), guaiacol-peroxidase (GPX), ascorbate peroxidase (APX) & glutathione reductase (GR) activities	Lee *et al*. (1997)
USA	Spreading dogbane (*Apocynum androsaemifolium*)	Foliar spray, 300ppm	60ppb, 5h mean for 3 months	Protection from foliar injury	Bergweiler and Manning (1999)
USA	Bean (*Phaseolus vulgaris*)	Foliar spray, 300ppm	55ppb for total of 355 hours	Increase in crop yield	Elagoz and Manning (2005)
USA	Cut leaf coneflower (*Rudbeckia laciniata*)	Foliar spray, 200ppm, 400ppm and 600ppm	23ppb, 12 h mean for 12 weeks 26ppb, 24h mean for 12 weeks	Reduced percent of leaves injured by EDU, but decreased root and total biomass	Szantoi *et al.* (2009)

Table 3.2: Examples of research conducted on EDU application to different crops in Europe

Location	**Crop**	**Method of application and concentration**	**Average O_3 (ppb) exposure**	**Response**	**Reference**
Italy	Bean (*Phaseolus vulgaris*)	Soil drench, 300ppm	50ppb, 6h mean for 4 weeks	Increase in the dry weight, photosynthetic capacity, total above ground biomass, chlorophyll a content and peroxidase activity	Astorino, *et al*. (1995)

Italy	Tobacco (*Nicotiana tobaccum*)	Soil drench, 300ppm	150ppb for 2 h	Increased in the level of antioxidant metabolites	Batini *et al*. (1995)
Germany	Bean (*Phaseolus vulgaris*)	Soil drench, 150ppm	0·98, 14·1, 14·98 and 31·56ppb, 24h mean for 14 days	Delay in foliar senescence, High soluble protein content, peroxidase activity increased, lower ascorbic acid content	Harti *et al*. (1995a)
Sweden	Clover (*Trifolium subterraneum*)	Soil drench, 150ppm	25 ppb (24 h mean) and 30 ppb (7 h mean) for 3 consecutive summer seasons	Reduced extent of visible injury	Karlsson *et al.* (1995)
Italy	Bean (*Phaseolus vulgaris*)	Hydroponic condition, 150ppm	300ppb for 3h	No visible or reduced leaf injury	Gatta *et al*. (1997)
Netherlands	Subterranean clover (*Trifolium subterraneum*)	Soil drench, 150ppm	58ppb, 7h mean for 4 weeks	Increase in biomass and less leaf injury	Tonneijck, and Dijk (1997)
Sweden	Radish (*Raphanus sativus*)	Soil drench, 200ppm	31ppb, 24h mean for 5 weeks	Increase in biomass and chlorophyll content	Pleijel *et al.* (1999)
Netherlands	Subterranean clover (*Trifolium subterraneum*)	Soil drench, 150ppm	46ppb, 7h mean for 8 weeks	Little foliar injury	Tonneijck and Dijk (2002b)
Ukraine	Subterranean clover (*Trifolium subterraneum*)	Soil drench, 300ppm	30ppb, 7h mean for 28 days	Less visible injury	Blum and Didyk (2006)

3.1.5.2. Asian and African EDU studies

EDU has been used in several countries in Asia over the last two decades to assess O_3 impacts on crops. The Asian studies are summarised in Table 3.3. In Pakistan, Wahid *et al*. (2001) carried out an experiment using EDU as a soil drench on a soybean crop at a suburban site in Lahore, a remote rural site, and a rural roadside site, and recorded an increase in yield (means of 47% and 113 % in post-monsoon and pre-monsoon seasons, respectively) in EDU-treated plants as compared to non-treated ones. Increases in yield were more pronounced at rural (182%) and roadside rural sites (285%) compared to suburban sites (94% at the remote rural site and 170% at the rural roadside site) in the post monsoon season. Tiwari *et al*. (2005) used EDU to investigate the effect of ambient O_3 concentrations on the yield of two Indian field-grown cultivars of wheat (*Triticum aestivum* L.) and found a positive response to EDU in a range of growth parameters. In another South Asian study, Agrawal *et al*. (2005) showed that non-EDU treated mung bean plants (*Vigna radiata* L.) exposed to ambient O_3 concentrations in a suburban area of Allahabad (India) had significant reductions in plant growth and yield compared to EDU treated plants of the same cultivar. Similarly, Bambawale (1989) and Varshney & Rout (1998) reported reduced effects of O_3 on several physiological parameters of EDU-treated Indian grown potato and tomato plants, respectively.

Few studies have been carried out using the anti-ozonant EDU on crops in Africa (Table 4.3). However, these studies have shown negative effects of O_3 on crops using EDU. For example, Hassan *et al*. (1995) subjected radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.) treatment with EDU in a field experiment, and concluded that the growth and yield of these two economically important crops was increased by EDU applications, and hence was being reduced by ambient O_3 levels. Similar effects were also examined for potato

in a second Egyptian study by Hassan (2006), in which the application of EDU as a foliar spray reduced foliar injury symptoms on potato plants.

Table 3.3: Examples of research conducted on EDU application to different crops in Africa and Asia

Location	**Crop**	**Method of EDU application and concentration**	**O_3 concentration (ppb) average exposure**	**Response**	**Reference**
Egypt	Radish (*Raphanus sativus*) and Turnip (*Brassica rapa*)	Soil drench, 500ppm	55ppb, 6h mean for 3 weeks	Growth increase	Hassan *et al.* (1995)
India	Tomato (*Lycopersicon esculentum*)	Soil drench, 400ppm	46ppb, 5h mean for four months	Increase in the root &shoot length along with biomass	Varshney and Rout (1998)
Pakistan	Soya bean (*Glycine max*)	Soil drench, 400ppm	75ppb, 6h mean for 4 months	Increased in seed weight/plant	Wahid (2001)
Egypt	Bean (*Phaseolus vulgaris*)	Soil drench, 200ppm	83ppb, monthly mean for 4 months	Stimulated carbohydrate and pigment contents the upper canopy	Ali and Hammad (2004)
Egypt	Soya bean (*Glycine max*)	Soil drench, 200ppm	52ppb, 4 weeks mean for 6 months	Increase in nitrogen content of the leaves and enhanced growth	Ali & Abdel-Fattah (2005)
India	Wheat (*Triticum aestivum*)	Soil drench, 300ppm	41, 8h mean for 4 months	Increase in root and shoot length along with biomass and number of tillers/plant	Tiwari *et al.* (2005)
Egypt	Potato (*Solanum tuberosum*)	Foliar spray, 300ppm	78ppb, 10h mean for 4 months	Reduced visible injury to leaves and increase in tuber weight	Hassan *et al.* (2006)
China	Rice (*Oryza sativa*) and wheat (*Triticum aestivum*)	Foliar spray, 150, 300 and 450ppm	50ppb, hr mean fro 10 months	Increase in yield, seed number/ plant, seed set rate and harvest index of wheat, no parameters were statistically significant for rice	Wang *et al.* (2007)
India	Spinach (*Beta vulgaris*)	Soil drench, 150, 300 and 450ppm	40, 50 and 60ppb, 8h mean for 4 months	Increase in lipid peroxidation, ascorbic acid, bio mass and Na, K, Ca, Mg and Fe content	Tiwari and Agrawal (2009)
India	Mung bean (*Vigna radiate*)	Soil drench, 300ppm	52-64ppb monthly mean for thee months	Increase in yield	Singh *et al.* (in press)

3.2. AIMS AND OBJECTIVES

These EDU studies indicate that it is a useful technique to assess the negative effects of O_3 on the growth, yield and visible injuries of crops in different part of the world. Therefore, EDU was applied to evaluate the effects of O_3 on the Peshawar region, using a protocol that has been specifically developed for such experiments in South Asia (Tiwari and Agrawal, 2009). The specific objectives were:-

1. To assess the impact of O_3 on the growth, yield and visible foliar injuries of winter spinach in the Peshawar region by using EDU.
2. To assess the effect of O_3 on the nutritional quality of winter spinach in the Peshawar region, using EDU.
3. To identify any toxic effects of EDU in the presence of low O_3 concentrations in winter.

3.3. MATERIALS AND METHODS

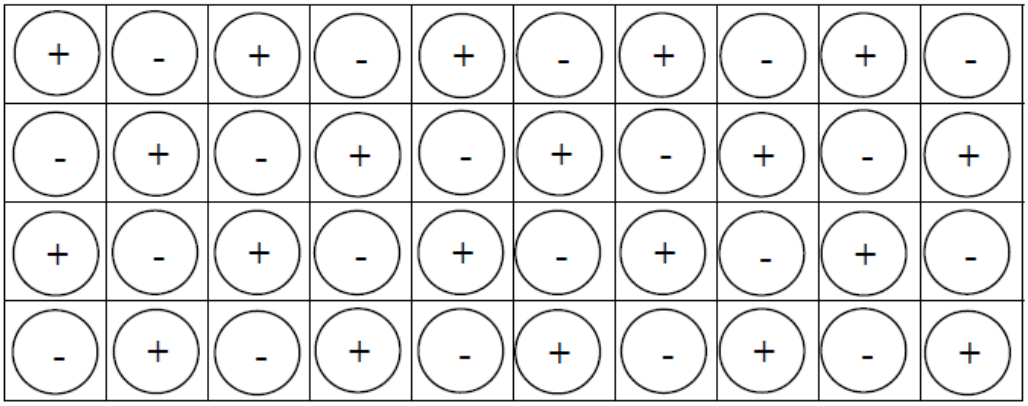
The EDU application experiment was set up in pots at ARI Peshawar (see Chapter 1) on 23rd October, 2008 according to the modified method of the Malé Declaration Network (Tiwari and Agrawal, 2009). The plot area (30x30m) was selected at ARI in an open field away from buildings and at least 200 meters away from main Great Trunk road. The area was fenced to keep the plants safe from mammals. The plot was situated in the middle of a vegetable garden (Figure 3.1).

Figure 3.1: EDU experimental plot in the middle of ARI farms protected by barbed wire fence.

15 litre volume pots were used with a surface diameter of approx. 30 cm and a height of 30 cm. These pots were made from mud that is resistant to over-heating and water logging. Locally available soil at ARI (silt + clay soil in a ratio of 1:2) was used in these pots. Silt was brought from the irrigation canal situated near the site.

The IVL passive samplers were used for measurement of O_3 concentrations in the air (details in Chapter 2). Relative humidity and temperature were recorded throughout the experiment near the site. The readings were taken twice a day at 08:00am and 05:00pm everyday. The number of replicate plants placed at the experimental site was 20 per treatment, i.e. 40 altogether. The pots were labelled to identify the treatment (EDU/non-EDU). One day before planting, pots were filled with soil. The soil was thoroughly mixed before putting it into the pots. The pots were arranged as alternate EDU-treatment and non-EDU-treatment pots in 4 rows of 10 pots with 0.5m distances within rows and 1m distances between rows (Fig 3.2).

Fig 3.2: Experimental design of chemical protectant study with 20 EDU-treated (+) and 20 non-EDU-treated (-) plants.



Seeds of a locally available spinach cultivar (*Nare palak*) of winter spinach (*Beta vulgaris*) were selected for the experiment from the main seed market in Peshawar. Care was taken to select the best quality seeds, free from diseases and pests. Five seeds were sown directly into each pot on 23 October, 2008. The soil was thoroughly wetted with deionised water after the sowing procedure, but care was taken to avoid over watering. One week after the emergence of seedlings, which occurred on 29th October 2008, they were thinned to one plant per pot, keeping the best developed and healthiest seedling.

Ethylenediurea (EDU) was supplied in sufficient quantities by Dr. Abdul Wahid, Government College University, Lahore. This EDU was originally produced and made by Prof. William Manning, University of Massachusetts, USA. Day 1 of the experiment was when the seedling emerged on 29 October, 2008. The first EDU application was made on day 7. EDU was applied only to 50% of the plants i.e. 20 pots only. The remaining pots were given only deionised water in same amount.

EDU solution was freshly prepared in deionised water a day before the application. There were a total of 6 applications, based on 10 day intervals up to the maturity of the plant. The EDU solution was applied as a soil drench in the early morning (7-8 am). A concentration of 300ppm EDU was selected on the basis of the study of Tiwari and Agrawal (2009), who conducted an experiment on Indian summer spinach (*Beta vulgaris* L.), showing that sensitive cultivars can be protected against O_3 at an EDU concentration of 300ppm. An EDU application gap of 10 days was kept because EDU can persist in the leaves for up to 10 days (Agrawal *et al.* 2003, Singh *et al.* 2005 and Tiwari and Agrawal, 2009).

A single application was applied to 20 pots (with concentration of 300ppm) on:

Day 7, 1st application 100ml/ pot (0.6g/2 litres of deionised water)

Day 27, 2nd application 100ml/pot (0.6g/2 litres of deionised water)

Day 37, 3rd application 150ml/ pot (0.9g/3 litres of deionised water)

Day 47, 4th application 150ml/ pot (0.9g/3 litres of deionised water)

Day 57, 5th application 200ml/ pot (1.2g/4 litres of deionised water)

Day 67, 6th application 200ml/ pot (1.2g/4 litres of deionised water)

Therefore, in total 5.4g of EDU was required in 18 litre of deionised water for the whole experiment for each pot. The pots were watered with deionised water every second day depending on the environmental conditions. The pots were always watered a day before the EDU application, to avoid adding EDU to dry soil. Further watering was given on the second day after EDU application. Weeds were removed in and around the pots on a weekly basis. Careful observations were made for pest/insect control twice a week. No pesticide applications were made during the experiment. Growth assessment was made by measuring the height of the plant every week.

The number of damaged fully expanded leaves was counted at weekly intervals, as well as the total number of fully expanded leaves. Each plant was graded as either healthy (H) or abnormal, with the cause of the abnormality graded as 1 (slight), 2 (moderate), or 3 (severe) using the key in Table 3.4:

Table 3.4: Summary of forms of visible injury that were recorded

Abbreviations	**Injury names**
S1, S2, S3	Stunted
D1, D2, D3	Diseased
I1, I2,I3	Insect damaged
SL1,SL2,SL3	Slug Damaged
A1,A2,A3	Animals (rabbits, goats, birds etc.)
V1,V2,V3	Virus

The spinach plants were harvested after 8 weeks, on day 58 (24th December, 2008). The entire above-ground biomass of each plant was put into a labelled paper bag (i.e. one bag per plant). The labelled bags containing spinach plants were brought to the laboratory of Department of Chemistry, NWFP Agricultural University, Peshawar as soon as possible. The biomass were dried at approx. 70°C for approx. 48 hours in an electric oven before being

weighed using a high precision balance. After drying the samples were enclosed in a paper bag and were sent to University of Nijmegen, Netherlands for the chemical analysis.

3.3.1. Determination of elemental content of spinach

The plant samples were analysed by inductively coupled plasma-mass spectroscopy (ICP-MS) and inductively coupled plasma-optical emission spectrometer (ICP-EOS) at Nijmegen University, Netherlands. The nutrients (e.g. K, Ca, and Fe) were analysed by ICP-MS and trace elements (e.g. Cr, Zn, Mn, Cd and Pb) by ICP-EOS.

200mg of dried ground plant sample was accurately weighed and was put into a pressure vial. 4ml of 6% HNO_3 and 1ml of 30%H_2O_2 were added to the pressure vial. The vial was covered tightly and was shaken to mix the reagents. The vial was then placed in a microwave. The microwave sample sequence was set at 1min at 250W, 2min at 0W, 5min at 250W, 5min at 400W, 5min at 400W and 5min at 500W. The sample was then taken out and was cooled for 30min at 4°C. After cooling, the vial was opened, the mixture was flushed with deionised water into a 100ml volumetric flask, and the volume was made up to 100ml with deionised water. The extract was then analysed by ICP-MS and ICP-EOS.

3.3.2. Statistical summary

The data summary (means and standard errors) was carried out using Microsoft Excel (2003) and the statistical analysis was carried out using SPSS 17.0. The dataset for all parameters was explored for skewness, kurtosis and normality, using the Shapiro-Wilk-test. None of the parameters showed major deviations from a normal distribution. An independent

sample t-test was then used for each of the parameters to determine whether these were significantly different at $p<0.05$.

3.4. RESULTS

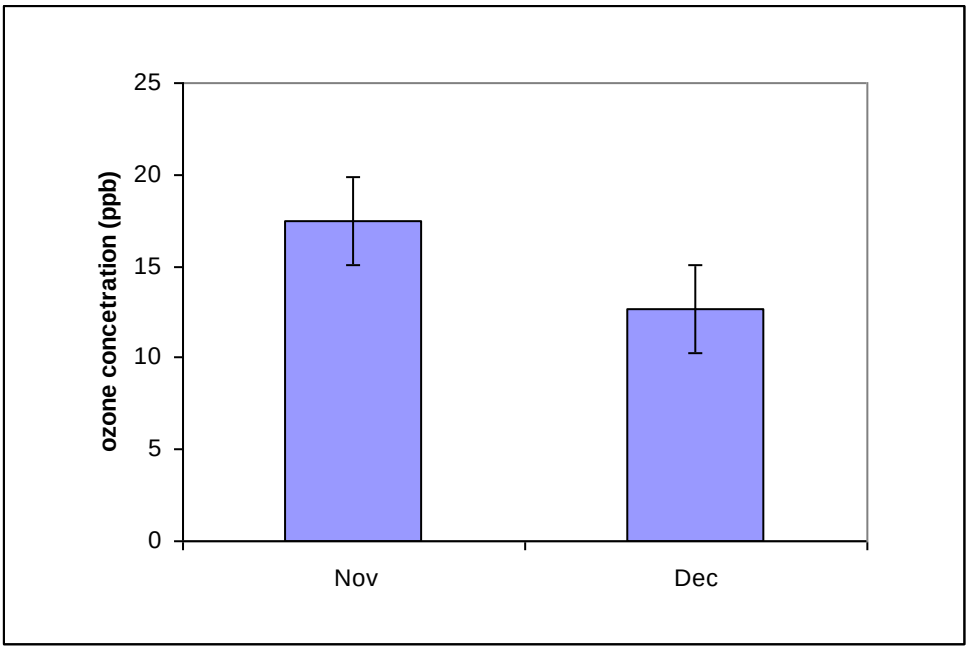
3.4.1. Meteorological data

The mean relative humidity for the first four weeks in November was 55.2%, with a range of 34-88%. The mean temperature recorded was 19.5°C, with a minimum temperature of 6°C in the morning and a maximum of 33°C in the evening. The relative humidity ranged from 24% to 89%, with a mean of 56.7%, during December, whereas the temperature ranged from 0°C to 26°C, with a mean of 13°C.

3.4.2. O_3 passive sampler data

Results from the O_3 passive samplers revealed that the mean O_3 concentration for the first four weeks was 17.5ppb and for the last five weeks was 12.7ppb. The mean concentration for the whole duration of the experiment, from 23rd October 2008 to 24th December 2008, was 15.1ppb (Figure 3.3).

Figure 3.3: Ambient O_3 concentrations (ppb) in winter (November-December) at ARI. Bars represent standard error of the values



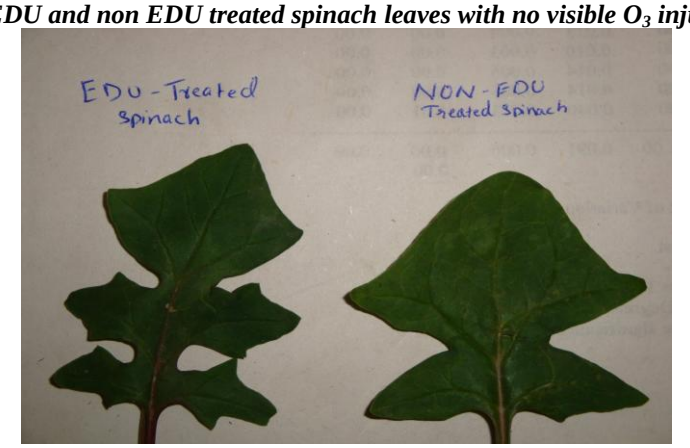
3.4.3. Injury assessment

The first inspection was made on 2nd November 2008, when all plants in every pot were healthy and there were no signs of any O_3 injury, disease or animal attack. However, in the second and third assessments, carried out on 25th November 2008 and 15th December 2008 respectively, 8 plants were affected due to attack by birds (mainly crows and sparrows). The 4th and last inspection, carried out on 24th December 2008, just before harvesting, showed that, apart from bird damage, seven other plants were damaged due a disease characterised by chlorosis on leaf surfaces. This disease appeared in the last week before harvesting.

In all, the results showed that birds destroyed 4 EDU treated plants and 4 non-EDU treated plants, while chlorosis affected 4 EDU and 3 non-EDU treated plants. There were no visible injury symptoms typical of O_3 recorded on spinach leaves, including no visible colour difference between the EDU and non-EDU treated plants at any stage of the experiment. Figure 3.4 and 3.5 show the EDU and non EDU treated spinach plants on the day of harvest, with no signs of O_3 injury.

Figure 3.4: EDU and Non-EDU treated spinach plants on the last day of harvest (9 weeks old)

Figure 3.5: EDU and non EDU treated spinach leaves with no visible O_3 injury symptoms



Subsequent data analysis only used data from those plants which were not affected by chlorosis and bird damage. This gave the total of 12 EDU treated plants and 13 non-EDU treated plants.

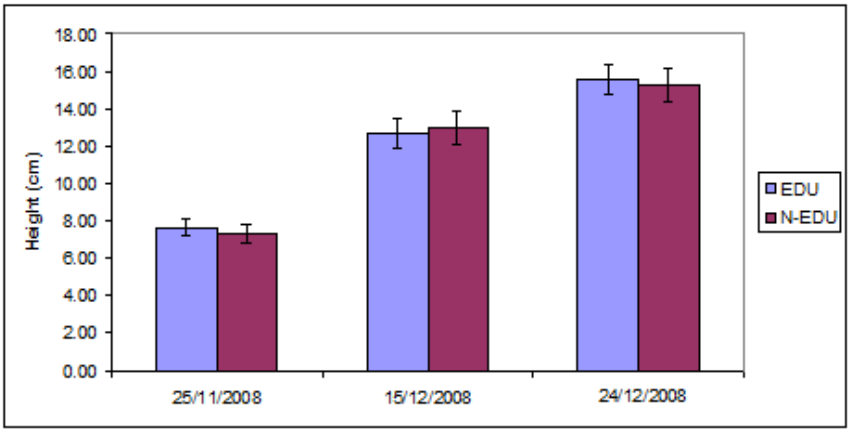
3.4.5. Plant height

Measurements of the spinach plant growth were made three times during the whole experiment. Plant height was recorded on each occasion by measuring the length of the longest mature leaf. There was no significant difference between the EDU and non EDU plant height during the entire experiment (Figure 3.6; Table 3.5).

Table 3.5: Independent sample t-test showing t values and probability values (p) for the effect of EDU on spinach plant height.

Date	t-value	P
25/11/2008	0.545	0.589
15/12/2008	-0.341	0.735
24/12/2008	-1.126	0.271

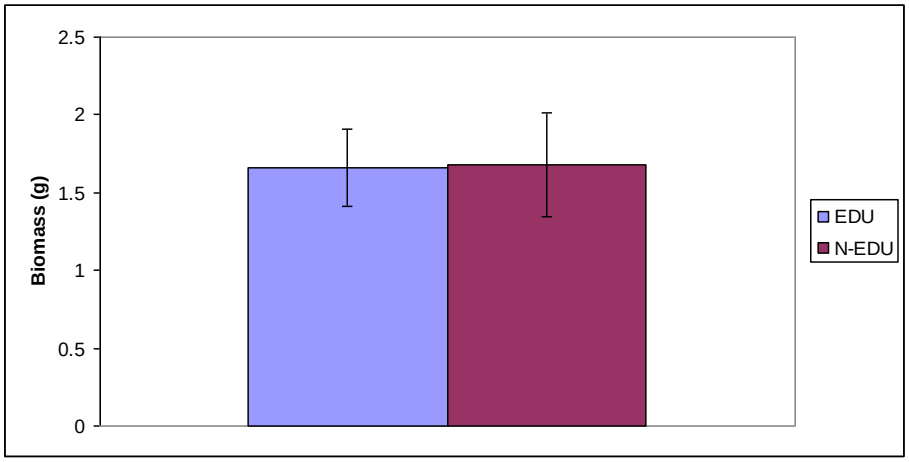
Figure 3.6: EDU effect on spinach height growth. Bars represent standard error of the values



3.4.6. Plant biomass

The average biomass of EDU treated plants (shoots+ leaves) was 1.66g and that of non-EDU treated plants was 1.68g. The EDU treated plants (Fig 3.7) did not show a significantly higher biomass than the non-EDU treated plants (independent sample t-test; t = 0.462 and p = 0.503).

Figure 3.7: Effect of EDU on spinach biomass. Bars represent standard error of the values.



3.4.7. Effect of O_3 and EDU on elemental content of spinach

The results revealed that leaf concentrations of the macro elements Mg, Si, K, Ca, Mn, P and Zn were significantly reduced, by 40%, 31%, 17%, 40%, 38%, 38%, 36.5% respectively, in EDU treated spinach compared with non-EDU treated spinach (Table 3.6). In contrast, Na, Al, S and Fe concentrations did not differ significantly between the treatments. The total base cation concentration (Na, Ca, K an Mg) was also significantly reduced, by 12.5%, in EDU treated plants compared to non-EDU treated plants (Table 3.6). Concentrations of the trace elements Pb, Cu, Cd, As, Se, Sr and Mo were significantly reduced, by 40%, 44%, 42%, 33%, 54%, 49% and 52% respectively, in EDU treated plants (Table 3.7).

Table 3.6: Independent sample t-test showing t values and probability values (these shown in bold are significant at p = 0.05) for the effect of EDU on macro element content of spinach.

Element	EDU (µg g^{-1})	non-EDU (µg g^{-1})	t-value	p
Na	644.13 ±47.6	740.43 ±52.9	-1.299	.206
Mg	608.57 ±33.2	854.13 ±47.8	-3.871	**.001**
Al	44.31 ±2.6	45.02 ±2.3	-.200	.843
Si	32.30 ±1.4	42.03 ±2.5	-2.996	**.006**
P	429.16 ±28.1	593.03 ±46.8	-2.716	**.012**
S	181.20 ±14.5	218.80 ±15.9	-1.654	.111
K	2864.83 ±92.5	3346.50 ±136.0	-2.683	**.013**
Ca	559.47 ±22.5	786.17 ±42.9	-4.187	**.000**
Fe	35.24 ±4.4	34.33 ±2.0	.198	.845
Mn	2.25 ±0.1	3.11 ±0.3	-2.427	**.023**
Zn	2.28 ±0.1	3.11 ±0.2	-2.691	**.013**
Base cations	4677 ±195.8	5727 ±279.6	-2.982	**.006**

****Positive t-value indicates that mean EDU value is higher than mean non EDU value. Bold values indicates significant reduction in metal content***

Table 3.7: Independent sample t-test showing t values and probability values (these shown in bold are significant at p = 0.05) for the effect of EDU on micro element content of spinach.

Element	EDU (*n*g g^{-1})	N-EDU (*n*g g^{-1})	t-value	p
Pb	5.76 ±0.44	7.61 ±0.39	-3.074	**.005**
Cr	106.45 ±21.15	160.10 ±29.83	-1.350	.190
Co	22.26 ±2.00	25.45 ±1.64	-1.219	.235
Ni	70.00 ±5.64	77.28 ±7.55	.771	.448
Cu	283.31 ±10.52	408.69 ±23.26	-4.344	**.024**
AS	16.47 ±0.86	24.55 ±2.07	-3.170	**.004**
Se	24.85 ±1.94	37.41 ±6.37	-2.777	**.010**
Sr	39.68 ±4.30	77.69 ±29.94	-5.106	**.001**
Mo	65.61 ±2.29	99.49 ±5.88	-4.705	**.019**
Ag	0.78 ±0.18	3.92 ±3.00	-.893	.381
Cd	5.96 ±0.38	10.35 ±0.80	-4.394	**.030**
Sn	4.60 ±0.72	8.36 ±2.38	-1.308	.203
Ba	70.07 ±7.10	91.12 ±9.66	-1.623	.118
Hg	0.71 ±0.13	0.47 ±0.05	1.847	.077

****Positive t-value indicates that mean EDU value is higher than mean non EDU value. Bold values indicates significant reduction in metal content***

3.5. DISCUSSION

No O_3 visible injury symptoms were found on spinach during the entire experiment. Surveys of O_3 visible injury were also made on the same spinach variety grown in the fields surrounding the ARI site during the winter season of 2008, at the same time as the experiment. No O_3 visible injuries were found. Suspected O_3 damage was limited to roadside vegetation, mainly to bottle brush (*Callistemon citrinus*), during the same winter period. In contrast to winter, suspected O_3 visible injuries in and around Peshawar city on different crops, especially on potato and onion, were identified during the spring and early summer survey of 2008 (see Chapter 2).

This was the first EDU experiment conducted in Peshawar and no previous data on ground level O_3 concentrations were available for the winter season in Peshawar. The experiment was carried out on the basis of experiments carried out in Lahore on different varieties of wheat in OTC filtration experiments (Wahid *et al*., 1995 and Wahid, 2006) that revealed relatively high O_3 concentrations during the winter season and significant reductions in wheat yields.

No visible O_3 injuries to spinach leaves, and no significant effect of EDU on height and yield (biomass), were found during the experiment. There might be several reasons for these results:-

1. The EDU batch used for the experiment might not be effective to protect spinach in the local conditions. However, the same EDU batch was used in the studies of Wahid *et al*.

(2001; 2006), in which EDU reduced the negative effects of O_3 on crops in the presence of high O_3 concentrations.

2. The O_3 concentrations in winter were too low to cause any negative effects on the spinach. The O_3 concentrations monitored by the passive sampler during the experiment were low compared to the concentrations recorded from February) to June 2008.

3. The fact that no negative effects of O_3 were observed on the growth and yield of this particular spinach variety may be due to the fact that it is relatively resistant to O_3 impacts.

Significant visible injuries were found on 60ppb treated plants of the same variety during the OTC experiment, but no negative effects were observed on 30ppb treated plants (Chapter 2). The mean monthly O_3 concentration was 15ppb during the EDU experiment and from this it is expected that the daytime peak O_3 concentrations might have reached 30ppb. Based on the OTC experiment, this concentration was not high enough to cause any significant damage to the spinach. This suggests that winter grown varieties of *Beta vulgaris* are not at risk of negative effects of O_3 in Peshawar, even through the effects of short term exposure to 60ppb suggest that they are sensitive to O_3.

Previous research carried out in different parts of the world suggests that spinach varieties of the species *Spinacia oleracea* are very sensitive to O_3 (e.g. Sun *et al.*, 1994). Two previous studies have examined effects on *Beta vulgaris*. Agrawal *et al.* (2003) and Tiwari and Agrawal (2009) both used EDU to study the effect of O_3 on spinach (*Beta vulgaris L. var* All Green) in summer in Varanasi, India, and showed that the O_3 levels were high enough to affect *Beta vulgaris*. The O_3 concentration, based on average monthly 8h mean

concentrations, ranged from 52ppb to 73ppb from 2002 to 2006 during summer at the suburban site of Varanasi, where EDU experiments revealed yield reductions of *Beta vulgaris*. Agrawal *et al*. (2003) and Tiwari and Agrawal (2009) used summer varieties, grown in spring and early summer with high O_3 concentrations, in contrast to the winter variety used here. The results of Agrawal *et al*. (2003) and Tiwari and Agrawal (2009) are consistent with the OTC spinach experiment described in Chapter 2, in which negative effects of O_3 were found in 60ppb treatments. This further supports the conclusion that summer varieties, but not winter varieties, of *Beta vulgaris* are likely to be affected by O_3 in South Asia.

Another important influence on the outcome of experiment was the effect of birds and aphid infestation on spinach plants. About 37.5% of plants were affected by these pests. Although there was a fence around the research plot, this did not completely prevent the birds from attacking the spinach plants. Disease appeared on the leaves of both EDU and non EDU treated plants in form of chlorosis and necrosis during the 8^{th} week, and may possibly be due to unexpected warm and moist conditions during the early winter season. These two conditions may have played a role in the results being non-significant.

In the current study, EDU did not have any effects on growth and yield but affected the element content of *Beta vulgaris*. Concentrations of base cations (Ca, Mg and K, but not Na) were significantly reduced in EDU treated plants. Concentrations of heavy metals (e.g. Cd and Pb) were also significantly lower in EDU treated plants. In contrast, the concentrations of other important elements, like Fe and Al, were not significantly affected by EDU treatment. These effects might be due to the high EDU dose (300ppm). Tiwari and Agrawal (2009) conducted an experiment on EDU effects on Indian summer spinach (*Beta vulgaris* L.) all green (Palak) and concluded that macro element concentrations (Ca, Na, K,

Mg and Fe) were lower in non EDU treated plants compared to EDU treatments. However, they did not observe any toxic effects of EDU on spinach by using a 300ppm EDU treatment. The mean 8h O_3 concentrations in the study of Tiwari and Agrawal (2009) ranged from 52ppb to 73ppb, and thus the effect on the element content was likely to be due to O_3 rather than EDU, with EDU protecting the nutrient content of spinach in the presence of high O_3 concentrations. In contrast, the mean monthly O_3 concentrations were very low (15ppb) during the EDU experiment at Peshawar. It is possible that EDU has some negative effects on the element content of spinach in the absence of high O_3 concentrations, but to date, no study of EDU effects on elemental contents of plants have been carried out in the absence of O_3 or at low O_3 concentrations. However, there are studies in which EDU caused phytotoxicity because of the high dose of EDU given to plants (e.g. Heagle, 1989; Krupa and Nosal; 1989; and Ecardt and Bell, 1996). The visible toxic effects of EDU include marginal necrosis and leaf curling (Kostka-Rick and Manning, 1992) but no such effects were observed during the current study.

The results of the open top chamber O_3 fumigation (Chapter 2) were in line with the EDU field study. The environmental conditions (e.g. day length) were not exactly the same during both studies, due to which the leaves did not expand as well as expected, and flowering of the plants also occurred prematurely during the OTC experiment. This spinach variety in Pakistan is known to be grown best in 12 hours of dry sunshine at 15-18°C and low temperatures at night (Waseem *et al*., 2001). There was not much difference between the atmospheric temperature and relative humidity in the EDU and OTC experiments, the mean temperature and relative humidity during the EDU experiment was 17°C and 57%, respectively, while it was 21°C and 61% during the OTC experiment. However, the extended light duration during the OTC experiment (17 hours) caused plants to bear flowers

prematurely and the leaves did not expand as during the EDU field study in Peshawar. In spite of the different environmental conditions between the two experiments, it is concluded that winter spinach grown in Peshawar is not at risk of O_3 damage because of the low ambient O_3 concentrations during the winter season. EDU should be applied to assess the impact of O_3 on summer crops, when the O_3 concentrations will be much higher than in winter.

CHAPTER 4

FLUORIDE AND SULPHUR SURVEY AT THE VICINITY OF BRICK FIELDS LOCATED AROUND PESHAWAR CITY

4.1. INTRODUCTION

It is widely thought that fluoride is one of the most toxic atmospheric pollutants along with ozone (O_3) and sulphur oxides (SOx) (Jha *et al*., 2008). It can be phytotoxic as well as a health risk to humans and animals. Aluminium smelters, ceramic manufacture, phosphorus fertiliser factories and brick kiln industries are the main sources responsible for fluoride pollution in the air, soil and water. Most of these factories use coal that emits sulphur and fluoride containing compounds. Coal may contain fluoride contents ranging from 40-295ppm, whereas bricks are made from clay that may contain fluoride at concentrations up to 500ppm depending upon its size and texture (Churchill *et al*., 1948; Jha *et al*., 2008). Bricks are produced in these brick kilns at a high temperature, ranging from 900°C to 1150°C. At this temperature, fluoride compounds are released into the atmosphere, generally in the form of gaseous hydrogen fluoride (HF) or silicon fluoride and particulate calcium fluoride (Suttie, 1980). SO_2 is also emitted by brick kilns.

Fluoride is very phytotoxic to plants. Fluoride compounds in the atmosphere are deposited to the vegetated surfaces either in gaseous form or in the form of particulates. The airborne gaseous fluorides can also enter directly into the leaf through stomata. This fluoride then dissolves in the apoplast, ultimately affecting plant growth and yield (Weinstein and Davison, 2003; Miller *et al*., 1999). Hydrogen fluoride is considered as one of the most phytotoxic fluoride compounds in the air. This form of fluoride is readily available to plants and is harmful to some plant species at very low concentrations (Weinstein and Davison,

2003; WHO, 1984). An HF concentration of 0.2µg F m^{-3} over 9 weeks or 0.52µg F m^{-3} over 21 days can be injurious to certain plants (Boese *et al.,* 1995), causing visible injuries, altering the photosynthetic process and reducing overall growth and yield (MacLean & Schneider, 1981). In addition, fluoride can also affect certain enzymes important for metabolic pathways inside the leaf, e.g. ATPase, RUBP carboxylase/oxygenase, and sucrose synthetase are known to be sensitive to fluoride, which can affect the whole photosynthetic process (Boese *et al.*, 1995; Quick *et al.,* 1989; Giannini *et al.,* 1985 and Parry *et al.*, 1984).

Previous research studies have revealed that fluoride compounds, such as HF, in the air are more dangerous to plant health than fluoride compounds in the soil, because HF can enter directly to the leaf via stomata and passes into the transpiration stream in which it is transported to the leaf tips and margins. If the concentration of fluoride in leaves exceeds a threshold of 20-30µg F g^{-1} then depending upon plant species, marginal and interveinal chlorosis or tip burn will appear as the first symptoms (Weinstein and Davison, 2003).

HF can also increase the fluoride concentration of soil via wet or dry deposition (Haidouti *et al.*, 1993). On the other hand, the availability of soil fluoride to the plant depends upon certain factors, such as pH, ionic strength, content of clay, organic matter, and concentrations of Al and Fe oxides/hydroxides (Arnesen, 1997; McLaughlin *et al.*, 2001; Loganathan *et al.*, 2006). Fluoride uptake of plants is greater in soils with pH between 4-6, as it is complexed with Al and Fe oxides and thus readily available to the plant (Arnesen, 1997).

4.1.1. Brick Kiln Fields in Peshawar

Approximately 400-450 brick kilns are situated in and around Peshawar city (EPA, 2004). More than 150,000 workers are associated with this industry, making it one of the biggest industries in the city. It is considered to be the backbone of the development process, as it contributes to the construction of roads, houses, hospitals and schools (Dawn, 2008). Therefore, it is also one of the main contributors to the rise in air pollution in Peshawar, together with vehicular emissions and rapid urbanisation (EPA, 2007). On a monthly average, a single brick kiln produces about 800,000 bricks, using large amounts of rubber tyres to start fires, and burns a total of 8 tons of low-quality coal or 20 drums of used vehicle oil (EPA, 2007).

For thousands of years, mankind has fired bricks, which are still the preferred choice for house construction material in most countries around the world. Suitable clays for manufacturing bricks exist almost everywhere, and the brick-making process can be done with simple manual methods. Brick kilns are generally found in clusters situated on the outskirts of main cities and towns, as in case of Peshawar.

There are two types of brick kiln factories, the Bull's Trench Kiln and the gas fired furnace. The most common type of brick kiln operating in Pakistan is the Bull's Trench Kiln (Fig 4.1). These brick kilns are mostly built on agricultural lands, which are rented from the farmers for a limited period of time; usually the contract runs for 3-5 years (EPA, 2007). The land is also the source of clay for the bricks. The land is then returned to the owners, once the

contract period is over. The other type is the more sophisticated industrial unit using a gas fired furnace. The bricks made in these factories are generally considered of good quality, but are also expensive compared to the bricks manufactured in Bull's trench kilns (EPA, 2007).

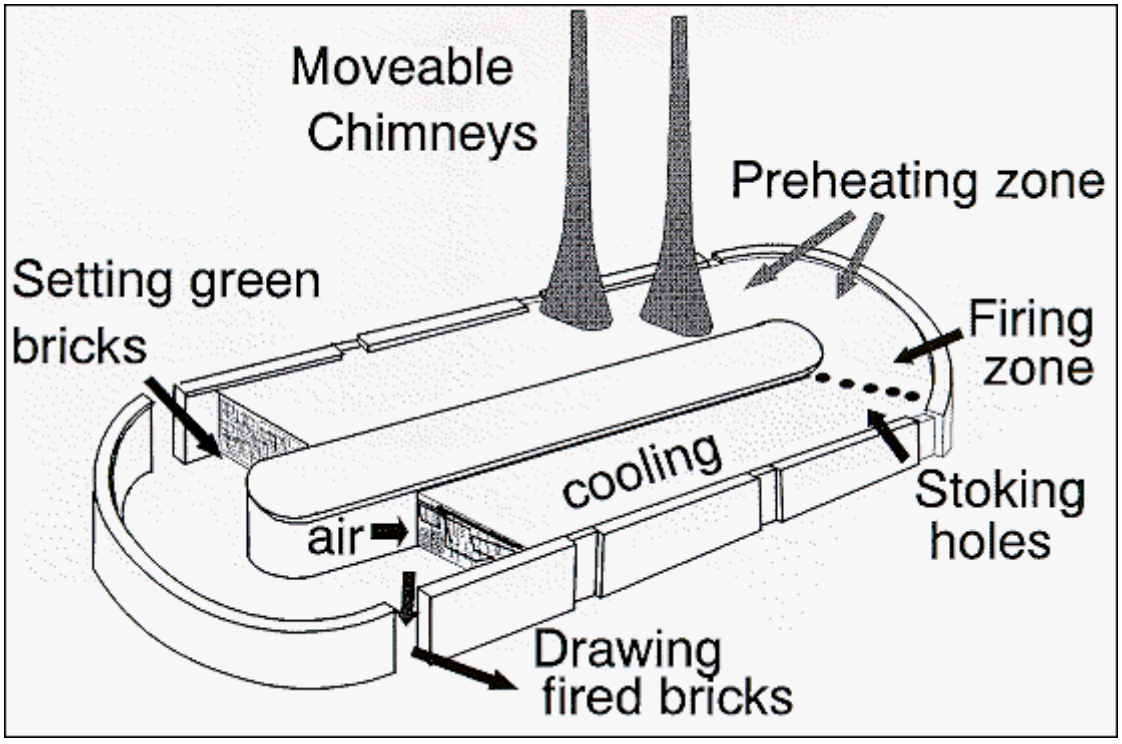


Figure 4.1: Typical Bull's Trench Kiln (EPA, 2007)

4.1.2. Brick kiln construction and working principle

The Bull's Trench kiln is constructed by first digging a trench in a circular or oval shape that may be 100-150m long, 6-10m wide and 2-3m deep depending upon the availability of the area. Small gates are built in the outer walls for easy access and for brick transportation. The green bricks are first made by mixing clay with water to form a paste. The paste is then put in moulds (Fig 4.2) to get unbaked (green) bricks, which are then dried in the sun (Fig 4.3) before bringing them into the trench via the gates. These bricks are then stocked in rows up to the level of the outer walls, two to three bricks wide, leaving holes between them for the adequate amount of coal and for air ventilation (Fig 4.4). In addition, two layers

of ash and brick dust are put on top of the bricks to seal the setting. The chimneys are made in the centre of the kiln and on top of the brick setting, and are made of metal sheet or bricks (Fig 4.5). The trench may contain 200-300,000 bricks at a time depending upon the size of the trench. The initial fire is started with used engine oil and rubber tyres; after the fire gets started, coal is continuously used throughout the day and night. The fuel channel is constructed in the centre of the kiln, and is connected with the chimney through which the exhaust gases flow. The fire temperature can reach from 900°C to 1100°C inside the kiln.

Figure 4.2: A worker preparing green bricks from clay paste near brick kiln factory in Peshawar.

Figure 4.3: Drying the green bricks in the sun before baking near brick kiln factory in Peshawar

Figure 4.4: Stocking of bricks inside the trench before firing

Figure 4.5: Chimney in the middle of the trench

There are typically two teams of three fire crew working in shifts to run the kiln continuously. They stoke the fire through the holes on top of the brick settings; the holes are covered with removable iron lids (Fig 4.6). Stoking of the fire is done 3-4 times, depending on the environmental temperature, e.g. in winter the fuel consumption can go higher. In Pakistan, in addition to coal, other materials e.g. lignite, peat, firewood, sawdust and agricultural waste (such as rice husk, brand or coffee shells) have also been reported to be used as fuel (EPA, 2007). Upon baking the bricks, they are then transported to the market.

Figure 4.6: A young worker is shown sitting near the fire holes. These have a removable iron lid through which the fuel is dropped in for brick baking.

4.1.3. Fluoride pollution in Peshawar

Despite the high fluoride emissions, and its potential threat to crops, no measurements of fluoride have been carried out to date and the impacts of brick kiln activity on crops in this region are unknown. It is important to assess the HF concentration during the summer and winter period, and its potential threat to local agricultural crops, because the area around Peshawar is heavily cultivated and might be at risk of HF toxicity emitting from brick kilns.

4.2. SPECIFIC OBJECTIVES

1. To assess the HF concentrations at ground level in and around Peshawar using HF passive samplers.
2. To assess the extent of visible injuries of different crops typical of hydrogen fluoride, and whether they are associated with elevated levels of foliar fluoride.
3. To determine the fluoride and metal content of different soil and plant samples collected from various areas of Peshawar.

Most of the brick kiln factories in Asia are poorly regulated and use low quality fuel and by burning clay that contributes to the rise in fluoride and sulphur emissions (Emberson *et al*., 2003). In china, one million bricks produce about 663kg of fluoride emissions (Emberson *et al*., 2003). The fluoride concentrations around brick kiln areas in Japan are 0.7μg m^{-3} (Inoue *et al*., 1995). The fluoride content of plant around brick kiln factories in Taiwan were consistently higher compared to F plant content away from the polluted area in two years of study (Lee *et al*., 1996). Air pollution from brick kilns has been also reported in Nepal and Bangladesh (Marshall, 2000), whereas, HF effects on vegetation in India has been reported mainly from aluminium factories and thermal power plants (Lal and Ambasht, 1981; Pandey 1981; Singh *et al*., 1990 and Pandey, 1985).

However, despite the high fluoride emissions, and its potential threat to crops, the impact of these brick kilns on agricultural production and farmers' livelihoods in the region is poorly understood. Here we report the results of a case study of these impacts in and around Peshawar city, in north-west Pakistan.

No measurements of fluoride pollution have been carried out to date in Peshawar and the impacts of brick kiln activity on crops in this region are unknown. The research focussed on the potential threat to local fruit trees, because the area around Peshawar is considered the best in Pakistan for apricot and plum fruits, and these species are known to be sensitive to fluoride toxicity emitting from brick kilns. The aims of the work were to assess the hydrogen fluoride concentrations using HF passive samplers, to determine the extent of visible injuries typical of hydrogen fluoride on fruit trees, and to determine whether they are associated with elevated levels of foliar fluoride. In addition, interviews were conducted with local farmers to assess their awareness of the problem, the severity of its impact, and to identify any measures that had been adopted to reduce the problem.

In this research study, the measurements of the fluoride content of plants, soil and air were carried out in field surveys in Peshawar at different sites. The details of the selected sites were given in Chapter 1. A greenhouse experiment on the effect of NaF on a Pakistani wheat variety (*Bakkar*) collected from a polluted site in Peshawar was also undertaken in alkaline soil conditions to assess the effect of soil fluoride on wheat. This experiment is described in detail in Chapter 5.

4.3. MATERIALS AND METHODS

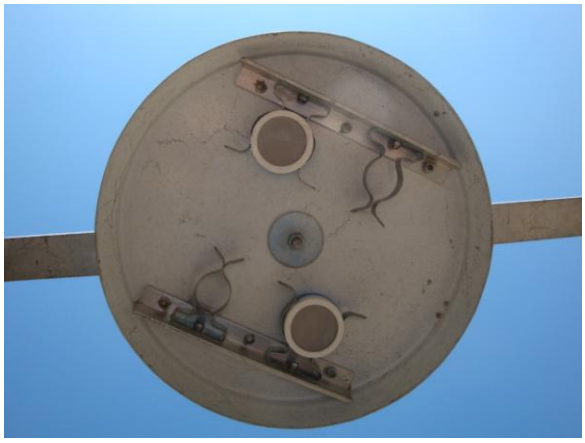
Four sites were selected in and around Peshawar city to investigate the effects of hydrogen fluoride (HF) and sulphur dioxide (SO_2) pollution on crops and vegetables. Sites at the Agricultural University (AUP), Tarnab Research Farm (ARI), Charsadda (control) and the vicinity of brick kiln factories (BKF) were investigated during the summer and winter survey. The detailed locations and reasons for location of these sites are given in Chapter 1. The first field survey was carried out from February to June, 2008, in and around Peshawar city, to gather data regarding fluoride pollution in the city. The second field survey was carried out from October to December, 2008. The second survey of foliar injuries were undertaken mainly due to visible foliar injuries to different crops and fruit trees being observed during the first survey in spring and summer, which were thought to be due to air pollution emitted from brick kilns in and around Peshawar.

4.3.1. Passive sampler installation

Pollution concentrations and climate variables were monitored at the survey sites in order to relate any observed foliar injury to the pollution climate and to provide information on the risk resulting from HF and SO_2. The HF and SO_2 concentrations were measured using a passive sampler developed by Swedish Environment Institute (IVL). The passive sampler has a propylene absorbent which absorbs strong acids (HF) and the oxides of moderately strong acids (SO_2) from air. After exposure, the filter is leached in water, fluoride and sulphate concentrations are determined by ion chromatography (Ferm *et al*., 2002). Passive samplers were installed for periods of four weeks at AUP, ARI and BKF from 2nd February 2008 to 9th June 2008 during the summer. For the winter survey, passive samplers were installed at AUP and BKF from 13 October 2008 to 14 December 2008. The passive samplers

arrived in small capped plastic containers sealed in a plastic bag. The containers were opened shortly before the start of the exposure at the site. The samplers were carefully removed from the container and fixed firmly to the tool holder with the grey net mesh pointing downwards under metal plates to avoid direct sunlight and rain. Careful measures were taken to avoid touching the net (Fig 4.7, 4.8).

4.7. Passive sampling set-up in the middle of the agricultural filed at ARI Date 14/12/2008



4.8. Inside view of the plate containing passive sampler with the help of hooks at ARI Date 14/12/2008

The date, start time and sampler location were noted. The plates were then attached to wooden poles at 2m height from the ground. Air temperature and humidity was monitored by the local weather station at the AUP and ARI sites, by taking readings at 8:00am and 5:00pm on a daily basis.

At the end of the four week exposure period, the samplers were carefully taken down and were placed in the corresponding plastic container. These were sealed tightly with a cap and the containers were placed in sealed plastic bags. The date, stoppage time, relative humidity and temperature were noted from all the three sites. The samplers were then posted back to the IVL laboratory in Gothenburg, Sweden, in a cushioned envelope for the chemical analysis.

4.3.2. Identification of visible injuries

The visible injury symptom surveys were carried out in May and June, and in November and December, of 2008. Observations on different crop leaves were carried out at the BKF, AUP, ARI and Charsadda (control) sites. The mature leaves of apricot (*Prunus armeniaca*), plum (*Prunus cerasifera*), mango (*Mangifera indica)*, lady finger (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), sugarcane (*Saccharum officinarum*), maize (*Zea mays*), clover (*Trifolium repens*) and summer spinach (*Beta vulgaris*) were investigated during the summer survey, whereas winter spinach (*Beta vulgaris*), wheat (*Triticum aestivum*) and clover (*Trifolium repens*) were examined during the winter survey. The crop species were selected for the survey because of their importance in the agricultural economy of the region. The injuries were identified as typical tip burn and leaf margin necrosis (Weinstein *et al*. 2003). The damage by air pollution was assessed on the basis of % injury to mature fully expanded leaves as follows:

No damage = 0% injury
Little damage = <30% injury
Significant damage= >30-70% injury
Severe damage = >70% injury

4.3.3. Soil sampling

Soil samples were collected from the AUP, ARI and BKF sites in Peshawar during the summer survey. Four field plots from BKF and five plots each from AUP and ARI sites,

cultivated with wheat crops, were selected for sampling of soil. Samples of soil in the brick kiln area (BKF) were taken less than 50m from brick kiln factories at two villages 'Urmar' and 'Achini'. Soil was sampled from two fields each from both villages. Five fields each at AUP and ARI were also selected for soil sampling with a distance of at least 100m between them at each site.

During the winter survey, five plots each were selected for soil sampling from spinach fields at BKF and Charsadda (control area). The plots at Charsadda were close to each other and about 5km away from the city centre. The sampling plots at BKF were close to the brick works in a similar position to wheat fields sampled in the summer. The control (Charsadda) area was selected because of the greater distance from the polluted site (BKF) compared to AUP and ARI sites.

A composite sample of about 1kg was collected from each plot using a 'W' sampling design (i.e. from each corner and the middle), taking the top soil to 20cm depth by inserting an auger into the soil and then pulling out the soil carefully using the lever. Each soil sample was carefully sealed in a labelled paper bag immediately to keep it fresh. The composite samples from each plot were shifted as soon as possible from the field to the laboratory at Tarnab Research Farm (ARI), Peshawar for the analysis of moisture and pH.

After bringing the soil samples to the laboratory, the composite samples were mixed thoroughly in order to homogenise the samples. Soil pH was measured first, before air drying, in order to avoid any chemical changes that can alter the soil and pH.

4.3.4. Wheat grain and spinach sampling

Wheat grain samples were collected in May 2008, from the same plots at the AUP, ARI and BKF sites from which soil was collected. 10 ears were harvested in the beginning of May, 2008 from each of the four corners and from the middle of each plot (using the same 'W' sampling design) in order to get homogenized sample. Spinach samples were also

collected during the winter survey in December 2008 from the same fields from which soil sampling was carried out. The samples were also collected in the same 'W' shape and each sample consisted of fully matured leaves including their shoots. Each sample was mixed together to form a composite sample. These samples were carefully put in already marked paper bags and transported immediately to the laboratory of Chemistry Department, NWFP, Agricultural University Peshawar.

Upon arriving at the laboratory, the composite samples were taken out of the sealed bags and each sample was weighed, and then dried at 70°C for 48 hours. After drying, the wheat ears were crushed in the palm to separate the grains from the ears. The spinach samples, including leaves and shoots, were sealed directly after drying in a paper bag. The wheat samples were sealed in a plastic bottle and then transported to the University of York, UK for further analysis of heavy metals and fluorides.

4.3.5. Sampling of apricot, plum and mango leaves

Leaves of apricot, plum and mango were collected in the last week of May 2008 from the BKF, AUP and Charsadda (control) sites to determine foliar concentrations of heavy metals and hydrogen fluorides. Only injured leaves from AUP and BKF were selected for sampling, for comparison with the healthy leaves collected from Charsadda (control).

Fresh leaf samples, each having a minimum 10g of fresh weight per sample, were collected from different orchards near the brick kiln factories. A single sample contained 8-10 leaves. At the BKF, eight apricot leaf samples were collected at a distance of about 50m from the brick kiln factories from 8 orchards, and 6 plum leaf samples were collected from 6

orchards. Four samples each of apricot and plum were collected from 4 orchards near AUP, which were about 10km from the brick kiln factories. Four control samples for apricot and plum were taken from 4 orchards in the rural area (Charsadda), 20 miles to the north of Peshawar. Mango trees were not found at BKF and AUP sites. Therefore, three mango leaves samples were picked from mango trees at three different places in the Hayatabad township area, situated 4km to the south west of BKF site. Three control samples for mango were also collected from the rural area (Charsadda). The leaves were picked with the help of clippers at the height of 2.2m for apricot and plum, whereas the leaf samples of mango were collected at a height of 4m from the ground to get a uniform sample. All samples from a location were mixed together to form a composite sample.

After harvesting, all the samples were sealed in a paper bag immediately, to reduce the loss of moisture, and were taken directly to the Laboratory of NWFP Agricultural University, Peshawar. Upon arriving at the laboratory, the samples were weighed, dried, packed and transported to the University of York, UK for further analysis of heavy metals and fluorides using the methods described in Section 4.3.10.

4.3.6. Determination of soil pH

In order to measure the pH of fresh soil, the composite sample was divided into two sub-samples. Soil pH was determined in a 1:1 soil-water suspension. 10g of soil was added to two replicate cups, and then shaken with distilled water for 30 minutes on the shaker. After filtration, the pH of the extract was measured using a ‘Fisher Scientific Accumet model 10’ pH meter.

The pH meter was installed in a place free of vibrations. The electricity supply was switched on for 30 minutes to allow the pH meter to warm up. The electrode assembly was rinsed with deionised water and was dried with clean tissue paper. The pH meter was calibrated first at pH 7 and then at pH 9 using a standard buffer solution. The electrode was washed again with deionised water and dried. The temperature was measured and the temperature dial was adjusted to the temperature of the test solution. The electrode was then fully dipped in the test solution to get accurate readings. The procedure was repeated for all soil samples.

After measuring the soil pH, each composite sample was air-dried at room temperature for 24 hours by spreading the sample on paper in a clean, warm and dry area. After air drying, twigs and stones were removed and the samples were passed through a 2mm sieve.

4.3.7. Determination of soil moisture

From each of the composite soil samples, 20g was taken for the determination of moisture content, as follows. A clean and dry beaker with an aluminium cover was weighed (W_1). 20g of fresh soil was then placed in the weighed beaker and the aluminium cover was replaced. The beaker was then weighed again (W_2). The cover was removed and the beaker, with contents, was placed in the oven for drying, at a temperature between 105°C and 110°C, for 24 hours. The beaker with contents was removed from the oven and the cover was replaced. The beaker was then put in the desiccator for cooling and then weighed again (W_3). The soil moisture content (MC%) was calculated as a percentage of the soil dry weight using the following equation:-

$$MC\% = \frac{(W_2 - W_3)\ x\ 100}{W_3 - W_1}$$

After drying, 50g of each sample was sealed carefully in labelled plastic bags and then transported to University of York, UK to carry out further analysis of metals and fluoride.

4.3.8. Determination of soil fluoride concentration

After transporting soil samples from Pakistan to the laboratory of the University of York, UK, the soil fluoride concentration was determined by the method of Larsen and Widdowson (1971). This involved extraction with $CaCl_2$ and water, and then analysis using a fluoride ion selective electrode.

4.3.8.1. Extraction of soil

The soil samples were taken out of the sealed envelopes and were mixed well to homogenise them again. 5g of soil from each sample was accurately weighed and was transferred to 150ml acid washed bottles, and was mixed with 37ml of 0.01M $CaCl_2.2H_2O$. The bottles were then closed firmly to avoid any leakage. The bottles were placed in a large box in a horizontal position and the box was then placed on the shaker. The samples were shaken for 16 hours at 120rpm at room temperature. After shaking, the mixture was filtered with Whatman's filter no. 4 paper. 5ml of the filtrate was then mixed with 5ml of anti-complexing agent, and was filtered again. The solution was then adjusted to pH 5-5.5 with the help of NaOH, and fluoride concentration was determined using a Thermo Scientific Orion fluoride ion selective electrode (Fisher Scientific Inc).

The anti-complexing agent was made from sodium chloride, glacial acetic acid and sodium citrate. 58g of sodium chloride (NaCl) was accurately weighed and was then transferred to a 500ml clean beaker. Glacial acetic acid (57ml) was pipetted into the beaker

containing NaCl. Finally, 0.3g of sodium citrate was also added to the beaker. The beaker was then stirred for five minutes with the help of a magnetic stirrer to get a uniform solution.

For the extraction of soluble fluoride from soil, deionised water was used instead of $CaCl_2$ as a solvent. The rest of the protocol was the same as for $CaCl_2$.

4.3.9. Determination of fluoride concentration using an Ion Selective Electrode (ISE)

The electrode was connected to the meter and the meter was set to the mV mode. The ISE was rinsed with deionised water. The ISE was first calibrated with standards of 0.25, 0.5, 1.0, 2.0 and 10ppm NaF. The concentration of the samples was determined by constructing a calibration curve from these standards. A typical calibration curve is shown in Fig 4.9. At room temperature, the ISE was placed in a beaker containing 5ml of standard solution, along with 5ml of anti complexing agent (1:1), and the solution was stirred slowly by magnetic stirrer. The value (mV) was noted when the meter reading was stable. The same process was repeated for the remaining standards.

The concentration of fluoride in each soil sample was then calculated as part per million (ppm) dry weight of soil material using the following equation

$$\text{ppm F } (\mu g\ g^{-1}) \text{ dry weight} = CV/W$$

Where: C = Fluoride concentration of the extract (ppm)
W = dry weight of the soil sample used (g).
V = volume of the extract (ml).

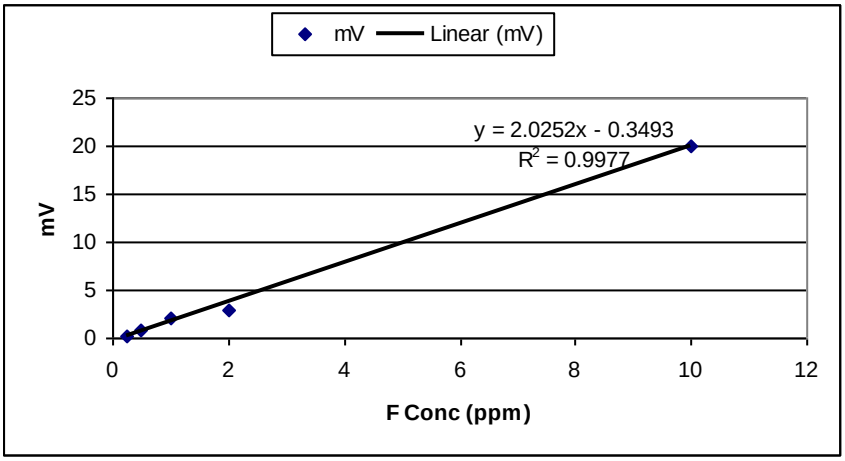


Figure 4.9: Sodium fluoride (NaF) standard curve from ion selective electrode

4.3.10. Determination of plant fluoride concentration

Plant fluoride was extracted by acid digestion (AOAC 1980) and alkali fusion methods (McQuaker and Gurney, 1977). The leaf samples from each orchard were analysed in duplicate by the method of AOAC (1980). Due to the limited amount of the sample, the number of leaf samples for alkali fusion analysis was reduced. Samples from different orchards of the same area were mixed together to form composite samples only for alkali fusion method, as shown in Table 4.1.

Table 4.1: The number of samples for apricot, plum and mango leaves that were mixed together from a composite sample for alkali fusion analysis. The digits showing the number of samples mixed to form a composite sample. Site codes are given in the text.

Apricot	Plum	Mango
BKF1,2,3	BKF1,2,3	BKF1
BKF4.5,6	BKF4.5,6	BKF2
BKF7,8		BKF3
AUP1,2	AUP1,2	
AUP3,4	AUP3,4	
Control1,2	Control1,2	Control1
Control3,4	Control3,4	Control2

4.3.10.1. Fluoride extraction from plants (AOAC, 1980)

The ground samples were taken out of the paper bag. Each of the samples was accurately weighted (0.25g) on an electrical balance and was then placed in an acid-cleaned 100ml beaker. 1ml of analytical grade acetone was carefully added to the beaker containing the dry plant material. The acetone was allowed to evaporate from the sample in the fume hood. 20ml of 0.05N nitric acid was then pipetted into the beaker. The mixture was mixed for 30 minutes with a magnetic stirrer inside the fume hood, after which 20ml of 0.1N KOH was pipetted and the mixture was stirred again for 30 more minutes. Finally, 5ml of 0.2N nitric acid, along with 5ml of 0.4M sodium citrate solution containing 1ppm fluoride was added. The mixture pH was adjusted to 5-5.5 with the help of a pH meter. The fluoride concentration of the extract was determined using a fluoride ion specific electrode (details given in Section 4.3.9). The fluoride content of the plant sample on a dry weight basis was calculated by the following equation.

$$\text{Fluoride content } (\mu g\ g^{-1}) = \frac{(C - 0.1)\ 50}{W}$$

Where: C = Fluoride concentration of the extract (ppm)
W = dry weight of leaf sample used (g)
0.1 = concentration (ppm) in the extract from the added sodium citrate solution
50 = total volume of the extract solution (ml)

4. 3.10.2. Plant fluoride extraction by alkali fusion method (VDI, 1981)

The alkali fusion analyses for plant sample were carried out at the University of Hoheneheim, Germany. One gram of ground plant sample was heated at 500°C overnight in a nickel crucible in a furnace. The ash was then fused with NaOH using a Bunsen burner. The crucible was then cooled down and was diffused with few ml of distilled water in a

50ml PE-flask. 12.5 ml of aqueous citric acid solution and 5 ml of hydrochloric acid (HCl) were added. The pH of the solution was adjusted to 6.2 using sodium hydroxide solution, and the final volume was made up to 50ml. 25ml of this solution were mixed with 25 ml of a total ionic strength adjustment buffer (TISAB for F determination, WTWWeilheim, Germany). The fluoride concentration of the extract was measured using a fluoride-sensitive electrode (ISE F 800 DIN, WTW Weilheim, Germany) coupled to an ion meter (Inolab pH/Ion 735WTWWeilheim, Germany). The calibration was made using five NaF standard solutions (Fig 4.10). The fluoride content of the plant samples on a dry weight basis was calculated by the equation shown in Section 4.3.10.

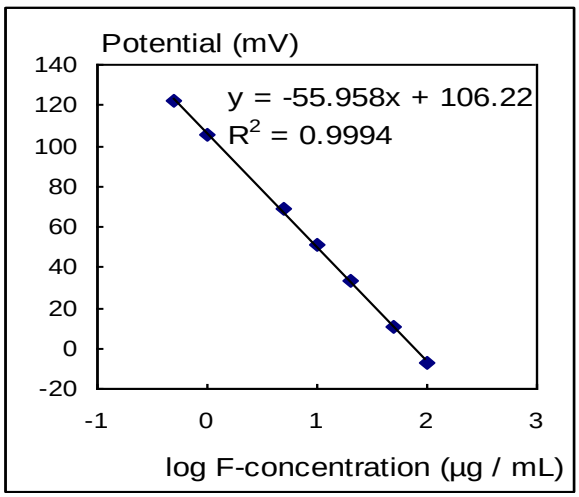


Figure 4.10: Sodium fluoride (NaF) standard curve from ion selective electrode using the method of VDI (1981)

4.3.11. Determination of metal content of plants and soils by ICP-MS

The plant and soil samples collected from Peshawar were also analysed for their metal content by inductively coupled plasma-mass spectroscopy (ICP-MS) and inductively coupled plasma-optical emission spectrometer ICP-EOS at Nijmegen University, Netherlands. The macro elements (Al, Fe) were analysed on ICP-MS and trace elements (Cr, S, Zn, Mn, Cd and Pb) on ICP-EOS.

200mg of dried ground sample of both soil and plant was accurately weighed and was put into a pressure vial. 4ml of 6% HNO_3 and 1ml of 30%H_2O_2 were added to the pressure vial. The vial was covered tightly and was shaken to mix the reagents. The vial was then placed in a microwave. The microwave sample sequence was set at 1min at 250W, 2min at 0W, 5min at 250W, 5min at 400W, 5min at 400W and 5min at 500W. The sample was then taken out and was cooled for 30min at 4°C. After cooling, the vial was opened, the mixture was flushed with deionised water into a 100ml volumetric flask, and the volume was made up to 100ml with deionised water. The extract was then analysed on ICP-MS and ICP-EOS.

4.3.12 Statistical summary

The data summary (means and standard errors) was carried out using Microsoft Excel (2003) and the statistical analysis was carried out using SPSS 17.0. The dataset for all parameters was explored for skewness, kurtosis and normality, using the Shapiro-Wilk-test. The parameters showing major deviations from a normal distribution were normalised by using $\log_{10}$ transformation. One-way-ANOVA was used for each of the parameters to determine whether there were significant differences between the sites. Tukey's HSD post hoc test was used for multiple comparisons at $p<0.05$. An independent sample t-test was used for mango leaf samples as these were only sampled for two sites.

4.4. RESULTS

4.4.1. Passive samplers

The mean HF concentration in the air during the spring and early summer (from February to May, 2008) was 0.22µg m^{-3} at the BKF site, whereas the HF concentration was below detection limit (<0.1µg m^{-3}) over the whole period at ARI (Fig 4.11). The HF concentration was 0.2µg m^{-3} in February and increased to 0.3µg m^{-3} in May at BKF (Fig 4.11). The HF passive sampler exposed from mid November to mid December, 2008 showed a mean HF concentration below the detection limit of <0.1µg m^{-3} at both BKF and AUP sites. No HF measurements were carried out at ARI during winter and at AUP during summer.

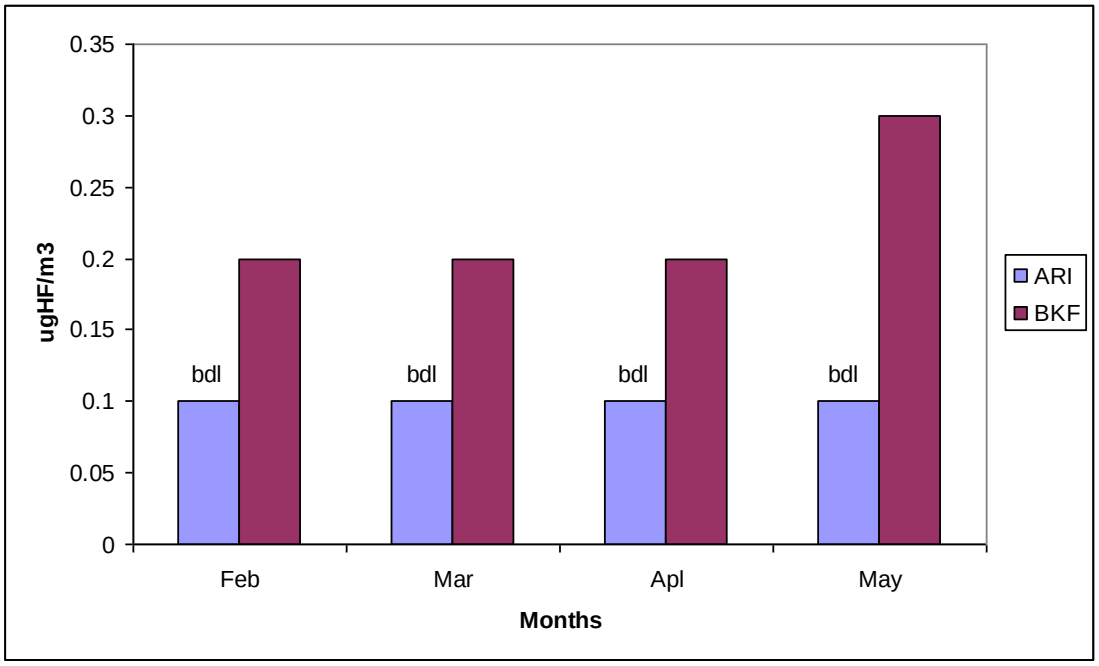


Figure 4.11: The HF air concentration during spring and summer seasons of 2008 at ARI and BKF, Peshawar (bdl is below detection limit of 0.1µg m^{-3})

Mean sulphur dioxide (SO_2) concentrations in the air during spring were 16.2µg m^{-3} at ARI and 8.0µg m^{-3} at BKF, respectively. Monthly mean values ranged from 7.9µg m^{-3} to 22.8µg m^{-3} at ARI, while at BKF, the monthly mean SO_2 concentration increased from

3.9µg/m^3 in February to 17.1µg m^{-3} in May 2008 (Fig 4.12). The mean concentration of SO_2 in November-December, 2008 was 4.7µg m^{-3} and 19.4µg m^{-3} at AUP and BKF, respectively (Figure 4.4.2). No SO_2 measurements were carried out at ARI during winter and at AUP during summer.

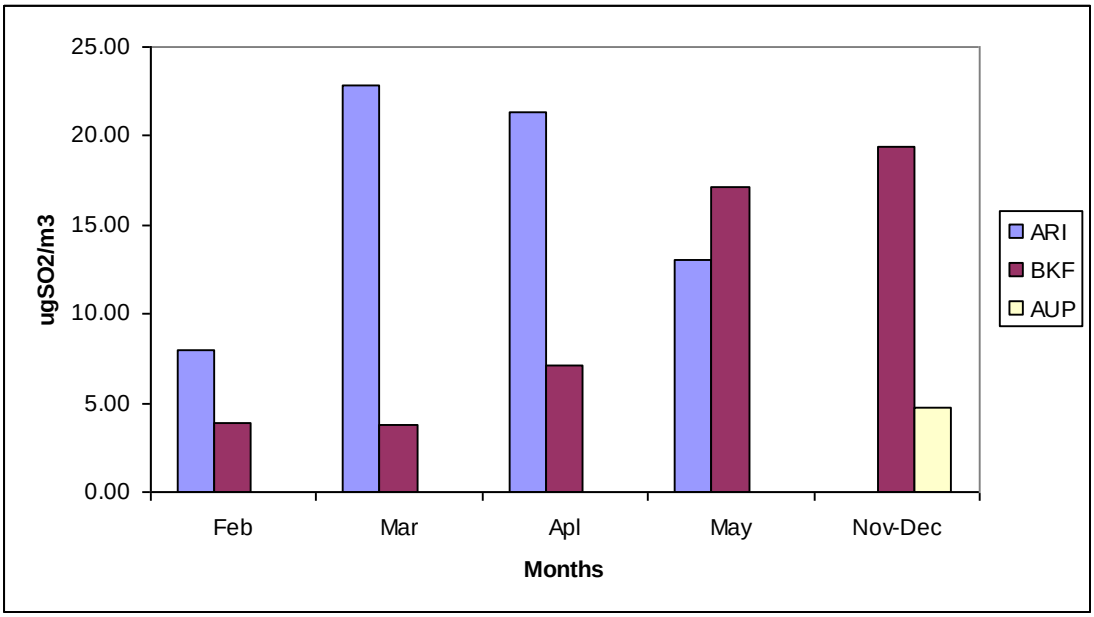


Figure 4.12: SO_2 concentration (µg m^{-3}) during spring and winter season of 2008 at ARI, AUP and BKF, Peshawar

4.4.2. Visible injuries to crops

Table 4.2: The extent of damage and symptom of summer (S) and winter (W) crop species by air pollution at BKF, AUP, ARI and Charsadda (Control) site. The injury to leaves were categorised into no injury, little injury, significant injury and severe injury to leaf.

Sites	Plant species	No injury	Little injury	Significant injury	Severe injury	Leaf injury symptoms
BKF	Apricot (S)				X	Margin & tip burn
	Plum (S)				X	Margin & tip burn
	Mango (S)				X	Margin & tip burn
	Peach (S)		X			Little Injury
	Tomato (S)			X		Necrosis tip burn
	Potato (S, W)	X				No Injury
	Onion (S)	X				No Injury
	Spinach (S, W)	X				No Injury
	Okra (S)	X				No Injury
	Cucumber (S)	X				Necrotic margins
	Sugarcane (S)		X			Necrotic margins
	Wheat (W)		X			Leaf tip burn
	Maize (S)			X		Necrotic margins
AUP	Apricot (S)		X			Margin & tip burn
	Plum (S)		X			Margin & tip burn
	Peach (S)	X				No Injury
	Tomato (S)	X				No Injury
	Potato (S, W)	X				No Injury
	Onion (S)	X				No Injury
	Spinach (S, W)	X				No Injury
	Cucumber (S)	X				No Injury
	Wheat (W)	X				No Injury
	Maize (S)	X				No Injury
	Clover (W)	X				No Injury
ARI	Apricot (S)		X			Margin & tip burn
	Plum (S)		X			Margin & tip burn
	Peach (S)		X			Little Injury
	Tomato (S)	X				No Injury
	Potato (S, W)	X				No Injury
	Onion (S)		X			Tip burn
	Spinach (S, W)	X				No Injury
	Cucumber (S)		X			Necrotic margins
	Wheat (W)	X				No Injury
	Maize (S)	X				No Injury
	Clover (W)	X				No Injury
Control	Apricot (S)	X				No Injury
	Plum (S)	X				No Injury
	Peach (S)	X				No Injury
	Clover (W)	X				No Injury
	Spinach (S, W)	X				No Injury

During the summer survey, air pollution damage to crops was observed in form of typical tip burn and necrosis on leaf margins at different sites. Table 4.2 describes the extent of visible injury damage to different fruit trees and crops at Peshawar. The plant species were categorised according to the form and severity of visible leaf injuries. In the spring-summer survey, apricot, plum and mango were severely damaged in the brick kiln area. Significant damage was observed to maize and tomato, with slight injury to wheat, sugarcane and wheat, at the BKF site in the month of May, 2008. However, no injuries were found on potato, spinach, onion and cucumber plants at the BKF or at any other sites for the same period. Visible leaf injuries to the affected crops, vegetables and fruits were higher at BKF compared to the AUP and ARI sites, during the spring-summer season (Table 4.2). No visible injuries were observed at the control site on any of the crops. Fruit tree crops were more damaged than other cash crops and vegetables. Orchards located at the BKF site were heavily affected (Fig 4.13a). The outer mature leaves of apricot (Fig 4.13b, c, d & e) and plum (Fig 4.14a & b) trees were more damaged than the young and inner leaves, which are shaded by the outer mature leaves. Visible injuries appeared in late March/early April when the trees of apricot and plum were at the vegetative stage. Apricot trees bear leaves in early spring (Feb) and bear flowers in late March. The fruit is matured by late April/early May. Premature shrinkage and scars on mature plum fruit were also observed at BKF (Fig 4.14c & d).

Figure 4.13a: Apricot trees severely damaged in the vicinity of BKF. Almost all apricot orchards were damaged and they seemed to be the most sensitive of the fruit crops at the BKF Date 21/05/2008

Figure 4.13b: A local farmer showing apricot damaged leaves on a branch cut from the top of an apricot tree in the vicinity of brick kiln factories Date 21/05/2008

Figure 4.13c: Severity of the damage to apricot at BKF in a closer view. Damaged leaf margins can clearly be seen Date 21/05/2008

Figure4.13d: Severe necrosis to apricot leaf tip and margins. Small interveinal necrotic spots are also visible on the leaf surface Date 21/05/2008

Figure4.13e: Apricot leaves were rolled inwards due to severe necrotic damage to leaf margin at BKF Date 21/05/2008

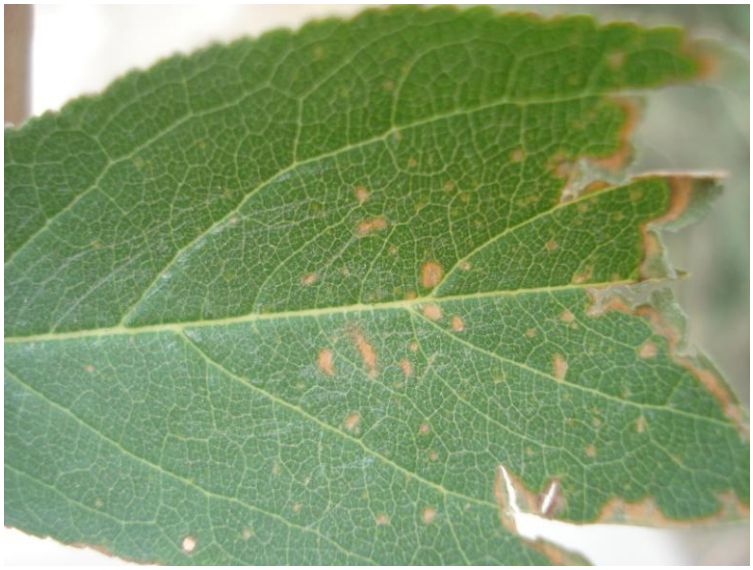


Figure 4.14a: Interveinal and margin necrosis to plum leaf at BKF. The necrotic part of the leaves was blown away by wind, unlike apricot leaves, in which the damaged parts were intact Date 21/05/2008

Figure 4.14b: Most of the outer plum leaves were damaged by air pollution at BKF Date 21/05/2008

Figure 4.14c: Shrinkage of plum fruit at premature stage at BKF Date 21/05/2008

Figure 4.14d: Scars appeared when the plum fruit matures at BKF Date 21/05/2008

Visible leaf injury symptoms were also found on maize, sugar cane and tomato at BKF during the summer season (Fig 4.15a, b, c & d). Maize was more damaged than tomato, while sugarcane was less affected (Table 4.2). Some crops, including potato, onion, okra, cucumber and summer spinach, showed no visible leaf injury symptoms. Mango trees and grape plants at Hayatabad Township (about 4 km from BKF area) were highly damaged (Table 4.2) and had necrosis and leaf tip burn. The symptoms were very similar to those of apricot and plum at BKF (Fig 4.16a, b, c, d & e).

Figure 4.15a: Typical tip burn injury and necrosis to leaf margin of maize leaf at BKF. Most of the mature fully expanded maize leaves were damaged, whereas young leaves did not show any injury symptoms Date 21/05/2008

Figure 4.15b: Maize leaf tip burn injury in the vicinity of brick kiln factories Date 21/05/2008

Figure 4.15c: Chlorosis to sugarcane leaf margins from top to bottom at BKF. There was no necrosis found on sugarcane leaves at BKF Date 21/05/2008

Figure 4.15d: Typical tip burn injury and necrosis to leaf margin of tomato leaf at BKF Date 06/05/2008

Figure 4.16a: Tip burn leaf injury and necrosis to mango at Hayatabad near BKF during the summer survey Date 24/05/2008

Figure 4.16b: Leaf rolling due to marginal necrosis of mango at Hayatabad Date 24/05/2008

Figure 4.16c: A closer view of the affected mango leaf at Hayatabad Date 24/05/2008

Figure 4.16d: Grape plant severely damaged at Hayatabad Date 20/10/2008

Figure 4.16e: The grape leaf with necrotic margins and also particulate matter on the leaf surface Date 20/10/2008

Less visible leaf injuries to crops was observed at AUP and ARI during the summer survey (Table 4.2) than in the BKF area, while no visible injury was found on these crops in Charsadda (control) area, which is situated 30 km north of Peshawar city (Table 4.2). Necrosis of leaf margins of apricot, plum, peach and cucumber was observed at ARI site

(Fig 4.17a, b, e & f). Small injuries to leaves of apricot and plum were found at AUP site (Fig4.17c & d). No leaf injuries were found on potato, tomato, onion, spinach and maize at ARI and AUP sites (Table 4.2). There was also no damaged observed at Charsadda site on apricot, plum, peach and spinach during the summer survey (Table 4.2).

Figure 4.17a: Visible injury to apricot at ARI, Peshawar. The leaves were less severely damaged than apricot leaves at BKF Date 21/05/2008

Figure 4.17b: Leaf margin necrosis of apricot leaf at ARI Date 21/05/2008

Figure 4.17c: Minor injury to apricot leaf at AUP Date 21/05/2008

Figure 4.17d: Injury to plum leaf at AUP Date d 21/05/2008

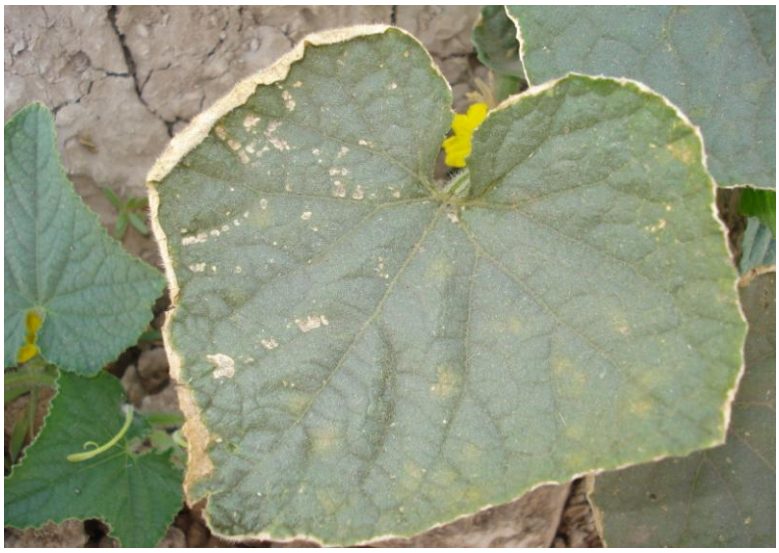


Figure 4.17e: Visible leaf margin injury to cucumber at ARI Date 21/05/2008

Figure 4.17f: Visible injury to peach at ARI Date 21/05/2008

In contrast to the summer survey, no visible injuries were observed during winter surveys conducted from November, 2008 to January, 2009 at AUP, ARI, BKF and Charsadda area. However, leaf tip burn injury to young wheat plants (2 months old) was found at BKF area in January, 2009 (Fig4.18a & b; Table 4.2). The injury was restricted to a few farms and not widely spread throughout the BKF area, in contrast to the symptoms observed in the summer.

Figure 4.18a: Chlorosis and tip burn injury to young wheat plants at BKF during winter season Date 14/12/2008

Figure 4.18b: Typical tip burn leaf injury to wheat at BKF Date 14/12/2008

4.4.3. Soil pH

There was no significant difference between the pH of the soil samples collected from wheat fields of ARI, AUP and BKF during the summer survey (Table 4.3). The pH of all soil samples were above 7.0. The mean soil pH of ARI, AUP and BKF was 7.82, 7.79 and 7.85, respectively.

Table 4.3: ANOVA results (those significant at p = 0.05 are shown in bold) showing the comparison of pH, and calcium and water extractable fluoride content of soil collected from BKF, AUP and ARI areas of Peshawar during summer survey of 2008. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	df	F	Sig.	Post hoc
pH	2,15	1.77	0.204	*ns*
Water-ext F	**2,15**	**5.12**	**0.02**	**AUP, BKF>ARI**
Calcium-extract F	**2,15**	**7.64**	**0.005**	**AUP, BKF>ARI**

4.4.4. Soil Fluoride content of wheat field

The water extractable soil fluoride content of wheat fields was higher in all soil samples compared to calcium extractable fluoride content. Both water and calcium chloride extractable soil fluoride content of AUP were significantly (28%) higher than that of ARI, but did not differ significantly from those at BKF (Table 4.3; Fig 4.19).

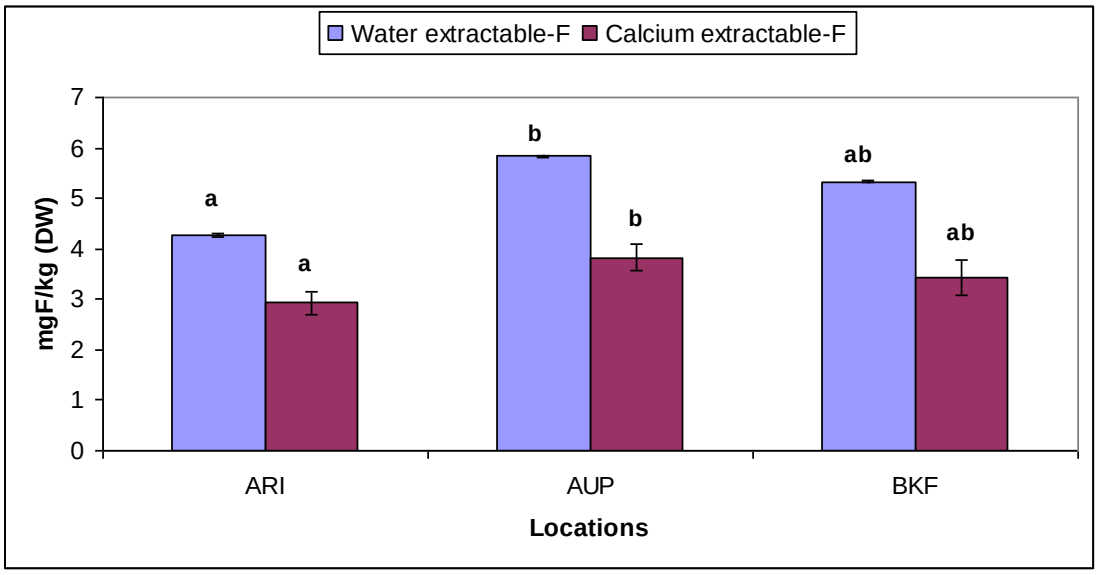


Figure 4.19: Water and calcium extractable fluoride content of soil samples collected from ARI, AUP and BKF sites during summer season. The values of BKF are the mean of 8 replicates, while values of AUP and ARI have the mean of 5 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly between locations at P=0.05

4.4.5. Fluoride content of soil collected from spinach fields

Water and calcium extractable fluoride concentrations of soils collected from BKF spinach fields in winter were significantly higher, by 15 and 9% respectively, than those of Charsadda (control) soil samples (Fig 4.20).

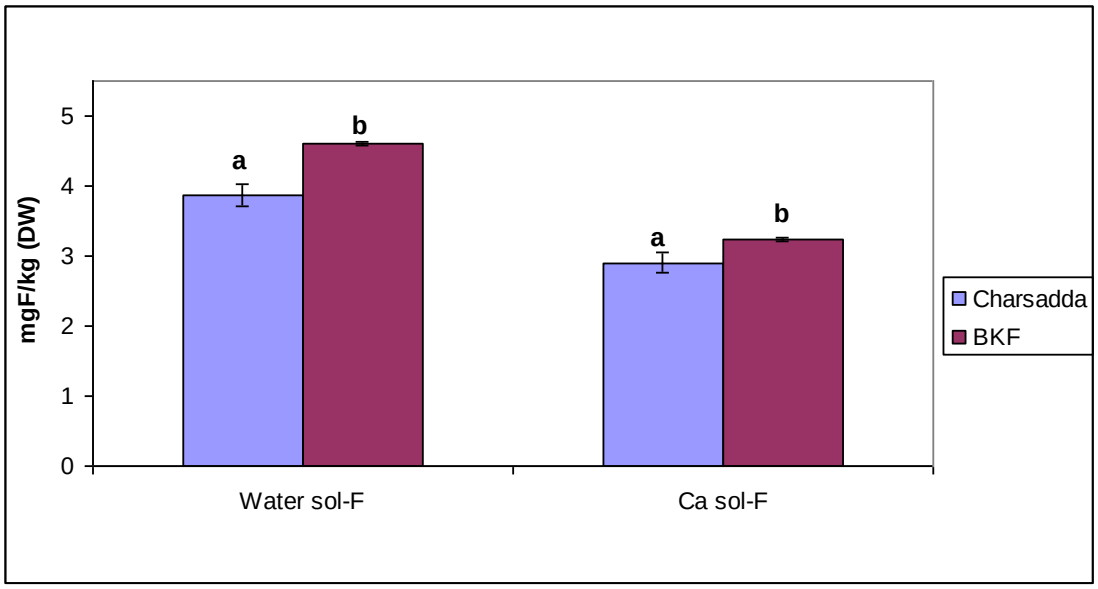


Figure 4.20: Independent sample t-test showing the difference between the total fluoride content of soil collected from spinach fields of BKF and Charsadda (control) sites during winter season. The values are the mean of 5 replicates. Bars sharing different letters differ significantly from each other at P=0.05

4.4.6. Wheat grain fluoride content

The total fluoride content of wheat grain samples from BKF was significantly higher, by approximately a factor of two, than that at the ARI and AUP sites (Table 4.4; Figure 4.21). There was no significant difference between the total fluoride content of wheat grain samples of AUP and ARI (Figure 4.21).

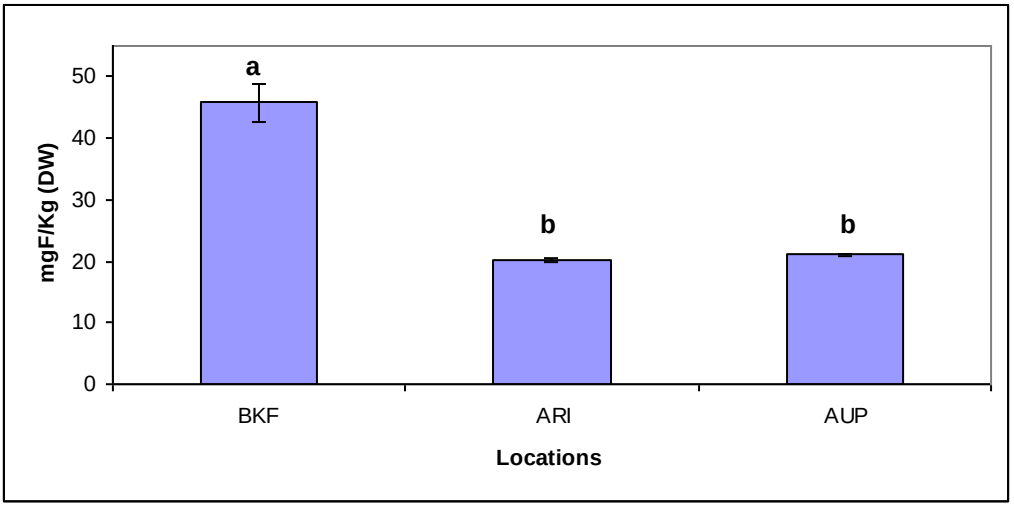


Figure 4.21: Total fluoride content of wheat grain samples collected from ARI, AUP and BKF sites during summer season. The values of BKF are the mean of 8 replicates, while values of AUP and ARI were the mean of 5 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05

Table 4.4: ANOVA results (those significant at p = 0.05 are shown in bold) showing the comparison between the fluoride content of apricot, plum and wheat grain collected from different areas of Peshawar during summer survey of 2008 by the method of AOAC, 1980. Plum and apricot leaves were also extracted for fluoride by alkali fusion method (VDI, 1981). Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Acid digestion (AOAC, 1980)				
Fluoride content	**df**	**F**	**Sig.**	**Post hoc**
Apricot	**2,13**	**7.93**	**0.006**	**BKF>AUP,ARI**
Plum	**2,11**	**19.9**	**0.000**	**BKF>AUP,ARI**
Wheat grain	**2,15**	**68.5**	**0.000**	**BKF>AUP,ARI**
Alkali fusion extraction (VDI, 1981)				
Apricot	**2,4**	**101.96**	**0.000**	**BKF>AUP, ARI**
Plum	**2,3**	**263.6**	**0.000**	**BKF>AUP, ARI**

Table 4.5: Independent sample T-test (those significant at p = 0.05 are shown in bold) showing the fluoride content by AOAC, 1980 method and sulphur content of mango collected from Peshawar during the summer of 2008.

Independent sample T-Test			
Parameters	**t**	**df**	**sig**
Fluoride	**4.034**	**4**	**0.016**
Sulphur	1.226	4	0.287

4.4.7. Fluoride content of fruit leaves

The total fluoride content of fruit leaves was determined by two extraction methods; the AOAC (1980) method, in which samples were extracted by acid digestion, and fluoride extraction through alkali fusion (VDI, 1981).

The concentration of fluoride at BKF for the three species was in the range of 46.5-63mg kg^{-1}, whereas the fluoride concentration at the other sites was in the range of 7.7-10mg kg^{-1} (Fig 4.22). Fluoride values at the BKF were overall 5 times higher than that of other sites and this effect was highly significant (Table 4.4). Fluoride content of mango leaves were also 5 fold higher than that of control site (Table 4.5).

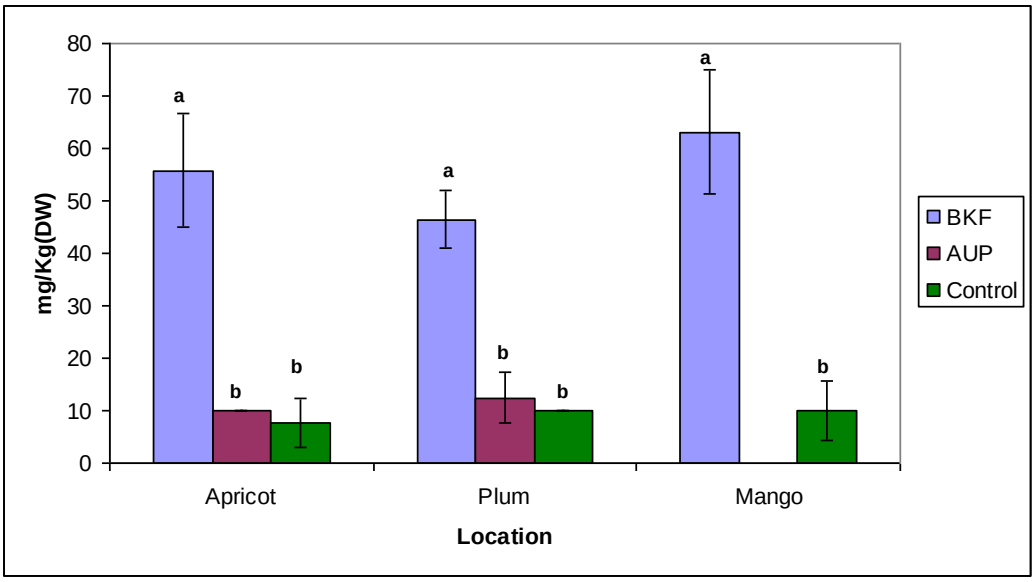


Figure 4.22: Total fluoride content of apricot, plum and mango leaf samples collected from AUP, BKF and control sites during summer season determined by acid digestion method. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

The fluoride concentration by alkali fusion extraction yielded similar results to those using AOAC (1980) method. But high fluoride concentrations were obtained at other sites (Fig 4.23). There were significantly larger concentration differences between the three species although, slightly smaller in size (Fig 4.23). The mean fluoride leaf content at BKF by alkali fusion method was 57.7mg kg^{-1} compared to 55.1mg kg^{-1} by acid digestion method. At AUP and Charsadda, the mean fluoride concentrations by acid digestion were 11.1 and 9.2mg kg^{-1}, while with alkali digestion was 19.4 and 16.6mg kg^{-1}, respectively.

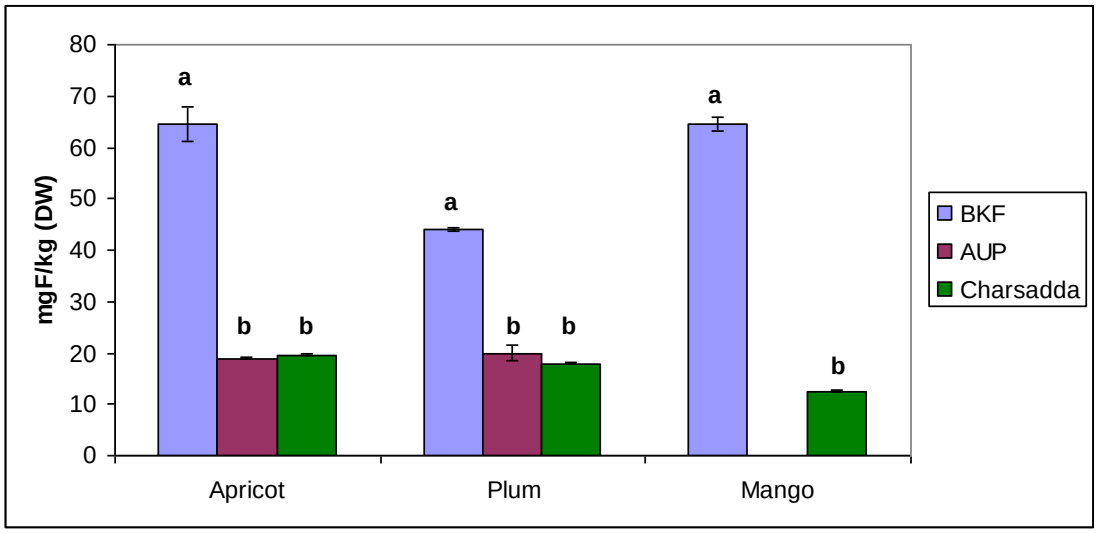


Figure 4.23: Total fluoride content of apricot, plum and mango leaf samples collected from AUP, BKF and control sites of Peshawar during summer season determined by alkali fusion method. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

4.4.8. Fluoride content of spinach

The total fluoride content in BKF spinach samples varied from 39.8 to 79.3mg kg^{-1} (DW), with a mean fluoride concentration of 64.8mg kg^{-1} that was significantly higher (by 70%) than that of control spinach samples. The total fluoride content of control sample was ranged from 10 to 39mg kg^{-1} (DW) with a mean fluoride concentration of 19.9mg kg^{-1}.

4.4.9. Sulphur content of soil and plant

The sulphur content of soil and wheat grains collected from wheat fields at BKF, AUP and ARI did not vary significantly (Table 4.6). The mean sulphur content at BKF, AUP and ARI was 5.0, 6.2 and 6.2mg kg^{-1} (DW), respectively.

Table 4.6: ANOVA results (those significant at p = 0.05 are shown in bold) showing the comparison between the sulphur content of soil, wheat grain, apricot and plum collected from BKF, AUP and ARI areas of Peshawar during summer survey of 2008. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Sulphur content	df	F	Sig.	Post hoc
Soil	2,15	1.88	0.187	*ns*
Wheat grain	2,15	2.5	0.12	*ns*
Apricot	2,13	1.85	0.19	*ns*
Plum	2,11	2.98	0.1	*ns*

The mean sulphur concentration was 41.9, 47.9 and 43.3mg kg^{-1} (DW) for BKF, AUP and ARI, respectively. Sulphur content (S) of soil from spinach fields also did not vary significantly between the BKF and control site (Table 4.6). The mean content of S was 5.95mg kg^{-1} (DW) at BKF site, whereas the mean sulphur soil content was 4.5mg kg^{-1} (DW) at the Charsadda site.

The sulphur content of leaf samples from fruit trees was not significantly different between the BKF, AUP and Charsadda sites during the summer survey (Table 4.6) including mango leaves (Table 4.5). The mean leaf S concentrations were ranged between 45 to 55 mg kg^{-1}.

4.4.10. Metal content of soil

The aluminium (Al) content of AUP soil of wheat fields was significantly higher than that of ARI soil samples by 16%, but the BKF soil was not significantly different from the AUP and ARI sites (Fig 4.24). Similarly, iron (Fe) content of BKF was significantly (13%) higher than that of ARI, but the AUP soil was not significantly different from the soils at BKF and ARI (Fig 4.25).

Table 4.7: ANOVA results (those significant at p = 0.05 are shown in bold) showing the comparison between the mean metal content of soil samples collected from BKF, AUP and ARI during summer survey of 2008. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Metal	df	F	Sig.	Post hoc
Al	**2,15**	**6.47**	**0.009**	**AUP, BKF>ARI**
Cr	2,15	1.405	0.276	*ns*
Fe	**2,15**	**4.746**	**0.025**	**AUP, BKF>ARI**
Mn	2,15	1.467	0.262	*ns*
Ni	2,15	1.428	0.271	*ns*
Cu	2,15	0.749	0.49	*ns*
Cd	2,15	0.895	0.643	*ns*
Zn	2,15	1.37	0.284	*ns*
As	**2,15**	**4.669**	**0.027**	**ARI, BKF>AUP**
Pb	2,15	2.726	0.098	*ns*

Other trace elements, like manganese (Mn) and nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn) and lead (Pb), did not differ significantly in concentration between sites, apart from arsenic (As) that was significantly reduced in ARI and BKF compared to AUP site (Table 4.7). There was no significant difference between the metal content of soil collected from spinach fields of BKF and Charsadda.

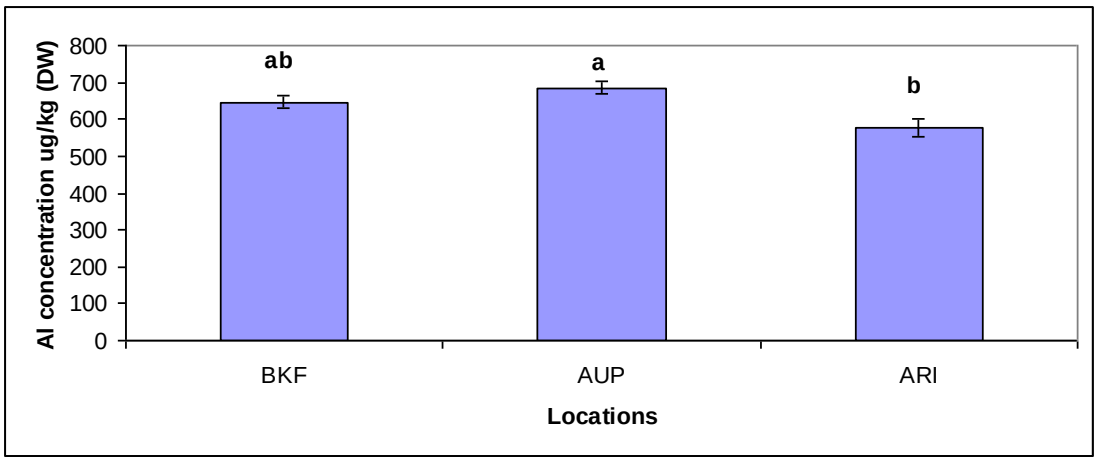


Figure 4.24: Al content of soil samples collected from wheat fields of BKF, AUP and ARI sites. BKF values are the means of 8 replicates while AUP and ARI values are the means of 5 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05

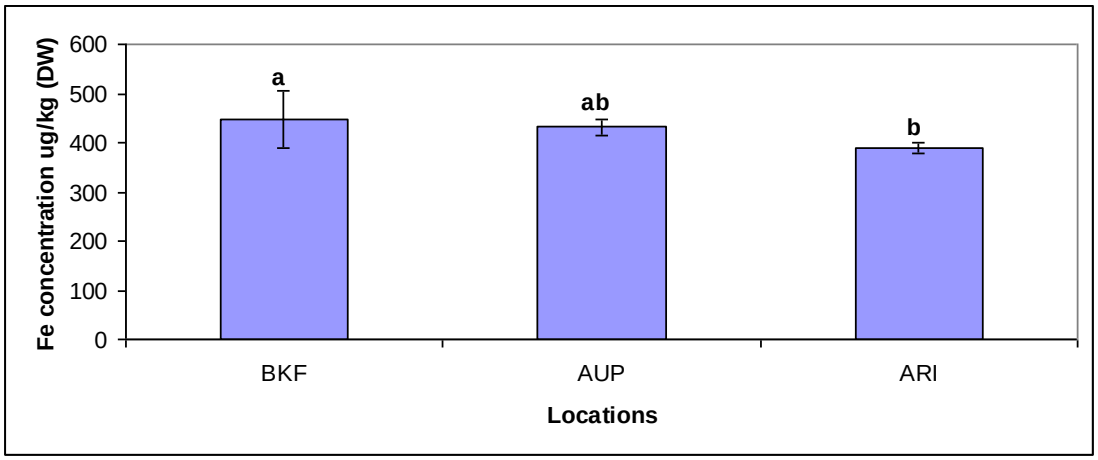


Figure 4.25: Fe content of soil samples collected from wheat fields of BKF, AUP and ARI sites. BKF values are the means of 8 replicates while AUP and ARI values are the means of 5 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05

4.4.11. Metal content of wheat grain

The aluminium (Al) and iron (Fe) content, and that of trace elements, did not vary significantly between BKF, AUP and ARI sites (Table 4.8).

Table 4.8: ANOVA results (those significant at p = 0.05 are shown in bold) showing the comparison between the mean metal content of wheat grain samples collected from BKF, AUP and ARI areas of Peshawar during summer survey of 2008. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Elements	df	F	Sig.	Post hoc
Al	2,15	0.865	0.444	*ns*
Cr	2,15	3.613	0.057	*ns*
Fe	2,15	0.878	0.439	*ns*
Mn	2,15	0.431	0.659	*ns*
Ni	2,15	1.433	0.274	*ns*
Cu	2,15	0.757	0.489	*ns*
Zn	2,15	0.098	0.907	*ns*
As	2,15	0.35	0.711	*ns*
Cd	2,15	0.65	0.538	*ns*
Pb	2,15	1.09	0.365	*ns*

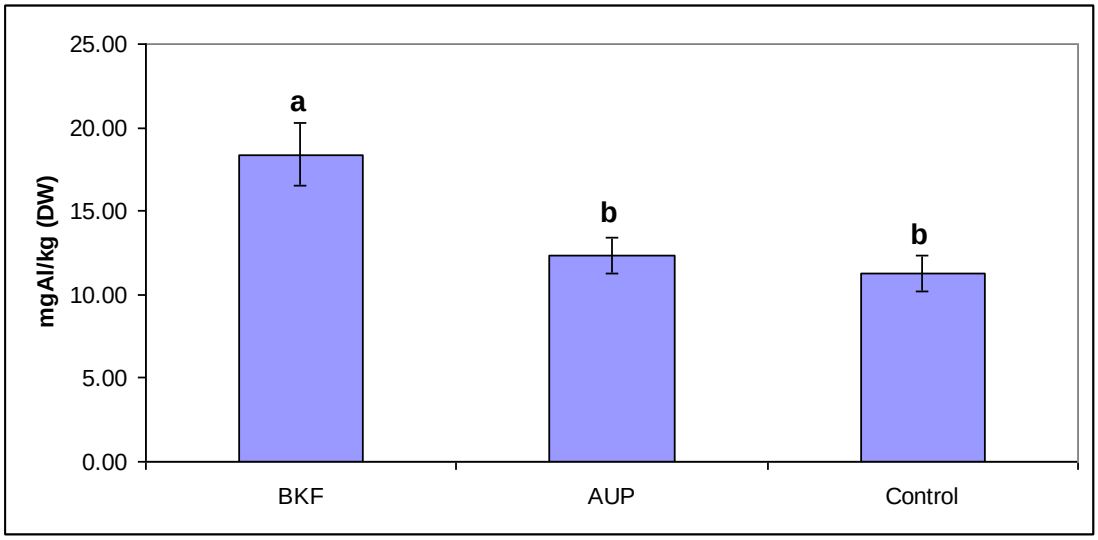
4.4.12. Metal content of fruit leaves

The metal analysis of fruit leaves (apricot, plum and mango) collected from BKF, AUP and control areas revealed that the aluminium (Al) content of BKF fruit crop leaves were significantly higher than those from AUP and Charsadda areas by 33% and 38%, respectively (Fig 4.26).

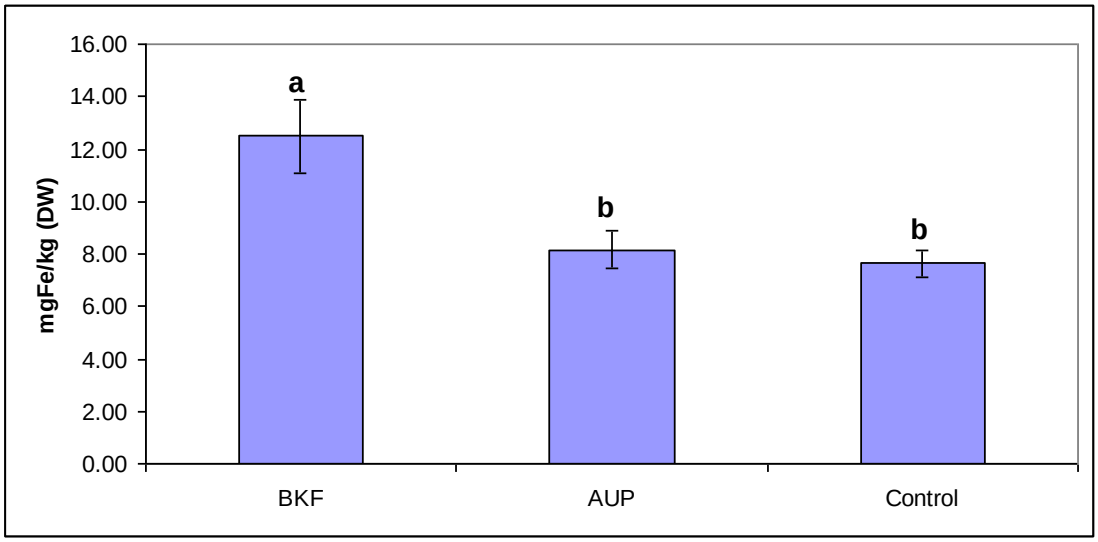
Table 4.9: ANOVA results (Probability values: those significant at p = 0.05 are shown in bold) showing the comparison between the mean metal content of fruit leaf samples collected from BKF, AUP and Control areas of Peshawar during summer survey of 2008. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Metal content	df	F	Sig.	post hoc
Al	**2,30**	**5.488**	**0.009**	**BKF>AUP, Charsadda**
Cr	2,30	0.12	0.887	*ns*
Fe	**2,30**	**5.161**	**0.012**	**BKF>AUP, Charsadda**
Mn	2,30	3.095	0.06	*ns*
Ni	2,30	2.714	0.083	*ns*
Cu	2,30	0.539	0.589	*ns*
Zn	2,30	2.245	0.123	*ns*
As	**2,30**	**8.565**	**0.001**	**AUP>Charsadda, BKF**
Ag	2,30	2.677	0.085	*ns*
Cd	**2,30**	**9.054**	**0.001**	**BKF>AUP, Charsadda**
Pb	**2,30**	**6.621**	**0.004**	**BKF>AUP, Charsadda**

The iron (Fe) content of fruit leaves of BKF site was also significantly higher, by 33% and 41%, than that of AUP and Charsadda sites, respectively (Fig 4.27). Apart from Al, Fe, As, Cd and Pb, the remaining metals were not significantly different among the sites (Table 4.9).



4.26: Al content of fruit leaves collected from BKF, AUP and ARI sites. BKF values are the means of 16 replicates while AUP are and ARI values are the means of 8 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differs significantly at p = 0.05.



4.27: Fe content of fruit leaves collected from BKF, AUP and ARI sites. BKF values are the means of 16 replicates while AUP are and ARI values are the means of 8 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differs significantly at p = 0.05.

4.5. DISCUSSION

4.5.1. Passive sampler

The HF passive sampler results showed higher HF concentrations at BKF site in summer than in winter the season, although only one measurement was made during the winter. The HF concentration was below the detection limit of 0.1µg m^{-3} during all seasons at all other sites. During the months of February, March and April 2008, the mean HF concentration in the air stayed at 0.2µg m^{-3} at BKF, but increased to 0.3µgm^{-3} in May, 2008. This suggested that HF concentration may have been influenced by the atmospheric temperature, light quality and intensity. The mean temperature increased from 24.8°C in February to 28.5°C in May, 2008 at BKF. The HF concentration may also be higher due to higher emissions in the summer. There are no local data on how many brick kiln factories are active round the year. The difference in HF concentration between summer and winter seasons may be directly related to the number of active brick kilns, or may be due to changes in temperature and light intensity and duration. This observation is supported by Brewer *et al*. (1957), who worked on fluoride accumulation in foliage and fruit of wine grapes growing in the vicinity of heavy industry, and concluded that high air temperature and light intensity will increase the amount of HF in the atmosphere. Similar conclusions were reached by Adams *et al.* (1956), Benedict *et al*. (1965) and Murray (1984).

Given the amount of brick work activity around Peshawar, it was expected that the HF concentration might be higher than the mean of 0.2µg HFm^{-3}. The data are based on 4 week mean HF concentration, and the HF concentrations could be higher at particular times of the day. The HF concentration just started to increase in the month of May, and could have been higher later in the summer. It is suggested that HF should be monitored during the entire

peak time and around the year in order to get more detailed information on HF concentrations in the area. The other important limitation was the monitoring of HF at only two sites in summer and in winter. As the BKF area stretches over 15km^2 to the east of Peshawar, taking data at one point in the BKF area is not enough, and there should be a multiple point risk assessment study in the BKF area for the whole 12 months.

The mean spring concentration of 0.2µg HF m^{-3} may still be damaging for certain sensitive crops. Long term exposure to concentrations above 0.2µg HF m^{-3} is phytotoxic to sensitive species according to WHO (1984). Another study showed that a concentration of 0.3µg HF m^{-3} for a longer duration (3 months) is enough to induce injury to plants (Cape *et al*., 2003). In the case of Peshawar, this is highly likely as the brick kilns have been operating for the last 10 years or so, according to the local farmers. The mean HF concentrations may be regularly exceeding the WHO threshold level of 0.2 µg HF m^{-3} at different time of the year, depending upon environmental conditions, but this can only be validated after more detailed HF risk assessments round the year at Peshawar.

The findings of this study were consistent with those of Franzaring (2007), who studied the effect of airborne fluoride near an HF producing factory using standardised grass cultures and passive samplers, based on five months study, and concluded that a mean concentration of 0.27µg HF m^{-3} can affect the performance of the grass cultures. Pandey (1985) also worked on the effect of HF on tree leaves around an aluminium factory in India and concluded that the HF effect on trees was negatively correlated with the distance from the pollution source. Murray (1983) exposed grapevine (*Vitis vinifera L.*) to 0.05, 0.13, 0.17 and 0.28µg HF m^{-3} for 64 days in open-top fumigation chambers and concluded that necrosis

occurred in plants exposed to 0.28µg HF m^{-3}, which accumulated a leaf fluoride content of 85mg kg^{-1} (DW). No injury was observed in the other treatments.

In contrast to HF concentrations, the SO_2 concentration in the atmosphere was higher (16.2µg m^{-3}) at ARI site than at the BKF (8.0µg m^{-3}) during the summer season. The higher SO_2 concentration at ARI site may be due to its close proximity to the main Great Trunk (G.T) road that runs from Peshawar to Islamabad. The heavy traffic, the use of low quality fuel with a high sulphur content, and low maintenance of the vehicles might be the main reasons for high SO_2 concentration at ARI compared to BKF. The SO_2 concentration at BKF increased to 19.4µg m^{-3} during the winter survey, but this was based on one month's measurement. SO_2 emissions may continue throughout the year as the brick kiln factories use coal and used engine oil as a fuel around the clock. The SO_2 concentrations at the AUP site in winter of 2008 were lower (4.7µg m^{-3}), this site is way from the G.T road and brick fields.

Khan and Khan (1996) concluded that SO_2 concentration from 43-348µg m^{-3} around power plant in India for 75 days can inhabit SO_2 injury to green plants. In contrast barley does not show injury to SO_2 in the concentration range 115-343µg m^{-3} (Navari-lzzo *et al*., 1991). The WHO (2005) gives a threshold of an annual mean concentration of 30µg m^{-3} SO_2 for the effects on the yield of crops. For visible injury, much higher concentrations are needed over short periods. In the current study the mean measurements were made for four weeks, so no conclusions about short term exposures can be drawn at this stage. Overall, the evidence suggests that, in contrast to HF, the SO_2 concentrations were not more elevated at sites showing more injury. However, SO_2 in combination with HF, and other pollutants like NO_2 and O_3 at low concentrations, may still be harmful to crops. This statement is supported by Mandl *et al*. (1980), who exposed sweet corn to SO_2 and HF in combination and

separately. They concluded that the two pollutants can reduce the yield of the plant when fumigated jointly. Foliar injury was lower in HF alone compared to the combination of SO_2 and HF. Of the two pollutants (HF and SO_2), HF is likely to be the most phytotoxic for the crops in Peshawar. However, this study was carried out for a short duration, in order to get an initial picture of pollution damage around Peshawar. It is suggested that risk assessment for all these pollutants should be conducted for a longer duration based on the seasonal trends and short term variation in concentrations of these air pollutants.

4.5.2. Visible injuries

Most of the severe visible injury on different crops was observed at the BKF site. The visible injuries were associated with necrosis to leaf margins and tip burn, which are typical HF visible leaf injuries (Weinstein and Davison, 2003). Severe HF injuries to apricot and plum at the BKF site suggested that they were the most sensitive species to HF pollution; almost all the mature leaves of apricot and plum orchards were affected. Solberg and Adams (1956) reported that apricot leaves are very sensitive to HF, while VDI (1987) also classified apricot and plum as very sensitive species to HF. According to the local farmers, this problem is so severe that most of them are forced to dig out these orchards, and plant other crops that do not show these symptoms. Therefore the number of apricot and plum orchards has been reduced in the past decade despite the fact that Peshawar and its surrounding areas are thought to be the best agricultural lands in Pakistan for apricot, plum, peach and pear fruit crops (Fig 4.4.18).

Figure 4.28: A sign on M1 (Motor way-1) welcoming the tourist to the best pear producing area. The photo is taken few km outside Peshawar. The orchards can be clearly seen on the left of the picture Date, 15/01/2009

The lower severity of injury symptoms to plum and apricot leaves at the AUP, ARI and Charsadda sites indicates that the severe injuries to these two fruit crops at BKF is mainly due to HF. The HF concentrations were the highest at the BKF site, and there were five fold higher fluoride concentrations in apricot and plum leaves collected from BKF than those of AUP and Charsadda leaf samples. In contrast, the sulphur content was not significantly different between ARI, AUP and BKF. Similar injuries to the leaves of mango in summer and grapevine in winter at Hayatabad also suggested that these plants were injured by HF, although Hayatabad is 4km to the south of BKF. The high content of fluoride in Hayatabad mango samples compared to Charsadda samples confirmed that the injury symptoms are due to HF emissions. DeOng (1949) found similar necrotic injuries to apricot orchards near an aluminium factory, and similar injuries were found by Griffiths (2003) and Bonte (1982) on plum leaves. Zhang *et al*. (1995) studied the cause of the mango black tip disorder in Guangdong, China and confirmed that mango fruits and leaves are highly sensitive to HF emitted from local brick kiln factories.

The visible leaf injuries to maize in summer and wheat in winter at BKF site also suggested that they were damaged by air pollution, causing marginal necrosis and tip burn on

the leaves. However, these crops showed less severe injury compared to apricot, mango and plum at the BKF site. MacLean (1990) termed maize less sensitive to HF, although Muhammad (1969) and VDI (1987) categorised maize as a fluoride sensitive crop. Wheat leaf injuries (tip burn) were only observed in January winter when the plants were two months old at BKF, but no injury was seen in the later stages of the plant development, during the months of March and April. This suggests that wheat might be sensitive to HF in the early stages of growth. Previous studies have not reported visible injuries on young wheat plants but suggest that the HF can alter the growth and yield of wheat at HF concentrations, which may be found in the BKF area. Davison *et al*. (1990) exposed wheat to HF (<0.03 and 0.30μg m^{-3} concentrations) in open top chambers for 120 days and found that HF did not induce foliar injuries, but reduced shoot, ear and grain weight, and increased fluoride content of the leaves. Maclean and Schneider (1981) exposed wheat (*Triticum aestivum* L.) for four days at 0.9μg HF m^{-3} and Maclean *et al*. (1984) fumigated wheat with 1·6µg HF m^{-3} for three days, and both studies found that HF did not cause visible injury but inhibited growth and yield of the crop.

No injuries to maize and wheat leaves were observed at AUP, ARI and Charsadda sites. This supports the hypothesis that these crops are not at risk from HF because of the low concentrations at these sites. Tomato and sugarcane were less sensitive to HF, as few of the plants at BKF showed leaf injury, with no injuries recorded at other sites. Weinstein (1961) worked on the effects of HF on metabolic constituents of tomato leaves by fumigating continuously for 24 hours at 5.1 and 0.5μg HF m^{-3} and concluded that HF did not induce injury to its leaves but can affect their growth. However the growth of the leaves recovered in the mature stage. Solberg and Adams (1956) and VDI (1987) also termed tomato as more

resistant to foliar injury from HF and categorised it as less sensitive to atmospheric fluoride. There is no literature available on HF and SO_2 effects on sugarcane.

Peach, onion, summer and winter spinach, cucumber and okra showed no injury due to air pollution at any site. The lack of injury to peach, onion and spinach leaves in the BKF area, adjacent to apricot and plum orchards, is not consistent with the literature because these plant species are reported to be very sensitive to HF pollution (VDI, 1987). The difference in resistance of these crops may be due to several environmental and genetic factors. Weinstein and Davison (2003) worked on native plant species suitable as bio-indicators and bio-monitors for HF for USA, Europe and Australia and concluded that the sensitivity of a species in one region might not be a reliable predictor of sensitivity in another region, because of genetic factors (cultivar and variety) and environmental factors e.g. soil composition, pH, air temperature and humidity.

The resistance of the plants to fluoride toxicity also depends on maintaining their calcium and magnesium levels in the leaf (Abdallah *et al*., 2006; Weinstein and Davison, 2003). Calcium (Ca) is an important component of the cell, and is found mainly in the walls (50%), outer plasma membrane and in vacuoles of the cell (Abdallah *et al*., 2006). Ca is exposed to fluoride as it is carried in the transpiration stream and precipitation of calcium as CaF will affect certain metabolic processes and also can loosen the strength of the cell wall (Weinstein and Davison, 2003). Magnesium is also part of the cell wall but most of it is present in chloroplast. The function of Mg is to act as a bridging element for phosphoryl groups in complexes (Marschner, 1995) and most importantly, it is the central atom in the chlorophyll molecular structure. The interaction of fluoride with Mg is most likely as the pollutant is located in the cell wall and chloroplast resulting in disturbing the function of

photosynthesis (Weinstein and Davison, 2003). Those plants which maintain high amounts of Ca and Mg inside the leaves at high fluoride concentrations will avoid fluoride visible injuries to the leaf (Abudallah *et al*., 2006). This suggests that the resistant plants in the area might maintain higher contents of calcium and magnesium content to avoid HF toxicity. However HF can still affect the yield and growth of the crops, regardless of the lack of visible injuries due to HF, which will result in reductions of crop yield and loss of economic value for the local farmers (Maclean and Schneider 1981; Maclean *et al*., 1984). The degree of HF visible injury was found to be in the order of mango >grapes >apricot >plum >maize >tomato >sugarcane for BKF.

4.5.3. Plant fluoride content

The fluoride content of wheat grains at BKF was also significantly higher than that of AUP and ARI, presumably because of the HF emissions from brick kilns. This suggests that fluoride accumulated in the grains but the main reason for the high content of fluoride in grains might be the direct deposition of fluoride from the atmosphere. The grains were directly analysed for fluoride and sulphur contents without washing them with deionised water, because there was no deionised water facility in Peshawar. They were also not washed with tap water as this might increase the fluoride content of the wheat grains. It was also important to analyse the grains without washing, because the grains are separated directly in threshing machines and during that process a lot of dust settles on the grains (Fig 4.4.19). After threshing the grains, they are then directly crushed in the mills. In this way, fluoride and metal contents might be increased in flour from atmospheric dust deposition during threshing.

Figure 4.29: Thrashing of wheat plant at BKF. Dust emitting by thresher machine can be seen settling on the heap of grains after threshing Date, 23/05/2008

Sulphur content was not significantly different between the sites for any of the crops. This suggests that SO_2 is not a significant factor damaging the local crops. The high content of fluoride in the shoot and leaves of spinach samples collected at BKF, compared to Charsadda samples, suggest that fluoride has accumulated in the leaves. Although fluoride did not cause any foliar injury to spinach it can reduce the photosynthetic activity and yield of the plant. Boese *et al*. (2000) subjected spinach to HF (5µg HF m^{-3} for 6 days) and concluded that spinach did not show any visible injury symptoms to fluoride but that the fluoride content of roots and leaves was increased, and the chlorophyll content was reduced.

Several studies have found that HF injured leaves have high fluoride contents compared to plant species grown in HF free environments. Brewer (1957) extracted 211mg Fkg^{-1} from spring cycle citrus leaves in California near industrial sites and found concentrations of 10mg Fkg^{-1} in the same species away from the industrial areas. Moreover, Haidouti (1993) worked on the effect of HF near an aluminium factory and concluded that the average fluoride concentrations in highly damaged plants were ranged from 257-621mg Fkg^{-1}, whereas it was 64-144mg Fkg^{-1} in the less damaged vegetation. The average fluoride

content of control samples were 8-15mg Fkg^{-1}. However, all these studies revealed high fluoride content compared to the current study.

4.5.4. Soil fluoride content

It was important to measure the soil fluoride content of these sites, because plants can accumulate fluoride from soil, which can affect plant growth (Jha *et al*., 2009). Ca and water extractable fluoride were determined from AUP, ARI and BKF soils. Ca and water extractable fluoride were measured, as these are the most bioavailable forms of fluoride in the soil (Arnesen, 1997). The Ca and water extractable fluoride concentrations were significantly higher at AUP and ARI sites compared to BKF. This might be due to the higher fertilizer applications at these research sites, because crops are subjected to different experimental treatments of both high phosphate fertilizers and pesticides that contain fluoride. Loganathan *et al*. (2001, 2006) worked on the fluoride accumulation in pastures of New Zealand and concluded that the fertilizer application can increase soil fluoride. However, the fluoride content of Peshawar soils could be irrelevant to plants because the soil pH of all samples was above 7.5, which suggests that very little fluoride is likely to be available to the plant for uptake via roots. Several factors influence the availability of fluoride in soil, such as, pH, content of clay, organic matter, Al and Fe oxides/hydroxides (Arnesen, 1997; McLaughlin *et al.*, 2001 and Loganathan *et al.*, 2006). The fluoride bioavailability to plants is high at pH below 6.0, as it forms complexes with Al and Fe, but above this pH, the solubility of Al/Fe oxides becomes lower, thereby increasing the concentration of free fluoride in the soil (Arnesen, 1997). Fluoride uptake by plants is easier when F is complexed with Al and Fe oxides, compared to free F-ion in solution (Arnesen, 1997).

Peshawar soils are generally calcareous, which means that soil fluoride is likely not to be a direct threat to plant growth and development in the region compared to atmospheric fluoride. However the total soil fluoride should be taken into consideration. The total fluoride content was not analysed because of the non availability of the necessary apparatus. The total soil fluoride content of Peshawar region may still be higher because of the continuous cycle from soil to air and the deposition again onto the soil surface via brick kiln factories. Apart from entering via the leaf stomata, fluoride can also be deposited back into the soil via rain or dry deposition and thus can increase the fluoride content of soil in the longer run.

4.6. CONCLUSION AND RECOMMENDATIONS

It was concluded that HF is the main pollutant in the BKF area, given that its concentrations in air are near threshold levels for effects, and the plant fluoride concentrations were generally elevated. Summer crops were more sensitive to HF compared to winter crops, which reflects the higher HF levels and sensitivity of spring and early summer crops. The passive sampler study should be expanded to multiple sites and for a longer time to get a clear picture of the concentrations of HF. Only the damaged leaves were chemically analysed, suggesting high fluoride content, but it is important to determine also the fluoride content in uninjured leaves. It is also important to analyse the fluoride content of irrigation water, as water soluble fluorides are easily available to the plants.

Currently is there is no threshold level set by the government in Pakistan for air concentrations of HF. It is therefore recommended that more research should be carried out on the HF and SO_2 concentrations, along with other pollutants, in Peshawar and the surrounding big cities of NWFP. All the major crops, fruits and vegetables of the area should be subjected to HF and other pollutants in controlled fumigations to determine their sensitivity and to set a threshold level for these crops. It is also very important to educate the local farmer and the community, as well as the government agencies, such as the EPA, about the damage these pollutants are causing to their crops. The government should prepare a policy to either discourage the building of new brick kilns in their area or the use of better structured brick kiln factories along with good quality fuel, in order to reduce HF concentrations in the atmosphere.

It is clear from the current study that the crops in the vicinity of brick kiln factories are at risk to HF emissions compared to the crops grown away from the brick kiln area. To date there has been no economic survey being carried out related to air pollution damage in the region, and hence it is important to see that how much damage has been done by the air pollution in terms of economic loss.

CHAPTER 5

EFFECTS OF SOIL FLUORIDE ON A PAKISTANI WHEAT VARIETY

5.1. INTRODUCTION

During the field survey for fluoride pollution effects to agricultural crops described in Chapter 4, it was noted that some of the young wheat plants in the brick fields (BKF) area outside Peshawar had typical tip burn leaf injury. An experiment was conducted at the University of York to find out whether the visible leaf injury was due to soil fluoride contamination. This experiment used wheat seeds collected from BKF area of Peshawar, which were grown in soil similar to that in the field in Peshawar. The experiment was conducted under controlled conditions in a heated greenhouse.

This experiment was important to conduct because wheat is the most important staple and cash crop of Pakistan. It is a Rabi crop sown in October/December and harvested in April/May. Wheat is cultivated in about 8.6 million hectares in Pakistan, with total national production of about 21.7 million tonnes in 2006-07; NWFP wheat annual production was about 1.184 million tonnes in 2006/07 (PARC, 2007). Although the production of wheat has increased in the last decade (FAO, 2009), the demand is still higher than the production in Pakistan, due to which the government still imports wheat from abroad to fulfil the domestic needs.

The total fluoride content of soil can range from 20-1000 mg kg^{-1} without any anthropogenic factors like phosphate, ceramic and brick kiln industries (Weinstein and Davison, 2004). Not all the fluoride content of soils is available to the plant, because its bioavailability is influenced by factors like soil pH, organic matter, aluminium and iron content, and the presence of ion-exchange materials such as clays. Only the readily available fluoride that complexes with several elements (Al, Fe and Ca oxides) are available to plants from the soil (Brewer, 1965). It is usually transported in the transpiration stream in complexes with aluminium, iron and calcium. The formation of these complexes depends on the soil pH (Cooke *et al*., 1976). Therefore only the soluble fluorides from the soil are biologically important to the plants (Weinstein and Davison, 2004). A soil pH below 5-5.5 greatly increases the uptake of fluoride by the plants, but fluoride availability at soil pH values above 6 is reduced, as it is in a free ion form and is strongly attached to the soil particles, making it unavailable to the plant (Arnesen, 1997).

However, fluoride can still be taken up by plants in alkaline soil conditions. Several experiments on soil fluoride effects have been conducted on different vegetables grown in high pH soils. Jha *et al.* (2008 & 2009) worked on soil fluoride effects on spinach and onion grown in soil at pH 8, and concluded that water and calcium extractable fluoride were taken up by the plant tissues. Similar results were also obtained by Singh *et al*. (1995) who studied the soil fluoride toxicity to okra (*Abelmoschus esculentus*) grown in alkaline soil.

The effect of soil fluoride on powdery mildew (*Erysiphe graminis f. sp. tritici*) infection of wheat leaves was also assessed during the experiment. It is well documented that cereals are susceptible to powdery mildew (Wiese, 1987), a fungal pathogen that is in white grey form and mostly occurs on the leaf surface (Daamen, 1989). It germinates in the form of

spores in favourable conditions, with an optimum range of 15-20°C air temperature and high humidity (Bennett, 1984). It affects the plant by reducing green leaf area, increasing respiration, reducing root growth, altering the photosynthetic process, and draining nutrients from the plant (Schafer, 1987). Under favourable conditions, powdery mildew can complete its life cycle very quickly (4 days at 20°C). The life cycle takes longer in unfavourable conditions, e.g. 30 days at -2°C and 12 days at 10°C (Ward and Manners, 1974).

Wheat was selected for this study, as it is one of the main cash crops cultivated in the vicinity of brick kiln factories in Pakistan. During the winter survey of 2008, foliar injury to young wheat leaves was observed at BKF. It was important to know whether the injury was caused by HF emitted by brick kilns or due to the soil fluoride toxicity that could have been increased due to the HF deposition from the atmosphere. The effect on powdery mildew is also important, because wheat is mainly sown in winter in Pakistan and powdery mildew can attack wheat at optimum temperatures (15-20°C) along with high humidity. This means that cereal plants are most likely exposed to powdery mildew infection in Pakistan during winter.

5.2. OBJECTIVES OF THE STUDY

There were three main objectives for this study:-

1. To assess what concentrations of soil fluoride can cause foliar injury to wheat.

2. To determine the effect of soil fluoride on wheat sown in alkaline soil conditions.

3. To assess the effect of different concentrations of soil fluoride on the growth and yield of wheat.

Although this was not a planned pot experiment, the opportunity also arose to observe the effect of elevated soil fluoride concentrations on powdery mildew infection of wheat.

5.3. MATERIALS AND METHODS

5.3.1. Facility and experimental design

The experiment was conducted in a heated greenhouse at the University of York, UK. Wheat grain seeds collected from BKF area of Peshawar was grown at different concentrations of fluoride in alkaline soil. The soil NaF concentration was based on 6 treatments (T1=0, T2=10, T3=20, T4=50, T5=100 and T6=200mg kg^{-1}), each with 4 replicates. Soil was provided in sealed bags by the Food and Environment Research Agency, Sand Hutton York (FERA). This soil was selected for the experiment because of the close resemblance to the soil properties of soil in Peshawar. The soil had the properties shown in Table 5.1.

Table 5.1: Properties of the soil used for the experiment

Soil property	**Analytical results**
Sand (%)	58.72
Silt (%)	22.62
Clay (%)	10.49
Texture class	Sandy loam
pH in water (1:5)	6.0
Total Organic Carbon (%)	1.4
EC in water (1:5)	71.5
Bulk density	1.2 kg l^{-1}

Because of the acidic nature of the soil, 7g of lime kg^{-1} were added to increase the soil pH values to between 7 to 8, which is the average pH of Peshawar soil, as described in Section 4.4.3. After adding the lime, the soil was mixed thoroughly and the initial soil characteristics (pH, EC, water and calcium extractable fluoride) were measured. The details of measurement methods are given in Section 4.3.8.

Pot sizes of 14cm depth x 15cm diameter were selected for the experiment. About 2 litres of soil was added to each pot. The soils were then further treated with different levels of NaF (0, 10, 20, 50, 100, 200 mg NaF kg^{-1}) by adding NaF to pots and thoroughly mixing it. The soil was then put into the respective pots and was transferred to the glasshouse where pots were put randomly on $1m^2$ tables that were 1m in height. Twenty seeds of a Pakistani wheat variety (*Bakkar*), collected from a single field in Peshawar in May 2008, were sown in each pot on 16th March, 2009 and were thinned to 10 plants/pots after germination 7 days later. Pots were irrigated with deionised water twice a week. Pot positions were mixed randomly every week in order to get uniform light and temperature. Plants were harvested after 7 weeks. The temperature was kept between 15-20°C throughout the experiment, by means of an automatic electric heater.

5.3.2. Observations of plant response

Each plant was checked every week for visible injury symptoms caused by fluoride and powdery mildew. Plant injury on fully expanded leaves was assessed by using the scale shown in Table 5.2.

Table 5.2: Scale used to assess the visible injury to fully mature leaves

I.D	***Injury scale***
0	No injury
1	Very slight injury, < 5 % of fully expanded leaves with slight injury
2	Slight injury, 5 -15 % of fully expanded leaves with slight injury
3	Moderate injury, 15-30 % of fully expanded leaves with injury
4	Heavy injury, 30-50 % of fully expanded leaves injured
5	Very heavy injury, 50-90 % of fully expanded leaves injured
6	Total injury, 90 -100 % of fully expanded leaves are injured

A leaf injury index for each pot was calculated by the following equation:

$$\Pi = {}_1\Sigma^{np} (i_i \times n_i)/N$$

Where,

Π = Leaf injury index
N = Total number of leaves in pot
i_i = mean injury percentage for each category in Table 5.2.
n_i = number of leaves on plant *i*
n*p* = number of plants in pots

Plant growth was determined every week by measuring the shoot height and number of leaves per plant. The number of ears per pot was also counted after they emerged in Week 4. The entire above ground plant biomass was harvested after 7 weeks on 5th May, 2009. Plants in each pot were divided into stem, ears, live leaves and dead leaves. The plant samples were then washed thoroughly with deionised water to remove dust and other particles and were transferred to the oven in bags for drying. The plant samples were dried in the oven for 48 hours at 70°C. The dried plant samples were ground and were kept in sealed vials at a cool dry place until analysis.

Soil from each pot was also collected. Each of these was transferred to marked paper bags and was transported immediately to the laboratory. Upon arrival, the soil samples were taken from the sampling bags and were sieved through a 2mm sieve to remove twigs and small stones. The soil was then air dried for two day at room temperature by spreading it on cardboard. After air drying, the soil samples were subjected to pH, EC, and moisture analysis (see Section 4.3.6 and 4.3.7). The dried soil samples were then stored in a cool dry place until fluoride analysis.

5.3.3. Plant analysis

Wheat ear, live and dead leaves were subject to fluoride and metal analysis. Ears were analysed instead of grain, as it was difficult to separate the grains from the ears because of their freshness. The plant samples were analysed for total fluoride content by the method of

AOAC (1980). The metal content of wheat samples were analysed on ICP-MS by the method of Wang *et al*. (2004). The detailed methods of analysis of fluoride and metal contents are described in Sections 4.3.10 and 4.3.11, respectively.

5.3.4. Soil analysis

The water and calcium soluble fluoride analysis for soil fluoride was carried out by the method of Larsen and Widdowson (1971) with the help of fluoride ion electrode for each of the soil sample. The details are given in Section 4.3.8.

5.3.5 Statistical summary

The data summary (means and standard errors) was carried out using Microsoft Excel (2003) and the statistical analysis was carried out using SPSS 17.0. The dataset for all parameters was explored for skewness, kurtosis and normality, using the Shapiro-Wilks-test. The parameters showing major deviations from a normal distribution were log transformed. One-way ANOVA was used for each of the parameters to determine whether overall effects of fluoride were significant. ANOVA repeated measures test was used for plant height and leaf number data. Tukey's HSD post hoc test was used for multiple comparisons of different fluoride concentrations at $p<0.05$.

5.4. RESULTS

5.4.1. Soil fluoride content

The mean water and calcium chloride extractable fluoride concentration before wheat germination was 6.45mg kg^{-1} (DW) and 3.97mg kg^{-1} (DW), respectively. The content of water and calcium chloride extractable fluoride increased among the treatments after wheat harvest (Fig 5.1). The water extractable fluoride was higher than calcium chloride extractable fluoride in all samples before and after the experiment. The mean water extractable fluoride after harvest ranged from 3.64mg kg^{-1} (DW) in T1 to 10.04mg kg^{-1} (DW) in T6. The mean calcium chloride extractable fluoride was 3.58mg kg^{-1} (DW) with the highest value of 5.11mg kg^{-1} (DW) in T6 and lowest value of 2.93mg kg^{-1} (DW) in T1, as shown in Figure 5.1. Although ANOVA showed a significant effect, the difference in calcium chloride extractable fluorides concentration between treated and control plants was only significant at 100 mg kg^{-1} and above. In contrast, the difference between water extractable fluorides was significant at the lowest treatments, 10 mg kg^{-1}.

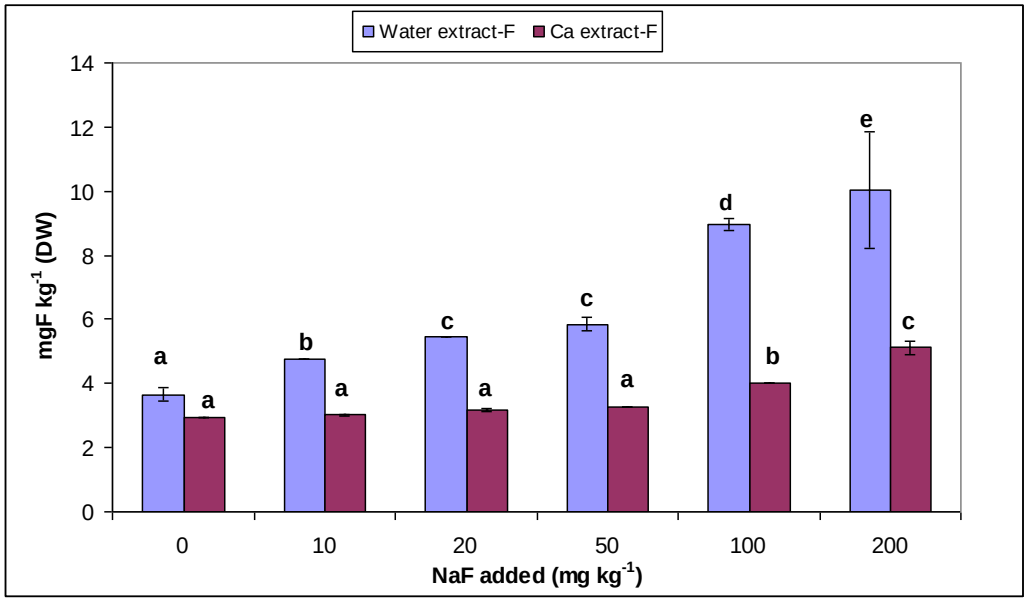


Figure 5.1: Water and calcium extractable fluoride content of soil with different levels of added NaF after wheat harvest. Values are the mean of 4 replicates. Error bars represent the standard error of the values. Bars sharing different letters varied significantly from each other at p = 0.05.

5.4.2. Soil pH and EC

The mean soil pH after adding NaF and lime before germination was 7.07, ranging from 6.92 in T2 to 7.2 in T4 (Fig 5.2). However, the mean soil pH after wheat harvest increased to 7.58, varying significantly from 7.52 in T6 to 7.64 in T4 (Fig 5.2; Table 5.3).

Table 5.3: ANOVA results (Probability values: those significant at p = 0.05 are shown in bold) for the effect of fluoride on soil pH and EC after harvest. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameter	df	F	p	Post hoc
pH	**5,18**	**5.811**	**0.002**	**T6, T5,T4>T3,T2,T1**
EC	**5,18**	**3.906**	**0.014**	**T1>T2,T3**

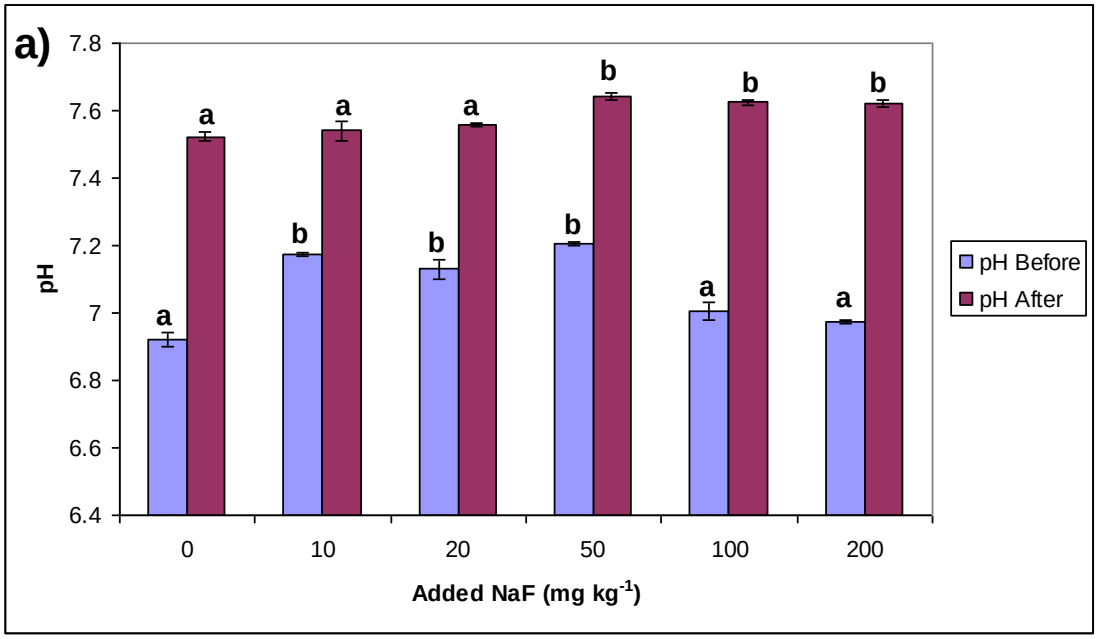


Figure 5.2: Soil pH after wheat harvest. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at p=0.05.

The mean electrical conductivity (EC) of soil was 466.5µs/cm before germination. This decreased significantly among the treatments to 153.1µs/cm after final harvest. The EC values from T1 were significantly higher than T2 and T3. However, EC values in T4, T5 and T6 were not significantly different from these in T1 and T2 (Fig 5.3; Table 5.3).

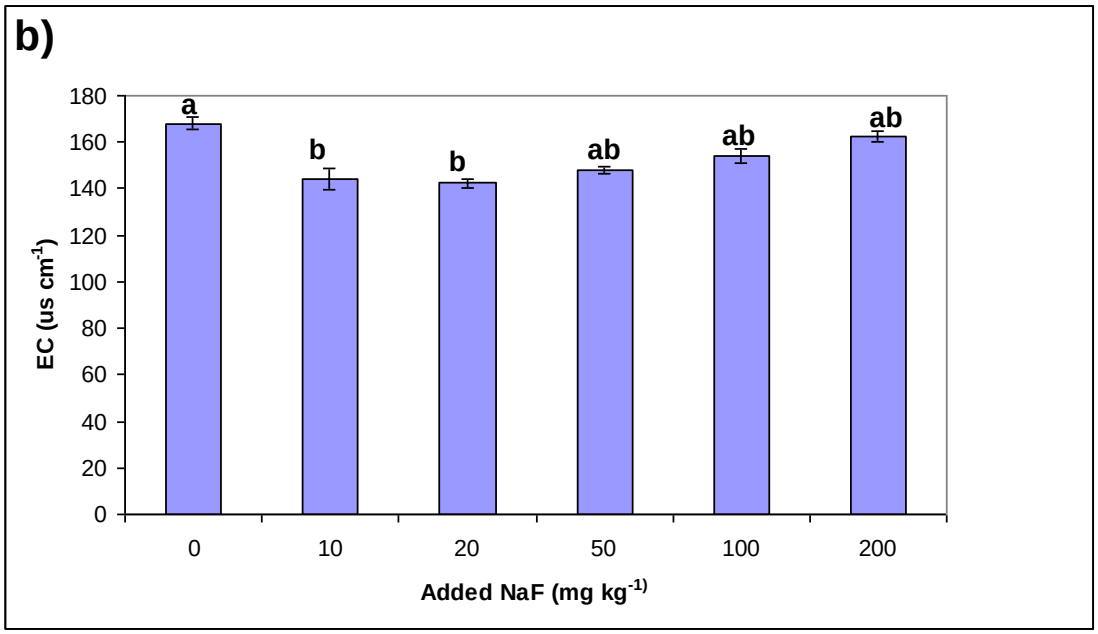


Fig 5.3: Soil EC after wheat harvest. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at p=0.05.

5.4.3. Plant height

The ANOVA repeated measures test found that the plant height significantly increased during the entire experiment (Table 5.4). However, there was no significant effect of the treatment, or the interaction between treatment and time (Table 5.3).

Table 5.4: The results of the ANOVA repeated measures test showing the effect of time and treatment and the time/treatment interaction. The tabulated values are the F ratios. The Tukey's B test shows the effect of soil fluoride on plant height and leaf number in each week. (* p <0.05, ** p <0.01, ** p< 0.001, ns = non significant).

Effect	*df*	*Plant height*	**Leaf number**
Time	5,18	**4993*** **	**9101*** **
Treatment	5,18	1.96 *ns*	**6.77*** **
Treatment x Time	5,18	1.51 *ns*	0.78 *ns*
Tukey's B test			
		Plant height	**Leaf number**
Week1		**T1> all other treatments**	**no data**
Week2		*ns*	**T1> T6**
Week3		*ns*	**T1> other treatments**
Week4		*ns*	**T1> other treatments**
Week5		*ns*	**T1> other treatments**
Week6		*ns*	**T1> other treatments**
Week7		*ns*	*ns*

The post hoc test showed that plant height of Treatment 1 one week after seed germination was significantly higher (21%) than in Treatments 2, 3, 4, 5 and 6 (Figure 5.4). However, the plant height was not significantly different between treatments after week 1 (Figure 5.4; Table 5.4).

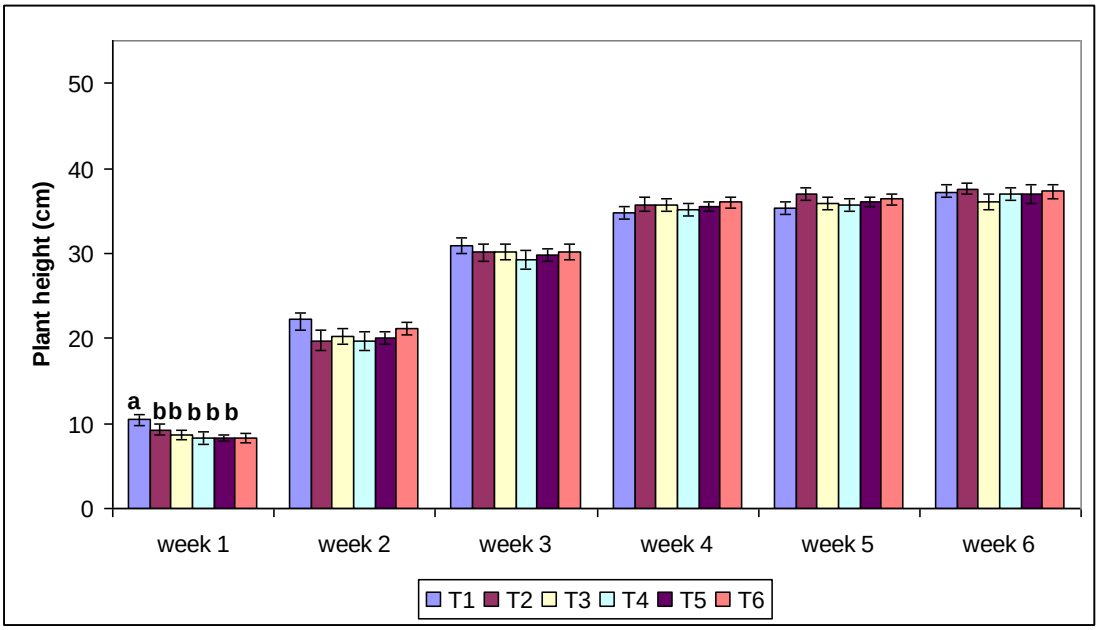


Figure 5.4: Mean plant height from week 1 to week 6. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

5.4.4. Leaf number

The ANOVA repeated measures test showed that leaf number significantly increased with time (Table 5.4). There was also a significant difference between treatments, but there was no significant interaction between treatment and time (Table 5.4). The post hoc test showed that the leaf number in Treatment 1 was significantly higher than the rest of the treatments from week 2 to week 6. The size of the difference between Treatment 1 and Treatment 6 declined with time, being 22%, 20%, 7%, 7% and 6% in weeks 2, 3, 4, 5 and 6, respectively (Fig 5.5). Treatments 2, 3, 4, 5 and 6 were not significantly different during the entire experiment. There was no significant difference between the treatments in the final week (Fig 5.5; Table 5.4).

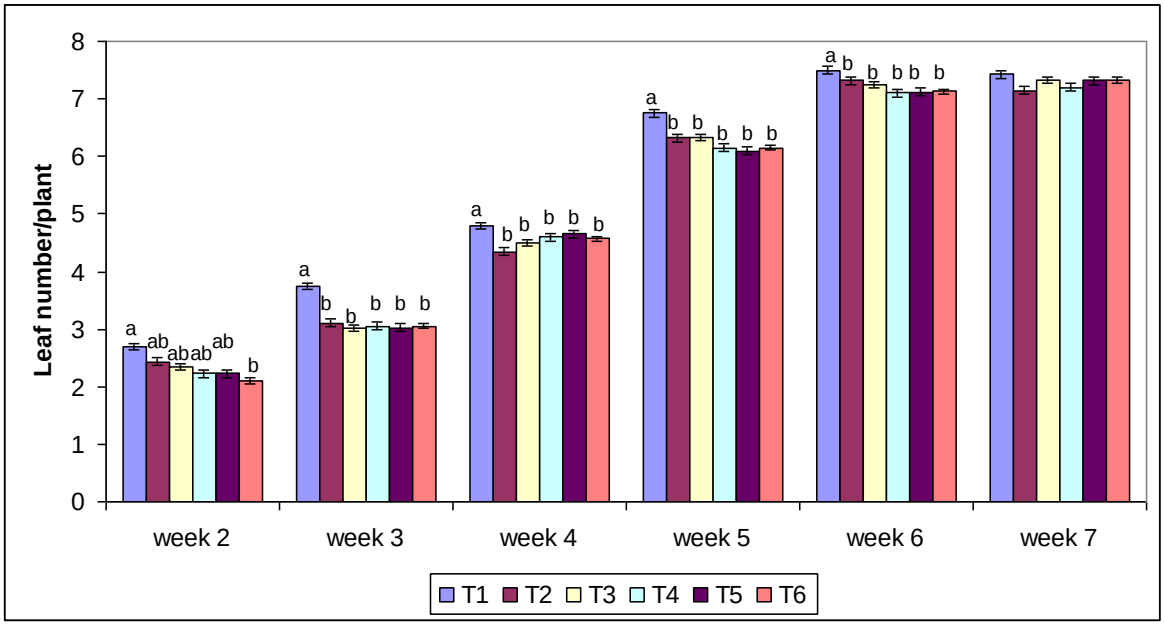


Figure 5.5: Mean leaf number/plant from week 1 to week 7. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

5.4.5. Visible leaf injury

No visible injury resembling that found on young leaves of wheat plants in the BKF, Peshawar area appeared on leaves during the entire experiment. However, injury due to powdery mildew (*Erysiphe graminis f. sp. Tritici*) appeared during Week 5 after wheat germination on mature leaves and the stem, and was characterised by a powdery white to grey fungal growth. The first injury assessment was carried out in Week 6.

Visible injury measurements, based on the number of leaves per treatment in each injury class, suggested that Treatments 1 to 3 had significantly higher numbers of non injured leaves compared to Treatments 4 to 6 (Fig 5.6a). The number of totally injured (90-100% of injured leaves) and very heavy injured leaves (50-90%) was significantly higher in Treatments 5 and 6 compared to Treatment 1 (Figure 5.6b and c). There was no significant difference between the number of leaves in the category of slight injury (5-15%), moderate injury (15-30%), heavy injury (30-50%) as shown in Table 5.5.

Table 5.5: ANOVA results (F ratios and probability values: those significant at p = 0.05 are shown in bold) for powdery mildew injury to leaves during Week 6. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Injury level	df	F	p	Post hoc
0%	5,18	**8.097**	**0.000**	**T1,T2,T3>T4,T5,T6**
<5%	5,18	2.574	0.063	*ns*
5-15%	5,18	2.329	0.085	*ns*
15-30%	5,18	2.184	0.102	*ns*
30-50%	5,18	0.827	0.547	*ns*
50-90%	**5,18**	**7.82**	**0.001**	**T6,T5>T1,T2,T3,T4**
90-100%	**5,18**	**6.73**	**0.001**	**T6>T1,T2,T3,T5**

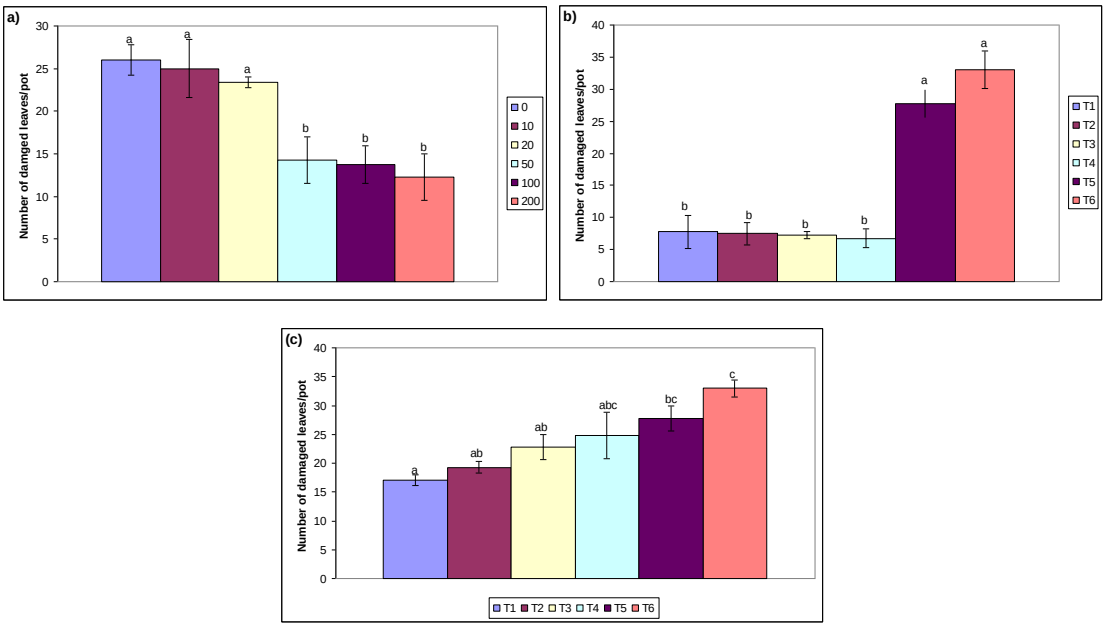


Figure 5.6: The number of powdery mildew damaged leaves in week 6. (a) no injury (0%) (b) Very heavy injury (>50-90%) (c) Total injury (>90-100%). Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

In order to control the powdery mildew disease, the glasshouse was fumigated with sulphur from 9pm to 2am from Week 6 until the plant harvest in Week 7. However, the plants did not show any significant improvement. During Week 7, the powdery mildew injury increased and it was very difficult to distinguish the leaves based on the injury scale used for Week 6 measurements. This was also the main reason for the experiment being terminated before plants were fully matured. Because of the heavy powdery mildew injury, leaves were separated into healthy (not affected by the fungus), diseased (affected by having chlorosis)

and dead (completely necrotic) leaves. However, there was no significant difference between treatments in the number of healthy, diseased and dead leaves (Fig 5.7).

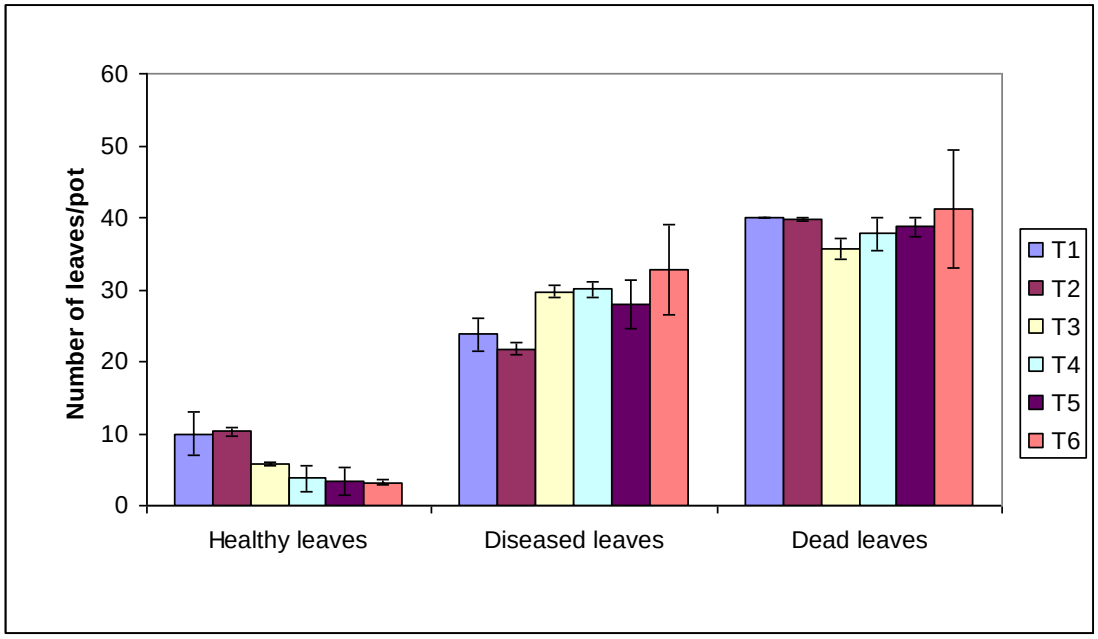


Figure5.7: The number of healthy, diseased and dead leaves of in week 7. Values are the mean of 4 replicates. Error bars indicate standard errors of the means at significant level of p=0.05.

5.4.6. Ear count

Ear numbers were counted in Week 6 on 27th April. The ears were distributed between the mature stage (fully developed ear), middle growth (half developed ear) stage and early growth (flag leaf) stage categories. Mature ears were only found in Treatment 1 (Fig 5.8a). Middle growth stage ears were found in Treatments 1 and 2. However, early growth stage ears were found randomly among all the treatments. During the final Week 7, on the day of harvest (5th May), all the ears were fully mature and there was no significant difference between treatments in the numbers of mature ears (Figure 5.8b).

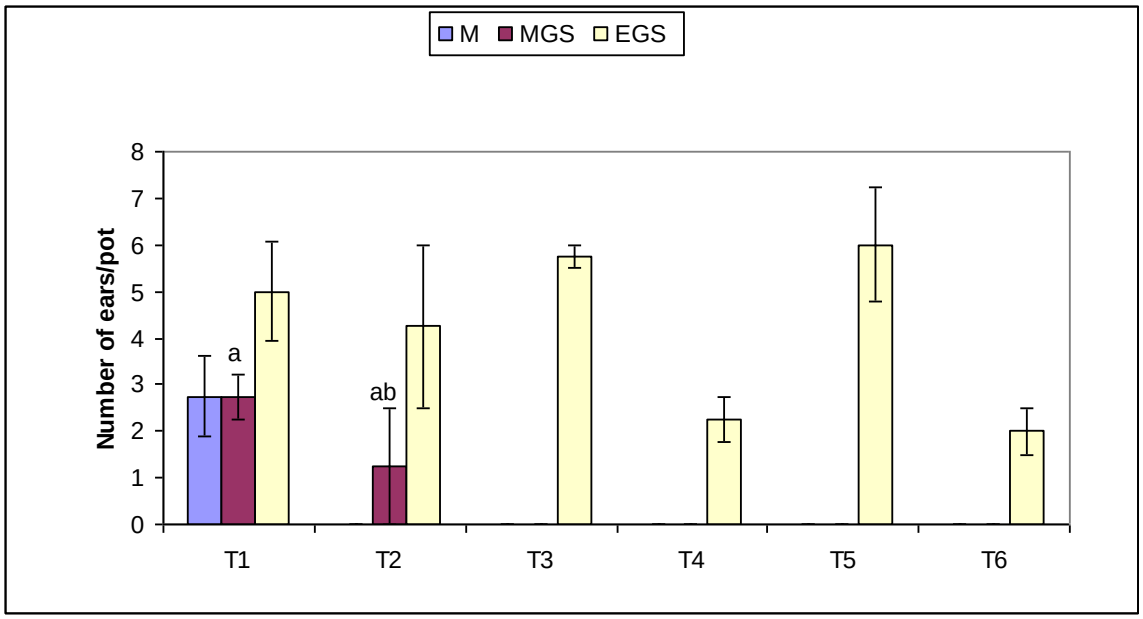


Figure 5.8a: The number of ears in the mature (M), middle (MGS) and early growth (EGS) stage in week 6. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

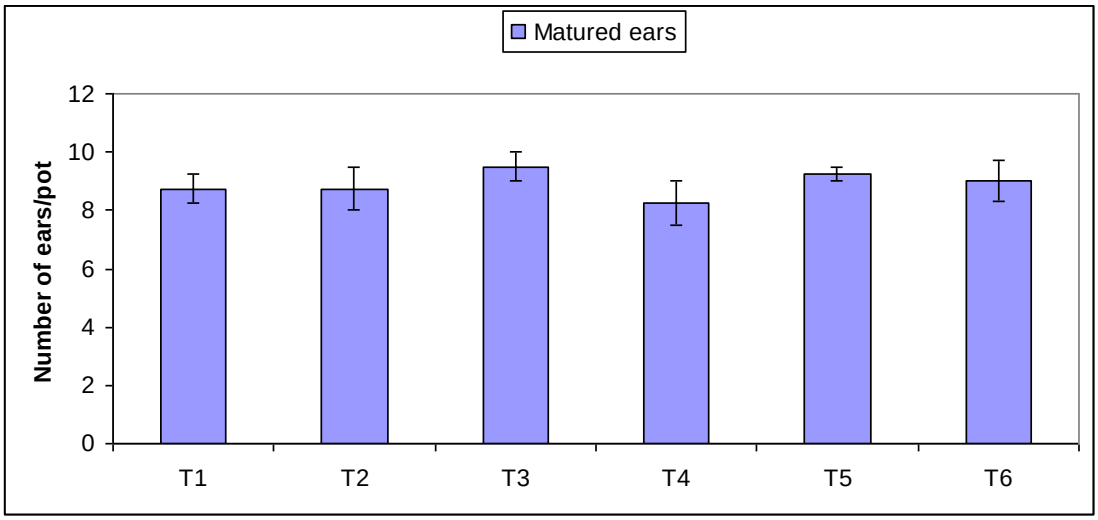


Figure 5.8b: The number of fully mature ears in week 7. Values are the mean of 4 replicates. Error bars indicate standard errors of the mean.

5.4.7. Plant biomass

There was no significant difference between the treatments in the biomass of dead leaves, live leaves, ears, stem and total biomass (Fig 5.9a, b, c, d & e). However, there was an apparent decrease in ear biomass of 19% in Treatment 6 compared to Treatment 1, as shown in Figure 5.9e.

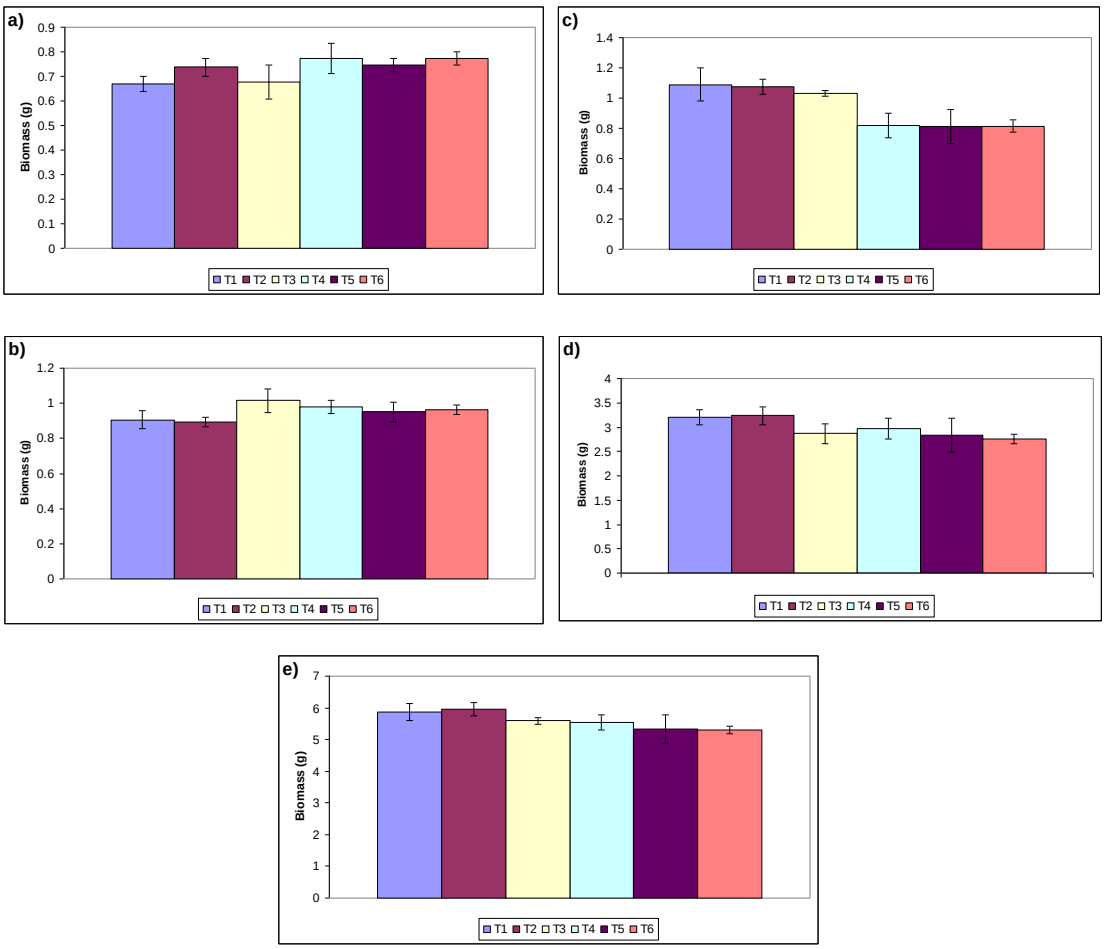


Figure 5.9: Biomass per pot of a) dead leaves (b) live leaves (c) ears (d) stem and (e) total plant biomass in week 7. Values are the mean of 4 replicates. Error bars indicate standard errors of the means.

5.4.8. Fluoride content of wheat

The results of the statistical analysis of the total fluoride content of live leaves, dead leaves and ears are summarised in Table 5.6.

Table 5.6: ANOVA results (F and probability values those significant at p = 0.05 are shown in bold) for the effect of soil fluoride on the fluoride content of live leaves, dead leaves and ears (* p <0.05 significant level)

Parameters	df	F	*p*	*Post hoc*
Live leaves	**5,18**	**127.6**	**0.000**	**T1<T4<T5<T6**
Dead leaves	**5,18**	**61.11**	**0.000**	**T1,T2<T3,T4,T5<T6**
Ears	**5,18**	**185.5**	**0.000**	**T1,T2<T3,T4<T5<T6**

The total fluoride contents of live leaves, dead leaves and ears were significantly different between the treatments (Table 5.6; Fig 5.10a, b & c). The fluoride content of plant increased with the increase in soil fluoride content. The highest fluoride concentration was

found in live leaves (71µg g^{-1}) compared to dead leaves (36.6µg g^{-1}) and ears (23.8µg g^{-1}). For all three parameters, fluoride content in Treatment 3 and above was significantly higher than in T1.

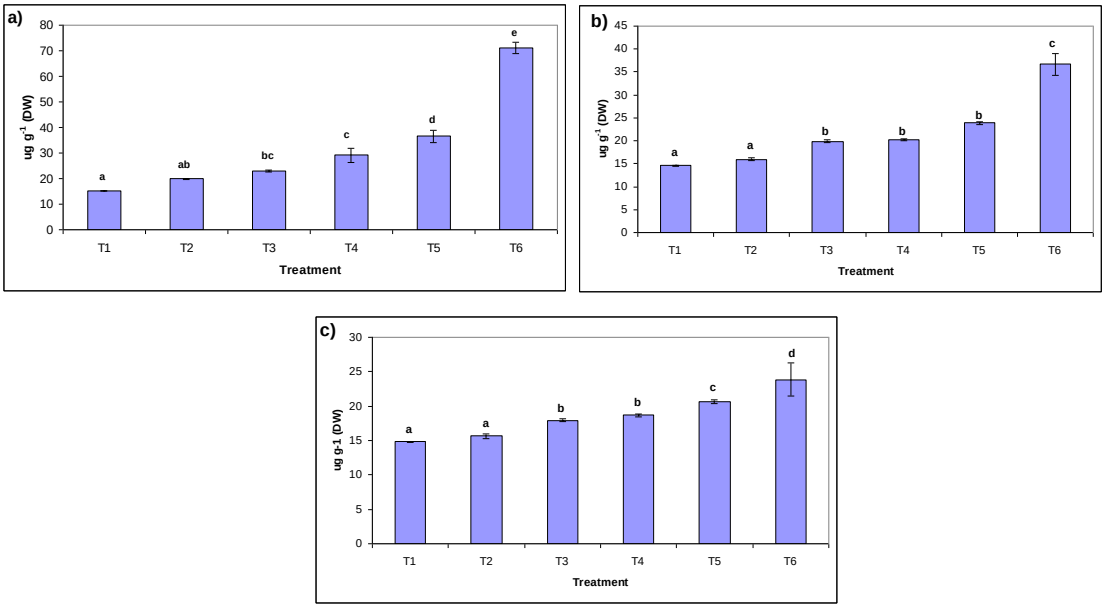


Figure 5.10: Fluoride content of a) live leaves, (b) dead leaves and (c) ear after harvest. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

5.4.9. Metal content of wheat

The results of the statistical analysis of metal contents of wheat are summarised in Table 5.7a, b & c.

Table 5.7a: ANOVA results (those significant at p = 0.05 are shown in bold) for the effect of fluoride on Al, Ca, Mg, Mn, P, S, Si and Zn content of live leaves. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	df	F	*p*	Post hoc
Al	5,18	1.579	0.295	*ns*
Ca	5,18	0.621	0.691	*ns*
Fe	5,18	4.088	0.058	*ns*
K	**5,18**	**9.797**	**0.008**	**T1>T2, T3, T4,T5 & T6**
Mg	5,18	0.361	0.859	*ns*
Mn	5,18	2.278	0.173	*ns*
P	5,18	3.051	0.104	*ns*
S	5,18	1.877	0.233	*ns*
Si	5,18	3.998	0.061	*ns*
Zn	5,18	0.605	0.701	*ns*

Table 5.7b: ANOVA results (those significant at p = 0.05 are shown in bold) for the effect of fluoride on Al, Ca, Mg, Mn, P, S, Si and Zn content of dead leaves. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	df	F	p	Post hoc
Al	5,182	0.816	0.579	*ns*
Ca	5,18	2.447	0.153	*ns*
Fe	5,18	4.56	0.056	*ns*
K	5,18	3.109	0.100	*ns*
Mg	5,18	1.647	0.279	*ns*
Mn	5,18	0.988	0.495	*ns*
P	5,18	1.244	0.393	*ns*
S	5,18	3.596	0.075	*ns*
Si	5,18	2.178	0.185	*ns*
Zn	5,18	1.081	0.455	*ns*

Table 5.7c: ANOVA results (those significant at p = 0.05 are shown in bold) for the effect of fluoride on Al, Ca, Mg, Mn, P, S, Si and Zn content of ear. Post-hoc differences were tested for significance at P=0.05 (ns = non significant)

Parameters	df	F	Sig.	Post hoc
Al	**5,18**	**9.99**	**0.007**	**T1>T2, T3, T4,T5 & T6**
Ca	5,18	3.03	0.104	*ns*
Fe	5,18	2.5	0.14	*ns*
K	**5,18**	**5.48**	**0.031**	**T4>T1**
Mg	5,18	2.69	0.13	*ns*
Mn	5,18	3.02	0.105	*ns*
P	5,18	2.26	0.175	*ns*
S	5,18	2.909	0.113	*ns*
Si	**5,18**	**8.29**	**0.011**	**T2, T5>T1**
Zn	5,18	1.91	0.226	*ns*

The Al content of live and dead leaves was not significantly affected by fluoride treatment but there was an apparent reduction, of 70% in live leaves and 34% dead leaves, respectively in T6 compared to T1 (Fig 5.11a, b). However, the ear content of Al in Treatment 1 (control) was significantly higher by a factor of 10, than in the rest of the treatments (Table 5.6c). The potassium (K) content did not vary significantly among the treatments in dead leaves but was significantly different in live leaves and the ears (Fig 5.12). The Si content also varied significantly in the ears (Table 5.7c). However, K and Si concentrations showed no obvious relationship with the fluoride treatments. Other element (Ca, Fe, Mg, Mn, P, S and Zn) also did not vary significantly between the treatments (Table 5.7).

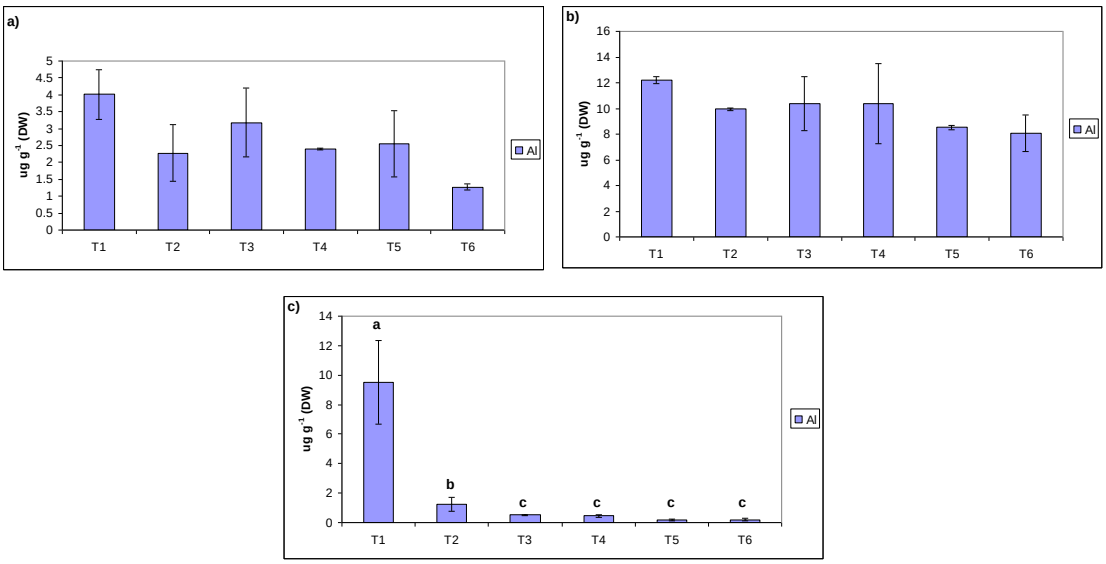


Figure 5.11: Al content of a) live leaves, (b) dead leaves and (c) ear after harvest. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

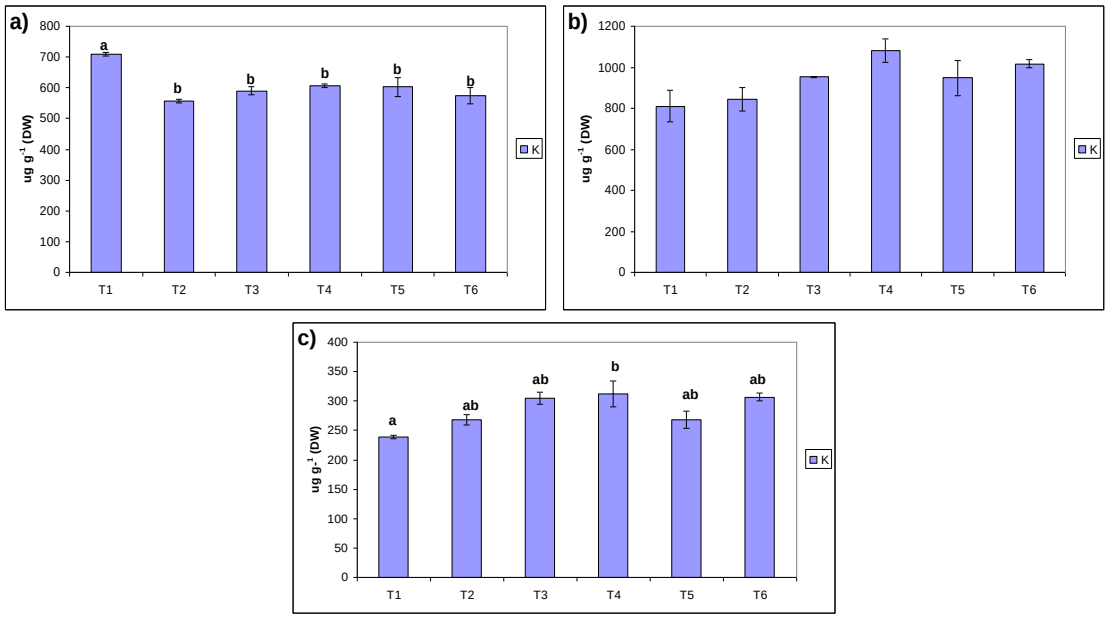


Figure 5.12: K content of a) live leaves, (b) dead leaves and (c) ear after harvest. Values are the mean of 4 replicates. Error bars indicate standard errors of the means. Bars sharing different letters differ significantly from each other at P=0.05.

5.4.10. Regression analysis

Linear regression analysis showed a greater increase of fluoride concentration in live leaves compared to dead leaves and ears, when plotted against both the water and calcium extractable fluoride content of soil (Fig 5.13a & b); all theses relationships were highly significant. However, a linear regression analysis showed no significant relationship between

plant biomass and water extractable soil fluoride concentration (Fig 5.14). Logarithmic regression analysis also showed that the leaf injury index increased between leaf fluoride concentrations of 10-30 µg g^{-1}, but showed little further increase at fluoride concentrations above 30 µg g^{-1} (Fig 5.15).

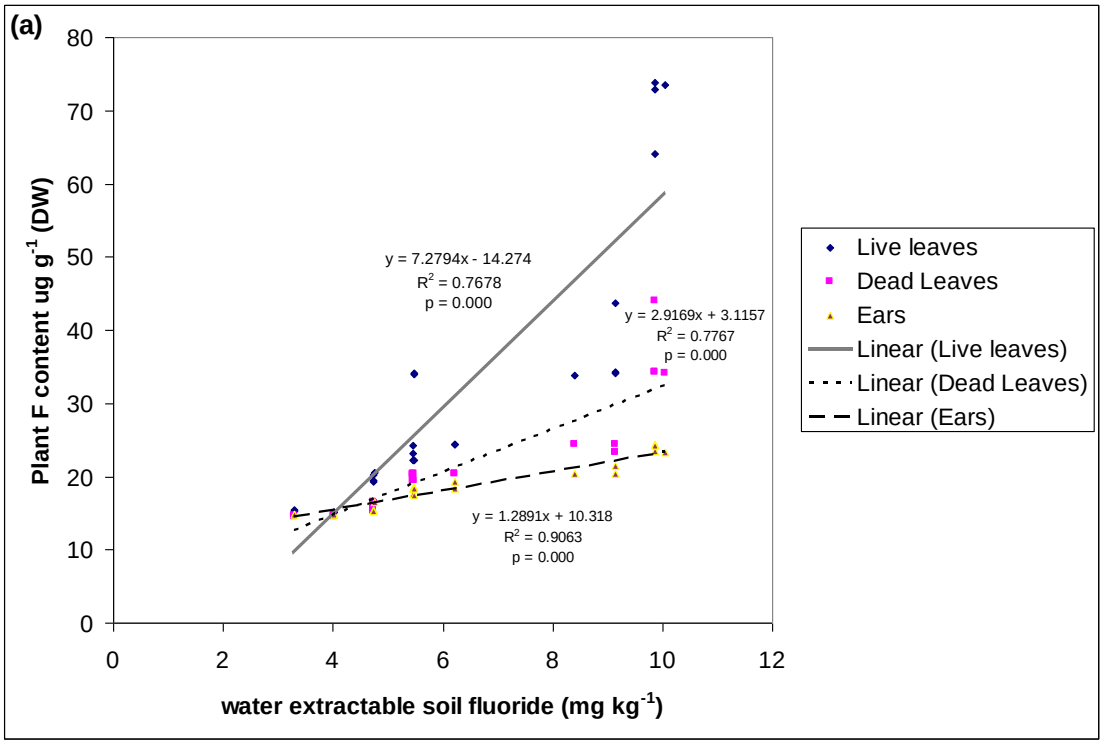


Figure 5.13a: Linear regression analysis showing the effect of water extractable soil fluoride concentration on the plant fluoride concentrations in live, dead leaves and ears. Plotted values represent individual plots.

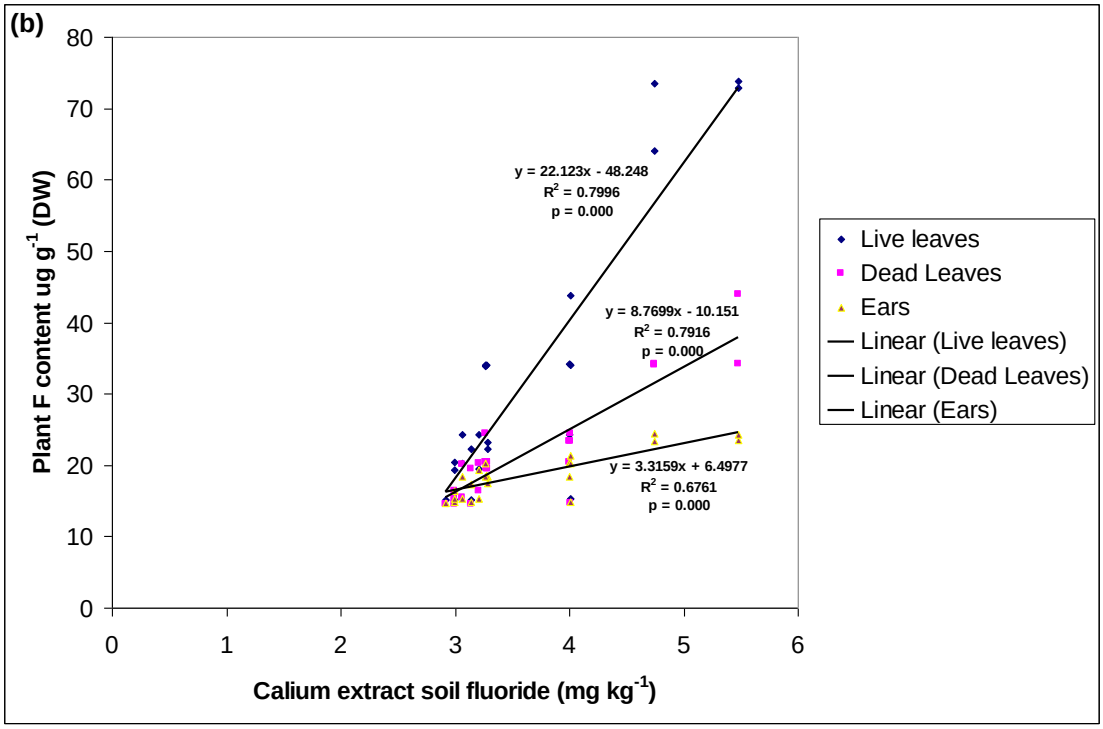


Figure 5.13b: Linear regression analysis showing the effect of calcium extractable soil fluoride concentration on the plant fluoride concentrations in live, dead leaves and ears. Plotted values represent individual plots.

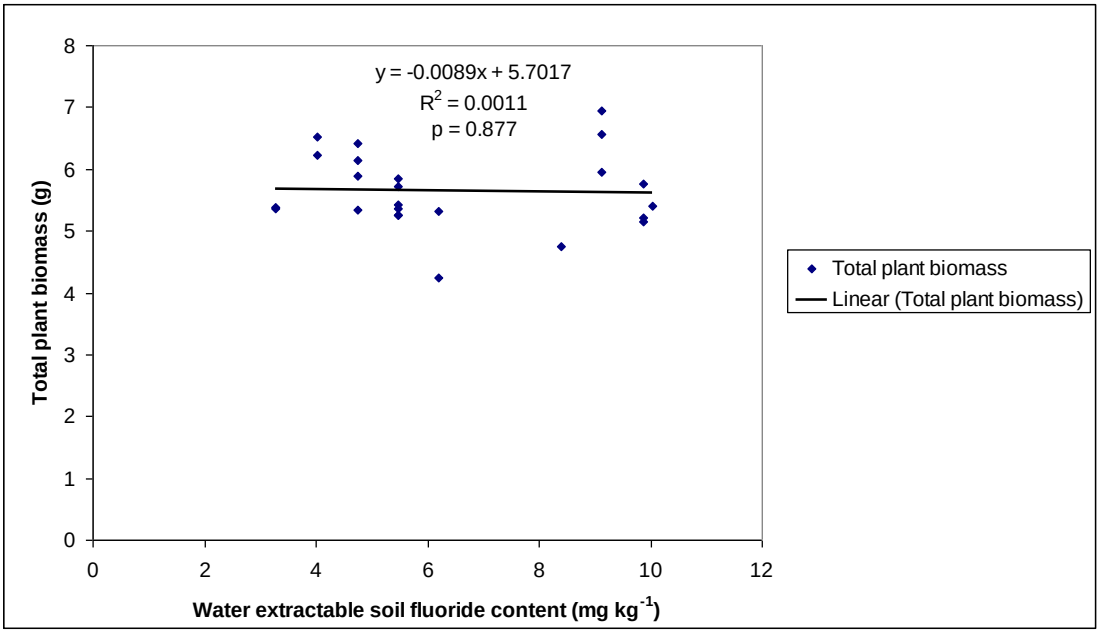


Figure 5.14: Linear regression analysis showing the effect of soil fluoride concentration on total plant biomass. Plotted values represent individual plots.

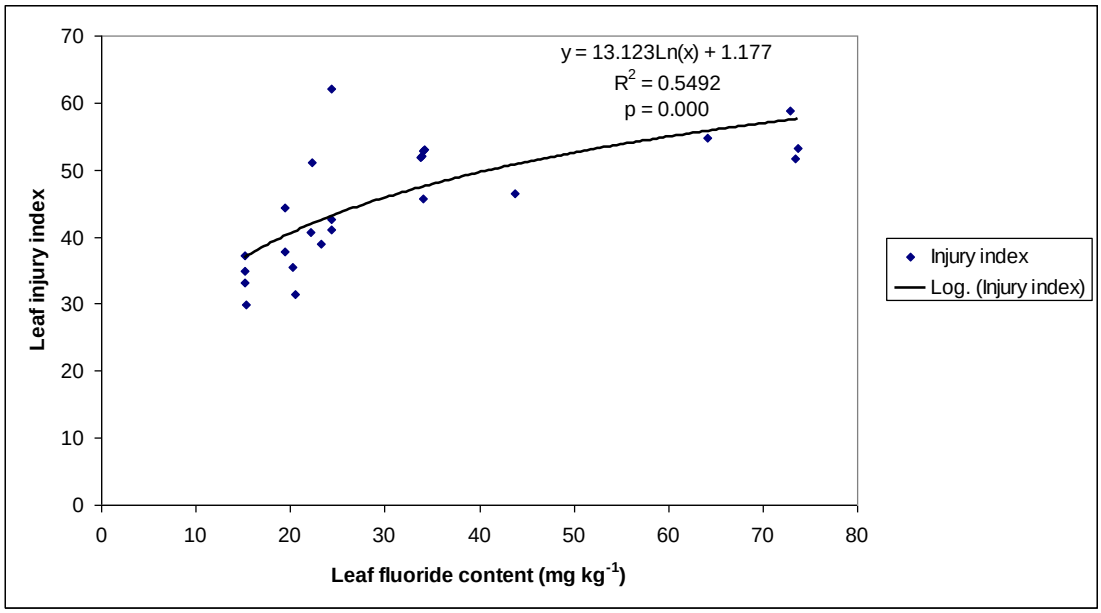


Figure 5.15: Logarithmic regression model showing the effect of live leaf fluoride content in week 6 on the leaf injury index. Plotted values represent individual plots.

5.5. DISCUSSION

The aim of the study was to determine the effect of soil fluoride on wheat variety '*Bakkar*' in alkaline soil under greenhouse conditions. The mean plant height was only affected in the first week after germination, when the plant height of the control seedlings was significantly higher than the rest of the treatments. However, in the remaining weeks until the harvest (Week 7), there was no significant difference between treatments in plant height. The average leaf number per plant in T1 was also significantly greater in the initial growth stages, and the size of the effect level declined with time. This suggests that the NaF soil application primarily affected plant growth immediately after the emergence of the seedlings. The lack of effect on plant height and leaf number in the later stages of the growth might be due to nutrients being deviated to the ears, and also because height and leaf number reached there maximum, i.e. the effect of NaF was primarily to delay development. This conclusion is supported by Pant *et al*. (2008), who studied the effects of concentrations of 0.001, 0.01, and 0.02 M sodium fluoride on early root and shoot growth of typical crop plants of India, in Petri dishes for 48 hours at 30°C. They concluded that fluoride inhibited the shoot growth of wheat (*Triticum aestivum L.*) seedlings. However, in the current study the initial plant content of fluoride was not measured due to the limited amount of plant samples. Bozhkov *et al.* (2009) also subjected winter wheat (*Triticum aestivum* L.) seedlings to different NaF solutions, at concentrations of 0.001, 0.005, 0.01 and 0.02M, in Petri dishes for 24 hours at 20°C, and found that NaF inhibits the growth of the seedlings. Although these studies were in solution cultures rather than soil, but they support the conclusion that NaF can be affect the early growth of the plants.

No fluoride induced injury was found on wheat leaves during the entire experiment, which shows that wheat leaves are resistant to fluoride foliar injury in spite of the fluoride

uptake from the soil. This result also suggests that the leaf tip burn injury to young wheat plants at BKF site in Peshawar was due to the HF emitted from the surrounding brick kiln factories, rather than the soil fluoride toxicity. The water and calcium extractable fluoride from soil samples collected from wheat fields were also not significantly higher in polluted area (BKF) compared to other sites in Peshawar (See Chapter 4). The water extractable fluoride in Peshawar ranged from 3mg kg^{-1} in ARI to 6mg kg^{-1} in AUP. These are the range of values found in T1 to T4 in this experiment, supporting the interpretation that soil concentrations in Peshawar were not high enough to cause visible foliar injury. However, previous studies have found that wheat is also resistant to HF in terms of foliar injury. Davison *et al*. (1990) exposed wheat to HF (concentrations <0.03 and 0.30μg m^{-3}) fumigation in open top chambers for 120 days and found that HF did not induce foliar injuries but reduced shoot, ear, and grain weight, and increased the fluoride content of the leaves. Maclean and Schneider (1981) exposed wheat (*Triticum aestivum* L.) for four days at 0.9μgHFm^{-3} and Maclean *et al*. (1984) fumigated wheat with 1·6µg HFm^{-3} for three days; both studies found that HF did not cause visible injury but inhibited the growth and yield of the crop. The wheat variety grown in Peshawar might be sensitive to HF pollution emitted from brick kiln factories, but not to soil fluoride. However, there is a possibility that the symptoms observed on young wheat plants at BKF might not be due to HF emissions. Weinstein and Daveison (2004) reported that species sensitive to HF in one region might be resistant in another region due to environmental conditions and genetic composition.

Leaves developed visible injury from powdery mildew attack during Week 6, due to favourable environmental conditions inside the greenhouse. The degree of powdery mildew infestation increased with the increase in fluoride concentration in the soil, and there was less injury to control plants compared to fluoride treated plants during Week 6. The logarithmic

regression model showed that there was sharp increase in the injury in T2 and T3 plants. This suggests that elevated foliar F concentrations enhanced powdery mildew infestation at the early stage of the fungal attack. However, in Week 7, the powdery mildew infestation spread to all treatments, despite the sulphur fumigation, due to which very few healthy leaves were left.

Very little is known about the effect of soil and leaf fluoride on powdery mildew infestations. Farkas and Kiraly (1955) worked on the effect of powdery mildew and stem rust on wheat respiration. They treated healthy wheat leaves and leaves infected with powdery mildew in Petri dishes with sodium fluoride at different concentrations. They concluded that fluoride inhibits oxygen uptake in the leaf tissues by increasing the rate of respiration in healthy leaves, as well as in diseased leaves. However, Garg and Mandhar (1976) treated lady finger (*Abelmaschus esculentus*) leaves infected with powder mildew in a Petri dish with sodium fluoride and found that oxygen inhibition was greater in infected leaves compared to control. This suggests that fluoride is a respiratory inhibitor inside the leaf tissue as it disrupts metabolic processes like glycolysis. However, further study should be carried out on how the leaf fluoride enhanced the infestation of powdery mildew. The literature also suggests that high HF in the air decreases plant microbial infestation by directly affecting the pathogenic organisms (Manning, 1975). However, soil and leaf fluoride cannot affect microbial metabolism of the leaf surface directly, unlike atmospheric fluoride, and hence these are two different processes.

The current experiment was carried out for 7 weeks, and was terminated due to severe powdery mildew infestation, so it is difficult to say how wheat plant growth and yield, in terms of ear production, would have responded to soil fluoride toxicity and powdery mildew

infestation in the long run. The ear emergence was delayed in all treatments except the control treatment, during week 6. Mature ears were only found in the control treatment and the middle growth stage ears were only found in Treatment 1 and 2. This suggests that either fluoride toxicity or the powdery mildew attack inhibited the early emergence of the ears in wheat plants grown on soil having high fluoride concentrations.

The water extractable soil fluoride content of soil in Peshawar was between 3-6mg kg^{-1}. The water extractable soil fluoride content in the 50mg kg^{-1} treatment was also near 6mg kg^{-1}. T this treatment, ear emergence was delayed and there was more powdery mildew infestation. This suggests that the early growth of the ears in wheat plants at Peshawar is at risk from soil fluoride toxicity, and there is also a risk of greater powdery mildew infestation. The experiment was stopped before the scheduled time because of the powdery mildew infestation.

The total fluoride content of the soil is not always available for plant uptake and this largely depends upon the soil pH (Arnesen 1997; Jha *et al*., 2008). Therefore, only the readily available fluorides are important to the plants (Jha *et al*., 2009) and hence, in this case, water and calcium extractable soil fluorides were analysed. The water extractable fluoride concentration was higher than calcium chloride extractable fluoride concentration in all treatments. This might be due to the Ca affinity to fluoride; CaF_2 precipitates could have been formed by the addition of lime, thus reducing the calcium extractable fluoride content of soil (Ruan *et al*., 2004). Lime addition increases pH and ion exchange, and thus can increase the availability of water soluble fluorides. The increase of water soluble F following lime application might be the result of the displacement by OH^- of F^- from adsorption sites, or from Al oxides/hydroxides into soil solution (Farrah *et al*., 1987).

The water and calcium extractable fluoride content increased with the added NaF content of the soil, showing linear trends. The water and calcium extractable soil fluoride was only significant at 100mg kg^{-1} and above. On contrast, the difference between water extractable fluoride was significant at the lowest treatment, 10 mg kg^{-1}. This significant increase might be due to the lack of absorption particles in the soil for the fluorides. This means that with an increase of alkalinity in the soil, the fluoride bioavailability to the plants will also increase. This statement is supported by Garber (1968) and Jha *et al*. (2008 & 2009) who studied the effect of soil fluoride on spinach and onion grown in alkaline conditions and reached similar conclusions.

The linear regression analysis showed that the plant fluoride content is linearly related to the available soil (water and calcium extractable) concentration. This supports the use of these extractions to indicate the plant available soil fluorides. Higher concentrations were found in live leaves than in dead leaves and ears, suggesting that fluoride has been allocated mainly to live leaves, presumably in the transpiration stream, and that fluoride is transferred from the live leaves before they die. This is consistent with the work of Ruan *et al*. (2004), who studied soil fluoride uptake by tea plants and found that fluoride from soil was readily taken up and transported to the leaves via the xylem, which resulted in elevated fluoride concentrations in older and mature leaves. Similar results were also reported by Fung *et al*. (1999), and Ruan and Wong (2001). The live leaves of Treatment 6 showed a fluoride content >40μg Fg^{-1}, which is the threshold level for animal feed to prevent adverse effects on cattle and other stock (WHO, 2000). Although wheat is not grown as a fodder crop, fodder crops like clover are also cultivated in the BKF area. Elevated amounts of fluoride in these crops might result in fluorosis to local livestock, but in the current study, no fodder plant samples were taken for fluoride analysis.

Ears showed lower levels of fluoride than live leaves. The ears at the time of harvest were still green (milky stage) and it is unclear how much fluoride would have been accumulated if the ears had fully matured. Major effect was observed on the metal content of the plants except for Al, which had a significantly higher concentration in control ears compared to other treatments. According to the literature, fluoride is readily taken up by plants in the form of Al/Fe complexes and is transported via the xylem (Ruan *et al*., 2004). It is then dissociated upon reaching the leaf apoplast, leaving fluoride in F^- form, which is the main reason for the fluoride in high amount in leaves and lower in other part because of its complexes with the metals (Takmaz-Nisancioglu and Daveison, 1988). The low amount of Al in the ears in polluted samples compared to the control might be due to the complexing of Al with fluoride that might have decrease the concentrations of both Al and fluoride in the ear.

5.6. CONCLUSION

From this experiment, it is concluded that soil fluoride cannot induce visible foliar injuries to wheat at the concentrations used in this experiment. However, the soil fluoride was taken up by plants, and accumulated in the leaves despite lack of visible injury.

The most important outcome of the experiment was the soil fluoride relationship to the powdery mildew attack on wheat plants, and the late emergence of ears in high fluoride treatments. It is suggested that these two mechanisms should be further investigated in order to clarify mechanisms underlying the soil fluoride enhancement of powdery mildew infestation. It is important to repeat this experiment in local field condition on multiple varieties, in order to get a better understanding of the significance of soil fluoride levels in the region.

CHAPTER 6

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The main aim of the study was to carry out a risk assessment of the threat posed by ground level O_3 and HF to crops in and around Peshawar city, and to observe whether these air pollutants pose any danger to local crops grown in the peri-urban areas in terms of growth, yield and visible foliar injury. This study was the first of its kind in the region; previously there had been no study in this region to determine the effect of air pollution on local crops.

6.1. AGRICULTURAL CONTEXT

Peshawar is the capital of NWFP (North West Frontier Province) and is one of the most heavily cultivated areas in Pakistan for a variety of crops, fruits and vegetables. The total area of NWFP is 18.4 million acres and the cultivable area is 6.55 million acres (http://www.khyberpakhtunkhwa.gov.pk). The soil is fertile but the province is not self-sufficient in staple foods to fulfil the needs of the local population for a number of reasons. Most of the NWFP area is mountainous and areas in the plain, including Peshawar, Mardan and Mansehra, are where most of the agriculture takes place. The majority of the land is irrigated from rivers from the northern Himalayan region. In the plain areas (e.g. in Charsadda and Mardan districts), the water table is high, leading to high alkalinity in some areas, due to which the soil is unfit for cultivation. The province is not self-sufficient in food production, because of which the province is dependent on Punjab province to fulfil its wheat needs. The main crops are maize, wheat, rice, sugarcane and tobacco, and popular fruits are apple, apricot, plum, almond, pear and peach.

Apart from the above factors, the rising air pollution situation in Peshawar could also affect agricultural crop production, because Peshawar, like other major cities of Pakistan, is facing a serious air pollution problem from a rapidly increasing population of 3 million, which is exerting pressure on its resources as a result of the rise of vehicular, industrial and domestic emissions (EPA, 2007). This study was conducted in one city of the region and therefore gives limited knowledge about the air pollution impacts and also only considered two air pollutants. There are two other big cities near Peshawar (Mardan and Nowshehra) and most of the province's population lives in these three cities. It will be very important to extend air pollution studies to these cities and to other pollutants in the future.

6.2. HYDROGEN FLUORIDE (HF)

Visible injury assessments, and plant and soil sampling, were carried out during the summer and winter surveys. The passive sampler results suggest that HF concentrations were not especially high, but were high enough to cause visible injuries to sensitive crops during the spring and early summer. HF concentrations were below detection limits for passive samplers during winter. Most of the visible foliar injuries were seen on the leaves of the local fruit trees at BKF during the summer survey. Almost 90% of the apricot, plum and mango trees were damaged by HF emissions from brick kilns, indicating that fruit trees are at particular risk from HF pollution in the vicinity of brick fields. Tomato, maize, and sugarcane were less sensitive to HF in terms of visible injuries. No HF leaf injuries were found during the winter survey apart from leaf tip burn injury to young wheat leaves at the BKF site. However, the wheat visible injuries were limited to a few fields in BKF. The fluoride contents of injured leaves of mango, apricot and plum of BKF were elevated compared to control sites, suggesting that the injuries to these fruit crops were due to HF emitted from brick kilns.

Tables 6.1 and 6.2 provide a comparison of literature classification of crop sensitivity to fluorides with field data. Visible injuries to apricot, plum, mango, maize and tomato leaves were in line with the previous literature, suggesting that they are at risk from HF in the vicinity of brick kilns in Peshawar. Maclean *et al*. (1984) reported peach to be sensitive to HF, but there was little injury to peach at BKF (Table 6.1). Wheat leaf injuries (tip burn) were only observed in January when the plants were two months old at BKF, but no injury was seen in the later stages of the plant development, during the months of March and April. This suggests that wheat might be sensitive to HF in the early stages of growth. Previous studies have not reported visible injuries on young wheat plants but suggest that the HF can alter the growth and yield of wheat at elevated HF concentrations, which may be found in the BKF area. Daveison *et al*. (1990) and Maclean and Schneider (1981) termed wheat resistant to HF visible injury, but stated that it can alter plant growth and yield. Onion, summer and winter spinach and okra were termed sensitive to HF by previous researchers but in the current study, no injury was observed on these plants. Cabbage, carrot, cauliflower, potato and sugarcane did not show any visible injury to HF at BKF (Table 6.2) and these crops have been reported to be resistant by VDI (1987). However, VDI (1987) also reported cucumber to be resistant to HF; although visible foliar injury in the form of necrotic margins was observed on cucumber in the BKF area, the symptoms were not severe.

The difference in resistance of some of these crops in the field compared with published classifications may be due to several environmental and genetic factors. Weinstein and Davison (2003) worked on native plant species suitable as bio-indicators and bio-monitors for HF for USA, Europe and Australia and concluded that the sensitivity of a species in one region might not be a reliable predictor of sensitivity in another region, because of genetic and environmental factors.

Table 6.1: Crops reported sensitive to HF in comparison with surveys of local crops of Peshawar

Crops	Sensitivity reported in literature	Evidence in Peshawar
Apricot (S)**	Solberg and Adams (1956)	Margin & tip burn
Mango (S)**	De Ong (1946)	Margin & tip burn
Plum (S)**	Griffiths (2003) and Bonte (1982)	Margin & tip burn
Peach (S)*	Maclean *et al*. (1984)	Little Injury
Tomato (S)*	Solberg and Adams (1956) and VDI (1987)	Necrosis tip burn
Onion (S)**	VDI (1987)	No Injury
Spinach (S, W)*	VDI (1987)	No Injury
Okra (S)*	VDI (1987)	No Injury
Wheat (W)*	Davison *et al*. (1990), Maclean and Schneider (1981)	Leaf tip burn
Maize (S)*	VDI (1987)	Necrotic margins

***Highly sensitive to HF*
**Sensitive to HF*
(S) Summer season crop
(W) Winter season crop

Table 6.2: Crops reported resistant to HF in comparison with surveys of local crops of Peshawar

Crops	Reported resistant in literature	Evidence in Peshawar
Cabbage (W)	VDI (1987)	No Injury
Carrot (W)	VDI (1987)	No Injury
Cauliflower (W)	VDI (1987)	No Injury
Cucumber (S)	Taylor (1973)	Necrotic margins
Potato (S, W)	VDI (1987)	No Injury
Sugarcane (S)	VDI (1987)	No Injury

(S) Summer season crop
(W) Winter season crop

The lack of visible injuries to some crops does not mean that these crops are completely resistant to HF, as HF can still reduce the yield of these crops, and hence the economic value. No assessment of effects of HF on the yield of both sensitive and resistant crops was carried out, due to the shortage of time, man power, lack of facilities and security. It is very important to assess the impact of HF on the yield of these crops.

The soil fluoride concentrations were not significantly higher in the BKF area compared to other sampled sites. However, the soil samples were collected from only 5 fields close to brick kilns from a vast area of 40km^2, within which 450 brick kilns are operating. The availability of soil fluoride largely depends on the soil pH. Since the pH of Peshawar

soils is alkaline, the plants are not at high risk of soil fluoride toxicity compared to HF. However, soil fluoride can still be taken up by plants as shown in the experiment described in Chapter 5. Therefore, the soil fluoride of BKF should be kept in check as it can be increased due to HF deposition from the brick kilns.

Wheat grain and fruit leaf samples showed significantly higher fluoride content in the BKF area. This might be due to both elevated soil fluoride and gaseous HF, although the visible damage may be mainly due to HF as it enters directly through the stomata and has low threshold levels of injury. Surface contamination could contribute to elevated plant fluoride in the current study, as the plant samples were not washed with deionised water due to the lack of a facility in Peshawar; they were not washed with tap water either, as the water may contain traces of fluoride.

It was concluded from the experiment described in Chapter 5 that soil fluoride cannot induce visible foliar injuries to wheat at the concentrations found in the field in Peshawar. However, soil fluoride was taken up by plants, and accumulated in the leaves despite the lack of visible injury. The experimental linear relationship between soil fluoride and powdery mildew infestation may not be significant in field conditions because the soil fluoride content was not significantly different between the sites in Peshawar. However, the powdery mildew infestation would be more directly related to the high fluoride content in the leaves, which means that any increase in the leaf fluoride content could enhance the infestation of powdery mildew, whether the fluoride originates from air, soil or water. However, this experiment should be repeated under field conditions on a range of local wheat varieties in order to get a clearer picture.

6.2.1. HF Conclusions and Recommendations

It was concluded from the current study that HF is the main pollutant in the BKF area which is phytotoxic to crops. Further research should be done on the impacts of HF pollution on crops, fruits and vegetables. Priorities should be focussed on more detailed surveys, to establish the area over which visible injury occurs, and the fluoride content of wheat grain and fruit tree leaves are elevated, compared to control sites. The concentrations of fluoride in other species also need to be assessed, especially those species which are grown as a fodder crops. An example is clover, which is widely grown in the BKF area and might have fluoride contents greater then 40 mg kg^{-1}, the threshold level of fluoride in livestock feed according to WHO (2000). The ingestion of high fluoride content in feed can lead to fluorosis in cattle. It is also very important to asses the concentrations of fluoride in irrigation water as water soluble fluoride is readily available to the plant, can be accumulated and can affect plant growth. Therefore, it is suggested that future studies should be focused on the determination of fluoride concentrations in irrigation water, fodder crops and in livestock.

Given the amount of damage, in terms of visible injury, done by HF at the BKF site, it is highly recommended to carry out an economic survey, in comparison to other control sites, as most of the farmers only rely on their agricultural products for survival. A temporal economic survey, based on the previous 10 years' profit from different cash crops and fruit trees of BKF site, in comparison with control sites, could give a clear idea of any changes in the economic damages being caused by brick work pollution.

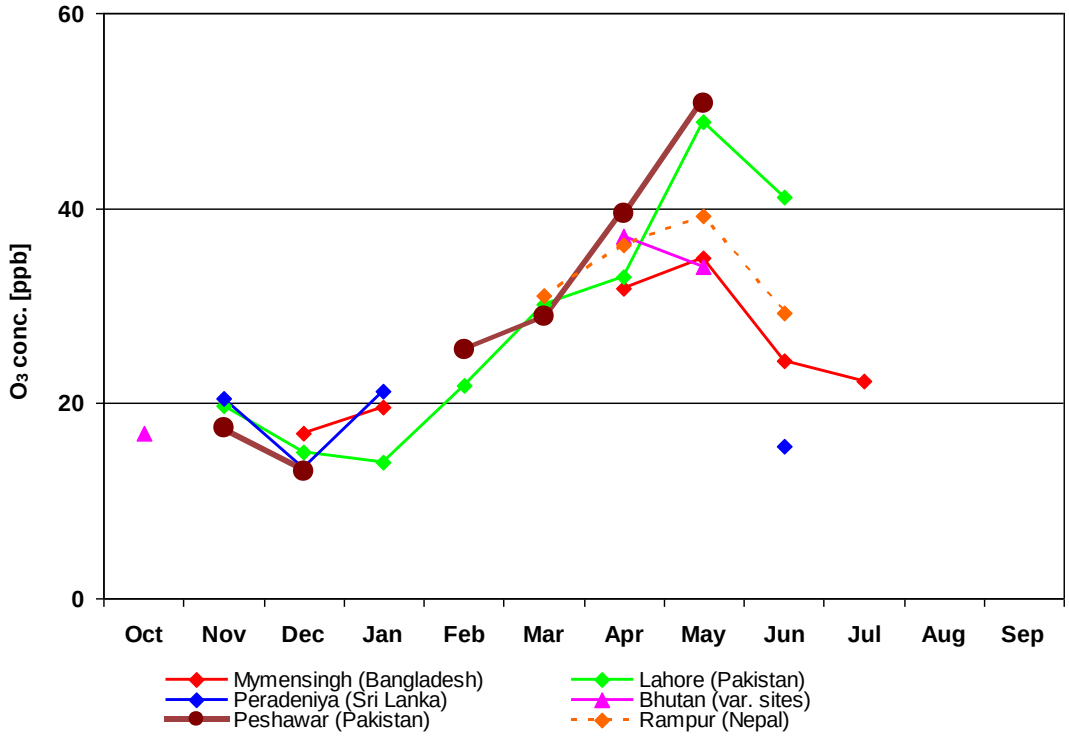
6.3. Ozone (O_3)

The O_3 survey was mainly carried out at the AUP and ARI sites from February to June and then from October to December, 2008. The survey was carried out for the first time

in this region and was mainly based on the previous research studies at Lahore, Pakistan in which different crops (wheat, rice, barley and soybean) were subjected to OTC filtration and EDU experiments to assess the effects of ambient O_3. These experiments revealed significant reductions in the growth and yield of these crops (Wahid *et al.*, 1995; Maggs and Ashmore, 1998; Wahid *et al*., 2001 and Wahid, 2006a & b). Since there were no previous O_3 data in and around Peshawar, it was important to measure the ambient O_3 concentrations and to compare the seasonal O_3 trend with that of Lahore O_3 data, and also to assess the O_3 foliar injuries on different crops at the AUP and ARI site.

The four week mean O_3 data were compared to that of the Male´ Declaration assessment report on air pollution effects on crops (2009), in which ambient O_3 was measured in different countries of South Asia in 2008 using the same passive sampler technique (Fig 6.1). The study was conducted from October to July in peri-urban areas in Bhutan, Maldives, Nepal, Pakistan and Sri Lanka, to assess the impact of O_3 on different crops. The results of the passive samplers were similar in terms of both the absolute values and the seasonal trends.

Figure 6.1: O_3 data of Peshawar measured by passive sampler (this study) in comparison with those of Male´ Declaration sites in South Asia during 2008.



The passive sampler comparison shows that the early summer (April-May) is the time of the year when O_3 concentrations are at their peak both in Peshawar and other South Asian sites. An important implication of this is that the visible foliar injury survey in this study was carried out during the period with peak O_3 concentrations (May). Visible injuries on different crops were observed during May. Leaf tip burn injury to onion and black flecking on potato at ARI supported the results of the passive samplers that O_3 concentrations were high enough to cause visible foliar injuries to crops.

The frequent and sensitivity of O_3 foliar injuries were lower than those of HF visible injuries during the summer surveys. However, this does not mean that the crops in Peshawar are not sensitive to high O_3 concentrations. Most of the South Asian studies have shown that

high O_3 concentrations can reduce the yield and growth of plants without inducing any visible leaf injury and can also affect the nutritional quality (e.g. Agrawal *et al*., 2005; Tiwari and Agrawal, 2009). Due to the short time, and shortage of facilities and manpower, no experiments on the effects of O_3 concentrations on the growth and yield of local crops were carried out during the summer period, and only crops that were reported to be sensitive in the literature were considered for visible O_3 injury assessment. Therefore it is very difficult to say at this point of time that O_3 is not more dangerous than HF in Peshawar. Moreover, O_3 is a regional pollutant (Emberson *et al*., 2001) and can travel hundreds of miles unlike HF, which is a local pollutant. For this reason, HF damage is likely to be limited to the local brick works area, whereas O_3 may affect larger areas of the Peshawar region depending upon the direction and speed of the wind.

The results of the O_3 fumigation of winter spinach variety (*Beta vulgaris*) and summer onion variety (Swat-1) in controlled conditions showed that both species are sensitive to high O_3 concentrations. Spinach was sensitive to high O_3 concentrations (90ppb), showing visible injury and a reduction in yield. At 60ppb, spinach plants only showed visible injury symptoms and no significant effect on the spinach biomass. However, the overall O_3 exposure and the duration of exposure were rather low due to malfunctioning O_3 generators. Nevertheless, on the basis of this study, and the EDU experiment in Peshawar, O_3 concentrations in winter are too low (mean monthly O_3 15ppb) to cause any significant damage to this variety. However, the nutritional quality of EDU treated spinach was affected compared to the non-EDU treated plants. It is clear from the OTC and EDU experiments on spinach that this variety is sensitive to high O_3 concentrations, but it is unlikely that this variety is at risk of O_3 toxicity given the low O_3 concentrations during winter in the Peshawar region. In contrast, onion (Swat-1) showed sensitivity to medium (60ppb) and high O_3

(90ppb) fumigation in the OTC experiment in terms of both visible injury and yield, suggesting that this variety is at risk from high O_3 concentrations in Peshawar during the summer season.

However, onion and spinach are not major cash crops in the region, although they are used as vegetables by the lower income population. There are other major crops that are grown in the early summer in Peshawar, e.g. pulses, rice, maize and sugarcane. Pulses and rice have been reported to be very sensitive to O_3 in Pakistan experiments (Wahid *et al*., 1995 and Wahid, 2006), whereas C_4 plants like maize and sugarcane are considered to be less sensitive to O_3 (Leitao *et al*., 2007; Grantz and Vu, 2009). Wheat is also harvested in May, so it is also likely to be affected by high O_3 concentrations in the later growth stages.

The O_3 concentrations measured in the current study were also similar to those measured in Lahore, using same method (Male´ Crop Report, 2009). At this site, summer spinach (*Spinacia oleracea*) and mung bean (*Vigna radiate*) were subjected to EDU experiments in pots during the early summer season, which revealed significant reductions in biomass of the non-EDU treated plants and high levels of visible foliar injury. The site was 2km away from all roads. The mean monthly O_3 concentration for the summer season (April-May) was 43ppb, while the value for winter (November-December) was 16.2ppb, which is very similar to the mean monthly O_3 concentrations of Peshawar of 45.1ppb in April-May 2008 and 15.2ppb in November-December (Male´ Crop Report, 2009). An EDU experiment with white clover (*Trifolium repens* cv. Regal) was carried out on Lahore in winter. The clover was less affected compared to spinach and mung bean, because clover is a winter crop. However, clover did show visible O_3 injuries at the end of the harvest season (March/May), when the O_3 concentrations were high. This suggests that the early summer season is very

important in Pakistan for crops, and crops in Peshawar may be at risk of significant O_3 damage during this period.

6.3.1. Ozone conclusions and recommendations

It was concluded from the current study, based on the two surveys and EDU experiment that O_3 is above threshold levels during the summer season in Peshawar and may cause significant damage to local summer crops in terms of growth, yield and visible injuries. However, although winter crops may also be sensitive to O_3, due to the low O_3 concentrations in winter it is unlikely that O_3 will cause any significant injury. Some crops are grown in both seasons. For example, wheat, which is the main staple crop grown in Peshawar, is a winter crop but is harvested in early summer. This means that it is at risk from high O_3 concentrations in spring from February to May that may affect its growth and yield in Peshawar, as was shown in OTC studies in Lahore (Wahid *et al*., 1995).

More research on the effects of O_3 is needed in this region. Passive samplers were used in the current study, because of which peak O_3 concentrations were not determined. It is recommended that active O_3 samplers should be used in Peshawar in order to determine the peak O_3 levels. The lack of chamber facility due its high costs, EDU studies should be conducted on summer crops, due to the high O_3 concentrations in summer. In the light of Male´ Crop Report, EDU studies on different varieties should be carried out in order to identify local variety that are resistant to both O_3 foliar injury and can produce high yield.

6.4. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The results of the current study provide evidence that HF and O_3 are a significant risk to some crops in Peshawar. Since this was the first study of its kind in this part of the country, there were limitations that need to be removed in future research studies. The surveys were carried out at a limited number of sites, and for a short duration, due to the limited resources and the fragile security situation. For example, risk assessment of O_3 and HF should be done at multiple sites in the region around the year in order to define the seasonal trends of these pollutants in more detail. Further trials at research stations with detailed assessment of foliar injuries on variety of crops in the region should be carried out on a weekly basis around the year, to identify sensitive and resistant crop species. One of the most important limitations was the lack of information on the impact of these pollutants on the growth and yield of different crops. Therefore, it is recommended that those crops which showed foliar injury should also be assessed for their biomass in order to quantify the impacts of air pollution in terms of yield. EDU experiments should be conducted for O_3 studies and yields of different crops should be compared at different site for HF studies.

The estimated rate of population increase of Pakistan is about 4% (Pakistan Economic Survey 2007). This suggests that in the absence of effective air quality management, air pollution concentrations will increase in the future, putting crop yields at risk. Other factors, such as global warming or the recent flooding may further worsen food security in Pakistan. Apart from O_3 and HF, there are other pollutants that may affect crops in Peshawar, e.g. NO_x and SO_2. SO_2 concentrations were measured through passive samplers during both surveys, but concentrations were below the threshold that has been set to prevent significant damage

to crops. However, it is recommended that detailed studies on the effects of NO_x and SO_2 should also be considered in future surveys.

During the field surveys, it was also realised that local people, both farmers as well as agriculture officers of Peshawar, had little knowledge about O_3 or other air pollutants and their negative effects on crops. The local people are aware of the negative impacts of pollution on their health but they do not know about the toxic effects of air pollution on crops. Hence, it is also very important to educate people about this danger and alert them to the consequences that can jeopardise their future food security.

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