MASS PROPAGATION OF BAMBOO, AND ITS ADAPTABILITY TO WASTE WATER GARDENS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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I give thanks to God for making this work possible, agreeing with Proverbs 3:5-8

(5) Trust in the LORD with all thine heart; and lean not unto thine own understanding.

(6) In all thy ways acknowledge him, and he shall direct thy paths.

(7) Be not wise in thine own eyes: fear the LORD, and depart from evil.

(8) It shall be health to thy navel, and marrow to thy bones.

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ABBREVIATIONS AND ACRONYMS

A	**Net Photosynthetic Rate**
AAS	**Atomic Absorption Spectrophotometry**
ANOVA	**Analysis of Variance**
C_a	**Atmospheric Carbon Dioxide**
CAM	**Crassulacean Acid Metabolism**
CDR	**Colour Developing Reagent**
CEC	**Cation Exchange Capacity**
Ci	**Intercellular Carbon Dioxide**
CSIRO	**Commonwealth Scientific & Industrial Research Organization (Australia)**
DOC	**Dissolved Organic Carbon**
E	**Transpiration Rate**
EDCs	**Endocrine Disrupting Compounds**
EPA	**Environmental Protection Agency**
FAO	**Food and Agriculture Organization (United Nations)**
gs	**Stomatal Conductance**
HF	**Hydrogen Fluoride**
IC	**Inorganic Carbon**
ICPMS	**Inductively Coupled Plasma Mass Spectroscopy**
IRRI	**International Rice Research Institute**
IRGA	**Infra Red Gas Analyser**
ITE	**Instantaneous Transpiration Efficiency**
JKUAT	**Jomo Kenyatta University of Agriculture & Technology**
kPa	**kilopascal**
LAI	**Leaf Area Index**

LAR	**Leaf Area Ratio**
MHz	**Megahertz**
MJ m^{-2}	**Megajoule per meter squared**
MS	**Murashige and Skoog**
ng	**Nanogram**
nm	**Nanometer**
PCR	**Polymerase Chain Reaction**
SD	**Saturation Deficit**
SED	**Standard Error of Deviation**
SLA	**Specific Leaf Area**
SLW	**Specific Leaf Weight**
SPAD	**Soil and Plant Analyzer Development (Japan)**
TC	**Total Carbon**
TE	**Transpiration Efficiency**
TOC	**Total Organic Carbon**
UNEP	**United Nations Environment Programme**
USDA	**United States Department of Agriculture**
USEPA	**United States Environmental Protection Agency**
VPD	**Vapour Pressure Deficit**
w	**Gravimetric Soil Water Content**
WUE	**Water Use Efficiency**
µl	**Microliter**
ψl	**Water Potential**

ABSTRACT

Mass Propagation of Bamboo, and its Adaptability to Waste Water Gardens

Unregulated and inappropriate disposal of wastewater poses serious pollution problems in many parts of the developing World. However, reuse of wastewater may help to ameliorate global water shortages, especially in developing nations where facilities for safe disposal of wastewater do not exist. Some bamboo species grow more rapidly than timber species and have numerous actual and potential applications for environmental conservation and income generation. In view of these attributes, bamboo was chosen for use in the present study of the potential utilisation of wastewater to improve water and nutrient supplies, while providing an environmentally compatible method for wastewater disposal and a fast-growing, non-timber source of woody material for subsistence farmers.

As the extremely long vegetative period before flowering occurs in many species limits seed supplies, it is vital to develop effective methods for mass propagation of bamboo to enable its widespread adoption by subsistence farmers in East Africa. Seven potentially important species were used in studies intended to develop suitable micropropagation procedures: these were *Dendrocalamus membranaceus*, *Dendrocalamus yunnanicus*, *Dendrocalamus strictus*, *Phyllostachys heteroclada*, *Oxytenanthera abyssinica*, *Phyllostachys pubescene* and *Dendrocalamus giganteus*. Multiplication rates differed between species ($P<0.001$) and these difference became apparent within five months of establishing the cultures. *D. yunnanicus* was the most promising in terms of multiplication rate, easily outperforming all other species ($P<0.001$), by increasing to 3,500 plantlets within eight months.

Three species (*Dendrocalamus giganteus*, *Bambusa vulgaris* and *B. nutans*) were grown in 100 litre tanks in a factorial experiment under field conditions. Sewage effluent or clean water was

applied daily according to the treatment involved. A second experiment contained 339 younger plants irrigated with three sources of water, including industrial wastewater. Subsequent analysis revealed that the wastewater did not contain toxic concentrations of nutrients or trace metals. Weekly and diurnal measurements of net photosynthesis, transpiration rate and stomatal conductance were made over a nine month period, while non-destructive measurements of plant height, collar diameter, number of leaves and leaf area were made over a 15 month period. Destructive harvests after 0, 9 and 15 months of treatment were used to determine leaf and stem fresh and dry weights.

When averaged over all species, irrigation with wastewater increased stem fresh and dry weight plant^{-1} by 30-40 % relative to plants receiving clean water ($P<0.05$), with *B. vulgaris* and *B. nutans* performing better than *D. giganteus*. A significant water*time interaction was apparent for plant height, branch number, leaf area plant^{-1} and biomass production for all species; values were greater for plants irrigated with wastewater than in those receiving clean water. Volumetric soil moisture content did not differ significantly between the clean and wastewater treatments between March and November 2006, but differed between the two measurement depths (20 and 60 cm; $P<0.001$).

The gas exchange and SPAD values (an indirect measure of chlorophyll concentration) revealed several significant effects. SPAD values varied with time ($P<0.001$), but not between species, and were greater in plants irrigated with wastewater than in those receiving clean water ($P<0.001$). Stomatal conductance, transpiration and net photosynthesis all showed significant effects of species, irrigation treatment, time and leaf position in the canopy ($P<0.05$). Instantaneous transpiration efficiency (ITE) was greater in plants irrigated with wastewater than in those receiving clean water ($P<0.05$).

Elemental analysis showed that the concentrations of trace metal nutrients in the wastewater supplies used in both experiments were not sufficiently high to elicit toxic responses, although Cu, Ni, Zn, K, N and total organic carbon concentration were all higher in wastewater than in clean water ($P<0.05$). Na concentration was sufficiently high to induce a 10 % reduction in plant growth. The uptake of specific elements (e.g. K, Ca, Mg, Fe, Zn, Ni, Mo, As and Al) varied between species and with time ($P<0.05$), suggesting possible genotype preferences and environmental influences. The concentration of individual elements within leaves was influenced by their position in the canopy ($P<0.05$).

The large size, rapid growth and water pumping properties of many bamboo species make them suitable candidates for phytoremediation, although selection of appropriate species for specific applications is important. The multiple and diverse uses of bamboos makes them an attractive proposition for environmental restoration and poverty alleviation in subsistence communities in developing nations. Future research should focus on uptake of nutrients and pollutants by roots, the suitability of agroforestry for wastewater treatment, and long term studies of wastewater uptake by bamboo with regard to potential environment benefits and biomass production by bamboo.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Background

The global human population has grown from 1 billion in the early 1800s to 6.5 billion today (United Nations, 2000), but has grown more rapidly since 1950 than at any time during the previous 4 million years. 74 m people yr^{-1} are born each year, mostly in developing countries, and the United Nations predicts that the global population will reach 9 billion by 2050 (Fig. 1). However, although it has just 5% of the world's population, the USA consumes 25% of global resources; people living in the USA have 280 times the environmental impact of people born in Haiti (Ehrlich and Ehrlich, 1990).

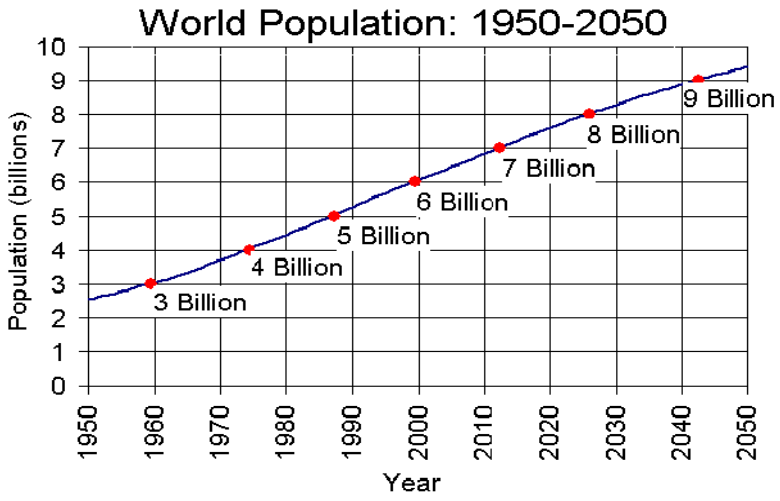


Figure 1.1. Trends in the world's population since 1950 and projections to 2050. (Source: US Census Bureau, International Data Base, July 2007).

Stabilising climate and population is essential to save the global environment (Worldwatch Institute, 2000; Showstack, 2005; Shindell, 2007) as this would increase the feasibility of reversing deforestation, stabilising water tables, preventing erosion and protecting plant and animal diversity more manageably (Vitousek, 1994). World Bank statistics show that more than 1.6 billion people depend on forests for their livelihoods (Nkem *et al*., 2007). Forest products are

worth US $270 billion yr^{-1} in global trade, with bamboo alone being worth over US $5 billion yr^{-1} (Hunter, 2003). Incorporation of bamboo in agriculture can help reduce CO_2 emissions by sequestering carbon in vegetation and in soils (Changzheng and Gang, 2003; Wang *et al*., 2003; Deep, 2007).

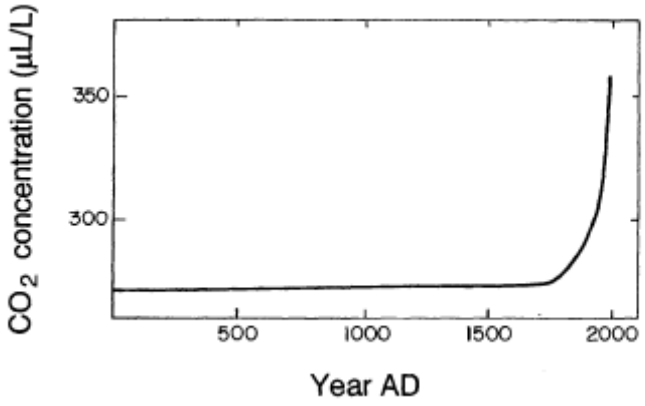


Figure 1.2. Atmospheric CO_2 concentration during the past 2000 years determined by analysis of air bubbles trapped in the Greenland and Antarctic ice caps (Raynaud *et al*., 1993).

As many as 160,000 people die each year from the side-effects of climate change (Asif and Muneer, 2007) and the world's poor are disproportionately affected by environmental degradation as their day-to-day living conditions and livelihoods are inextricably linked to environmental quality (De Souza *et al*., 2003; Wisner *et al*., 2003; Wong *et al*., 2005; Adams, 2006). In Kenya, for example, habitat loss and degradation are affected by smallholder agriculture, tree plantations, selective and clear-cutting, wood extraction, livestock rearing, infrastructure development and introduced pathogens (Wong *et al*., 2005), and this has also affected wildlife (Ross, 2004). Release of sewage to rivers adversely affects water quality, while improper disposal to the environment increases the chances of human contact, which may cause serious health problems resulting from pathogens and toxic compounds present in the water (EPA, 2004; Bynum, 2007). Reuse of wastewater in developing countries provides the potential advantages that water is conserved, low-cost disposal of sewage is provided, pollution of rivers, canals and surface water is

prevented, nutrients are conserved, avoiding the need for artificial fertiliser, crop yields are increased and a reliable water supply is available to farmers, thereby improving income to small scale farmers (Raschid-Sally *et al*., 2001).

Phytoremediation may provide an inexpensive, relatively low technology approach for decontaminating wastewater, groundwater and contaminated soils.

This approach involves using plants to remove, immobilise and/or neutralise contaminants including heavy metals, organic compounds and radioactive material via physical, biological and chemical processes which result in contaminants being sequestered, metabolised, degraded or absorbed (Hinchman *et al*., 1996). This process is carried out either by the plants themselves or by symbiotic organisms living in association with their roots. The root system plays a key role in uptake by mediating an assortment of free living organisms and root exudates (Pilon-Smits, 2005). Plants which transpire rapidly are best suited for phytoremediation as they transport large quantities of dissolved solutes, including soil pollutants, from the soil solution through the plant to the shoot; thus, the greater the volume of water which plants transport from the soil to the atmosphere, the greater their capacity to remove pollutants from wastewater and contaminated soil (Hinchman *et al*., 1996).

Hyperaccumulator species accumulate trace elements to much higher concentrations than non-hyperaccumulators (Galeas *et al*., 2008), and so might initially appear well-suited for phytoremediation; however, their biomass accumulation is often small, limiting their abilty to remove soil contaminants. An alternative strategy is to use non-hyperaccumulator species in which the low concentration of pollutant is offset by their much greater biomass production and transpiration rates.

A study of wastewater reuse in Nairobi, Kenya, and Kumasi, Ghana showed that field conditions differed greatly between these cities, making it difficult to provide any general guidelines for its use (Scott *et al*., 2004), although it is clear that rapid urbanisation is placing further pressure on already inadequate sanitation and treatment infrastructures. In developing countries, the cost of investments in water supply, sanitation and treatment facilities far exceed their current economic potential (Niemczynowicz, 1996; Scott *et al*., 2004). The use of untreated wastewater in such countries is increasing as populations grow, although the lack of guidelines based on effective risk assessments poses a serious problem (Parameswaran, 1999; Scott *et al*., 2004). Many urban and peri-urban irrigation farmers around Nairobi and Kumasi are already using various types of untreated wastewater to irrigate their crops under unregulated and informal arrangements. Wastewater may be regarded as a reusable resource which may be used to increase available water supplies (Sharma and Ashwath, 2006). Bamboo being one of the fastest growing groups of plants known (Janssen, 1991; Liese, 2003), and an efficient water pump, appears ideal for the cleanup of wastewater.

Estimates of depletion of natural forest resources usually reflect only the value of the timber removed and ignore all other non-timber benefits (Hamilton and Clemens, 1998). Forest ecosystems provide habitats for numerous species from all levels of organisation (Miller, 2006), oxygen and water, recreational facilities, economically valuable commodities and many of the resources required for human life, such as timber for building houses, cooking or heating. Forest products also have numerous uses at home and in industry. Demand for these cannot be sustained in the face of the rapidly increasing global population, with the result that forests are being destroyed in many parts of the world. Deforestation to provide agricultural land or for other reasons also limits oxygen production and CO_2 sequestration, adversely affecting air quality,

disrupting the carbon cycle and contributing to global climate change as a result of the reduced mitigating effect of plants on CO_2 emissions from motor vehicles, power plants and industry (Vitousek, 1994).

Plants and soil in tropical forests are estimated to contain 460-575 billion metric tonnes of carbon worldwide (McKane *et al.*, 1995). However, between 1850-1990, deforestation released 122 billion metric tonnes of carbon to the atmosphere, with the current rate being *c*. 1.6 billion metric tonnes yr^{-1} (Skole *et al*., 1998); by comparison, fossil fuel combustion (coal, oil and gas) releases *c*. 6 billion tonnes yr^{-1}. Thus deforestation has a significant role in determining global climate change and air quality relative to all other sources of pollution. Deforestation, whose detrimental effects may be alleviated if countries make appropriate economic decisions (Miller, 2006), may render many important species extinct (Lawton and May, 1995). Due to rapid population growth, the Earth's natural environment is changing in ways which differ fundamentally from those experienced at any other time in our history (Adams, 2006).

Phytoremediation makes use of species such as *Typha* sp., *Myriophyllum* sp., *Elodea* sp., *Azolla* sp., *Lemna* sp., *Eichhornia crassipes*, *Spartina* sp., *Populus* sp. and *Salix* sp. (Pilon-Smits, 2005). Rapidly transpiring trees maintain an upward flow, discouraging downward leaching, while grasses (including bamboo) prevent wind erosion and lateral runoff with their dense root systems (Pilon-Smits, 2005).

1.2 Growing bamboo

Many bamboos are herbaceous in nature and diploids, unlike the larger tetraploid and hexaploid bamboos (Calderon and Soderstrom, 1980). All genera comprise a single subfamily, *Bambusoideae*, containing both woody and herbaceous members.

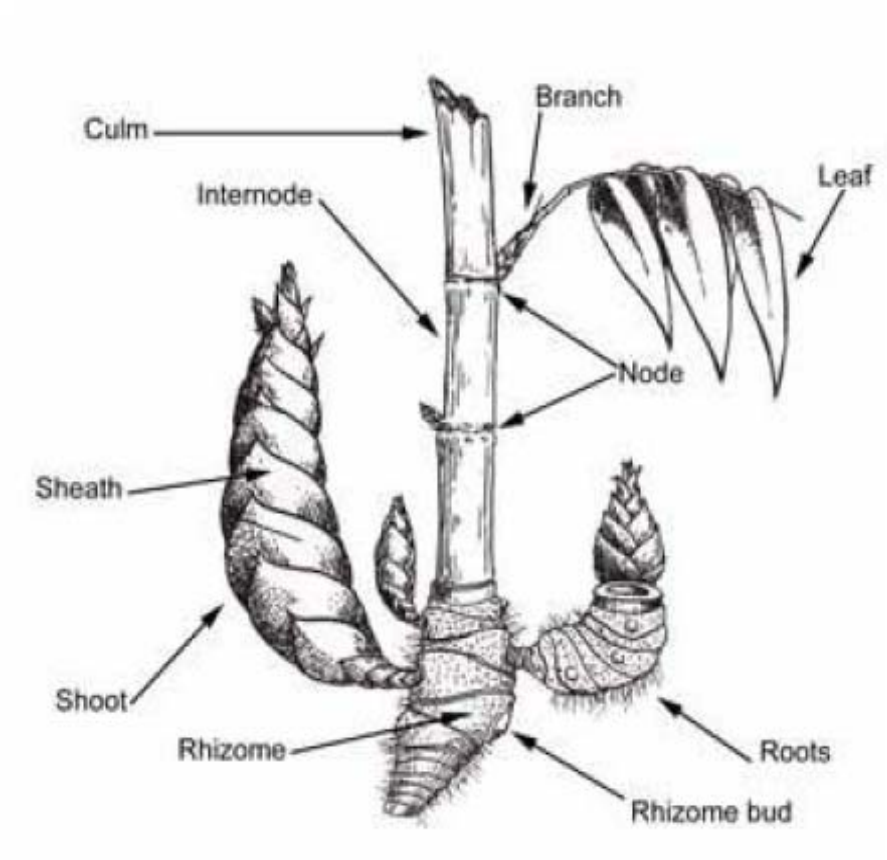


Figure 1.3. Morphology of bamboo plants (Kigomo, 2007).

Woody bamboos represent one of the few groups of arborescent monocotyledons which can compete with dicotyledonous trees in terms of their size and biomass production (Liese, 1998). Unlike most grass species, woody bambusoid species have biological peculiarities which have hampered their study, and collectors have shied away from these due to their size and complexity and because they rarely flower, making most collections of bamboo incomplete. The morphology and architecture of branches arising from the culm or stem nodes are important in bamboo taxonomy (Triplett *et al*., 2006). The culms of woody bamboo species grow more rapidly than in any other species (Janssen, 1991; Liese, 1998; Pizzolato, 2002; Wu *et al*., 2005; Lybeer *et al*., 2006) and there are records of tropical bamboos increasing in height by 31 m within three months,

or rates of up to 0.32 m h^{-1} (Janssen *et al*., 2005); culm diameter may exceed 20 cm in the largest species.

Bamboos constitute a peculiar and distinctive group with regard to their growth form (Soderstrom, 1981; Franklin, 2005). Their subterranean rhizomes consist of internodes and nodes and are responsible for anchorage and storage of substantial food reserves (McClure, 1938; Stapleton, 1998). The rhizomes extend widely in a horizontal direction and produce more or less widely spaced culms from lateral buds immediately distal to the nodes (McClure, 1966; Kochhar *et al*., 1998; Liese, 1998). When the rhizomes form new culms, numerous unsegmented adventitious roots are also produced to absorb water and nutrients (McClure, 1966; Lybeer, 2006), the culms have already reached their final diameter when they appear above ground. The type of culm leaves is characteristic of the genus involved, while differences in the foliage or culm leaves serve to distinguish between species (Stapleton and Rao, 1995). Bamboo genera are classified into clumpers and runners; the former includes *Bambusa*, *Chusquea*, *Dendrocalamus*, *Drepanostachyum*, *Fargesia*, *Himalayacalmus*, *Otatea* and *Thamnocalamus*, whereas *Chimonobambusa*, *Indocalamus, Phyllostachys*, *Pleioblastus*, *Pseudosasa*, *Sasa*, *Semiarundinaria*, *Shibatea* and *Sinobambusa* are runners (McClure, 1966). The occurrence of two growth forms in bamboos does not occur elsewhere among grass species.

As flowers are rare in bamboo, vegetative traits must be relied upon for systematic purposes. Flowering may be divided into three basic types: gregarious, sporadic and continuous (Li, 2003; Bystriakova *et al*., 2004). When gregarious flowering occurs, the entire population flowers over a period of 2-3 years and the shoots then die, although the rhizomes may survive (Janzen, 1976; Stapleton and Rao, 1995; Singh, 2000; Kumar and Divakara, 2001). During sporadic flowering, individual plants flower seasonally or occasionally and only the flowering culms die while the

rhizomes survive (Ramanayake and Yakandawala, 1998; Bhattacharya *et al*., 2006). Continuously flowering species flower throughout the year and culms producing flowers do not die (Makita, 1998). In many cases, the development of inflorescences is not limited to the distal parts of branches or culms, but occurs in all parts of the plant where the presence of active meristematic tissue permits. The complexity of bamboo inflorescences is increased by the different basic types of partial inflorescences, which exhibit either limited (determinate) or unlimited (indeterminate) growth (McClure, 1966; McClure, 1973; Calderon and Soderstrom, 1980). If bamboo culms are excised before they have begun to elongate they die, but if cut after elongation has begun, they branch from the nodes and produce new foliage; herbaceous species continue to grow even after mowing (McClure, 1973). As noted above, some bamboo species bloom simultaneously and then die, while others are greatly weakened or die back after flowering but then recover after a period of several years and may even bloom again (Janzen, 1976).

1.3. Bamboo as a natural resource

1.3.1 Distribution

Because bamboo is classified as a non-timber forest product, it is not routinely included in resource inventories; statistics on bamboo resources are therefore scarce (Bystriakova *et al*., 2003). With a total of 1575 species, subfamily *Bambusoidaeae* (of the family *Poaceae* or *Graminea*) contains both woody (1 tribe, *Bambuseae*) and herbaceous species (1-3 tribes; (Barker *et al*., 2001). Of these, 400 species of woody bamboos are distributed in Northern, Central and South America and Africa (Appendix 4). The main centres of diversity of bambusoid grasses are the Atlantic coastal regions of South America and the monsoon belt of South-East Asia and south China (Ohrnberger, 1999). Although Madagascar has the greatest diversity of bamboo species in Africa, the number of species present is low compared to South America and Asia, where species richness may reach 35 and 144 species km^{-2} respectively (Bystriakova *et al*., 2003). Bamboo has

great socio-economic importance in Asia but has little significance in America (Dransfield and Widjaja, 1995; Londono, 2001) with the exception of Colombia, Ecuador and Brazil. *Guadua angustifolia* is the only Latin American species of economic importance (Rao, 1998).

1.3.2 Ecology

Bamboos have an extremely important role in soil conservation (Stapleton, 1994) and provide a low-cost means of stabilising hillslopes while providing non-timber products (OTA, 1984). They also provide supplies of poles and fodder throughout the year (Li, 2003). The profitability of bamboo depends on its high productivity (Hunter and Wu, 2002; Hunter, 2003), which is within the range of production of woody biomass by conventional tree species within the same ecoclimatic region (Kleinhenz and Midmore, 2001), except that culm biomass does not achieve the very high values attainable by trees in favourable environments. There is little published information regarding the productivity of bamboo plantations and quantification of biomass production is complicated by the fact that bamboo may not grow in pure stands. Leaf fall in established stands is balanced by the production of new leaves (Shanmughavel and Francis, 1996). Below-ground biomass for bamboo may be no greater than in many tree species (Gaston *et al*., 1998; Kobayashi *et al*., 2000).

1.3.3 Bamboo as a timber alternative

Bamboos belong to the Gramineae family, subfamilies Bambusoideae and Graminsoideae. Bambusoideae are perennial species with hard woody stems, whereas Graminsoideae are mostly annual herbs with herbaceous stems (Zhang, 2003; Zheng *et al*., 2003). Bamboo has considerable potential as a wood substitute as it grows rapidly, has excellent mechanical properties, is eco-friendly, has a wide range of applications and is over 20 times more sustainable in terms of its mechanical properties than timber, steel and concrete (Hunter, 2003; van der Lugt *et al*., 2003; van

der Lugt and Otten, 2006). Because of its high nutrient content (starch, protein, sugar and hemicellulose) compared to timber, it requires greater protection against insect and fungal attack (Zhang, 2003). As an alternative to timber, bamboo can impact positively in many areas, with over 5,000 known uses, including production of paper, scaffolding, diesel, aircraft 'skins', desalination filters, aphrodisiacs, musical instruments, medicine and food (Bansal and Zoolagud, 2002).

The high fibre density of bamboo culms greatly enhances their strength (Tommy *et al*., 2004). Bamboo has many properties that help to control or prevent soil erosion (Chun-lin *et al*., 2003; Hunter, 2003) and is one of the strongest known building materials (Velez *et al*., 2000; Singh and Bhati, 2003; Bansal and Prasad, 2004) as its tensile strength is 190 MPa compared to 156 MPa for steel. Bamboo has a higher raw material output than trees and is a high yielding renewable resource (Bansal and Zoolagud, 2002; Hunter and Wu, 2002). There are almost 1600 known species of bamboo and this diversity makes them adaptable to a wide range of environments; for example, bamboo tolerates extremes of annual rainfall ranging from 762-6350 mm (Yuming *et al*., 2004) and can be harvested within 3-5 years of planting, compared to 10-20 years for most softwood tree species.

Industrial activities based on bamboo provide income, food and housing for over 2.2 billion people worldwide (Fu, 2001; Hunter, 2003; Embaye *et al*., 2004). In the violent earthquake in Limon, Costa Rica in 1992, only bamboo houses remained standing afterwards because of the flexible nature of their construction material (Anson *et al*., 2002; Hunter and Wu, 2002; Hunter, 2003). Just like trees, bamboo is a beautifying component of landscape design, providing shade, wind breaks, acoustic barriers and aesthetic beauty (Oprins and van Trier, 2006). In environmental restoration, bamboo wastewater gardens may be used to detoxify waste and produce marketable

products during this process (del Porto, 2002). The internode length of bamboo culms differs between species and long internodes are preferable for furniture manufacture (Liese, 2003). Death after flowering has a great impact on utilisation of the masses of dying culms (Mohmod and Liese, 2001).

1.4 Wastewater use

Although irrigation using wastewater is as old as agriculture (Braatz and Kandiah, 1996), it was only in the last century that large-scale 'sewage farms' were established in locations such as Cairo, China, USA, Mexico City and India (Arlosoroff and Bartone, 1987; United Nations University, 2000; Bradford *et al*., 2003; Petri and Tapio, 2005; Hamilton *et al*., 2007). Only 35 % of wastewater is treated in Asia, 14 % in Latin America and almost none in Africa (Martijn and Redwood, 2005); the rest is discharged untreated into rivers, lakes and oceans (Parr *et al*., 2002). Increases in harvestable commodities of up to 37 % are possible with wastewater irrigation compared to irrigating with clean water or chemical fertilisers (Shende, 1985; Scott *et al*., 2000; Scott and Shah, 2004). In Pakistan, land with access to a wastewater source has a value 3.5 times greater than comparable land without a wastewater source (van der Hoek *et al*., 2002). Trees have been irrigated with wastewater for centuries in countries such as Egypt and Kuwait (Armitage, 1985; El-Lakany, 1995; Braatz and Kandiah, 1996; Walter *et al*., 2001; Ioannis *et al*., 2004). In Egypt, sewage is applied to forest trees used to produce fuelwood and poles for sale at local markets (El-Lakany, 1995; Khaled, 1998; Walter *et al*., 2001), while in Kuwait, irrigated forests were established and effluent-irrigated agriculture programmes have been running for many years (Armitage, 1985; Simon, 2006).

The use of wastewater to irrigate trees, as opposed to crops, is more socially acceptable as it poses less serious health and environmental risks (EPA, 2004). Wastewater treatment normally involves

primary, secondary, tertiary and disinfection stages (Manoli and Samara, 1999). In the primary stage, sedimentation of organic and inorganic solids occurs, reducing biological oxygen demand by 25-50 %, total suspended solids by 50-70 % and grease content by 55-65 % (Manoli and Samara, 1999). Heavy metals, organic nitrogen and phosphorus are also removed (Braatz and Kandiah, 1996; Pena *et al*., 2002). At this stage, the water quality becomes acceptable for irrigation of trees and some food crops (Pena *et al*., 2002; Mara, 2004). Secondary treatment removes the remaining organic matter and suspended solids biologically using aerobic bacteria (Mara, 2004), while the tertiary stage removes nitrogen, phosphorus, suspended solids, heavy metals and dissolved solids to prevent pollution when the treated effluent is released to water bodies. This stage is detrimental for crop production as nutrients are removed. Disinfection involves the addition of chlorine to kill pathogens in the water and is done at the secondary or tertiary stage. Cost increases from the primary to the tertiary stages (Ujang and Buckley, 2002; Mara, 2004).

Irrigation of food crops with wastewater instead of discharging it into rivers may increase groundwater recharge (Drewes, 2004), the main source of water in arid and semi-arid areas. Because effluent is rich in nutrients, its use for irrigation increases crop productivity (Thomas *et al*., 2007) and the nutrients present in wastewater represent a valuable resource relative to the cost of fertiliser. Assuming nitrogen and phosphorus concentrations in wastewater of 20 and 7 mg L^{-1} respectively and an annual waste water application rate of 8000 m^3 ha^{-1}, the total annual input of nitrogen (N) and phosphorus (P) would be 160 and 56 kg ha^{-1}, respectively. As young eucalyptus plantations may absorb 120-150 kg N ha^{-1} yr^{-1} and *c*. 12 kg P ha^{-1} yr^{-1}, the nutrient supplies provided by wastewater treatment may be sufficient to support optimum plant growth (CSIRO, 1995; Lars and Gyula, 2001; Landesman *et al*., 2005). Many governments and industries are

cashing in on the possibility of reducing their carbon footprint by sponsoring the planting of forests in various parts of the world (Braatz and Kandiah, 1996; Hussain *et al*., 2002).

There are several examples of forest irrigation using municipal wastewater. In the USA, Spain, India and Australia, spraying forests with wastewater resulted in almost complete removal of nitrogen, phosphorus and other constituents after one year, making the water drinkable (Marilyn *et al*., 1997); 95 % of the effluent applied was recharged to the groundwater reservoir (Po *et al*., 2003), while nutrients in the effluent increased tree growth by 80-186 % (Marilyn *et al*., 1997).

1.4.1 Risks associated with wastewater use

There are many different types of wastewater, which impacts on the level of treatment required and the economic viability of its reuse (Toze, 2004). Pollution of water by toxic chemicals present in industrial effluents is now a worldwide problem (Gadallah, 1995; Bradford *et al*., 2003). Because of the perceived risks, some communities favour reusing wastewater (Western Australia State Water Strategy, 2003) only if this is done in remote locations where there is no possibility of physical contact with themselves (Nancarrow *et al*., 2002; Crute *et al*., 2003; Hartley, 2003; Marks, 2003; Po *et al*., 2003). However, the potential risks involved are demonstrated by observations that farm workers in India became anaemic and developed gastrointestinal symptoms because the untreated sewage used for irrigation contained numerous pathogenic microorganisms (Feachem *et al*., 1983; Bradford *et al*., 2003; Seuri *et al*., 2005). Exposed farm animals may also become weak and sickly, with reduced milk output and fertility (Crute *et al*., 2003; Seuri *et al*., 2005). Research in India showed that wastewater irrigation increased the growth of weeds and attack by pests, leading to the increased use of pesticides, decreased soil porosity and a decline in soil fertility associated with the high chemical concentration of the wastewater applied (Hofmann, 2005). The use of pesticides may lead to groundwater pollution, putting the community at large at

risk (Raschid-Sally *et al*., 2001). As most viruses present in recycled water can only infect humans (Haas *et al*., 1999), only human faecal contamination of water needs to be regarded as a concern in terms if direct viral risks to human health. Bacteria are the most common of the microbial pathogens found in wastewater (Toze, 1999; Toze and Hanna, 2002). Although many bacterial pathogens are enteric in origin, bacterial pathogens which cause non-enteric illnesses (e.g. *Legionella* spp., *Mycobacterium* spp. and *Leptospira*) have been detected in wastewater (Wilson and Fujioka, 1995; Fliermans, 1996; Neumann *et al*., 1997). The mode of transmission is via contaminated water and food or by direct person-to-person contact (Haas *et al*., 1999).

The most common enteric protozoans detected in wastewater are *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum* (Toze, 1997; Gennaccaro *et al*., 2003; Carey *et al*., 2004). Helminth parasites commonly detected in wastewater also pose a significant health risk (Toze, 1997). Secondary treatment of sewage removes trace organic contaminants and heavy metals from the effluent (Staples *et al*., 1998; Wang *et al*., 2003; Angelova *et al*., 2004). Endocrine-disrupting compounds (EDCs) interfere with the endocrine system, causing adverse health effects in an organism or its progeny (Lim *et al*., 2000). *Plutella xylostella* (diamondback moth) and *Helicoverpa armigera* thrive in fields irrigated with sewage, feeding on and damaging most *Brassica* and vegetable crops as a result of continuous cropping (Bradford *et al*., 2003). High salt levels in wastewater lead to salt accumulation in the root zone of crops and consequent yield losses (Surapaneni and Olsson, 2002).

Dissolved organic carbon (DOC) is the most common organic constituent in wastewater and its presence stimulates the activity of soil microorganisms (Sheikh *et al*., 1987; Magesan *et al*., 2000; Ramirez-Fuentes *et al*., 2002). Chakrabarti and Sdoodee, in 1995, observed that crops irrigated with wastewater produced higher yields than those receiving clean water. The physical

characteristics of wastewater which may affect the environment include its pH, dissolved oxygen content, suspended solids and salinity (Peasey *et al*., 2000). Salinity affects the soil, as well as the growth of irrigated crops and, when in the form of sodium, may directly affect soil properties through the phenomena of swelling and dispersion (Bond, 1998; Halliwell *et al*., 2001). Salinisation reduces the hydraulic conductivity of the soil, impacting on the ability of water to infiltrate into the soil profile and reducing water availability to irrigated crops (Balks *et al*., 1998; Halliwell *et al*., 2001; Surapaneni and Olsson, 2002). The presence of suspended solids (Magesan *et al*., 2000), nutrients which cause excessive growth of micro-organisms in the soil (Magesan *et al*., 2000) or interactions involving dissolved organic matter within the soil profile all affect hydraulic conductivity (Tarchitzky *et al*., 1999). Sodium in wastewater reduces the yields of some crops and its impact may involve interactions between soil characteristics and species sensitivity (Asch *et al*., 2000; Katerji *et al*., 2003). Increases in soil salinity following sustained application of wastewater may be problematic, requiring periodic leaching of sodium by irrigating with fresh or rain water (Surapaneni and Olsson, 2002).

1.4.2 Potential benefits of wastewater reuse

The global increase in the quantity of municipal effluent produced poses potentially serious health and environmental problems (Scott *et al*., 2000; Bradford *et al*., 2003) which must be addressed by the development of safe, environmentally sound and cost-effective means of treating and disposing of wastewater. Increased attention is also being focussed on the role that agriculture and forestry can play in improving urban and peri-urban environments. An opportunity to combine these two issues involves the use of wastewater to irrigate forests, farm plots and fish ponds (Bradford *et al*., 2003; Bosma *et al*., 2005).

Wastewater has the potential advantage that there is usually a constant and reliable supply, particularly for sources such as sewage effluent and industrial discharges (Toze, 2004). The discharge of such effluents to the environment may cause severe degradation of waterways, which is often related to the presence of organic and inorganic nutrients which cause problems associated with eutrophication and the formation of algal blooms (WHO, 2006). Appropriate reuse of these effluents may reduce or remove this environmental threat and alleviate pressure on clean water supplies (US EPA, 1992; Gregory, 2000) as water shortages are already being experienced throughout the world (Bushnak, 2002; Western Australia State Water Strategy, 2003; Toze, 2004). Irrigation with wastewater containing significant concentrations of nutrients has been reported to increase the activity of soil micro-organisms (Meli *et al*., 2002; Ramirez-Fuentes *et al*., 2002; Bradford *et al*., 2003).

1.5 Plant response to wastewater irrigation

In affluent communities, sewage effluent is generated at a rate of about 262 L person^{-1} d^{-1} (United Nations University, 2000) and has high concentrations of nitrogen (10-30 mg L^{-1}) and phosphorus (4-10 mg L^{-1}), and is slightly salty and alkaline after primary and secondary treatments (CSIRO, 1996). Unless wastewater gardens are properly managed, the problem is simply shifted below ground (Chia, 2000). Excessive amounts of wastewater may contribute to rising water table levels, run-off to watercourses and erosion, reduce soil productivity, threaten plant health through waterlogging and root decay, and increase the risk of plants lodging during periods of high winds or heavy rainfall by encouraging shallow root growth and weak stems (Helmer and Hespanhol, 1997). Problems associated with insufficient irrigation include reduced growth rates, poor plant health resulting from water and salt stress, and increased susceptibility to attack by insect pests and diseases (CSIRO, 1996). A key objective when irrigation is applied is to achieve the optimum balance between insufficient and excessive irrigation. The effectiveness, environmental

limitations and sustainability of using plants to treat wastewater need to be determined, including effects on plant growth and yield quality, nitrogen and phosphorus dynamics in soil and vegetation, water use and site water balance, impact on groundwater, and species and genotype responses to irrigation with wastewater.

Poor nutrition is a major reason why plants may not achieve their full growth potential (Mitchell and Ford-Robertson, 1992; Guo and Sims, 2000). Wastewater contains high levels of nutrients such as nitrogen and phosphorus and can provide at least a partial substitute for commercial fertilisers (Marecos do Monte *et al*., 1989; Hussain *et al*., 2002) and conventional irrigation practices. Soil properties such as pH and nutrient concentration are affected by long-term irrigation with wastewater (Waly *et al*., 1987; Russell *et al*., 1988; Falkiner and Smith, 1997; Guo and Sims, 2003), with implications for the sustainable use of the land. Although rainfall may provide sufficient leaching to protect the root zone, the risk of damage to the roots and entire plant by excess salt and/or nitrate needs to be minimised by proper management practices (FAO, 1992). Exposure of non-adapted plants to saline conditions may restrict their growth due to a range of stress factors including drought induced by the reduced water potential of the rooting medium, ion toxicity resulting from excessive uptake of chloride and sodium, and imbalances of mineral nutrients, particularly calcium (Marschner, 1995; Schuch, 2005). The susceptibility of plants to salinity varies between species (Bernstein, 1980; Maas, 1986; Francois and Maas, 1994) and tends to be greater in hot, dry climates than in cool, humid environments.

Salt accumulation in the soil occurs when evapotranspiration persistently exceeds leaching to deeper horizons, leading to the deposition and accumulation of salts in the rooting zone (Maas, 1986). Schuch, (2005), reported that the visual symptoms of salt injury were greatest during periods when air temperature exceeded 40°C on a daily basis, but almost vanished when maximum

daytime temperature was lower than 40°C. Wastewater induces mild physiological drought, conditioning plants to tolerate water deficits (Bernstein, 1980). Salt-induced water stress may inhibit growth and reduce yield due to osmotic inhibition of water uptake, oxidative stress and inhibition of important physiological processes by specific ions (Dudley, 1994; Orcutt and Nilsen, 2000). The principal components of soil water potential, i.e. osmotic and matric potential, are additive in their effect on water availability and may reduce both transpiration and yield (du Plessis, 1985; Shalhevet, 1994).

Nutritional disorders and retarded crop growth may occur under saline conditions characterised by low nutrient ion activities and high Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+} and Cl^-/NO^{3-} ratios (Grattan and Grieve, 1999; Orcutt and Nilsen, 2000). Because of their effects on nutrient availability, competitive ion uptake and the transport or partitioning of ions, nutrient imbalances may develop within plants. Salinity also directly affects the absorption of ions as a result of competition for uptake through cell membranes; for example, Na^+ and Cl^- respectively decrease the uptake of K^+ and NO^{3-} (Grattan and Grieve, 1999; Orcutt and Nilsen, 2000). It is difficult to distinguish between the relative contributions of water deficits, ion toxicity and nutritional imbalances to salt-induced injury in plants (Shannon, 1987; Munns *et al*., 2002). The older leaves initially exhibit salt-specific responses and die because of the rapid rise in salt concentrations in the cell walls or cytoplasm when the vacuoles can no longer sequester incoming salts (Bernstein, 1980). If the rate of leaf death approaches the rate of new leaf production, there is eventually a substantial decrease in assimilate supplies to growing leaves or changes in the supply of growth regulators, further reducing growth (Bernstein, 1980; Boland *et al*., 1993; Catlin *et al*., 1993). However, despite the potential problem of salinisation, irrigation with sewage effluent has been shown to increase the yields of crops and trees significantly (Al-Nakshabandi *et al*., 1997; Balks *et al*., 1998; Shannon *et al*., 1999; Fonseca *et al*., 2007).

The changes observed in soils receiving effluent were attributed to increased nutrient inputs rather than additional water supplies (El-Nennah *et al*., 1982; Schipper *et al*., 1996; Guo, 1998). Guo and Sims, 2000, and Guo *et al*., 2002, observed that nutrient recovery was greater at higher temperatures, with a substantially greater proportion of nitrogen (60 %) being recovered than phosphorus (30 %) at 15 ^{o}C. Waly *et al*., in 1987, found that soil pH in the surface horizons gradually decreased when soil was treated with sewage water, an effect attributed to the production of CO_2 and organic acids by soil micro-organisms; the nutrients and organic matter in effluent may enhance these activities. Guo and Sims, in 2000, reported that nitrogen and phosphorus concentrations were slightly higher in soils irrigated with effluent (5.36 mg g^{-1}) than when clean water was used (5.18 mg g^{-1}). Soil phosphorus concentration was significantly affected by temperature, irrigation type and rate of application; the uptake of nitrogen and phosphorus was greater in plants irrigated with effluent than in those receiving clean water. Biomass (foliage, branches and roots) was significantly greater in plants irrigated with effluent than in those supplied with clean water, an effect which reflected the greater concentration of nutrients in the effluent and was influenced by temperature. At 25 °C, water supplies became the key limiting factor for growth due to the greater transpiration rates under the prevailing higher temperature and lower humidity conditions (Guo and Sims, 2000).

To achieve sustainable land use in cropping systems, soil nutrients absorbed by plants must be replaced through the application of organic or inorganic fertilisers (Vigneswaran and Sundaravadivel, 2004). The high rate of recovery of nutrients by plants grown in sewage gardens demonstrates their success in removing nutrients (CSIRO, 1996; Guo and Sims, 2000). When effluent is discharged to rivers, the phosphorus contained within it promotes algal growth. However, phosphorus has lower mobility in soil than nitrogen, which is easily leached (Pereira and

Chaves, 1993) if the rate of application exceeds the capacity of the soil to immobilise it. The release of large amounts of nitrogen to groundwater makes the water unsuitable for human consumption, causing damage that persists for much longer than the harmful effects of effluent discharge to rivers (Guo and Sims, 2003).

Wastewater gardens provide a good storehouse for nitrogen, which is largely sequestered in the leaves (CSIRO, 1996), and by 2-5 years after planting, the canopy closes and nitrogen returned to the soil by fallen leaves increasingly meets growth requirements; the quantity of nutrients supplied to the system must therefore be reduced or, alternatively, the trees must be harvested and replanted to restore the rapid uptake stage. However, if stands contain trees at different growth stages (short rotation), annual demand for nitrogen and water may be relatively constant (CSIRO, 1996). Sopper, 1980; Pereira *et al*., 1989; White *et al*., 1994; Guo and Sims, 2000, all reported that irrigation of trees with effluent significantly increased leaf area index, leaf area ratio (LAR; ratio of leaf biomass/leaf area), specific leaf area (SLA; ratio of leaf area/leaf biomass) and biomass production, presumably due to improved nutrient and water supplies. Estimates of the ability of sewage gardens to use wastewater may facilitate calculations of how much water can be supplied to speific systems and minimise the risk of over- or under-irrigation (CSIRO, 1996). Water use in sewage gardens is influenced by monthly and annual balances between evapotranspiration and rainfall, the leaf area of the chosen species and the proportion of rainfall and irrigation lost by interception and evapotranspiration. Fast-growing plantations may use water at rates of up to 8 mm d^{-1}, equivalent to 80,000 L ha^{-1} d^{-1} during warm weather. During cooler seasons, irrigation is generally unnecessary as rainfall is likely to exceed evapotranspiration (CSIRO, 1996; Guo and Sims, 2000).

1.5.1 Plant growth responses to wastewater irrigation

Phytoremediation involves the uptake of contaminants from wastewater by plants and the transpiration of the great majority of the absorbed water to the atmosphere (Bolan *et al*., 2005). Plant responses to nutrients or pollutants present in wastewater vary greatly (Kiziloglu *et al*., 2007). The most effective species for phytoremediation are those which are inefficient at conserving water and so absorb and transpire large quantities of water; in so doing, they may absorb large quantities of dissolved nutrients and pollutants (Hinchman *et al*., 1996). Plants absorbing wastewater are therefore likely to absorb dissolved contaminants which they subsequently degrade, metabolise and/or sequester (Environment Canada, 2001). Rapid transpiration maximises the transport of water and dissolved contaminants from the soil to the shoot (Environment Canada, 2001). Hyperaccumulators are a group of generally small plants which accumulate unusually high concentrations of trace metals such as Zn, Ni and Cd in their shoots and are often members of the family *Brassicaceae* (mustard family). However, larger plants may compensate for their lower tissue concentrations of pollutant elements as a result of their high transpiration rates and much greater biomass production (Westphal and Isebrands, 2001).

As nutrients are stored in the greatest quantities in the foliage of most species, monitoring plant health by foliar analysis is important (Guo *et al*., 2002). Singh and Bhati, in 2003, reported that increases in the rate of application of municipal effluent increased tree growth compared to irrigation with canal water; leaf number and biomass both increased in proportion to the quantity of effluent applied. Singh and Bhati, and 2003, concluded that municipal effluent could be recommended as a suitable source of water and nutrients for tree biomass production to fulfill fuel requirements in suburban areas.

Chloride (Cl^-) is the dominant anion in soil and water and the main anionic contributor to salinity and consequent yield effects (SalCon, 1997; Unkovich *et al*., 2004). Sulphate (SO_4^{2-}) ions may have no characteristic toxic effect except to contribute to the total salt content and osmotic potential of the soil (USDA, 2000); similar effects may be induced by any salts providing an equivalent osmotic potential. This sensitivity might be related to interference with calcium uptake and the converse enhanced uptake reported for sodium and potassium in soils with high sulphate concentrations (Grattan and Grieve, 1999; Patterson, 2006). Grattan and Grieve, in 1999 examined the relationship between salinity and mineral nutrition in horticulture, while Bernstein, 1975, 1980 concluded that horticultural crops are more tolerant of salinity associated with sulphate as compared to chloride. Zelensky, (2000), reported an interaction between the effects of sulphate and chloride in saline soils on *Dubovsky 129*, a Russian rice cultivar. Abnormally high concentrations of sulphate esters may interfere with hormonal regulation of crop growth and development (Howarth and Stewart, 1992; Grattan and Grieve, 1999). Responses to salinity vary between species as some may increase water uptake from saline soil by osmotic adjustment whereas others cannot (Shannon, 1987; FAO, 1992).

The yield of almost all crops is not affected by salinity levels below 0.7 dS m^{-1}. Sewage generally falls within the 0.7-3.0 dS m^{-1} salinity range, for which the full yield potential may still be achieved if periodic leaching is carried out to maintain salinity within the tolerance range of the crops involved (Troubled Waters, 1996; Tanwar, 2003). Ions present in sewage (*cf*. Appendix 3), such as chloride, sodium and boron, are common toxins which may induce damage individually or in combination (Surapaneni and Olsson, 2002). The presence of high concentrations of trace metals in wastewater (e.g. Zn, Ni, Cu, Pb and Cd) may lead to substantial accumulation in plant tissue, thereby reducing crop growth or rendering the harvestable component unfit for human consumption (Kiziloglu *et al*., 2007). Specific ion toxicity is difficult to correct for sensitive

crops, other than by changing the water source or crop species involved, and high evapotranspiration during hot dry weather may worsen the problem by concentrating ions in the soil solution (MEDAWARE, 2005). N, P, K, Zn, B and S may pose problems to plants when present in excess. The concentration of nitrogen in wastewater may exceed crop requirements (McGuire *et al*., 2003; MEDAWARE, 2005), causing delayed or uneven crop maturity and reducing crop quality. Excess nitrogen supplies to agricultural crops may also lead to contamination of groundwater (McGuire *et al*., 2003).

1.5.2 Stomatal conductance

Stomata are pores situated on the external surfaces of plants which allow gas exchange with the atmosphere (Cañamero *et al*., 2006) and are important pathways for the loss of water and uptake of CO_2 (Lawson *et al*., 2002). They provide the primary control system for the short-term regulation of transpiration and photosynthesis, although the mechanisms controlling their movements are still not fully understood and are likely to vary depending on species and the prevailing environmental conditions (Jones, 1998). Stomatal conductance varies with irradiance, leaf temperature, leaf to air vapour pressure difference (VPD), atmospheric CO_2 concentration and turgor pressure in the guard cells and neighbouring epidermal cells in a complex response to environmental and physiological factors (Spence, 1987; Franks, 1999; Mencuccini *et al*., 2000; Comstock, 2002; Tuzet *et al*., 2003). Regulation of turgor in these cells requires metabolic energy (Blatt, 2000; Gao *et al*., 2002; Yordanov *et al*., 2003; Wakrim *et al*., 2005) and also depends on the balance between water supply from soil to the leaves and water lost by transpiration (Mansfield and Davies, 1985; Franks *et al*., 1997; Maier-Maercker, 1999; Mott and Franks, 2001).

In experiments with cocklebur (*Xanthium strumarium*), Messinger *et al*., in 2006, suggested the existence of at least two mechanisms by which stomata respond to CO_2. The first one depends on photosynthetic electron transport and is therefore sensitive to the balance between the light and

dark reactions of photosynthesis, whereas the second is independent of photosynthetic electron transport and may therefore occur in darkness. The dynamic response of stomatal conductance to plant water status and environmental variables such as ambient humidity, light and wind speed complicate measurements and interpretation of the results obtained (Soar *et al*., 2004; Messinger *et al*., 2006; Soar *et al*., 2006). Measurements of stomatal conductance are characterised by high levels of error resulting from the fact that each leaf is able to modify its stomatal behaviour independently of others, or even different sections of the same leaf (Cañamero *et al*., 2006).

Favourable water supplies provide a mass flow pathway for the uptake of water and nutrients and help to maintain the high level of turgidity required for growth and optimal stomatal opening to supply CO_2 for photosynthesis (Kirschbaum, 1988; Bond and Kavanagh, 1999; Mediavilla and Escudero, 2004; Coyle and Coleman, 2005). The strong correlation between photosynthesis rate and foliar Cl^- and Na^+ concentrations and the maintenance of turgor in salt-stressed plants means that biochemical limitations dominate the reduction of photosynthesis observed in salt-stressed trees (Munns *et al*, 2002; Paranychianakis and Chartzoulakis, 2005). Stomatal regulation has been attributed to the biosynthesis of abscisic acid and its subsequent transfer to shoots and the accumulation of carbohydrates, K^+, Ca^{2+} and Cl^- in guard cells (Bañuls and Primo-Millo, 1995; MacRobbie, 1998; Dietrich *et al*., 2001; Paranychianakis *et al*., 2004; Paranychianakis *et al*., 2006).

Increasing the atmospheric saturation vapour pressure deficit (SD) has been shown to stimulate stomatal closure in *Commelina communis* and *Tradescantia albiflora* (Lawson *et al*., 2002); similar responses have been observed for many other species, both herbaceous and woody. As the soil dries, plants respond by closing their stomata to reduce transpiration and avoid desiccation (Karlberg, 2002), with the result that water uptake also decreases. Because soil water deficits

promote reductions in stomatal conductance, transpiration rate and CO_2 assimilation, crop growth is also reduced (Shalhevet, 1994; Pankovic *et al*., 1999; Yordanov *et al*., 2001; Lawson *et al*., 2003). As the mechanisms mediating the effects of salinity stress are not yet fully understood, it is unclear whether the associated reductions in transpiration are directly responsible for decreasing growth (Shalhevet, 1994; Zhu, 2001). Stomata also play a vital role in the avoidance of heat stress in crop plants as any increase in stomatal conductance enhances the cooling effect of transpiration, enabling photosynthesis and respiration to continue unimpaired (Lu *et al*., 1998; Sperry, 2000; Rahman, 2005). Little is known about the influence of genetic factors on stomatal responses, even though the responses of plants to environmental factors are well documented (Wartinger *et al*., 1990). Several reports suggest that midday water potential (Ψ_{md}) was unaffected by irrigation with recycled water (Walker *et al*., 1981; Prior *et al*., 1992; Gibberd *et al*., 2001) and therefore may be unsuitable for the detection of water deficits (Patakasa *et al*., 2005).

The existence of negative correlations between gas exchange and foliar Cl^- concentration (Downton, 1977; Walker *et al*., 1981; Prior *et al*., 1992) suggests that non-stomatal factors are dominant in reducing gas exchange in plants exposed to salinity stress (Paranychianakis *et al*., 2004). Grapevines grown under field conditions or in pots showed a reduction in photosynthesis of *c*. 15 % when foliar Cl^- concentration increased from 100 to 200 mmol kg^{-1} (Downton, 1977; Prior *et al*., 1992). These findings suggest that there is no particular threshold foliar Cl^- concentration above which reductions in foliar gas exchange occur, although factors such as the composition of irrigation water, nutrient availability and cultural practices may influence the responses induced (Chow *et al*., 1990; Zhu, 2001). Gas exchange by leaves is known to decline with decreasing irrigation (van Zyl, 1987); for example, studies of the effects of water stress on grapevines in South Africa showed that photosynthesis and stomatal conductance were significantly correlated with soil water content and predawn leaf water potential (van Zyl, 1987;

Keller, 2005). Plants frequently exhibit reductions in photosynthesis when exposed to salinity (Bongi and Loreto, 1989; Tattini *et al*., 1995; Chartzoulakis *et al*., 2002), although its effect on CO_2 assimilation varies with salt concentration and genotype (Chartzoulakis, 2005). The greatest inhibition is observed in cultivars with inherently high photosynthetic and stomatal conductance values (Al-Nakshabandi *et al*., 1997; Loreto *et al*., 2003). Exposure to Na and Cl stress reduces photosynthesis in many species (Kaoa *et al*., 2006), although the relationship between CO_2 assimilation and Na^+ and Cl^- concentrations in *Frantoio* leaves was poor and changed drastically between salinity and stress relief periods (Tattini *et al*., 1997).

1.5.3 Photosynthesis

Photosynthesis may be measured at the level of individual leaves or estimated for whole canopies or areas of vegetation (Sellers, 1985; Rosati and Dejong, 2003; Goff *et al*., 2004). Specific leaf area (SLA), the ratio of leaf surface area to dry mass, may be used to estimate the leaf area of entire canopies from measurements of leaf number, area and dry weight (Poorter and Evans, 1998; Vande Walle, 2007). It is also possible to extrapolate measurements of photosynthesis by individual leaves to the canopy level using leaf area index (LAI); (Eriksson *et al*., 2005). Leaf characteristics such as water potential (ψ_l), net photosynthetic rate (A), stomatal conductance (g_s) and transpiration rate (E) are important in determining the flow of carbon within ecosystems (Subrahmanyam *et al*., 2006; Vande Walle, 2007). Forests offer the possibility of enhanced growth and carbon sequestration (Drake *et al*., 1997; Miller, 2006). The photosynthetic capacity of the leaves of tree species is determined mainly by their photosynthetic characteristics (Mendis and Openshaw, 2004).

Water stress reduces transpiration rate, stomatal conductance, net CO_2 uptake and plant growth (Scheuermann *et al*., 1991; Yordanov *et al*., 2001; Flexas *et al*., 2004). Water use efficiency

(WUE) is often increased by water stress as photosynthesis is affected less strongly by reductions in stomatal conductance than transpiration (Gu *et al*., 2005; Kaipiainen and Pelkonen, 2007); WUE may therefore increase with increasing severity of drought (Karlberg *et al*., 2006) or salinity stress (Brugnoli and Björkman, 1992). Severe water deficits may affect photosynthetic capacity, altering both the C_i:C_a ratio and stomatal conductance (Yordanov *et al*., 2001). During severe water stress, photosynthesis may also be limited by non-stomatal factors such as the capacity of chloroplasts to fix CO_2 rather than by increased diffusive resistance to CO_2 uptake (Chaves and Oliveira, 2004).

1.5.4 Transpiration

Transpiration may be defined as the evaporation of water from plants to the atmosphere (Koning, 1994). Plants must remain highly hydrated if they are to continue growing, and have evolved mechanisms such as waxy cuticles to limit water losses (Thut, 1939; Boyer, 1985; Pritchard, 2002; Lendzian, 2006; Maseda and Fernández, 2006). The simultaneous exchange of water vapour and CO_2 between leaves and the atmosphere occurs by diffusion through stomata, which make up *c*. 1 % of the total leaf surface area (Uddling *et al*., 2005). As plants are often exposed to a highly desiccating external atmosphere, they transpire between 100 and 1000 water molecules for every CO_2 molecule assimilated (Maseda and Fernández, 2006); this water has to be replaced by absorption from the soil and transported to the shoot by mass flow. Less than 2 % of the water absorbed by plants is used for photosynthesis and growth, and the remainder evaporates from the leaves and other above-ground organs as they attempt to capture CO_2 from an atmosphere containing only *c*. 0.038 % CO_2 (Pielke *et al*., 2007). Hsiao and Xu, in 2000 stated that plants transport 200-1000 times their own dry mass of water during their lifetime. The relative rates of photosynthesis and transpiration at any point in time depend on the concentrations of CO_2 and H_2O in the external atmosphere and intercellular spaces (Boyer, 1985). The terms transpiration

ratio or water use efficiency (WUE) describe the ratio of the quantity of CO_2 assimilated by photosynthesis per unit of water transpired and are strongly influenced by environmental conditions. However, when plants limit the flow of water vapour from their leaves, they automatically restrict the uptake of CO_2 for photosynthesis. This has been described as the dilemma of opposing priorities or the transpiration compromise (Canny, 1998).

The long distance transport of water through plants occurs in the vessel elements and tracheids of the xylem at velocities of up to several metres per hour when transpiration is rapid (Passioura, 1988). It has long been known that the hydraulic conductance of roots is variable depending on factors such as water supply, salinity and demand for water by the transpiring shoot, as well as nutrient deficiency, anoxia, temperature and the presence of heavy metals (Ma *et al*., 2001; Steudle, 2001; Kundt and Gruber, 2006). The diameter of the xylem tissue in roots increases acropetally as it collects water from lateral roots which contribute to the stream flowing through the larger roots; this flow reaches a maximum at the base of the stem (Koning, 1994; Shimizu *et al*., 2005). Atmospheric demand and, to a much smaller extent, osmotic pumping mechanisms are responsible for raising water from the roots to the top of the tallest trees (Kundt and Gruber, 2006). As the rate of water movement through plants depends on the rate of transpiration, it is strongly influenced by air temperature, SD, wind speed and irradiance (Vose *et al*., 2003). Thus, the cohesion-tension mechanism is dominant in transpiring plants, while root pressure becomes important at night (Canny, 1998; Kundt and Gruber, 2006).

Environmental variables such as irradiance, atmospheric CO_2 concentration, air temperature and SD, water supplies and endogenous growth regulators interact to regulate stomatal development and aperture, enabling plants to control their gas exchange (Hetherington and Woodward, 2003). Stomata typically open in the morning and close during the afternoon or early evening, with

variation in solar radiation usually considered to be the primary driving variable (Dodd *et al*., 1996). However, partial dehydration occurs when transpiration exceeds absorption (Hsiao, 1973) and affects a range of plant processes, including photosynthesis, transpiration and growth in various ways. Despite the unquestionable role of light in driving CO_2 assimilation, water deficits often limit the use of this resource (Steudle, 2001; Portes *et al*., 2006).

As noted previously, increased soil salinity may decrease transpiration and reduce growth due to its osmotic effect; plants which are adversely affected by salinity therefore grow more slowly and become stunted (Bernstein, 1975). Transpiration is a key process in the phytoremediation of soil and groundwater pollutants. For phytoremediation to be successful, vegetation must transpire sufficient contaminated water to accumulate meaningful quantities of the target pollutant; biotic and abiotic drivers of transpiration must therefore be considered when choosing appropriate site and species combinations (Vose *et al*., 2003). Potential transpiration is a function of windspeed, net radiation, atmospheric saturation deficit and stomatal resistance to the transport of water vapour, and may be calculated using Penman's combination equation as given by Monteith, (1993), and Karlberg *et al*., (2006).

Previous studies have failed to detect any effect of trace metals on transpiration rate (Tani and Barrington, 2005; Salah and Barrington, 2006). In experiments with wheat (*Triticum aestivum*) grown at two levels of SD, Salah and Barrington, (2006) found that Cd and Zn concentrations in the shoot depended on their concentration in the irrigation water and transpiration rate, whereas the Cd:Zn ratio in the shoot was more closely related to the adsorption capability of the soil and adsorption synergy between Cd and Zn. Grifferty and Barrington, in 2000 reported that time and transpiration rate affected Zn uptake significantly, with absorption being enhanced at higher

transpiration rates, while Gibberd *et al*., (2001), and Tani and Barrington, (2005), concluded that transpiration rate was influenced mainly by SD and growth stage rather than plant size.

1.5.5 Transpiration efficiency

WUE is widely used to describe the ratio between net photosynthetic and transpiration rates (Gibberd *et al*., 2001; Masle *et al*., 2005) or the ratio of dry matter production per unit of water transpired for longer term measurements (g kg^{-1}; (Jørgensen and Schelde, 2001; FAO, 2007). WUE may be calculated as the ratio of biomass accumulation, expressed as carbon dioxide assimilation (*A*), total crop biomass (*B*), or crop grain yield (*G*), to water consumed, expressed as transpiration (*T*), evapotranspiration (E_t) or water input to the system (*I*), and may be calculated over instantaneous, daily or seasonal intervals (Sinclair *et al*., 1984). The duration of the measurement period is important as daytime measurements do not take account of overnight respiration losses or variations in WUE during the growing season (Hui *et al*., 2001). The terms, water use ratio (Monteith, 1993) or transpiration efficiency (TE), may be more appropriate than WUE, which may be misleading as this implies the potential to achieve 100 % efficiency (Jørgensen and Schelde, 2001). Many approaches have been used to estimate WUE (Jørgensen and Schelde, 2001) and examine the relationship between water use and biomass production. Anwar *et al*., in 2003, and Karlberg *et al*., in 2006, reported a linear relation between transpiration and biomass production, suggesting that WUE was constant, while research on peanut (*Arachis hypogaea* L.) established a correlation between TE and specific leaf weight (SLW, the inverse of specific leaf area; (Byrd and May, 2000; Nigam *et al*., 2001; Sheshshayee *et al*., 2006), suggesting that SLW may be useful in predicting TE. Extensive research has shown that TE displays significant genetic variation within and between species (Masle *et al*., 2005).

Sheshshayee *et al*., in 2006, observed significant relationships between plant development, chlorophyll meter readings (SPAD) and TE after correction for seasonal differences in atmospheric saturation deficit (SD) in peanut, providing the first evidence of a significant positive correlation between chlorophyll content and TE. Correlations between yield and transpiration rate persisted throughout the life span of tomato (*Lycopersicon esculentum* Mill.) for two growing seasons (Sheshshayee *et al*., 2006), although Ben-Gal *et al*., in 2003, reported that WUE increased with plant size due to changes in the surface area:volume ratio. Transpiration efficiency is higher for C4 or crassulacean acid metabolism (CAM) species than for C3 species (Caird *et al*., 2007). Indeed, the International Rice Research Institute (IRRI) is attempting to develop rice plants which utilise the C4 rather than the more common C3 pathway (FAO, 2007). Colmer *et al*., in 2005, reported that low stomatal conductances enhance TE and so reduce the rate of depletion of soil water per unit of dry matter production, although plants may not use all the available water, with the result that they do not achieve their full yield potential. Plants close their stomata to save water and avoid desiccation when faced with water stress (Karlberg, 2002), decreasing their water uptake and transpiration. Stomatal closure also reduces CO_2 intake, decreasing photosynthesis and growth. As a result of the greater effect of salinity on stomatal resistance than on CO_2 uptake, WUE was markedly improved (Brugnoli and Björkman, 1992; Ben-Gal *et al*., 2003; Bhattarai, 2005).

1.6 Micropropagation

The propagation of bamboo may be achieved using seeds, clump divisions or rhizome and culm cuttings (Jimenez and Guevara, 2007). However, the development of other methods of propagation has proved necessary due to gregarious flowering, low seed viability, high costs and problems associated with long-distance transport of rooted plant material (Gielis *et al*., 2001; Guangchu *et al*., 2003). *In vitro* micropropagation constitutes a feasible alternative to mass-

propagation of individuals for bamboo (Zamora, 2003). Somatic embryogenesis (Gillis *et al*., 2007) and propagation using axillary buds (Jimenez and Guevara, 2007) have effectively been used to multiply bamboo *in vitro* and the latter procedure has been implemented successfully in several bamboo species to produce high multiplication rates (Zamora, 2003). According to Nadgir *et al*., (1984), and Gielis and Oprins, (2002), this will be the method of choice for mass propagation of bamboo because the regenerated plants are genetically uniform. While mass propagation of a single clone may adversely affect genetic diversity (Guangchu *et al*., 2003), it is impossible to implement a single protocol for micropropagation of all bamboo species because of their vast diversity (Gielis and Oprins, 2002). Juvenile bamboo has been found to be easier to micropropagate than mature plants (Guangchu *et al*., 2003). The mass propagation of bamboo is an easy and quick method of supplying subsistence farmers with planting material within a short time period. Tissue culture of bamboo has previously been carried out in Kenya by the author with varying degrees of success (Kigomo, 2007).

1.7 Bamboo species examined

1.7.1 The Poaceae Family

The grasses (Poaceae), which mainly comprise herbaceous species, are the fourth largest family of flowering plants, with about 500 genera and 11,000 species, and have a worldwide distribution (Saarela, 2003). Grass genera have been divided into two major taxa by some classifications (Mathews' and Sharrock, 1996). Five or six major subfamilies are recognised, namely the Bambusoideae, Centothecoideae, Pooideae, Chloridoideae, Panicoideae and Arundinoideae (Watson and Dallwitz, 1992). Woody bamboos are fast growing and high yielding, and are used widely for construction, food, hunting and the manufacture of paper (Li, 2003). Among the morphological characters useful in the taxonomy of bamboo are inflorescence type (McClure, 1973), although difficulties in determining the type of inflorescences of some bamboo genera

complicates their classification (Soderstrom and Ellis, 1987; Chao and Renvoize, 1989; Stapleton, 1994). The presence or absence of lodicules is an important generic character (Li, 1999).

The terms, sympodial and monopodial, initially used to describe the growth habit of bamboo rhizomes were later redefined as pachymorph and leptomorph (McClure, 1966; McClure, 1973). Floral characters are crucial in the classification of flowering plants, as is information on their underground components, branching patterns and culm sheaths in the case of bamboo (Soderstrom and Young, 1983). Flowering in bamboo may occur at intervals of up to 120 years, which greatly complicates their classification at species level and documentation of their diversity (Li, 1999). Molecular studies of the phylogenetic relationships among the Poaceae have led to the conclusion that re-evaluation of intrafamilial classification is necessary (Clark *et al*., 1995; Guo *et al*., 2001).

1.7.1.1 Dendrocalamus giganteus

Dendrocalamus giganteus belongs to the order Poales and genus *Dendrocalamus* (Schmidel, 1763). *Dendrocalamus* is a genus which contains the largest of all bamboo species and forms clumps up to 30 m in height (Stapleton and Rao, 1995). Its culms are thin-walled and covered with thick furry wax when young. Branches are usually absent on the lower parts of the culm and are highly variable in size, some being over 5 cm in diameter. The bracts at the base of each spherical inflorescence have one ciliate keel. Most species are from subtropical to warm temperate areas, and can only withstand a few degrees of frost (Stapleton, 1994).

Dendrocalamus giganteus has many applications as its large diameter culms may be used as pillars, the manufacture of storage containers or for special purposes such as road barriers. However, as the culms are too large for most purposes, this species is not widely cultivated although its very large leaves may be used as animal fodder. Propagation of *D. giganteus* is not

straightforward and the large size of its rhizomes makes it difficult to use traditional techniques such rhizome splitting and culm burial (Stapleton, 1994), emphasising the importance of the current study.

1.7.1.2 Bambusa vulgaris

Bambusa vulgaris (Schrad. ex J.C. Wendl) belongs to the order Cyperales and genus *Bambusa* Schreb. Bambusa is a genus which contains large bamboos that grow up to 26 m in height and several smaller species which reach heights of 10 m or less (Stapleton, 1994). These clump-forming bamboos have straight culms with thick walls which provide an important source of construction material and are sometimes used for weaving (Stapleton, 1994). They are an Asian introduction to Africa found at low altitudes where rainfall exceeds 1000 mm. *B. vulgaris* performs well in moist forest zones as well as wooded savannah regions and grows well at altitudes between sea level and 1000 m. It is widely distributed in tropical areas throughout the world and is widely cultivated as its shoots are edible (Rao, 1998). Its stems are bright green, mottled with yellow, and the distance between the inferior knots is 25-45 cm; the culm wall is relatively thin. Its culms reach a height of 6-15 m and have a basal diameter of 5-10 cm. *B. vulgaris* does not tolerate compacted clay loam or saline soils. There are few instances of generative reproduction and the common method of regeneration is through the use of cuttings (Stapleton, 1994).

1.7.1.3 Bambusa nutans

Bambusa nutans is the most common cultivated bamboo (Stapleton, 1994) and can be identified by its weakly cupped and persistent culm sheath blades and brown culm sheath hairs. Small culms are often flattened on one side above each bud or branch cluster. Its culms reach a maximum diameter of 10 cm and heights of up to 23 m and are strong and used for construction and weaving.

Its shoots are bitter and are therefore not eaten. *B. nutans* tolerates dry sites well and can lose most of its leaves during periods of spring drought without harm. Sporadic flowering of individual clumps is common, but seed has never been found; the spikelets are often filled with an orange or black fungus (Stapleton, 1994).

1.8 Statement of the problem

Serious shortages of water are affecting human livelihoods and wildlife globally. Developing and resource-poor countries do not have the means of safely disposing of wastewater because of the cost. Moreover, forests are being destroyed and encroached on due to rapidly increasing human populations and industrialization. This has interfered with natural ecological cycles without producing any significant improvement in the lives of the world's poor populations. There is therefore an urgent need to halt forest destruction and provide safe and employment-generating systems for disposing of wastewater.

1.9 Justification of the study

Reuse of wastewater may help to alleviate global water shortages by diverting to productive use, water that is normally discharged into the environment where it may act as a pollutant. Wastewater used in this way would replace clean water and so increase the availability of water. Developing nations currently lack the necessary resources for the safe disposal of wastewater. Additionally, the infrastructure, technology and chemicals required for effective treatment and disposal of wastewater are beyond the reach of developing countries. However, wastewater disposal systems which improve biomass production in subsistence farming systems may be self-financing, sustainable and provide additional income and employment for impoverished people. Previous reports suggest that bamboo grows faster than conventional tree species and is superior to steel, timber and concrete products in terms of its tensile strength (Bansal and Zoolagud, 2002).

Its rapid growth makes it a suitable substitute for timber, while its mechanical qualities provide a potential advantage over steel and timber.

1.10 Objectives

General objectives:

1. identify the most suitable bamboo species for wastewater reuse
2. develop ‘clean’ methods for wastewater disposal
3. provide a fast growing non-timber alternative for subsistence farmers

Specific objectives:

1. develop methods for the mass propagation of bamboo
2. assess physiological growth response of different bamboo species under wastewater treatment
3. effect of different types of wastewater on growth of bamboo
4. assess the balance of nutrients in the soil and plant tissue following wastewater treatment

1.11 Hypothesis

Fast growing bamboo species may provide an adequate substitute for tree species which may be used to convert wastewater into biomass suitable for use by humans and animals, so reducing human pressure on forests.

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction: Site description

The experimental site was located within the experimental nursery of the Department of Horticulture at Jomo Kenyatta University of Agriculture and Technology (JKUAT). JKUAT is situated at Juja, 30 km north of Nairobi, Kenya, in Thika District. Thika is one of the seven districts which form Central Province and was separated from the larger Kiambu and Murang'a Districts in 1995. Thika District lies between latitudes 3^o 53' and 1^o 45' south of the equator and and longitudes 36^o 35' and 37^o 25' east. Thika District borders Nairobi City to the south, Kiambu District to the West, Maragwa District to the north and Machakos District to the east and has a total land area of 1960 km^2. It has six administrative divisions, with Ruiru being the largest and Thika Municipality the smallest (Table 2.1). It has two constituencies, namely Juja, with 23 electoral wards covering Ruiru and Thika Municipality Divisions, and Gatanga constituency, with two wards, including Gatanga and Kakuzi Divisions.

2.1.1 Physiographic and natural conditions

Climatic conditions in Thika District are affected by topographic features which include highlands, valleys and riverbeds. The district has a diverse topography and its altitude ranges from 1060 to 3550 m above sea level (asl). The highlands in the west act as water catchment areas and watersheds for most of the rivers, which flow towards the lowlands in the southeast of the district. These rivers flow from the Aberdare Range in the west to the River Tana, forming part of the Tana and Athi River Drainage system. The higher areas to the west are characterised by deeply dissected topography with numerous slopes which are prone to landslides. The lowlands are located in the Ruiru, Thika Municipality and Kakuzi Divisions in the eastern part of Thika District.

Table 2.1* Administrative units and their land area in Thika District.

Division	Area (km^2)	Locations**	Sub-locations**
Thika Municipality	220.2	2	7
Kakuzi	481.2	4	12
Gatanga	251.1	4	17
Ruiru	526.6	2	7
Total	1960.2	-	-

*Source: Kenya National Mapping

**Constituencies are made up of divisions, locations, sub-locations and wards, in that order.

2.1.2 Climate and vegetation

Thika District has a bi-modal rainfall pattern, with the long rains occurring between March and May and short rains between October and November. Mean annual rainfall ranges from 965-2130 mm and is lowest in the eastern parts of the Thika Highway, Samuru, Mitumbiri, Juja, Gatuanyaga and Ithanga. The eastern part of Thika District is semi-arid and receives a mean annual rainfall ranging from 116-965 mm. The flat topography characterised by low rainfall and well drained soils makes this area suitable for irrigated plantation systems, mainly of coffee, pineapple, and beef farming. Production of cut flowers is also an increasingly important economic activity and the numerous streams and rivers are a vital source of water for such activities. Thika District has a high potential for the abstraction of water from underlying aquifers (Kenya Land Alliance, 2007) and there are already many scattered boreholes in locations such as Ruiru division. The importance of underground water as a source of water supplies, especially in Ithanga, Kakuzi and Gatuanyaga, cannot be underestimated.

With the exception of irrigated farming, agricultural activities and the types of crops grown in Thika District are determined primarily by rainfall patterns. In the northern and western parts, which receive at least 1500 mm of annual rainfall, tea, coffee and dairy farming are the dominant economic activities. By contrast, cattle rearing and production of drought-resistant crops are the main agricultural activities in the semi-arid areas to the east where rainfall is low and unreliable. Cotton can do well in this area, but its potential is not currently being exploited.

Thika District has a mean annual temperature of 20 ^{o}C, with the coldest months being June, July and August, while the hottest are February, March and April (Kenya Meteorological Department, 1984). Mean minimum and mean maximum temperatures are respectively 8 and 30 ^{o}C. With the exception of the Ndaka-ini dam, the source of water for Nairobi City, Thika District does not contain any significant bodies of water. JKUAT lies at a latitude 1^{o} 18' N and longitude of 37^{o} 12' E, at an altitude of 1416 m asl and experiences a bimodal rainfall pattern. The long rainy season starts in mid-March and ends in May, while the short rainy season lasts from October to November. The monthly mean rainfall for the period between March and May 2005 was 161 mm, and for the period between October and November 2006 was 107 mm. Mean monthly rainfall in 2006 was 84 mm, giving an annual total of 1008 mm. August and September 2005 had the lowest rainfall during the experimental period, with monthly totals of 2 and 6 mm, respectively. Monthly rainfall was also extremely low in January 2006 (2 mm) and July 2006 (8 mm). The annual mean air temperature in 2006 was 20 ^{o}C, with the highest and lowest values being 30 and 9 ^{o}C, respectively. July was the coolest month and February-March the warmest. Mean maximum temperature was 26 ^{o}C, while mean minimum temperature was 15 ^{o}C (Fig. 4.1).

Daily climatic information including daily maximum and minimum temperature, relative humidity and solar radiation at 0600 and 1200 h and daily precipitation were obtained for the duration of the

experimental work reported here from JKUAT meteorological station and the National Meteorological Station in Thika (*cf*. Section 3). Air temperature was determined using a maximum and minimum thermometer (BS 2841, Gallenkamp; JKUAT), with readings being recorded at 1200 h. Relative humidity was measured using the wet bulb depression method (Isuzu Seisakusho Co. Ltd; JKUAT) at 0600 and 1200 h. Rainfall was recorded using a rain gauge (Tipping Bucket type, Oota Co. Ltd; National Meteorological Station, Thika). Shortwave solar radiation was measured using a thermopile sensor (Prede Co. Ltd; National Meteorological Station, Thika).

2.1.3 Experimental site

The topography of the experimental site within the Department of Horticulture nursery area was flat. A three storey building located *c*. 30 m from the southern boundary of the site was sufficiently far away to avoid shading. The west and northern boundaries of the site were bounded by uncultivated tall grasses, while student experiments were located to the east. Although there were a few trees within the nursery area, none were close to the experimental site. The red soil within the nursery area was imported from other locations around 1982.

2.1.4 Experiment 1: Irrigation of bamboo with wastewater

Three bamboo species (*Dendrocalamus giganteus* Wall. ex Munro, *Bambusa vulgaris* Schrad. J.C. Wendl. and *Bambusa nutans* G.C. Wall. Munro) were grown in a split plot design with substrate (source of irrigation water) as the main plot and species as subplots (Fig. 2.1). Plant material was obtained from the World Agroforestry Centre, Nairobi, in the form of six month old rooted cuttings *c*. 50 cm in height; these were selected for uniformity from a larger population. Individual plants of each species were grown in 100 litre plastic tanks, the base of which was perforated to allow excess water to drain away and avoid unwanted waterlogging of the root system. Tanks

were used because their large capacity would allow extensive root growth during the experimental period. They were embedded in the ground (Plate 2.1) so their tops were slightly above the soil surface to avoid entry of water during periods of heavy rain when the surrounding soil might become flooded, prevent excess drainage from the base and heating of the roots due to exposure to the sun. The tanks were spaced at 5 m intervals to form a grid pattern (Plate 2.1).

The tanks were filled with soil transported from Kamai Forest by lorry, a distance of about 30 km. Forest soil was used as the plants were to be grown for 18 months, and forest soil is more nutrient-rich than locally available soil. The forest soil was black in colour and contained traces of tree leaves and roots; these were removed by passing the soil through a 6 mm sieve. The tanks were filled to the brim with approximately 100 litres of soil. This was not compacted as it was added to the tanks, but their subsequent transfer to the holes dug in the ground to accommodate them caused the soil to settle to an equal depth in all tanks. Soil was then removed from the centre of each tank to create a hole large enough to accommodate the root ball of the bamboo saplings. The tanks were watered after planting the saplings before adding more soil to fill the space created by the slumping effect caused by the addition of water. The soil surface in each tank was covered with black polythene sheeting to prevent evaporation and minimise penetration of precipitation.

The experiment was set up in August 2005 and the plants were allowed to establish for one month before the irrigation treatments began. Baseline data were obtained by carrying out destructive analyses of 15 plants on 29 August 2005 (Harvest 1), i.e. five replicates for each species, before commencing the irrigation treatments. These were applied to the remaining 60 plants, 30 for each irrigation treatment. A second harvest of 30 plants was taken on 25 July 2006 (Harvest 2), involving five replicates of each of the six species x treatment combinations. The remaining 30 plants were harvested on 17 November 2006 (Harvest 3).

Plate 2.1. Experimental site at JKUAT with bamboo growing in large plastic tanks sunk into the ground in Experiment 1.

The watering treatments initially comprised 5 L d^{-1} of either clean or wastewater depending on the treatment involved, but this was increased to 10 L d^{-1} midway through the experiment as the plants grew larger. Clean water was obtained from the mains tap in the nursery area, while wastewater discharged from JKUAT was collected at the outlet of the treatment ponds where it is held for micro-organism breakdown before being released. Wastewater collected from the JKUAT sewage outlet was added into five 100 L tanks and transferred to the experimental site by tractor once or twice each week. Water was applied gently to the surface of the soil in each tank using a 20 L bucket with a 10 L mark, being given sufficient time to drain into the soil without overflowing the top of the tank. Plants receiving wastewater had a red string attached to their stem to distinguish them from those receiving clean water. Samples of the clean and wastewater used in Experiment 1 were retained for elemental analysis at the University of Nottingham using the procedures described in Sections 2.7.5 and 2.8.

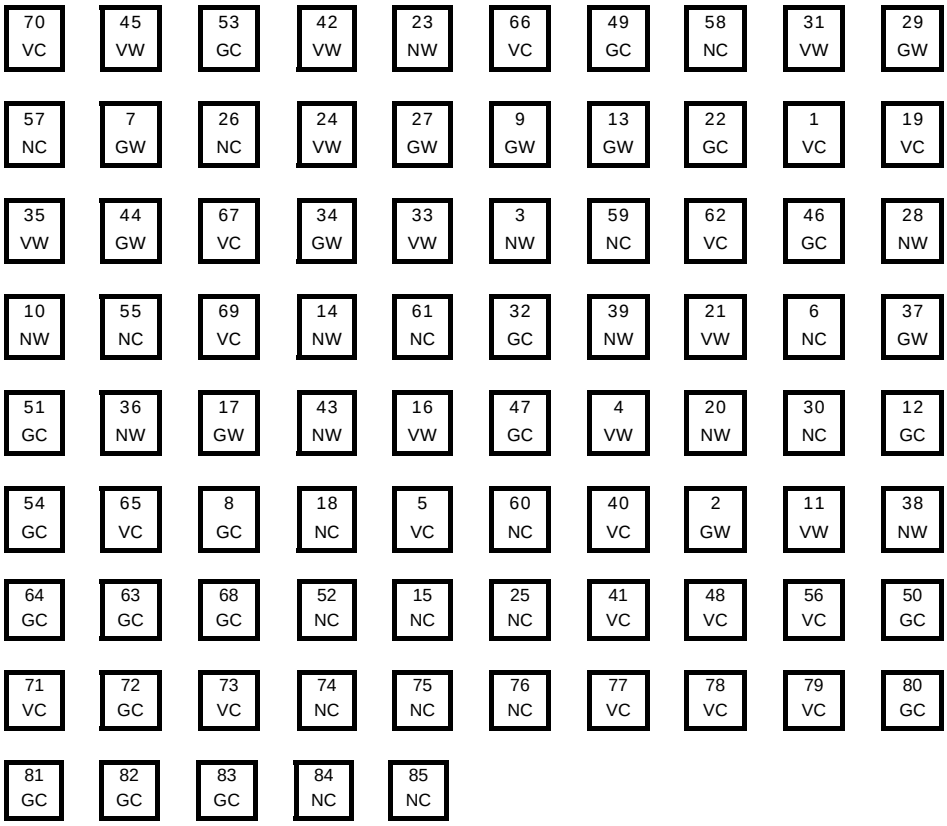


Figure 2.1. Layout of Experiment 1. G, V and N respectively denote *Dendrocalamus giganteus, Bambusa vulgaris* and *Bambusa nutans*; C and W denote clean and wastewater treatments.

2.1.5 Experiment 2: Irrigation of bamboo with alternative wastewater sources

Experiment 2 was conducted between mid-October 2006 and March 2007 using two month old rooted cuttings provided by the World Agroforestry Centre as planting material. These had been grown in black polythene pots with soil capacity of 3 kg and were *c*. 30 cm in height at the time of planting. The total number of plants used was 351. Similar procedures were carried out as for Experiment 1 above except that the treatments in Experiment 2 also involved industrial wastewater sourced from the Thika industrial area. Thirteen replicates of each species x treatment combination were used to compensate for any possible loss of plant material due to irrigation with industrial

wastewater. For each irrigation treatment, a trough 20 cm deep x 90 cm wide x 270 cm long was made in the ground and lined with a thick polythene sheet to prevent drainage. 39 pots containing 13 plants from each of the three bamboo species were randomly arranged in each trough. Water was then added to the trough and taken up by the plants through the perforations at the base of their pots. The irrigation treatments were applied daily to provide a depth of 10 cm of the relevant water type within each trough. The three irrigation treatments comprised JKUAT sewage water, industrial waste from the Thika industrial area or tap water from the JKUAT nursery. The amount of water applied varied from day to day depending on the quantity lost by evaporation or transpired by the plants on the previous day. It was not possible to cover the soil surface with polythene to prevent direct evaporation in this experiment because the close proximity of the plants left no space to do so. The heavy rainfall received between October and December 2006 may have influenced the impact of the irrigation treatments applied by diluting the concentration of pollutants present in the wastewater. By contrast, little rainfall was received between January and March 2007. The duration of the experiment was four months.

2.2 Non-destructive growth analysis

In Experiment 1, non-destructive measurements of plant growth, including height, culm diameter, branch number and the area of representative individual leaves were made at monthly intervals, beginning four months after transplanting. Plant height was determined using a metre rule, while culm diameter was measured 10 cm above ground level using vernier calipers. The culm with the largest diameter on each plant was measured on each measurement date throughout the experimental period. The numbers of brown and green leaves, branches and shoots were counted and plant height determined (*cf*. Section 2.2.4).

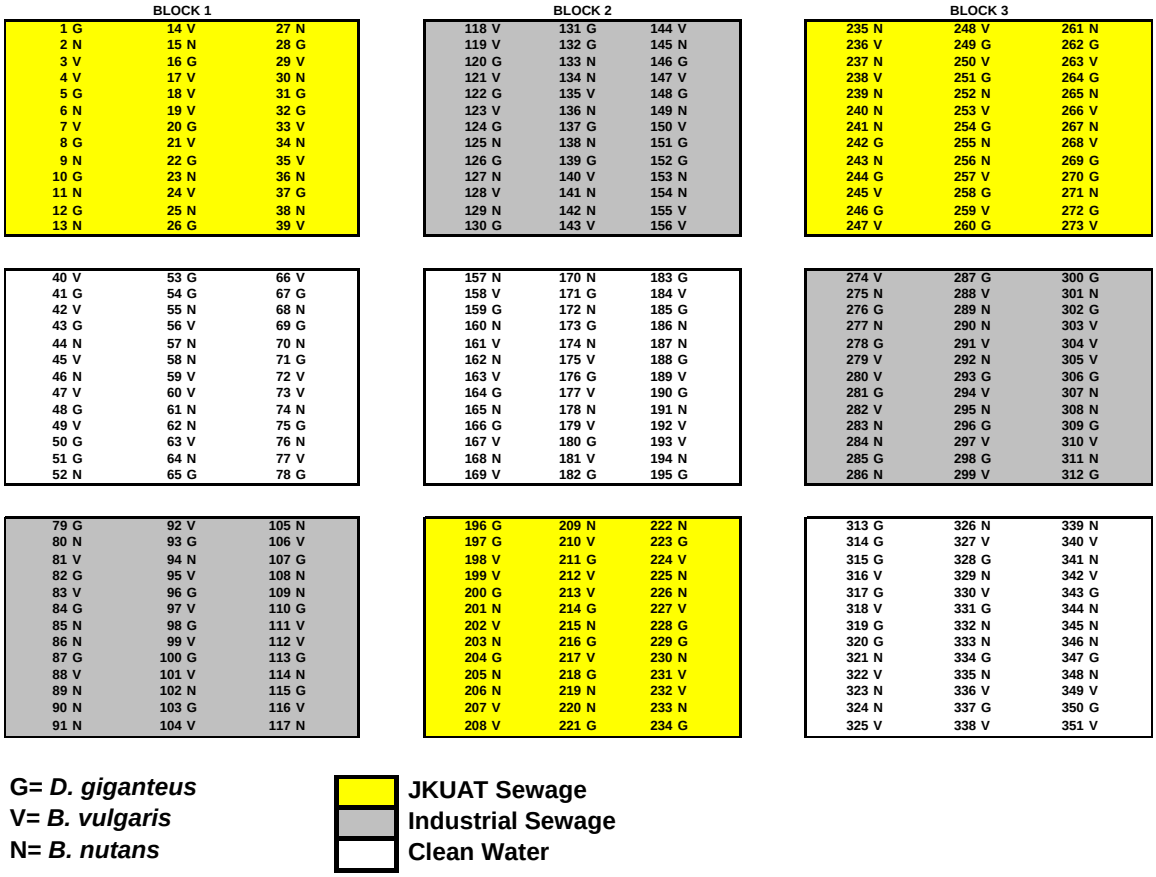


Figure 2.2. Design of Experiment 2 showing the position of individual species and irrigation treatments. The different types of shading represent the three water types used.

The area of selected leaves was estimated by measuring their width at the widest point and length. Ten leaves were measured for each plant in all treatments, avoiding the youngest and oldest leaves. 375 leaves for each species and irrigation treatment were subsequently harvested and similar measurements made before passing them through a leaf area meter (Li-3100 Leaf Area Meter, Li-Cor, Lincoln, Nebraska, USA) calibrated to 0.01 cm^2 to establish the relationship between leaf width and length and leaf area. By correlating the width and length of these leaves with their area, it was possible to formulate equations relating these characters for the whole population. The relationship for the various treatments is shown below :

***D. giganteus*:** clean water, y = 0.7159x - 1.3047; wastewater, y = 0.708x - 1.9334

***B. vulgaris*:** clean water, y = 06395x + 08574; wastewater, y = 0.6613x + 0.5171

***B. nutans*:** clean water, y = 0.6695x + 0.5691; wastewater, y = 0.6239x + 1.979

These measurements were intended to provide estimates of the growth in leaf area for each species to assist in identifying the species best adapted to the wastewater treatment. Destructive measurements are described in Section 2.3.

2.3 Destructive growth analysis

Destructive harvests were carried out on three occasions in Experiment 1 (i.e. at two months (29/08/05), 13 months (25/07/06) and 17 months after planting (17/09/06). 15 plants were harvested at Harvest 1 (5 replicates for each bamboo species), followed by 30 plants at 13 months and a further 30 plants at 17 months (5 replicates for both irrigation treatments and all three bamboo species). Harvest 1 provided baseline values for each species as the watering treatments had not yet been imposed. The leaves were removed from each harvested plant in the field and placed in polythene bags to prevent transpiration; their fresh weight was determined before placing them in an oven at 75 ^{o}C for three days and then determining their dry weight (Ohaus Precision Advanced GT 2100 balance with sensitivity of 1/100 g (i.e. 2 decimal points)). The stems were then severed immediately above ground level before being sub-divided into their upper, middle and lower stem portions; fresh weight was determined before placing the samples in an oven at 75 ^{o}C for one week. After determining their dry weights, leaf and stem samples were passed through a tractor-driven mill (Nogueira Disintegrator, Chopper and Grinder, Model DPM-4, RPM 3000, Irmaos Nogueira S/A, Brazil) for pulping.

Experiment 2 was harvesting four months after planting on 15 February 2007. The entire shoot was harvested and oven-dried at 75 ^{o}C for one week before being ground in readiness for analysis. The ground material was stored in labeled manila bags prior to elemental analysis (*cf*. Section 2.7).

2.4 Infrared gas analysis (IRGA) measurements

Measurements of CO_2 uptake provide a direct, instantaneous and non-destructive method for determining the net rate of photosynthetic carbon assimilation. Such measurements have been facilitated by the development of portable infrared gas analysers (IRGA), which provide the most common method for determining the photosynthetic and transpiration rates and stomatal conductance of plants under field conditions. This approach enables gas exchange to be determined for individual leaves temporarily enclosed in leaf chambers, while the on-board microprocessor allows gas exchange characteristics to be rapidly analysed and recorded (Parkinson and Day, 1990). Determination of CO_2 concentration by infrared gas analysis uses a process whereby radiation from an infrared source is passed through the analysis cell to the detectors. As CO_2 strongly absorbs infrared wavelengths, CO_2 in the analysis cell depletes infrared radiation passing through the cell to an extent related to its concentration. The reduction in CO_2 concentration between the inlet and outlet air streams is used to calculate net photosynthetic rate. The CIRAS 1 IRGA (PP Systems, Hitchin, Herts, UK) used in the present study employs the open or steady state approach, which involves passing air of known CO_2 and H_2O concentration through a chamber containing a known area of intact leaf and uses an algorithm to calculate intercellular CO_2 concentration (C_i) within the leaf. The instrument uses a standard protocol to minimise the time required to determine a wide range of photosynthetic information while optimising accuracy and precision (Parsons *et al*., 1997).

The CIRAS 1 portable photosynthetic system was used in combination with a broad Parkinson leaf cuvette with external dimensions of 20 x 20 mm and a cuvette area of 2.5 cm^2. This instrument uses four infrared gas analysers, two each for determining CO_2 and H_2O concentrations. One pair of CO_2 and H_2O analysers have common air inlets and outlets and are defined as reference analysers, while the other pair are used to analyse the modified air stream passing through the cuvette (PP Systems, 1994). Gas exchange by the enclosed leaf tissue depletes the CO_2 concentration of the air passing through the chamber and enriches it with water vapour relative to the air entering the chamber. The values upon which calculations of CO_2 and H_2O are based are the absolute concentration of these gases in the reference air stream and the difference between the reference and analysis concentrations (PP Systems, 1994).

Accuracy is maintained during operation by frequent zeroing of the CIRAS 1 by passing dry CO_2-free air through the analysers. A second zeroing whereby reference air is passed through all four analysers is used to balance readings for each pair of cells. Leaf temperature is measured using the energy balance approach, while a sensor situated beside the cuvette measures photosynthetic photon flux. Regular maintenance of the IRGA included renewal of the air-conditioning chemicals (soda lime, drierite and molecular sieve) when colour changes indicate exhaustion. Chamber seals were also checked for leaks by breathing around the closed cuvette with all CO_2 removed from the inlet air. The following parameters were set according to the manufacturer's specifications: flow rate 210 cm^3 min^{-1}, leaf area 2.5 cm^2, boundary layer resistance 0.21 m^2 s^{-1} mol^{-1} and transmission coefficient 0.14. Procedures for calibrating the various sensors and measuring boundary layer resistance in the cuvette were as described in the User's Manual (PP Systems, 1994).

Before each set of daily measurements, the CIRAS 1 system was allowed to warm up for 30 min to allow the sensors to stabilise and flush stagnant air from the air-conditioning columns and air-lines. Gas exchange measurements were made between 1000-1200 h for one (or three leaves for canopy level data) fully expanded leaf for each species and irrigation treatment. The leaves were tagged to allow measurements to be repeated on successive sampling dates. Calculations of gas exchange rates and stomatal conductance by the CIRAS 1 system require accurate information on the leaf area enclosed within the cuvette; in the present study, the leaves chosen were all broad enough to cover the entire cuvette area.

Net photosynthetic rate, stomatal conductance, transpiration rate and intrinsic water use efficiency were measured after attaching the cuvette to each sampled leaf for 10-20 s to allow conditions to stabilise before completing the measurement. The inlet air supply to the cuvette was conditioned to provide a CO_2 concentration of 400 ppm, while the water vapour concentration was set at 80 % of ambient relative humidity. Net photosynthetic rate was determined by measuring the difference in CO_2 concentration in the air stream before and after passing through the cuvette. As CO_2 concentration in the air stream is depleted by the photosynthetic activity of the enclosed leaf tissue, the outlet CO_2 concentration is lower than the inlet concentration to an extent related to photosynthetic rate. Measurements were made twice per week in Experiment 1 except when recent rainfall prevented measurements because the leaf surfaces were wet. The leaves used were healthy, young and sufficiently large to cover the cuvette window. Three approaches were used:

- measurements were made for all 60 plants (30 plants after Harvest 2 in Experiment 1) on each sampling date for one leaf which was intermediate in position between the top and bottom of the plant. Measurements commenced at 1130 h on consecutive days and were repeated at weekly intervals provided the leaves were dry.

- measurements were made at three levels within each plant (top, middle and lower leaves) to evaluate changes in gas exchange with leaf age. Measurements commenced at 1130 h on consecutive days and were repeated at weekly intervals provided the leaves were dry.
- measurements to capture the diurnal trends in gas exchange were made for 30 plants on eight occasions during the experimental period. These measurements were repeated at hourly intervals between 0800-1700 h for one healthy leaf of intermediate age on each plant.

2.5 SPAD estimates of chlorophyll content

SPAD measurements of chlorophyll concentration in healthy young leaves were made at weekly intervals except when rain prevented these from being done. The leaves used were intermediate in age, being neither juvenile nor senescent. Different leaves were selected at each measurement date. The SPAD 502 meter (Minolta, Japan) is a small portable instrument which uses measurements of the quantity of light transmitted through the leaf in the red and near-infra-red regions to assess greenness and hence foliar chlorophyll concentration (Fontes and Ronchi, 2002; Fontes and de Araujo, 2006). These estimates are instantaneous and non-destructive. As nitrogen is a major constituent of chlorophyll structure, its concentration is often also correlated with SPAD values. Factors affecting chlorophyll concentration and leaf colour such as leaf age, nutrient supplies, drought and disease also affect SPAD values. Mean values were obtained for five sampled leaves for each bamboo species and irrigation treatment on each measurement date.

2.5.1 Calibration of SPAD meter

As the correlation between SPAD values and chlorophyll concentration may vary between and within species (Barwinsky and Remphrey, 2007), it is necessary to construct a calibration curve to enable chlorophyll concentration to be estimated from the SPAD values obtained. Paired

measurements of SPAD values and chlorophyll concentration were made for 5-6 leaves for all bamboo species and watering treatments. Although several types of chlorophyll exist with differing absorption spectra (French, 1971), total chlorophyll concentration can be determined by spectrophotometry using equations based on the absorption spectra of the individual pigments. The procedure used was as described by (McKinney, 1941).

After first determining their SPAD value, fresh bamboo leaves were excised, weighed, cut into small pieces and placed in a mortar before being soaked in 20 ml 95 % ethanol for 30 s to dehydrate them. Once the ethanol had evaporated, approximately 20 ml of 80 % acetone was added before grinding the leaves using a pestle. A pinch of acid-washed sea sand was added to break up the tissue. The resulting suspensions were filtered into test tubes through filter paper in a funnel. Additional acetone was used to wash out the mortar and filter paper until the extract was colourless. The volume of 80 % acetone extract was determined using a graduated glass measuring cylinder before pouring a sub-sample into a 10 ml cylindrical quartz spectrophotometer cuvette. The absorbance of the extract was measured at 645 and 663 nm (UV-Mini Model 1240 spectrophotometer, Shimadzu, Japan) using an 80 % acetone blank (McKinney, 1941). Chlorophyll concentrations were calculated as:

$$\textit{Chl a } (\mu g\ g^{-1} FW) = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \textit{ml acetone/mg fresh leaf tissue}$$

$$\textit{Chl b } (\mu g\ g^{-1} FW) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \textit{ml acetone/mg fresh leaf tissue}$$

$$\textit{Total Chl} = \textit{Chl a} + \textit{Chl b}$$

Measurements made in Experiment 2 were similar to those in Experiment 1; plant height, leaf number and area, branches number and collar diameter were all recorded at monthly intervals, while gas analysis measurements were made for 81 plants at weekly intervals using the CIRAS 1.

SPAD values were recorded at monthly intervals for the entire population. Destructive measurements of leaf area, fresh and dry weight and tissue elemental concentrations were made at the end of the experiment. The entire above-ground component was collected for 81 plants (9 replicates of each of the 9 species x treatment combinations), as well as samples of the soil on which the plants were grown. To avoid subjecting the young plants to unnecessary shock through potting out, irrigation treatments in Experiment 2 were applied to the original red soil for plants transferred directly from the nursery.

2.6 Soil moisture measurements

The Moisture Meter type HH2 is a readout unit designed to be used with the PR1 Profile Probe Soil Moisture Sensor and ATS1 access tubes (Delta-T Devices Ltd, Burwell, Cambridge, UK). The meter is powered by a 9 V alkaline battery to facilitate field use. The Profile Probe measures moisture content at defined depths (10, 20, 30, 40, 60 and 100 cm) in the soil profile and comprises a sealed composite rod, 25 mm in diameter, with electronic sensors consisting of pairs of stainless steel rings at each of the designated measurement intervals. To determine moisture content, the probe is inserted into access tubes installed vertically in the soil. These purpose-designed, thin-walled fibre glass tubes maximise the penetration of the electromagnetic field emitted by the probe into the surrounding soil. The output from each sensor is a simple analogue DC voltage which is easily converted to soil moisture content using the generalized soil calibrations supplied with the instrument; the soil moisture probe may also be calibrated for specific soils using paired probe and gravimetric measurements across a range of soil moisture contents, preferably extending over the entire range between field capacity and permanent wilting point (Muthuri, 2004).

The principle underlying the Profile Probe is as follows: when power is applied, the probe generates a 100 MHz signal similar to FM radio. This is applied to pairs of stainless steel rings to generate an electromagnetic field which extends *c*. 100 mm into the surrounding soil; the signal passes easily through the access tube walls, but less easily through air gaps. The water content of soil determines its dielectric properties, a measure of the response of materials to polarisation by an electromagnetic field. Water has a dielectric constant of *c*. 81, compared to soil (*c*. 4) and air (»1). If the dielectric properties of the soil differ from those of the probe, some of the 100 MHz signal emitted is reflected back. The reflected component combines with the applied signal to form a standing wave whose voltage provides a simple and sensitive measure of soil moisture content. The sensitivity of the Profile Probe to soil water content adjacent to each pair of stainless steel rings is biased towards the soil closest to the rings. Air gaps immediately adjacent to the access tube wall therefore reduce measurement accuracy in two ways: firstly, the values obtained are affected by the presence of air as opposed to soil adjacent to the access tube; and secondly, air gaps also reduce the extent to which the electromagnetic field penetrates into the surrounding soil. Care was therefore taken to minimise air gaps around the access tubes during installation.

The HH2 is a compact, hand-held unit and readings are displayed on an LCD display. This system was used with the Profile Probe to monitor soil moisture content in all tanks containing bamboo plants in Experiment 1 using a single access tube installed at the beginning of the experiment. Before installing these, it was necessary to auger a clean, straight vertical hole to the correct depth in the soil. Although the manufacturer suggests that 28 mm diameter holes will accommodate the access tubes perfectly, 25 mm diameter holes were found to be acceptable in the present study. These slightly narrower holes were preferred to oversize ones as air gaps affect measurement accuracy to a greater extent than limited soil compaction adjacent to the probe sensors (Delta-T Devices Ltd., 2001). The Profile Probe may be used with access tubes with (a) intermittent

measurements using a Profile Probe attached to an HH2 moisture meter; this is the most common approach; (b) intermittent measurements with the top of the access tube flush with the soil surface in instances where machine cultivation is to be used to avoid potential damage to the top of the tube; and (c) semi-permanent installations, e.g. for continuous logging of soil moisture. The first option was used in the present study.

Moisture meter measurements were made biweekly. As the maximum depth to which the 1 m long access tubes could be installed was limited by the depth of the plastic tanks, the upper 40 cm projected above the soil surface. Thus readings could not be taken for the top rings and the meter only returned values for the sensors located below the soil surface (i.e. 60 and 100 cm below the top of the access tube, or 20 and 60 cm from the soil surface). The access tubes were fitted with black PVC caps to prevent dirt and moisture from entering and avoid errors associated with differential flow of water down the sides of the tubes. As the caps must provide an effective seal to prevent rain from penetrating, a small amount of silicone grease was smeared around the inside of the top of the access tube before fitting the cap. Each access tube was checked for the presence of any moisture or dirt, which was wiped away when necessary, before beginning measurements.

On each measurement date, the PR2 Probe was inserted sequentially into each of the 28 access tubes which had been installed in selected pots at the beginning of the experiment. Pressing the 'Read' button on the HH2 produced an instantaneous display of soil moisture content. With regard to field sensitivity, the signal is applied to the lower ring of each pair as the electromagnetic field is stronger around this ring. Although this field extends a considerable distance into the soil (*c*. 10 cm), it is strongest close to the rings, and so the soil close to the access tubes which contributes most to the output.

2.6.1 Calibration of soil moisture meter

Two tanks, identical to those in which bamboo was grown in Experiment 1, were filled with soil and suspended 20 cm above the ground so that free drainage of water could occur. Copious supplies of water were added over a two day period until the soil reached field capacity. Soil moisture content was then determined using the profile probe before taking soil samples at the same depths using an auger on six occasions over a nine day period. The soil samples were weighed before being dried in an oven at 75 ^{o}C for three days, after which they were weighed again. The difference between fresh and dry soil weights was used to calculate gravimetric soil water content (*w*); (mass of water per unit mass of fresh soil) using the following relation (Okalebo *et al*., 2002; Pikul Jr, 2003):

$$w = M_{water}/M_{soil\ fresh\ weight} \qquad \textbf{Equation 2.1}$$

2.7 Inductively Coupled Plasma Mass Spectrometry (ICPMS)

Inductively Coupled Plasma Mass Spectroscopy (ICPMS) is a technique which allows quantitative multi-elemental analysis, provides information on stable isotopic ratios, and is becoming increasingly important in biotechnology, nanotechnology, biochemistry and analytical chemistry (EPA, 2007). Its major advantages over previous elemental analytical techniques such as atomic absorption and atomic emission spectroscopy are its greater sensitivity, detection limits, dynamic range, multi-elemental capability and isotopic ratioing capability (Connor *et al*., 2007). Multi-element analysis for soil and plant samples was undertaken using ICPMS (Thermo-Fisher Scientific X-Series^{II}) with a hexapole collision cell (7 % hydrogen in helium) upstream of the analytical quadrupole. Internal standards were Sc (50 ng ml^{-1}), Rh (20 ng ml^{-1}) and Ir (20 ng ml^{-1}) made up in 2 % HNO_3. All external calibration standards were in the preferred range of 0-100 ng ml^{-1} (Connor *et al*., 2007). Samples were introduced via an autosampler (Cetac ASX-520) through a concentric glass venturi nebuliser (Thermo-Fisher Scientific; 1 ml min^{-1}). Sample processing

was undertaken using Plasmalab software (Version 2.5.4; Thermo-Fisher Scientific) set to employ separate calibration blocks and internal cross-calibration where required.

2.7.1 ICPMS procedures

Plant and soil samples were ground and digested prior to analysis using ICPMS. Plant samples were finely ground at JKUAT, but the soil samples were ground at the University of Nottingham using an Agate Ball Mill (Planetary Ball Mill Model PM 400 Retsch, UK) as described below.

2.7.2 Agate ball mill

The working principle of planetary ball mills depends on the relative rotational movement between the grinding jar and sun wheel. In addition to sun wheel diameter and speed of rotation, speed ratio is decisive for the energy input and hence the effectiveness of the grinding process as more energy is generated at the higher speed ratios. For example, a ratio of 1:-1 means that each time that the sun wheel rotates, the grinding jar rotates exactly once in the opposite direction as indicated by the minus sign. With a speed ratio of 1:-2, the grinding jar rotates twice for each sun wheel rotation. The model PM 400 Planetary Ball Mill Model has four grinding stations with a nominal volume of 12 to 500 ml and can grind up to eight samples simultaneously down to the submicron range, thus generating high sample throughput. It is also available with two grinding stations and speed ratios of 1:-2.5 and 1:-3. The freely selectable speed from 30 to 400 rev min^{-1} and effective sun wheel diameter of 300 mm provide a particularly high energy input.

Agate balls and jars were used as they are composed of pure silica and so could not introduce any impurities; the maximum milling speed was 300 rpm to avoid damaging the balls. Each jar contained four or five balls depending on the initial soil aggregate size i.e. four balls are used when mean soil aggregate size is <2 mm and five balls when they are >2 mm. Each jar was one-

third filled with soil before adding the balls and leaving the remaining volume as free space. The minimum and maximum quantities of soil placed in each jar are respectively 10 and 40 g, with 20 g being ideal. Once the soil sample and balls have been placed in the jars, they are inverted once to mix the contents before being clamped in place in the mill. This is normally operated at 290-300 rpm in the uni-directional mode, although the bi-directional mode can be used if the soil is sticking to the jar. Samples are normally milled for 10 min for sandy soils and 4 min or less for organic soil. In the present study, milling for 2 min at 200 rpm provided optimal results for the experimental soil. Milling was regarded as complete when the soil was floury to touch and no longer gritty.

2.7.3 Soil digestion using a block digester

The digester (VAO Anton Paar, Austria) is programmable, depending on the type of sample being digested (Filho *et al*., 2007). As hydrofluoric acid is used for the digestion, extreme care is required to avoid spillages and protective clothing is essential, including a face mask, neoprene gloves and heavy duty apron. All glassware and digestion pots were rinsed in 5 % HNO_3 Aristar made up in milli-Q water before use to ensure their cleanliness. The HF digestion was carried out in plastic beakers. After dispensing the required volume (2.5 ml) of acid into a beaker, the acid bottle was returned to its storage cabinet. A Finn pipette with an appropriate tip was used to remove acid from the beaker and 2.5 ml aliquots were dispensed into several digestion pots, after which the pipette tip was rinsed and discarded; the used beaker was also rinsed with deionised water. All operations were carried out in a fume cupboard equipped with a backwash facility.

2.7.3.1 Use of Finn pipette with multiple tips

Owing to the extremely dangerous nature of HF, elaborate methods for measuring desired quantities had to be used. The Finn pipette that was used to dispense the acid allowed the volume

of fluid released to be adjusted using a combination of settings and tip volumes, as shown in Table 2.2. To dispense 2.5 ml of acid, a 25 ml tip and setting of position 5 were used; to dispense 3 ml, a 25 ml tip with setting of 4 or a 50 ml tip with the setting of 2 were used; and to dispense 1.0 ml (1000 µl), a 25 ml tip and a setting of 2 or a 50 ml tip with setting of 1 were used.

Table 2.2 Settings for the Finn pipette used to dispense HF acid.

Setting	1	2	3	4	5	Tip/ml
Strokes	44	22	15	11	9	
Dispensing	10	20	30	40	50	0.5
Volume	25	50	75	100	125	1.25
(µl) 1 ml =	50	100	150	200	250	2.5
1000 µl	100	200	300	400	500	5
	250	500	750	1000	1250	12.5
	500	1000	1500	2000	2500	25
	1000	2000	3000	4000	5000	50

When a 25 ml tip was used, the pipette took up 25 ml of acid each time it was filled. The right hand lever was then slowly depressed completely to avoid splashing and then released until it clicked. The pipette dispensed the required volume of acid (2.5 ml in this case) into the digestion pot each time this process was repeated. The Finn pipette was refilled and the procedure repeated until acid had been added to all the samples. The use of a Finn pipette minimised the dangers of splashing or spilling HF.

2.7.3.2 HF digestion with block digester

A 0.2 g soil sample was placed in each block digester pot before transferring these to the block digester, which was set to Programme 2. 2.5 ml of hydrogen fluoride (HF) was added to each pot using a 25 ml pipette tip with a setting of 5 (Table 2.2). 2.0 ml of HNO_3 was added, followed by 1 ml of perchloric acid ($HClO_4$). The pipette tips were discarded after dispensing HF. The setting on the Finn pipette was then adjusted to position 4 to add the 2 ml of HNO_3 and finally to position

2 to add 1 ml of $HClO_4$. After dispensing the acids, the plastic beakers were rinsed in deionised water and left to dry in the fume cupboard. The block digester was left running overnight.

On day 2 of the digestion, the temperature was set to 50 ^{o}C before adding 2.5 ml HNO_3 and 2.5 milli-Q water; the samples were incubated for 1 h before switching off the block digester. The digested samples were allowed to cool before transferring them to 50 ml volumetric flasks. The digestion pots were rinsed with milli-Q water and this was added to the corresponding volumetric flask before making the volume up to 50 ml. The flasks were sealed with clean tops and inverted to mix the extract before using a portion of each sample to rinse the corresponding Universal bottle; this was discarded before filling the Universal bottle with fresh sample. The bottles were labeled and stored for analysis.

2.7.4 Microwave digestion of plant material

Finely ground plant material, (0.2 g), was weighed into a digestion tube. Batches of 48 tubes were prepared as the microwave digester (Microwave Model 3000, Anton Paar) holds 48 tubes in two racks each containing 24 tubes. The racks were placed in a fume cupboard before pipetting 6 ml of concentrated HNO_3 into each tube. The digestion tubes were then inserted into sleeves and caps screwed on before placing them in the microwave rotor holder, which has positions numbered from 1 to 48. The samples were arranged uniformly to ensure the rotor was balanced; if fewer than 48 samples are used, they must be arranged so their weight on the rotor is balanced. A pressure sensor was inserted into the digestion tube situated at Position 1 and the sleeve was hand-tightened into position before being loosened by half a turn. All tubes were placed in the rotor and covered tightly before placing the rotor in the microwave. Digestion was then started by accessing the microwave program, selecting library, choosing method P1 and pressing the start button. On completion of the digestion, the sleeves containing the tubes were carefully opened in the fume

cupboard one at a time by unscrewing the cap to release the internal pressure, making sure the tube was facing away from the operator. The samples were then made up to 20 ml as follows: i) 4 ml milli-Q water was pipetted into each digestion tube and the sample transferred to a clean universal sample bottle; ii) 5 ml of milli-Q water was added to the digestion vessel; iii) a second aliquot of 5 ml milli-Q water was added to the digestion tube and again transferred to the universal sample bottle; iv) the sample bottles were covered and shaken to mix the contents.

Milli-Q water (4 ml) was used to rinse the cap of each bottle and added to the digestion tube, before finally transferring the sample to the universal bottle. The tube was again rinsed by adding 5 ml of milli-Q water to the tube and pouring the contents into the universal bottle. This was repeated twice, to give a final volume of 20 ml. The sleeve covers were rinsed and dried before being replaced in the rotor, which was returned to the microwave.

2.7.5 ICPMS

1 ml of each sample (soil, plant, or water) was added to 9 ml of milli-Q water using a Compudil automatic dilutor to give a final dilution of 10 % and the analysis commenced after completing the following steps:

- the air conditioner was switched on and room temperature set at 20-22 ^{o}C
- argon pressure, cones and torch were all within the manufacturer's specified range
- there was an adequate supply of 2 % Aristar HNO_3 for washing the auto-sampler probe
- tubing to the instrument peri-pump and auto-sampler peri-pumps was connected
- 50 ml tubes containing 2 % Aristar nitric acid wash were present at auto-sampler positions 0, 1 and 9

- 50 ml tubes containing 5 ppb tune solution in 2 % HNO_3 were present at auto-sampler Positions 0, 1 and 10
- the 'internal standard' line was connected to a bottle containing 2 % Aristar HNO_3 wash
- all mains power lines were connected, computer and chiller switched on, Plasmalab software opened, 'Instrument/accessories' selected and autosampler probe sent to positions 0, 1 and 9 (2 % acid)
- plasma was switched on and the correct operating conditions file was opened (e.g. normal or CCTED)
- the instrument switched and left to warm up for 15 min

All other settings were as specified in the User's Manual

The auto-sampler probe was sent to position (Rack, Row, Column = 0, 1 and 10), which contained a 5 ppb Tune solutions in 50 ml tubes. A bottle containing 5 ppb tune solution was supplied to the 'internal standard' line and 3-5 min were allowed for stabilisation. When the CeO:Ce ratio was >0.02, the nebuliser was adjusted, and the performance check program was run; this had to be successful before the analysis could proceed otherwise it was necessary to run 'Correct Autotune' before repeating the performance check. The autosampler probe was sent to positions 0, 1 and 9 (2 % nitric acid) and 2 % acid wash was supplied to the internal standard line. To run a batch of samples, it was ensured that standards (rack zero) and samples (racks 1-4) were in position and the 'Template' was opened in Plasmalab on the control console. Care was taken to ensure that the correct tune conditions had been applied, a sample list constructed, and all conditions including standard concentrations had been checked. The 'internal standard' line was placed in the internal standard solution and the auto-sampler probe was sent to the wash position while ensuring that the auto-sampler wash pump was working effectively. The system was allowed to stabilise for 2-3

min. The samples were queued, ensuring the instrument was set to vacuum when the queue was empty. To shut down, the auto-sampler probe was sent to wash when necessary and the instrument switched off, allowing the switch-off procedure to complete before proceeding. The chiller was then switched off and both peri-pumps released and the tubing slackened off. Samples and standards were removed from the auto-sampler unless it was intended to re-run them. Data were recorded in spreadsheet and a report printed before switching the computer off.

2.8 Analysis using atomic absorption spectrophotometry (AAS)

2.8.1 Digestion of plant samples and analysis of water

One kg of soil from each species x watering treatment combination, 1 L of wastewater sterilised by being autoclaved, and samples of plant stems and leaves were brought to the University of Nottingham for chemical and physical analysis. As noted previously, the plant material was oven-dried at 75 ^{o}C at JKUAT for three days for leaves and one week for stem tissue immediately after being harvested. Stems from each replicate plot were sub-divided into upper and lower (September 2005) or upper, middle and lower sections (September 2006) before being passed through the tractor-driven grinder (Nogueira Disintegrator, Chopper and Grinder) as described earlier. Representative sub-samples were kept for analysis.

These samples were subsequently ashed at 450 ^{o}C in a muffle furnace (Sibata Ceramic Muffle Furnace SCM-300) before being acid-digested for analysis. A muffle furnace was used so that only CO_2 and H_2O were evolved and no material was lost as smoke. One g of ash for each replicate sample was used for digestion and analysis, corresponding to approximately 100 g of oven-dry material. Plant samples were weighed into 50 ml conical flasks and 20 ml of concentrated HNO_3 AR 70 % was added to each flask using a 25 ml measuring cylinder. The flasks were placed on a digestion block and heated at 1300 ^{o}C in a fume cupboard until the

digestion mixture was reduced to about 5 ml. Digested samples were transferred to 50 ml volumetric flasks and made up to the mark by rinsing the contents of the conical flask into a 7 cm diameter filter funnel. Samples were filtered through type DA210 filter paper using deionised water. For water samples, the steps mentioned above were omitted, and samples were simply transferred into the 50 ml volumetric flasks.

Preparation of solutions:

1. Zn standard bulk solution: 4 μg ml^{-1} zinc standard was prepared by pipetting 2 ml of 100 μg ml^{-1} Zn solution into 50 ml volumetric flasks which were then half-filled with deionised water and swirled to mix the contents. Concentrated HNO_3 AR 70 %, (2.5 ml), was added using a pipette before making the solution up to the 50 ml mark with deionised water.

2. Preparation of matrix: 10 % HNO_3 was used as a matrix (blank). A 1000 ml volumetric flask was half-filled with deionised water before slowly adding 100 ml HNO_3 down the side using a 100 ml volumetric flask. The 1000 ml flask was swirled gently to mix the contents before being made up to the mark with deionised water. The flask was then sealed with a stopper and inverted several times to mix the contents thoroughly. Before using the AAS, standard solutions for all the elements to be analysed were prepared and used to calibrate the instrument as described below.

3. Preparation of standard solutions: To prepare a standard containing 10 μg ml^{-1} Zn, 1 ml of solution containing 100 μg ml^{-1} Zn was diluted with 9 ml of deionised water using a Compudil sampler to provide 10 ml of calibration solution. To prepare a standard containing 1 μg ml^{-1} Zn, 1 ml of solution containing 10 μg ml^{-1} Zn was diluted with 9 ml of deionised water; the same procedure was used to prepare a standard containing 0.1 μg ml^{-1} Zn, and repeated for Pb, Cu, Ni and Cd.

2.8.2 Operation of Varian SpectrAA model 220 FS A in flame mode

Set-up: The AAS was initially checked to ensure the acetylene burner was in place before placing the SIPS pump in front of the instrument, connecting the sample capillary tube to the nebuliser and checking the flame guard and chimney were in place. Hollow cathode lamps were inserted at positions 1, 2, 3 and 4 for Pb, Cd, Cu and Ni, respectively before attaching the sampler capillary tube to the SIPS pump. The flow rate through the nebuliser was checked using a stop clock and found to be 8 ml s^{-1} (slightly higher than the normal 6 ml s^{-1}).

Powering up: Power to the AAS, SIPS, computer and autosampler was switched on before opening the master gas supply valve and adjusting the pressure of the acetylene gas supply to 11 psi. Power to the air compressor was switched on at the wall socket before lighting the flame by holding down the black ignition button on the front of the instrument. The extractor fan was then switched on.

Program Set Up: The SPECTRA program was opened on the computer. A new worksheet was opened and named appropriately. After the program had loaded, the 'Develop', then 'Edit methods' settings were chosen. The program was checked to ensure the set parameters were correct; these included Zn, Pb, Ni, Cu and Cd. Burner height was set at 15 cm and the atomic concentrator tube was put in place to optimise absorbance for the trace elements being analysed. The lamps were optimised as follows: Cu - 26 %, Ni - 78 %, Cd - 48 %, Pb - 48 %, Zn - 24 %.

Working conditions: Wavelengths were set as follows for the hollow cathode lamps:

Zn 213.9 nm; slit width 1.0 nm; lamp current 5 mA

Pb 217.0 nm; slit width 1.0 nm; lamp current 5 mA

Cd 228.8 nm; slit width 0.5 nm; lamp current 4 mA

Cu 324.0 nm; slit width 0.5 nm; lamp current 4 mA

Ni 232.0 nm; slit width 0.2 nm; lamp current 4 mA

Once optimisation was complete, labels were inserted by clicking on the 'Labels' icon. The sample labels were pasted into the worksheet from a text file saved in Excel as a txt.file. The samples contained on the sample rack were then placed on the auto-sampler (SPS5).

A background matrix of 10 % HNO_3 was prepared and placed in the bottle attached to the centre tube of the SIPS before pressing the 'Start' button to initiate analysis. A mixed calibration standard containing Pb, Ni, Cu and Cd, also known as bulk solution, was prepared by placing 1 ml of solution containing 100 µg ml^{-1} Pb, 1 ml of 100 µg ml^{-1} Cu or 1 ml of 100 µg ml^{-1} Ni in a 100 ml volumetric flask; 0.5 ml of a solution containing 100 ppm Cd was also added. The solutions were added separately using a Finn pipette. Deionised water was used to half fill the volumetric flask before adding 10 ml of concentrated HNO_3. The solution was made up to the mark with deionised water.

Analysis of plant digests by AAS: Using standards prepared as described above; a random selection of five plant samples was used to determine the concentration range to be expected in the extracts for each element examined. Calibration curves for each element were constructed using the various standards. The AAS was recalibrated after analysing each tray containing 36 samples and an automatic reslope of the calibration curve was carried out after each batch of 12 analyses.

Data processing and analysis: The data obtained were entered into Excel spreadsheets and total elemental concentrations in dry plant material (mg kg^{-1}) were calculated using Excel. Elemental concentration per unit plant dry weight was determined as:

$$\textbf{Concentration (mg kg}^{-1}\textbf{)} = \frac{\textbf{Volume of digest (l) x AAS value (mg l}^{-1}\textbf{)}}{\textbf{Mass of element in digest (kg)}} \qquad \textbf{Equation 2.2}$$

Appropriate dilution factors were taken into consideration when using this equation, depending on whether plant or soil samples were being analysed.

2.9 Phosphate concentration

The molybdate blue, or ascorbic acid technique for determining phosphate concentrations was used (Crouch and Malmstadt, 1967) in which 1 ml of reagent was added to 10 ml of sample. The reagent possesses a ligand which, when it comes in contact with phosphate ions, generates a blue colour whose intensity increases with phosphate concentration. Absorption was measured within the near infrared waveband (880 nm), where the phosphate/methylene blue complex absorbs most strongly. Mixed Reagent (MR) was prepared as follows: 6 g of ammonium molybdate was dissolved in 250 ml of Milli-Q (MQ) water, a form of deionised water which has been purified using an ion exchange cartridge to minimise its ionic concentration. The use of purified water is important for many chemical applications where a high level of sensitivity is required. The purity and quality of such water is determined by measuring its conductivity, or its reciprocal, resistance. The higher resistance of purified water is associated with its very low concentrations of ions which normally serve as charge carriers; resistivity values exceeding 18.2 MΩ cm at 25 °C are desirable. As a final step, the water is normally dispensed through a 0.22 μm membrane filter). 0.1454 g of antimony potassium tartrate was dissolved in 500 ml of N sulphuric acid (73 ml of concentrated sulphuric acid in 427 ml of MQ) to provide antimony potassium tartrate in solution; as this is an

exothermic reaction, great care was required. When both reagents were completely dissolved, they were mixed together and stored in a fume cupboard.

The colour-developing reagent (CDR) for phosphate determination was prepared by adding 0.37 g of ascorbic acid to 70 ml of MR and shaken thoroughly on the day of the analysis. 1 ml of CDR was added to 10 ml of sample solution and the colour was allowed to develop for 30 min before measuring absorbance using the spectrophotometer. More dilute standards were prepared from the 100 μM phosphate standard. To prepare 10 ml of 1 μM standard, 100 μl of phosphate standard was added; for 2 μM, 200 μl was added etc. The CDR and samples to which CDR had been added were only usable on the day of preparation.

Samples:

1 ml of CDR was added to 10 ml of sample and shaken; samples were then left in darkness for 30 min to allow the colour to develop. While the samples were developing, blanks and standards were prepared and CDR was added before leaving them to develop. A cuvette filled with distilled water was then placed in the sample compartment of the spectrophotometer and the top closed. The 100 % knob was used to adjust the absorbance reading to 100 % before removing the water-containing cuvette and placing it aside. Sample solutions were sequentially added in another cuvette before reading their absorbance. The calibration curves obtained were used to calculate phosphate concentrations for all experimental samples.

2.10 Loss on Ignition

Six equivalent volumes (approximately 8 g) of air – dry soil were placed into 15 mL crucibles. Samples were oven dried at 105^0C overnight, cooled in a dessicator, and weighed. The samples were then combusted at 360^0C for 2 hrs in a muffle furnace, before being transferred to an oven at

105^0C overnight. The samples were then cooled in a dessicator and weighed. Loss on ignition was calculated using the following equation (Jacobsen and Lorbeer, 1998; Ryan *et al*., 2001):

LOI (g kg^{-1}) =

$$\left[\frac{\textbf{oven dry soil weight – soil weight after combustion}}{\textbf{oven dry soil weight}}\right] \quad \textbf{x} \quad \textbf{1000}$$

Equation 2.3

3.0 Statistical analysis

Statistical analysis for destructive measurements at individual harvests (e.g. growth data and elemental analysis) and repeated measurements (CIRAS measurements, SPAD and growth measurements) was carried out by analysis of variance (ANOVA), mean separation, and correlation using Genstat 8th edition (Rothamsted Research, Harpenden, Herts, UK).

CHAPTER 3

MICROPROPAGATION OF BAMBOO

3.1 Introduction

Micropropagation begins with the selection of plant material to be propagated, followed by collection of explant(s) which can include seeds, stem tips, anthers, petals, pollen and other tissue sources. The explant material is then surface-sterilised using a series of bleach and alcohol washes and finally rinsed in sterile water (Kubota and Tadokoro, 1999; Chand and Singh, 2003). The explant, a small portion of plant tissue, is then placed on a growth medium containing sucrose as an energy source and one or more plant growth regulators. Some plants grow easily on simple media while others need more complicated media for successful growth. Growth media may include vitamin, mineral and amino acid supplements. Multiplication involves bulking up the number of the explants produced during the first stage of growth. Through repeated cycles of this process, a single explant can be increased from one to produce hundreds or thousands of plants. Hardening involves preparing plants for growth under field conditions.

Micropropagation of bamboo is necessitated by the fact that many species rarely flower (Janzen, 1976), making it extremely difficult to produce large numbers of seedlings due to the unreliability of seed supplies. This reflects the peculiar flowering habits of bamboo as many species only flower once after remaining vegetative for 30-70 years, while others do not flower at all or die after flowering. Only a limited number of species flower and produce seed regularly (Janzen, 1976; Li, 1999). To avoid this difficulty, cloning may be attempted, which relies on the ability of sections of rhizomes, branches or culm buds to generate plantlets with roots. This occurs naturally when explants possessing buds are brought into contact with soil (Stapleton, 1994). The plants produced through this process are genetically identical copies or clones of their mother plant. The

advantage of cloning is that the mother plant's qualities are transferred to the offspring. In addition to the traditional method of 'clump division', several new cloning methods have been developed (Stapleton, 1994). These include clump division, propagation of rhizomes, whole culm or culm-segment cuttings, layering, branch cuttings and macroproliferation (Kleinhenz and Midmore, 2001). However, such propagation methods destroy the mother plants, present problems during transport of plant material, are labour-intensive and have a low success rate (McClure, 1966).

It has been reported that bamboo plantlets may be generated using tissue culture approaches (Nadgir *et al*., 1984). Such approaches have been perfected with time and now provide a suitable alternative for scaling up propagules within a relatively short period. Tissue culture requires reasonably advanced laboratory facilities and strict levels of hygiene which may not be achievable by typical smallholder farmers in developing countries. For this reason, most tissue culture laboratories in developing countries are operated by government institutions. The studies described in this Chapter were intended to validate the hypothesis that tissue culture approaches may be used for the mass propagation of bamboos and thereby solve the problem currently faced by Kenyan farmers in achieving ready access to large quantities of good quality planting material.

3.2 Materials and Methods

Procedures were developed to multiply bamboo germplasm with the objective of providing a sustainable year-round supply of tissue-cultured material. The species used were selected based on the ease of acquiring seed and their potential economic value (including fuel, construction, furniture-making and other uses) in alleviating poverty. Seed of the following species were used to produce material for *in vitro* culture studies:

Dendrocalamus membranaceus, D. yunnanicus, D. strictus, D. giganteus, Phyllostachys heteroclada, P. pubescenes, Oxytenanthera abyssinica.

Culture material was obtained both from germinated seeds and shoot buds of juvenile (<10 years from initial seed germination) and mature bamboo (>25 years old). Bamboo seed was obtained from collectors in China, UK and Germany, and its quality varied widely depending on the source (i.e. seed from some sources was clean and healthy whereas others exhibited insect damage, were rotten or consisted of empty husks). To reduce contamination, seeds were removed from their husks and treated with 10 % Savlon disinfectant solution for 5 min before being washed under running tap water and soaked in tap water for 30 min. Fifty seeds of each species were then surface-sterilized by placing them in beakers containing 100 ml 10 % Jik (sodium hypochlorite, a sterilizing agent) and two drops of Tween 80 (a surfactant or wetting agent); these were placed on a magnetic stirrer for 20 min to remove contaminants. After being rinsed three times with distilled water in a sterile hood and removing surface moisture using sterile filter paper, the seeds were placed in groups of five on solid MS (Murashige and Skoog, 1962) culture medium in Petri dishes. The Petri dishes were then transferred to a growth chamber to allow germination to take place. Daily checking was done to remove contaminated seeds and prevent bacterial or fungal growth from reaching uncontaminated seeds. As an alternative source of propagation material, shoot buds excised from seedlings grown in pots or taken from bamboo stands in the field were placed in bottles containing distilled water before being taken to the laboratory. This material was surface-sterilized by washing it in running tap water, followed by a 20 min soak in 10 % Jik (sodium hypochlorite) containing two drops of Tween 80 wetting agent. The material was then rinsed three times in distilled water under aseptic conditions in a laminar flow hood. Both the shoot buds and the seeds were placed on MS medium and transferred to a constant temperature growth room (25

^{o}C, 24 h photoperiod). Aseptically germinated seedlings were used for multiple shoot formation from nodes and propagated by micro-division.

The culture medium used was MS supplemented with 1 mg l^{-1} NAA and 4 mg L^{-1} 6-BAP, as preliminary studies had shown that this was the most appropriate formulation for plantlet establishment and shoot multiplication in bamboo. The medium used for root induction was further modified by adding 1 g L^{-1} of activated charcoal. Activated charcoal has been found to stimulate rooting (Reddy *et al*., 2001). These experiments were repeated three times with five replicates on each occasion, and were grown in a growth chamber providing a 24 h photoperiod under four 40W fluorescent tubes (cool-white daylight type) throughout the establishment, multiplication and rooting stages. The plantlets were transferred to fresh medium at two week intervals and sub-divided for multiplication whenever necessary.

The final laboratory phase for all cultures involved *in vitro* induction of rooting. The agar was washed from the roots of plantlets by holding them under running tap water prior to transfer to a poly-tunnel for acclimation. The plantlets were then dipped briefly in a mild solution of Bayleton, an antifungal formulation for plants, made up at 1 % of the normal concentration of 1.56 g/11.35 L water. This was mixed with Bayfolan foliar feed at 1 % of the normal rate of 4 l/0.404 ha and added to 20 x 60 cm trays containing a 10 cm depth of heat-sterilised forest soil. Forest soil was chosen because it is an inexpensive cheap starter medium which is freely draining and has a high organic matter content. The purpose of dipping the plantlets was to provide prophylaxis against soil pathogens and provide a foliar boost. To sterilise the soil, a 100 L metal barrel containing 25 L of water was filled with soil and boiled for 15 min. Following transfer to the poly-tunnel, the plantlets were sprayed with water periodically (twice a day for 3 d, then once a day for 7 d) to maintain high humidity and prevent them from becoming dessicated as their leaves had not yet

developed an effective waxy cuticle. After two weeks, humidity was gradually reduced and the plantlets were transplanted into 15 x 23 cm perforated polythene sleeves before being transferred to a lath house (an open-sided structure used to provide shade to tender or young plants) to allow them to harden to more extreme environmental conditions. The duration of the period in the lath house stage varied between 1-2 months depending on how quickly the plants hardened. Plants were considered to be hardened when they had developed 2-3 new leaves with a waxy covering. When the plantlets appeared sufficiently robust, they were moved outdoors.

3.3 Results

Figure 3.1 shows the change in plantlet number following division at subculture for all seven bamboo species examined. The difference between species was significant ($P<0.001$) and became apparent after five months (December 2005). Figure 3.2 shows the monthly mean number of plantlets produced by each species during the same period; the difference between species was again significant ($P <0.001$).

Plates 3.1a and b show tissue cultured bamboo plantlets in glass culture vessels with aluminium foil tops; the young bamboo plantlet shown in Figure 3.1b has produced multiple shoots. Plates 3.2a and b show culture vessels infected by bacterial and fungal contamination introduced by tiny insects (micro-arthropods, mites, thrips) which entered under the aluminium foil cap; this is a widespread problem in tissue culture (Leifert and Cassells, 2001).

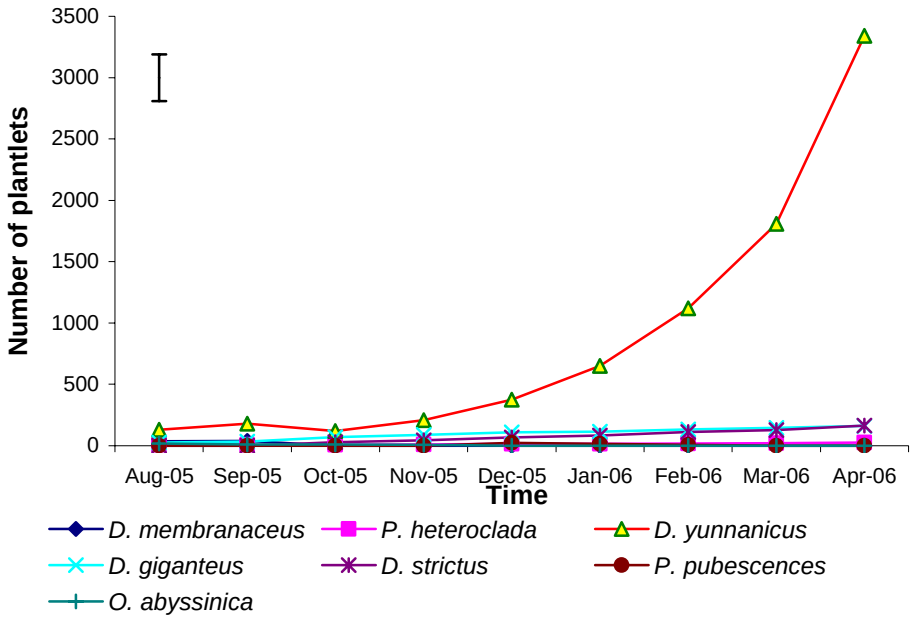


Figure 3.1. Monthly increase in the number of plantlets following division and sub-culture of the seven bamboo species *in vitro* over a nine month period. The vertical bar represents the standard error of the difference (SED) for comparing species.

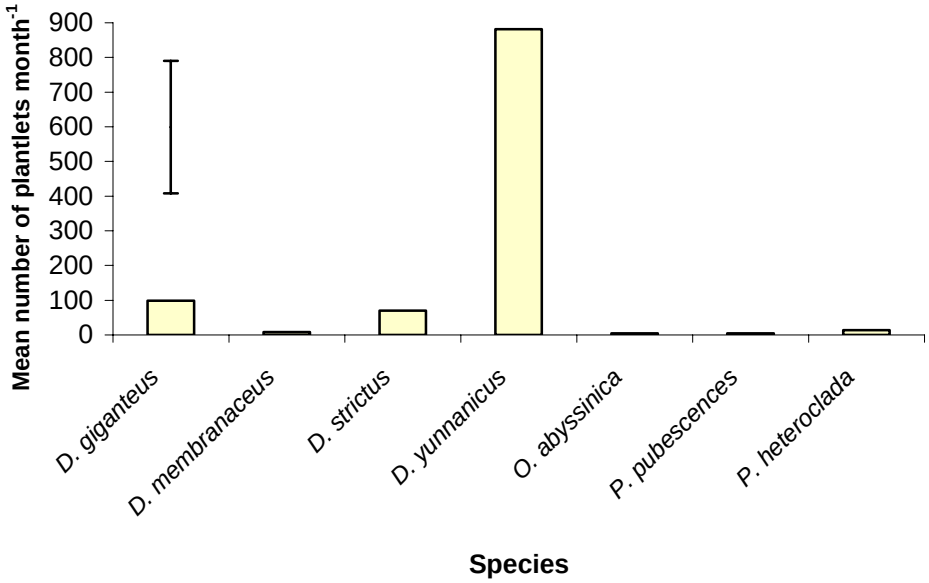


Figure 3.2. Monthly mean number of plantlets produced during the nine month sub-culture period. The vertical bar represents the standard error of the difference (SED) for comparing species.

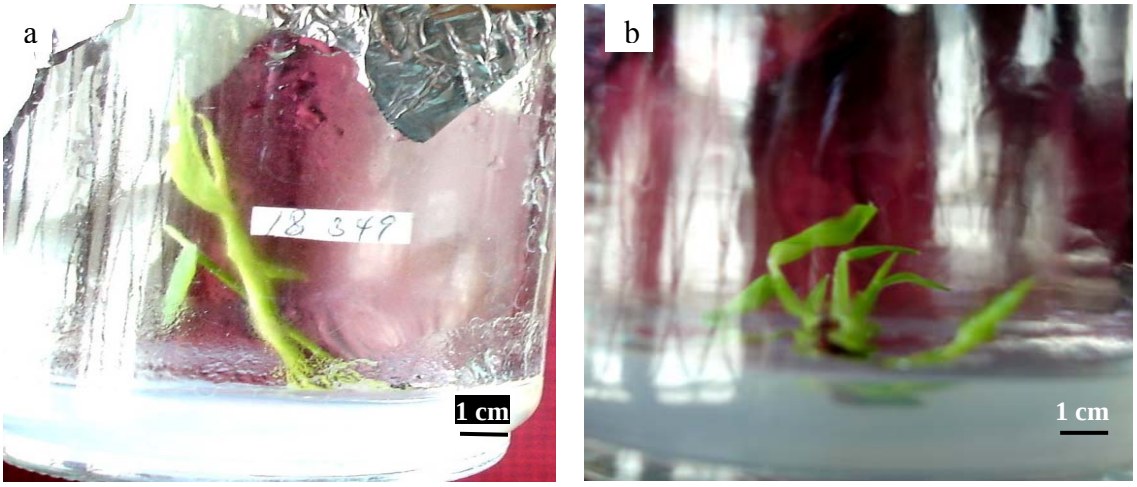


Plate 3.1. a) Tissue cultured bamboo plantlets in glass culture vessels with aluminium foil tops; b) a young bamboo plantlet producing multiple shoots.

In this particular case, the situation could have been prevented if better closures for the culture vessels had been available or the culture room had been completed sealed and ventilated with filtered air to keep out insects and fungal and bacterial contaminants.

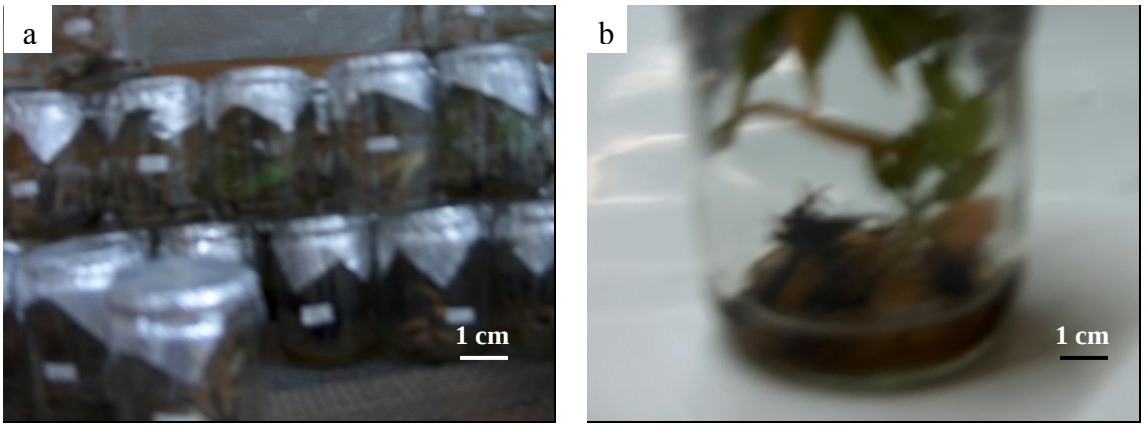


Plate 3.2. a) Contaminated bamboo plantlets in culture vessels stacked in the growth chamber; b) contaminated bamboo plantlet in a culture vessel.

3.3.1 Potting out

Plantlets were transplanted into forest soil in pots and transferred to a polythene tunnel when they reached a height of 6-8 cm. They were initially maintained under high humidity by spraying them daily with tap water, taking care to avoid the outbreak of diseases associated with the humid

conditions. Spraying with Bayleton, at the recommended rate at two week intervals helped in this respect. Humidity was gradually reduced by opening one side of the tunnel slightly after two weeks and increasing the size of the opening at two day intervals. This procedure resulted in almost complete plant survival.

After completing the acclimation stage, the plants were transferred to a lath house to undergo hardening. Plates 3.3a & b show the presence of several well expanded leaves during the hardening stage which followed *in vitro* germination, multiplication and acclimation. Provided adequate watering was given, survival in the field proceeded successfully. The number of plants transplanted to pots containing soil was not uniform for all species or months during the propagation period because the rate at which material was produced varied greatly between species and it was important to maintain the maximum possible numbers in the laboratory to avoid total loss of some species during the experimental period. The numbers of plants transferred to soil during the nine month propagation period were: *D. membranaceus* (1), *P. heteroclada* (7), *D. yunnanicus* (80), *D. giganteus* (20), *D. strictus* (20), *P. pubescences* (5) and *O. abyssinica* (10).

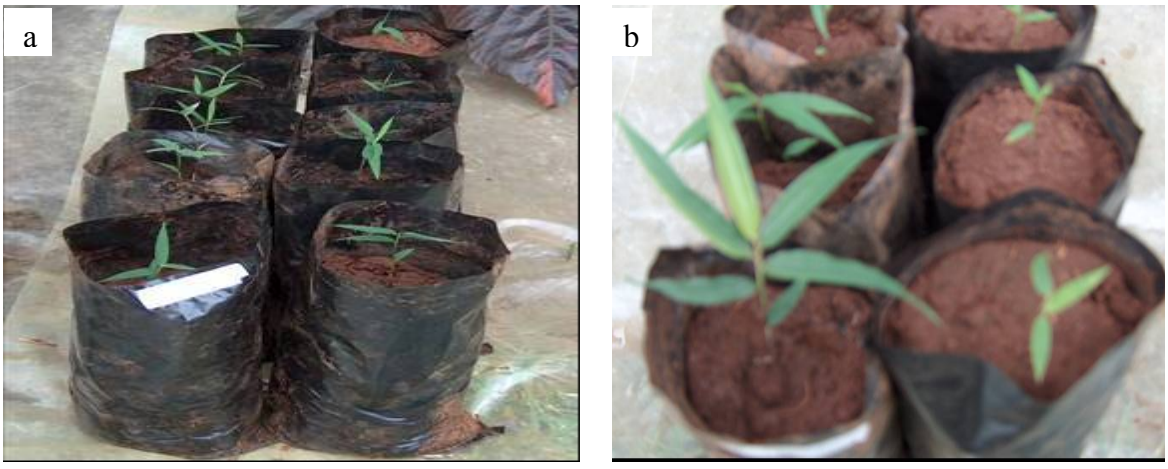


Plate 3.3. a) and b) bamboo seedlings undergoing hardening in a lath house, where they remained for four months before being ready to plant in the field.

3.3.2 Manipulation of bamboo materials under in vitro conditions

D. membranaceus, *D. yunnanicus*, *D. strictus, O. abyssinica* and *D. giganteus* all had large seeds which were easy to work with and relatively free of contamination following surface sterilisation. By contrast, *P. pubescenes* and *P. heteroclada* had small hairy seeds which were generally impossible to decontaminate. Tissues obtained from plants growing outdoors became severely contaminated by fungal and bacterial infections after inoculation and none survived.

Differences between species exhibiting clumping and non-clumping growth habits were apparent under *in vitro* conditions. As a running phenotype, *D. yunnanicus* grew quickly and its shoots elongated around the interior walls of the culture vessels. Multiplication of this species involved cutting between the internodes while leaving pieces that were sufficiently large to continue growing. By contrast, as *D. strictus* has a clumping phenotype, its multiplication required splitting at the base; care was vital to avoid damaging the stem base as this resulted in death. Pieces with an area less than $25mm^{-2}$ did not survive as well as larger ones under *in vitro* conditions.

3.3.3 Multiplication rates

Multiplication rates, i.e. the increase from the initial number of plantlets established following each subculture, differed between species ($P<0.001$) as *P. pubescens* and *P. heteroclada* grew very slowly and showed poor survival rates after being cut (Fig. 3.1.) whereas *D. membranaceous* and *O. abyssinica* initially started off well to produce 38 and 10 plantlets respectively by the second month, before succumbing to contamination. *D. giganteus* and *D. strictus* grew slowly but showed promise, producing 161 and 164 plantlets by the end of the nine month propagation period. Determining the appropriate size at which to split tissue-cultured plants of these species was critical for their continued survival as this appeared to differ depending on the species involved and its mode of growth i.e. whether monopodial or sympodial. *D. yunnanicus* was the

most promising in terms of multiplication rates, easily outperforming all other species examined ($P<0.001$).

3.4 Discussion

Contamination is the biggest source of losses in the tissue culture industry (Kubota and Tadokoro, 1999; Leifert and Cassells, 2001). Contamination carried by thrips and mites which entered under the aluminium foil used to cover the culture vessels greatly reduced the number of surviving plantlets, from 3340 to 113 in *D. yunnanicus*, from 161 to 15 in *D. giganteus* and from 164 to 20 in *D. strictus* within two months of the problem becoming apparent (Fig. 3.1; Plate 3.2). By the third month, most plantlets had become contaminated and were dying. A wide range of microorganisms including filamentous fungi, yeasts, bacteria, viruses and viroids and micro-arthropods (mites and thrips) have been identified as important contaminants of plant tissue cultures (Leifert and Cassells, 2001). Anti-fungal or anti-bacterial treatments (Kubota and Tadokoro, 1999) could not have been used in the present study because, apart from their cost and restricted availability, the bacterial filters and vacuum pumps or syringes needed to facilitate their inclusion in the media were not available. More effective seals and an air-conditioned growth room with an effective air filtration system would have helped to avoid this problem, but were again not available. Use of general and semi-selective microbial growth media or serological and PCR-based molecular techniques for specific pathogens is recommended for detection of latent contamination in tissue culture laboratories (Leifert and Cassells, 2001).

D. yunnanicus is a fast growing bamboo species which is well-suited for mass propagation. Its growth habit was of the runner type rather than the clumping type exhibited by all other species examined. It is important to know the relationship between bamboo species as this may help to explain some of the observed differences in their performance. However, it is also important to

note that fast growth under *in vitro* conditions may not necessarily translate into rapid growth in field environments. *D. giganteus* and *D. strictus* also showed good growth and it is possible that the performance of these slower growing species could be enhanced by manipulating the media conditions. These changes might include trial-and-error alteration of the concentrations of some of the macro- and micronutrient salts (e.g. Fe, Ca, Bo, Mg, Mn), changing the vitamin constitution, including or excluding additives such as coconut milk, among many others. Testing different types of media formulations might also be helpful. Improved methods of sterilising difficult material for tissue culture, such as using mercuric chloride and/or antibiotics exist but involve a significant cost element and some are potentially dangerous to users (Guangchu *et al*., 2003). It has also been suggested that using photoautotrophic sugar-free medium might help to reduce the incidence of contamination and also the inclusion of $AgNO_3$ in the medium (Kubota and Tadokoro, 1999).

3.5 Conclusions

As many bamboo species flower only after extremely long periods of vegetative growth and die after flowering (Janzen, 1976; Wang *et al*., 2007), tissue culture propagation programmes based on germplasm sourced from seeds offer end-users the most effective opportunity to replace aging bamboo plantations or plant new stands. However, traditional methods of propagation (including seeds, clump division and cloning), although involving relatively straightforward low technology approaches, have disadvantages in terms of the transport of material and transmission of diseases. Clumps and clones are bulky and likely to have attached soil, providing ideal conditions for the development and spread of diseases; their weight also makes it inconvenient to transport them for long distances. Tissue culture approaches using seed offer, at least for bamboo, the best way of producing large quantities of planting material with a guaranteed longevity. As an example, it is worth noting that in the present study, the numbers of *D. yunnanicus* doubled every month and had

reached *c*. 3500 plants by the end of the nine month propagation period. If this had continued, the populations would have reached 28,000 and 56,000 after eleven and twelve months respectively. This huge monthly increase cannot be achieved through traditional propagation means and tends to offset any losses that might occur at the beginning of the process (Nadgir *et al*., 1984). Tissue culture techniques are expensive but applicable in situations where alternatives do not exist. Application of tissue culture for such species offers a comparative cost advantage, justifying use of this technology. Bamboo represents such a case (Nadgauda *et al*., 1997) as its seeds are difficult to obtain and raising large nursery populations through conventional means is virtually impossible in the African context as large plantations do not exist in the first place. Seed needs to be sourced from overseas and its quality is not always good. The argument for starting from seed in this instance is that bamboo takes so long to flower (Janzen, 1976) that propagation of plants from seed using tissue culture technologies provide the best assurance of a long lifespan in these species.

It is worth noting that the genotype of seed-derived plantlets is not known because genetic segregation ensures that the resultant plant material will not perform like its parents, in contrast to material that has been vegetatively propagated. Associated risks include poor performance of the plants produced; by the time this problem is noted, plants produced using this approach may already have been transplanted to the field as it may take years for bamboo to reach maturity, whereas the initial multiplication using tissue culture takes only a matter of months (Guangchu *et al*., 2003). To avoid this, and problems associated with monocultures, tissue culture methods should be used in conjunction with the traditional methods of propagation described earlier (Saxena and Dhawan, 1995; Amalu, 2004).

CHAPTER 4

GROWTH OF BAMBOO

4.1 Introduction

Wastewater reuse in developing countries may provide advantages such as water conservation and disposal of sewage in a relatively safe and low-cost way (UNEP, 2003). Reliable supplies of wastewater may help to prevent pollution of surface water and enable cash-poor farmers to avoid having to buy artificial fertiliser, while increasing crop yields and improving incomes (UNEP, 2003). It has been reported that plant growth is improved when plants are irrigated with wastewater rather than clean water (Singh and Bhati, 2003), with leaf number and biomass increasing proportionately to the quantity of effluent applied. Wastewater usually contains higher concentrations of N, P, Cu, Fe, Mn and Zn than clean water, all of which are essential plant nutrients, and consequently has been found to be a superior source of water and nutrients for biomass production (MEDAWARE, 2005).

4.2 Materials and Methods

Three bamboo species (*Dendrocalamus giganteus*, *Bambusa vulgaris* and *B. nutans*) were grown in 100 litre tanks in a factorial experiment with an initial population of 75 plants (*cf.* Section 2.1.4). The plants were raised from stem cuttings for 3-4 months. 15 plants were harvested in August 2005 immediately before the irrigation treatments began to establish base-line values for plant growth parameters. The remaining 60 plants were grown until July 2006, when a second harvest of 30 plants was made. The final harvest was carried out in November 2006. Between the first and final harvests, half of the plants were watered with tap water; while the other half were irrigated with wastewater from the University sewage treatment ponds (*cf.* Section 2.1.4). Air temperature, relative humidity, solar radiation and rainfall were measured daily using standard

meteorological equipment (*cf.* Section 2.1.2). Plant height, number of branches, collar diameter and leaf area were recorded at monthly intervals (*cf.* Section 2.2) and plant biomass was determined at each harvest (*cf.* Section 2.3).

4.3 Results

4.3.1 Climatic conditions

Juja has a bi-modal rainfall pattern and the rains normally occur from March to May and October to December; the intervening months are relatively dry. During 2005-2006, appreciable rainfall was received in November 2005, March to May 2006 and November 2006 (Fig. 4.1). The heaviest rainfall events occurred on 4 May 2006 (75.2 mm) and 15 November 2006 (67.3 mm); heavy rainfall events approaching these values also occurred on several other dates.

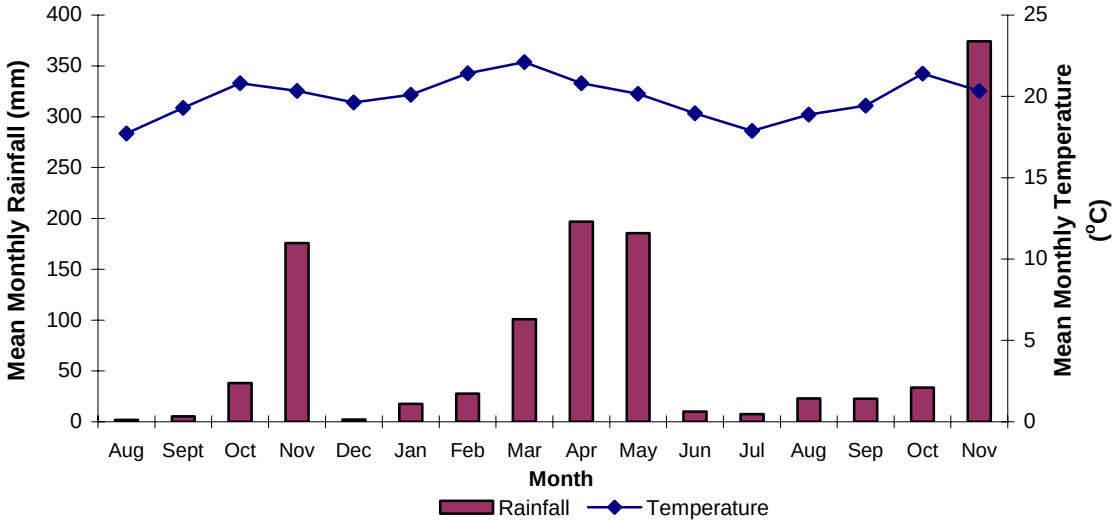


Figure 4.1. Monthly mean rainfall and air temperature for the JKUAT experimental site between August 2005 and November 2006.

Long term records show that the seasonal variation in daily mean air temperature seen in Figure 4.1 is typical of the area. The highest air temperature was recorded on 25 February 2006 (32.1 ^{o}C), while the lowest was on 30 August 2006 (6.8 ^{o}C). Monthly mean maximum temperatures

were lowest between April and September 2006, ranging between 22 and 25 ^{o}C, while monthly mean minimum temperatures for the same period ranged between 12 and 16 ^{o}C.

Incident shortwave solar radiation was relatively low in August 2005, increased to a peak in March 2006 and then declined to a minimum in July 2006 which was followed by a secondary peak in October 2006. This trend is consistent with that for air temperature (Fig. 4.1). The period of greatest solar radiation therefore occurred between October 2005 and March 2006, when daily mean values ranged between *c*. 20-30 MJ m^{-2} (Fig. 4.2).

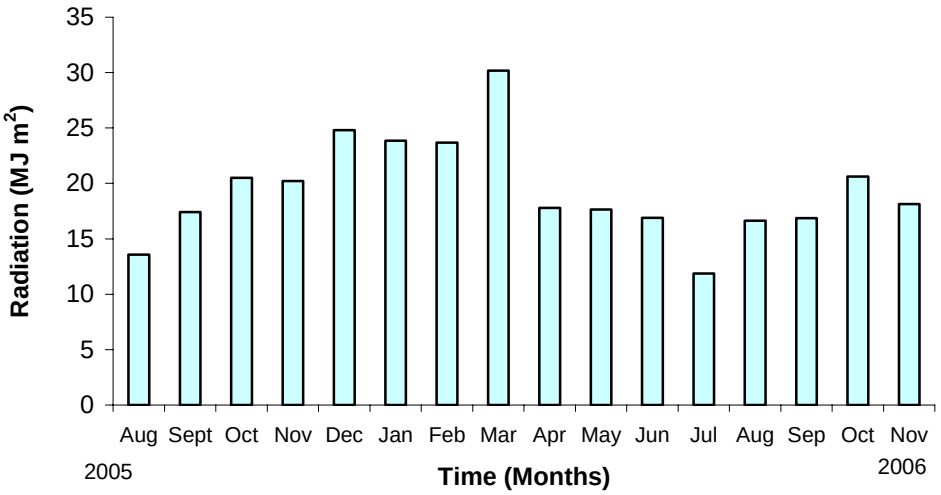


Figure 4.2. Monthly mean total incident shortwave radiation for the JKUAT experimental site between August 2005 and November 2006.

As expected, relative humidity was much higher at 0600 than at 1200 h (Fig. 4.3), reflecting the lower air temperatures at this time. Seasonal variation was apparent at both measurement times as the values tended to be lowest in October of both years and highest between March and August, and in November. These trends corresponded with the timing of the rainy and dry seasons.

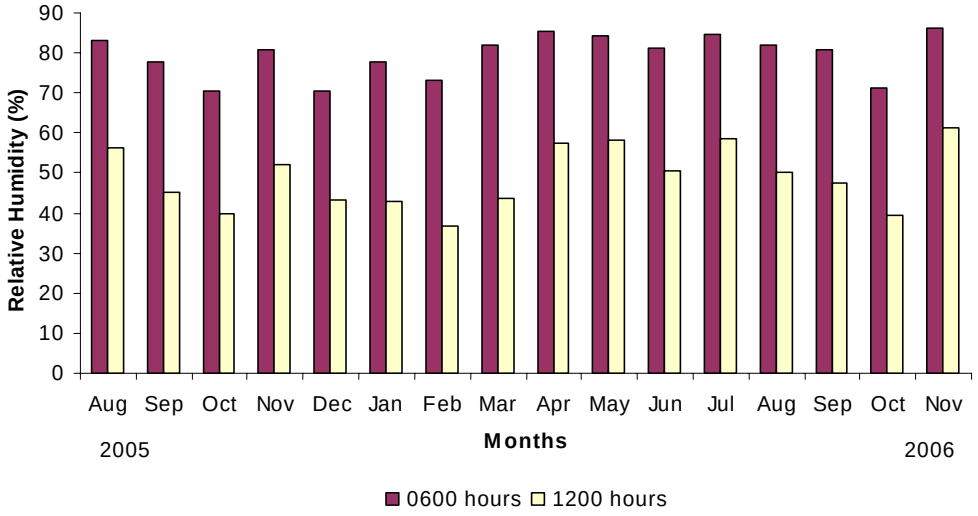


Figure 4.3. Mean monthly relative humidity values at 0600 h and 1200 h at the JKUAT experimental site between August 2005 and November 2006.

The maximum moisture holding capacity of air at 100 % relative humidity is known as its saturated vapour pressure (SVP) and has units of pressure (kPa). The difference between SVP and the actual vapour pressure of air is defined as saturation deficit or SD (Anwyl Bromeliads, 2007) and provides a measure of the ability of the air to absorb water. SD differs from relative humidity in that it increases with temperature and may be calculated as:

$$SD = \frac{(100 - RH)}{100} \times SVP \qquad \textbf{Equation 4.1}$$

where *RH* represents relative humidity and *SVP* is the saturated vapour pressure, which may be obtained from meteorological tables and is specific for any given combination of temperature and relative humidity (Anwyl Bromeliads, 2007). SD is a key factor in determining the potential for transpiration.

SD values between August 2005 and November 2006 are shown in Figure 4.4. These showed considerable variation, being low in August 2005, increasing between September and October 2005, decreasing in November 2005 and increasing to a maximum in February 2006. Values

decreased again between May and September 2006, before increasing in October 2006 and subsequently declining.

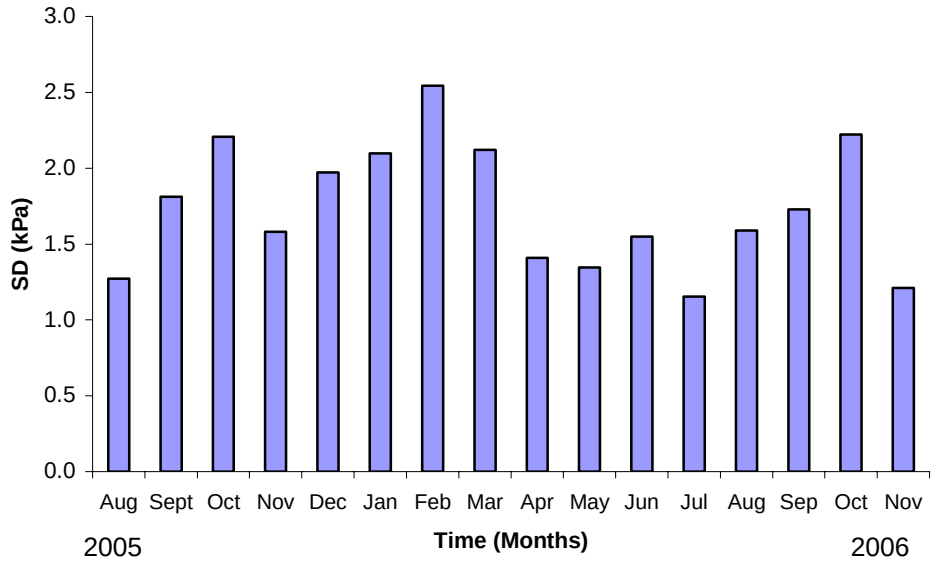


Figure 4.4. Mean monthly saturation deficit (SD) values at the JKUAT experimental site between August 2005 and November 2006.

The correlation between saturation deficit (SD) and incident shortwave radiation was strong (r^2 = 0.64; Fig. 4.5), demonstrating the importance of irradiance and air temperature in determining SD.

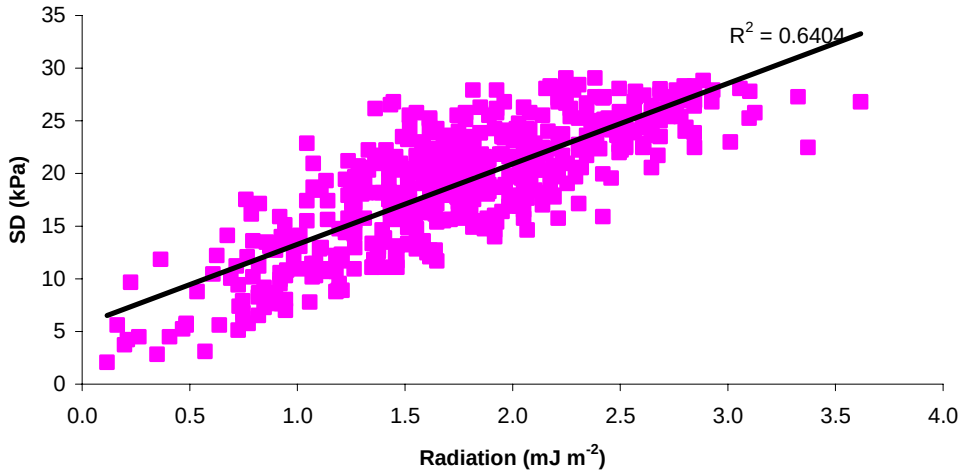


Figure 4.5. Correlation between atmospheric saturation deficit (SD) and incident short wave solar radiation at the JKUAT experimental site between August 2005 and November 2006.

4.3.2 Plant height

As noted previously, *D*. *giganteus*, *B*. *vulgaris* and *B*. *nutans* were irrigated with clean or wastewater in a population that initially comprised 60 plants spanning all species and treatments between Harvests 1 and 2, but was reduced to 30 plants after Harvest 2 (*cf*. Section 2.1.4). Half of the plants of each species received wastewater while the other half received clean water. The plants were arranged in five blocks, each initially containing four replicates of each species*irrigation treatment; the number was reduced to two replicates of each species*irrigation treatment after Harvest 2. Plant height was determined monthly for all species*watering treatments.

Plant height showed no significant species*irrigation type*time interaction ($P<0.065$; Fig. 4.6), although this approached significance. However, a significant effect of time on plant height and significant species*time interaction were detected ($P<0.05$; Fig. 4.7). Height increased with time in all species, but increased more rapidly in *B*. *nutans* than in the other species during the first four months of growth (Fig. 4.7); this situation persisted until April 2006, when height in *D*. *giganteus* increased sharply to become equal to *B*. *nutans*. Although the increase in height with time was significant for all species, the differences between species were never significant. The influence of irrigation treatment approached significance ($P<0.065$) and plants irrigated with wastewater were slightly taller (Fig. 4.6). After 12 months, *G*. *giganteus* was still marginally taller than *B*. *nutans*, while the relative position of *B*. *vulgaris* was unchanged (Fig. 4.7).

Plant height did not differ significantly between species irrespective of whether they received wastewater or clean water ($P<0.46$; Fig. 4.8), although the values were marginally greater in the former treatment of *B*. *nutans*.

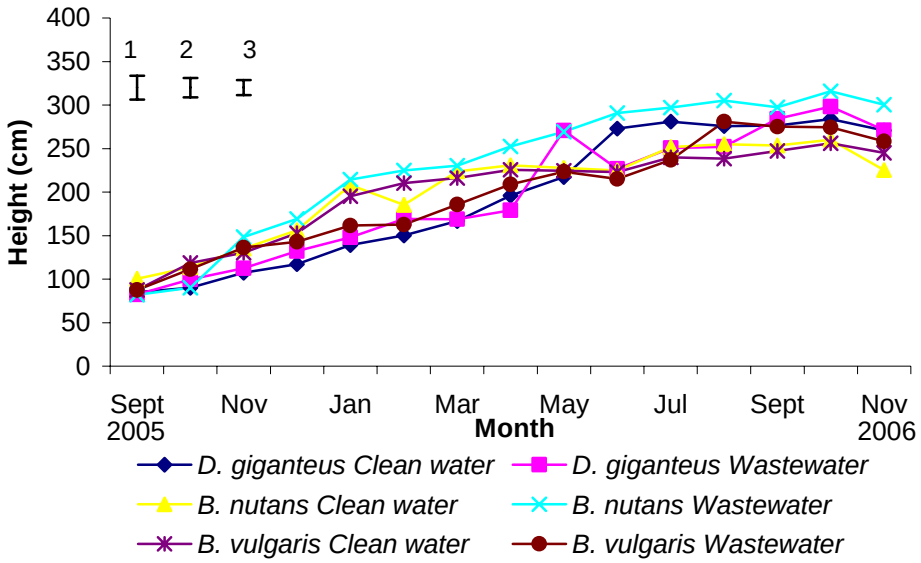


Figure 4.6. Timecourses of plant height for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing measurements for different species, times and irrigation treatments respectively. n=5.

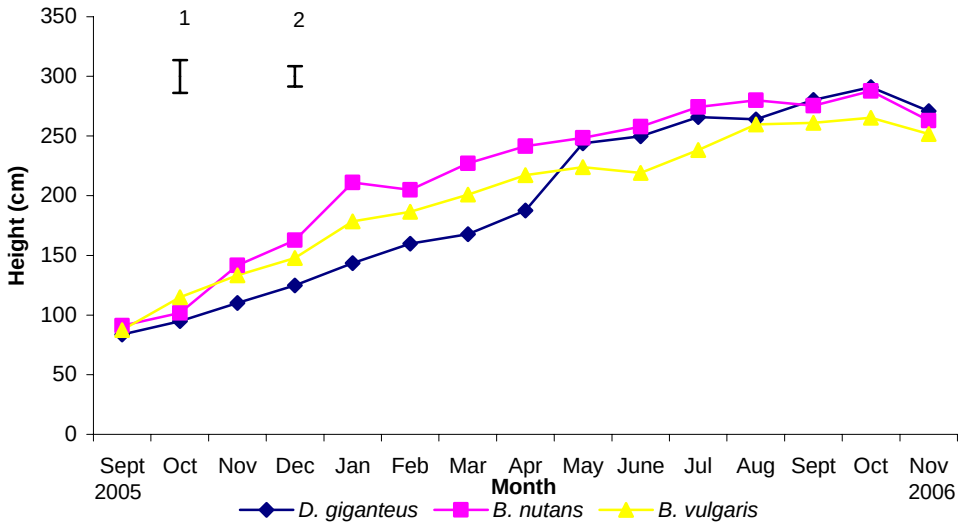


Figure 4.7. Timecourses of mean plant height for each bamboo species averaged over both irrigation treatments. Vertical bars 1 and 2 show SED values for comparing differences between species and with time respectively. n=10.

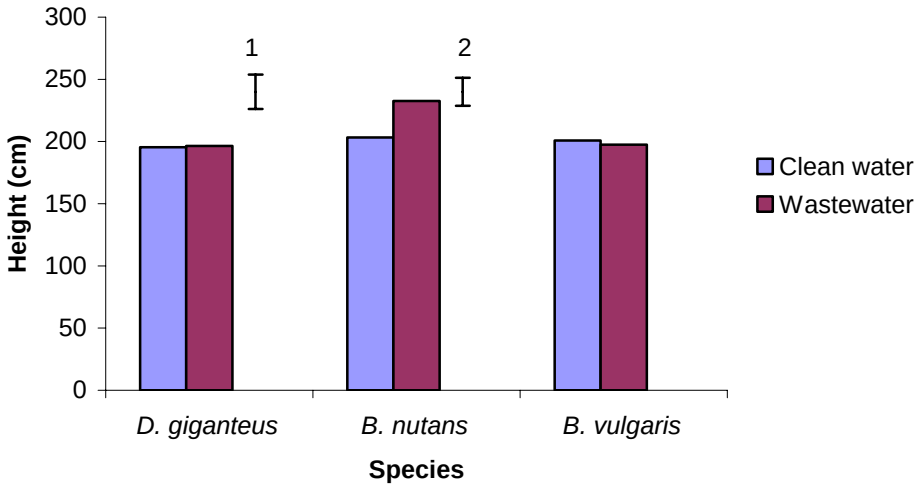


Figure 4.8. Mean plant height for three bamboo species irrigated with wastewater or clean water. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

4.3.3 Collar diameter

Measurements of collar diameter 10 cm above the soil surface did not provide reliable information regarding growth differences between species or with time as new culms emerged from the soil with a specific diameter which persisted for several weeks before decreasing in diameter. Collar diameter did not differ significantly between species over the entire observation period ($P<0.70$; Fig. 4.9), but was greater for plants receiving wastewater than for those receiving clean water during the first four months when averaged over all three species ($P<0.05$); the values were subsequently comparable (Fig. 4.10). The influence of species*irrigation type was not significant ($P<0.311$; Fig. 4.11).

4.3.4 Leaf area

As noted previously, the leaf areas of 10 leaves per plant were estimated non-destructively at monthly intervals between September 2005 and November 2006 from measurements of length and width at the widest point of the leaf blade (*cf.* Section 2.2).

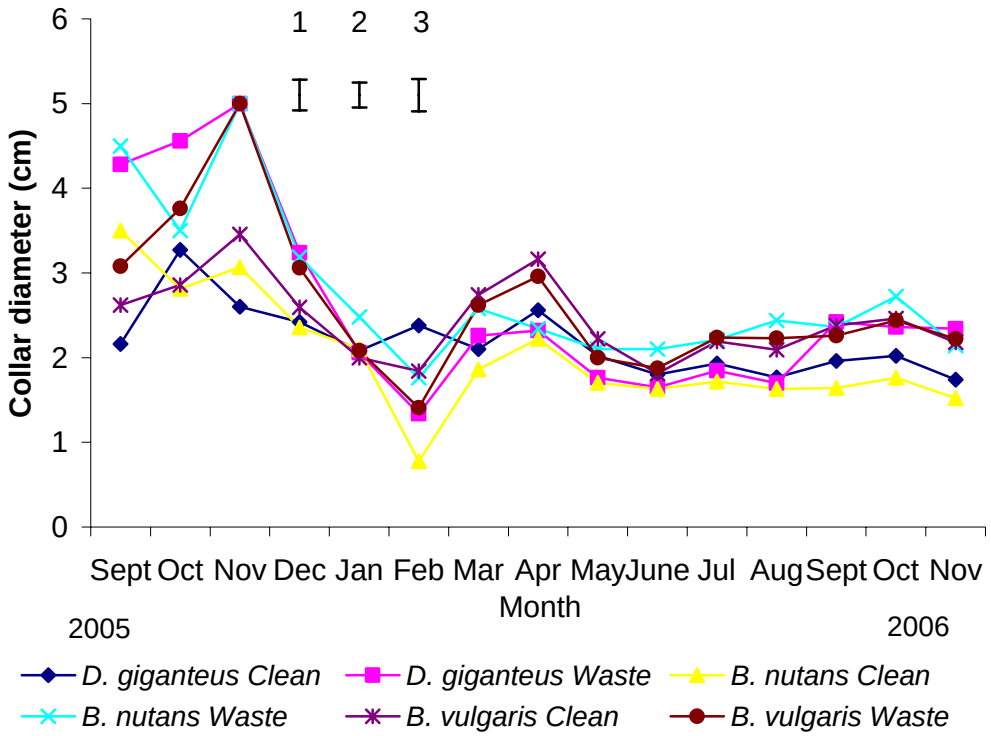


Figure 4.9. Timecourses of mean changes in collar diameter for species*treatment combinations. Vertical bars numbered 1, 2, and 3 show SED values for comparing differences associated with species, irrigation treatment and time.

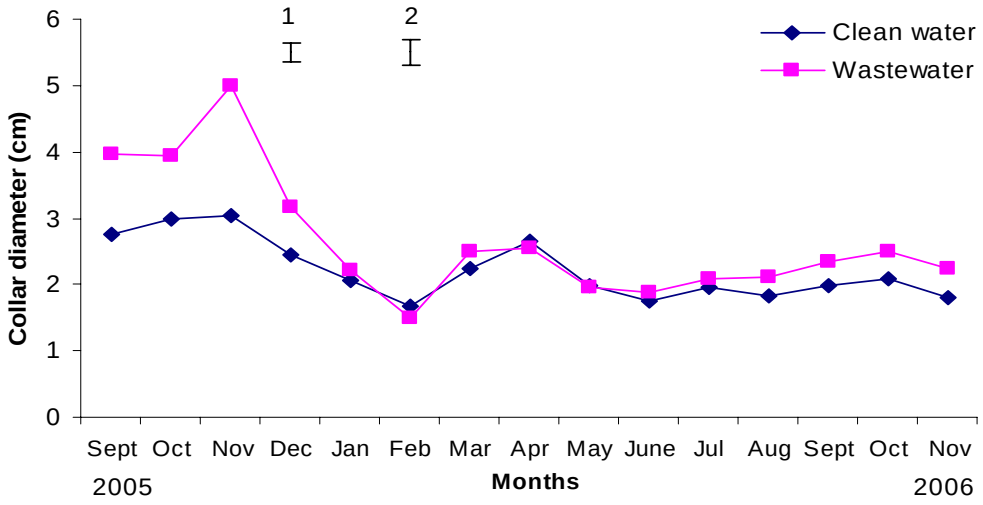


Figure 4.10. Timecourses of mean collar diameter averaged over all three bamboo species for each irrigation treatment. Vertical bars numbered 1 and 2 show SED values for comparing differences associated with irrigation treatment and time.

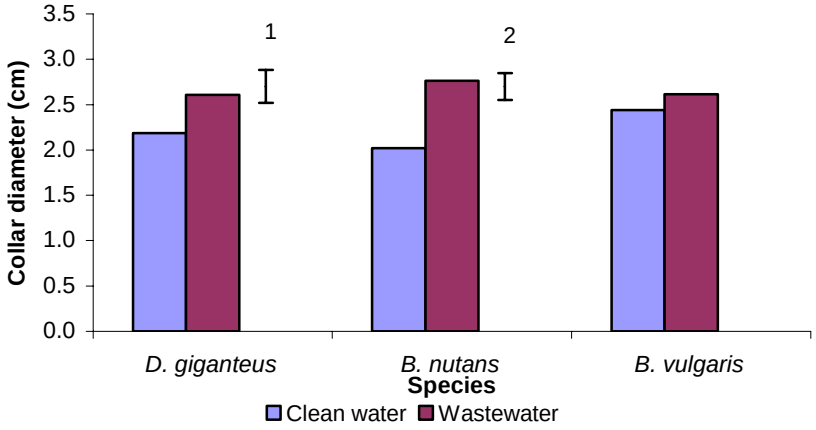


Figure 4.11. Mean collar diameter for three bamboo species irrigated with clean water or wastewater. Vertical bars numbered 1 and 2 show SED values for comparing differences caused by species and type of irrigation treatments.

This procedure was calibrated by passing 200 sample leaves of each species for which length and width had been recorded through a leaf area meter (*cf*. Section 2.2). These measurements revealed significant differences between species and irrigation treatments and with time ($P<0.05$; Fig. 4.12). The area of individual leaves was greatest for *D*. *giganteus*, particularly in the wastewater treatment whereas the values were generally similar for all treatments of *B*. *nutans* and *B*. *vulgaris*. When the values were averaged for all three species, leaf area was found to be greater in plants irrigated with wastewater than in those receiving clean water ($P<0.05$; Fig. 4.13). Similarly, when the values were averaged over both irrigation treatments of each species, the mean individual leaf area for *D*. *giganteus* was more than double the corresponding values for *B*. *vulgaris* and *B*. *nutans* ($P<0.05$; Fig. 4.14), for which the values were comparable.

4.3.5 Branch number

Branch number plant^{-1} increased greatly between September 2005 and October 2006 in all species and both irrigation treatments ($P<0.05$; Fig. 4.15), although there was a slight decline in most treatments between October and November 2006.

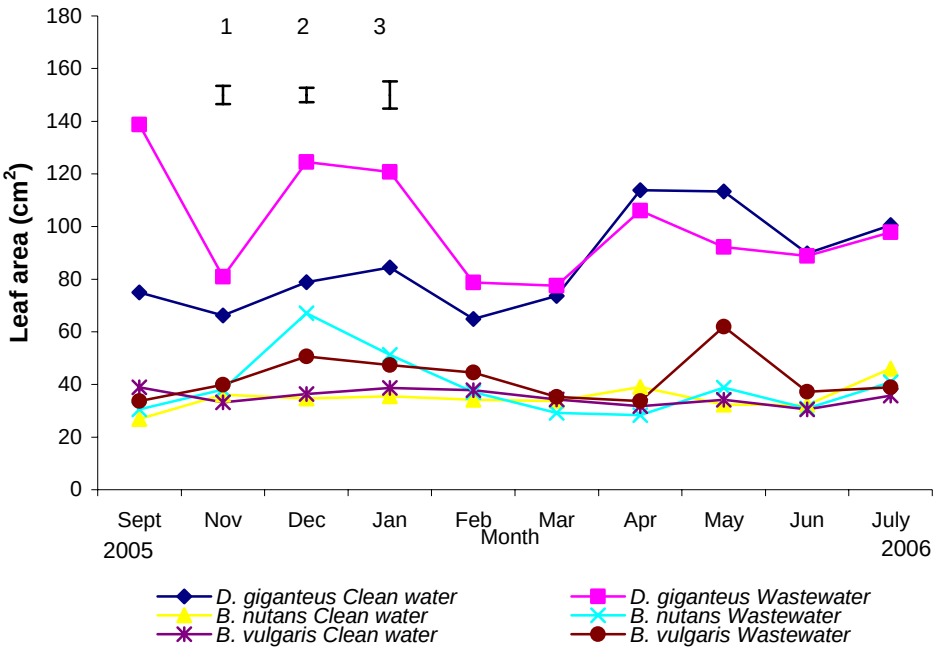


Figure 4.12. Timecourses of individual leaf area for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing measurements for different species, irrigation treatments and times. n=5.

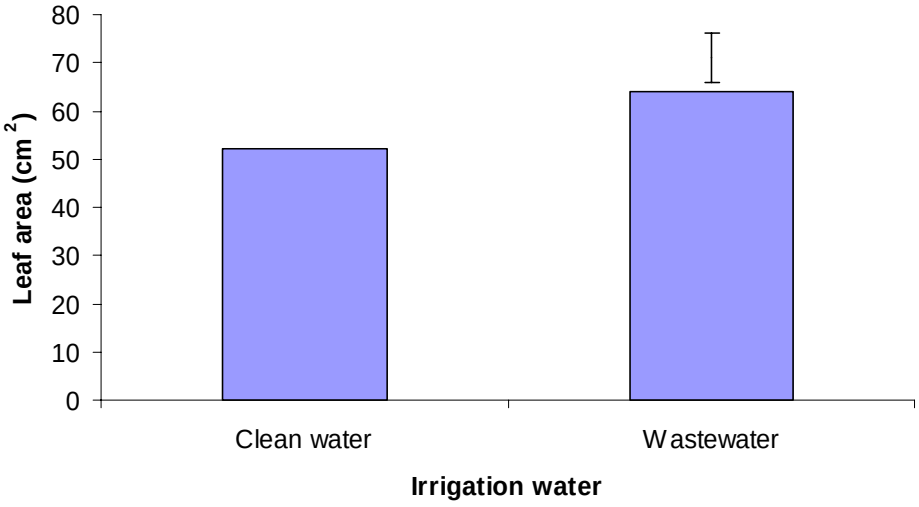


Figure 4.13. Mean individual leaf area for the period from September 2005 to November 2006 of the three bamboo species irrigated with clean water or wastewater. The vertical bar shows the SED value for comparing irrigation treatments. n = 5.

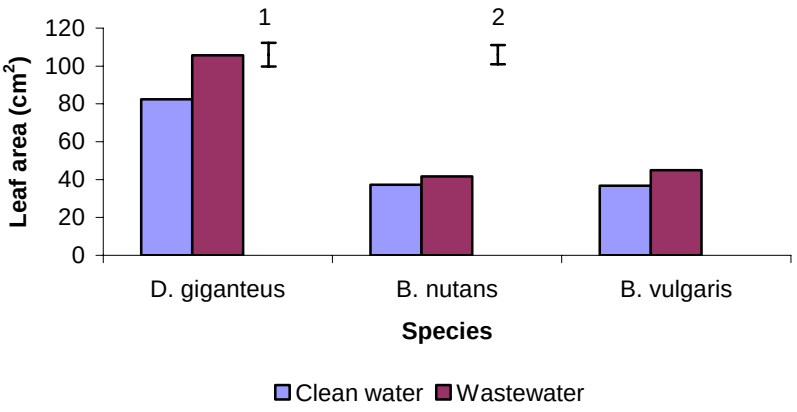


Figure 4.14. Mean individual leaf area for each bamboo species and irrigation treatments for the period between September 2005 to July 2006. Vertical bars 1 and 2 show SED values for comparing measurements for different species and irrigation treatments. n = 5.

Branch number plant^{-1} differed between species ($P<0.05$) but not between irrigation treatments ($P<0.3$; Fig 4.16). Significant species*time, water*time and species* water*time interactions were detected, but there was no interaction between species and irrigation type. Mean branch number plant^{-1} peaked in October 2006 and was greatest in *B. vulgaris*, followed by *B. nutans* and *D. giganteus*. Irrigation with wastewater greatly increased mean branch number compared to plants receiving clean water; the increase in branch number plant^{-1} associated with wastewater treatment was greatest in *B. nutans* (141.8 *vs*. 71.7), followed by *B. vulgaris* (119.4 *vs*. 107.1) and *D. giganteus* (54.6 *vs*. 40.8).

4.3.6 Leaf fresh weight

A significant irrigation treatment*time interaction was detected for leaf fresh weight, as shown by the much greater increase between Harvests 1, 2 and 3 in plants irrigated with wastewater compared to those receiving clean water ($P<0.05$; Fig. 4.17). Clean water values were greatest in *B. vulgaris* at Harvest 1, while the *B. nutans* wastewater treatment was greatest in Harvest 3.

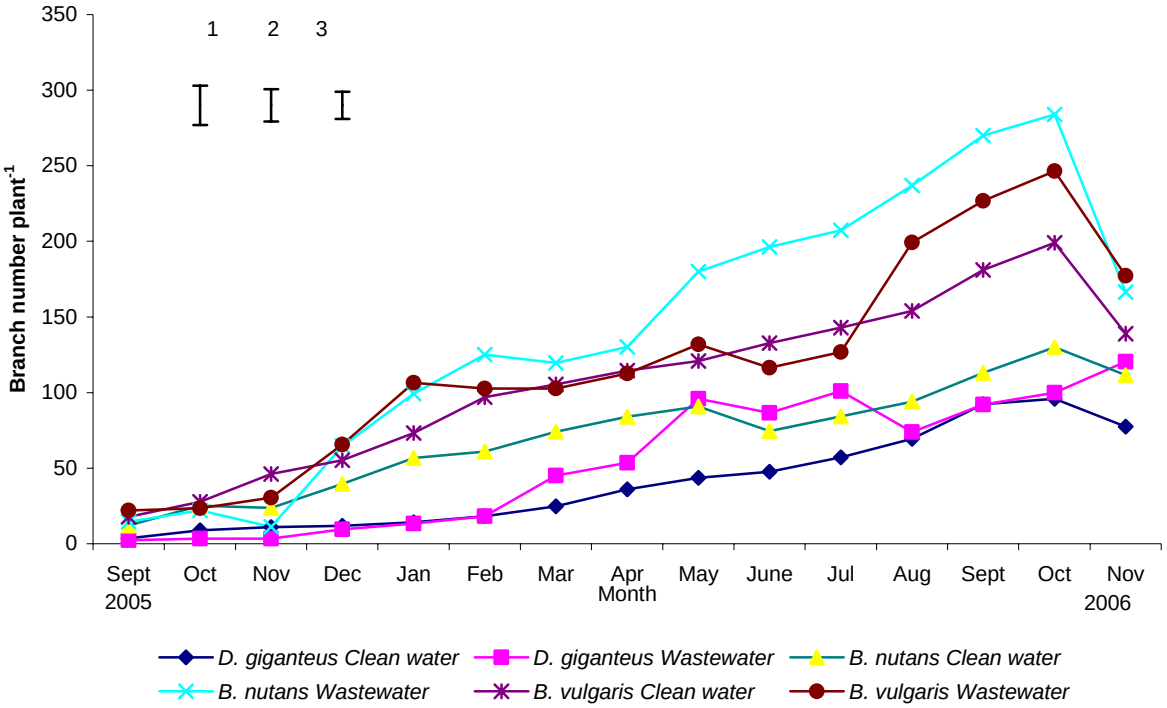


Figure 4.15. Timecourses of branch number for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing measurements for different species, irrigation treatments and times. n=5.

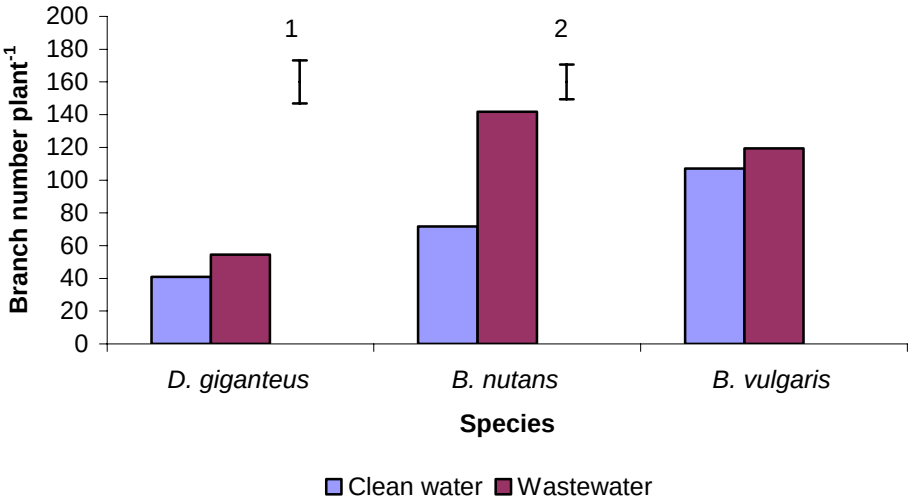


Figure 4.16. Mean branch number for the entire observation period (September 2005 to July 2006) for three bamboo species irrigated with wastewater or clean water. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

Leaf fresh weight plant^{-1} averaged across both irrigation treatments was *c*. 40 % greater in *B*. *vulgaris* and *B*. *nutans* than in *D*. *giganteus* (P<0.05; Fig. 4.17), while the value for the wastewater treatment averaged across all three species was much greater than that for plants receiving clean water in all Harvests (Fig. 4.18). Analysis of the pooled results for both irrigation treatments of all three species confirmed the existence of the significant irrigation treatment*time interaction referred to above (Fig. 4.18) as the increase in leaf fresh weight plant^{-1} with time was much greater in the wastewater treatment (P<0.05).

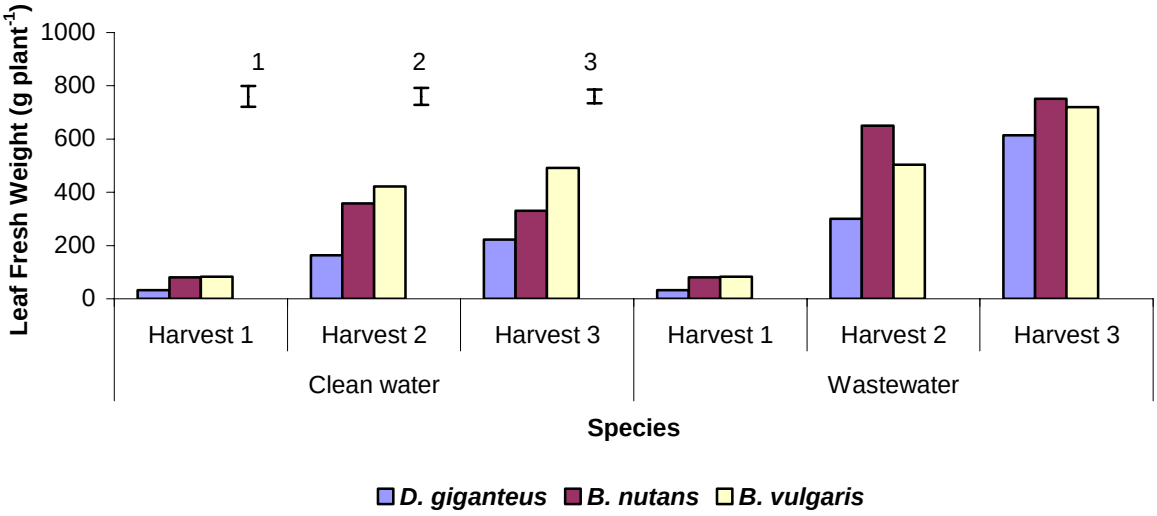


Figure 4.17. Mean leaf fresh weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

4.3.7 Leaf dry weight

Leaf dry weight plant^{-1} increased greatly between Harvests 1 and 2, particularly in *B*. *nutans* and *B*. *vulgaris* (Fig. 4.19), both of which had more numerous and smaller leaves than *D*. *giganteus*. The increase in leaf dry weight plant^{-1} between Harvests 1 and 2 in plants irrigated with wastewater was almost double that in plants supplied with clean water (P<0.05). Leaf dry weight plant^{-1} also increased between Harvests 2 and 3, particularly in the wastewater treatment (P<0.05).

Figure 4.18. Influence of irrigation treatment on leaf fresh weight plant^{-1} averaged over all species for Harvests 1, 2 and 3 (August 2005, July 2006 and November 2006). Vertical bars numbered 1 and 2 show SED values for comparing irrigation treatments and changes with time.

The mean values for both irrigation treatments and all harvests was substantially greater in *B. vulgaris* and *B. nutans* than in *D. giganteus* ($P<0.05$). Calculation of the mean values for all species revealed that irrigation with wastewater increased mean leaf dry weight plant^{-1} by 40 % (307 *vs*. 185 g plant^{-1}; $P<0.05$) between Harvests 2 and 3. There was a significant irrigation treatment*time interaction as the increase between Harvests 1 and 3 was much greater in plants irrigated with wastewater than in those receiving clean water.

4.3.8 Stem fresh weight

A significant species*irrigation interaction was apparent for stem fresh weight plant^{-1} (Fig. 4.21) as the response to irrigation with wastewater shown by *B. vulgaris* was much smaller than those of *D. giganteus* and *B. nutans*. Mean stem fresh weight plant^{-1} averaged across all three harvests was significantly greater in both *B. vulgaris* and *B. nutans* than in *D. giganteus* ($P<0.05$; Fig. 4.22).

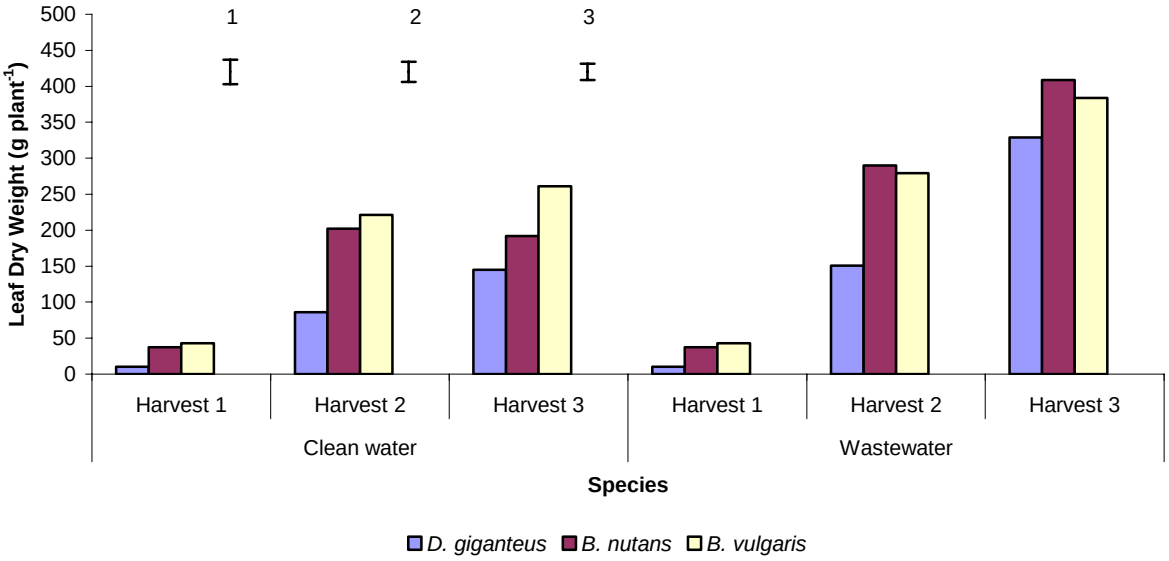


Figure 4.19. Mean leaf dry weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

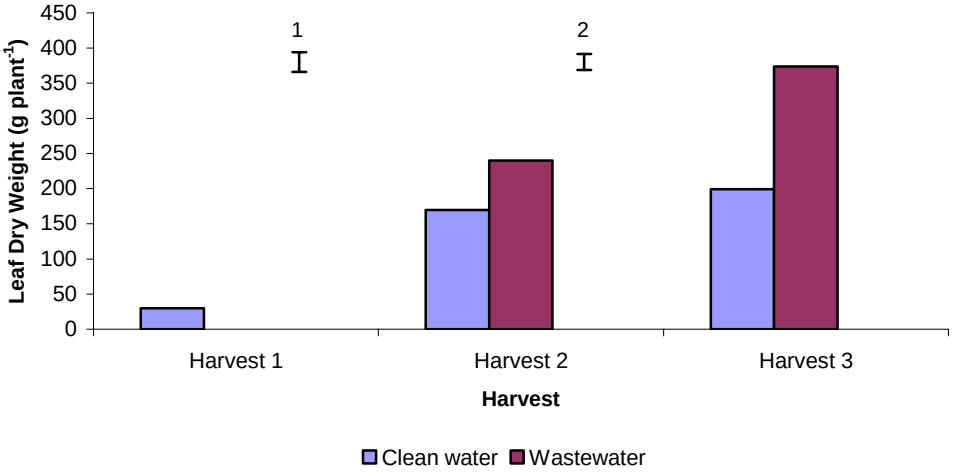


Figure 4.20. Influence of irrigation treatment on leaf dry weight plant^{-1} averaged over all species for Harvests 1, 2 and 3 (August 2005, July 2006 and November 2006). Vertical bars numbered 1 and 2 show SED values for comparing irrigation treatments and changes with time.

When averaged across all species, stem fresh weight plant^{-1} was found to be 30 % greater (960g and 1227 g at H2, and 1040g and 1593g at H3) in plants irrigated with wastewater than in those receiving clean water ($P<0.05$; Fig. 4.23). A significant irrigation treatment*time interaction was

apparent (P<0.05), as shown by the greater increase in stem fresh weight plant^{-1} weight between Harvests 2 and 3 (Fig. 4.23) in plants irrigated with wastewater.

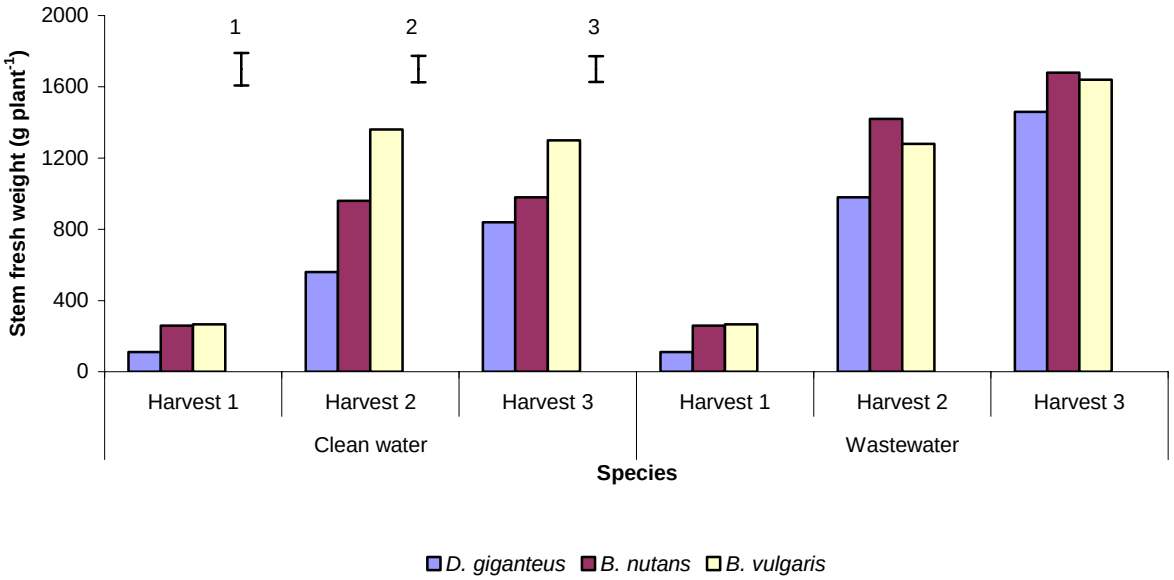


Figure 4.21. Mean stem fresh weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

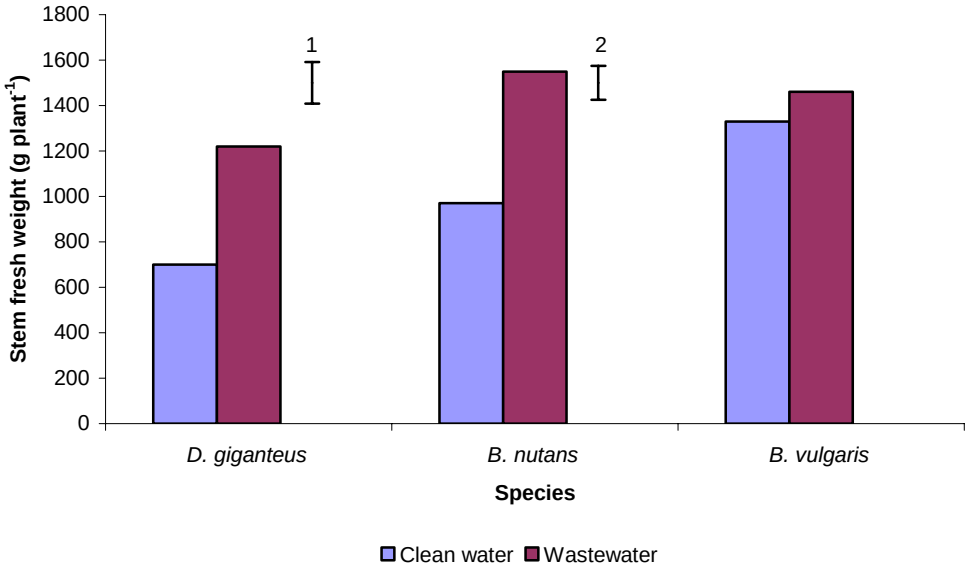


Figure 4.22. Influence of irrigation treatment on mean stem fresh weight plant^{-1} for Harvests 1, 2 and 3 (August 2005, July 2006 and November 2006). Vertical bars numbered 1 and 2 show SED values for comparing irrigation treatments and changes with time.

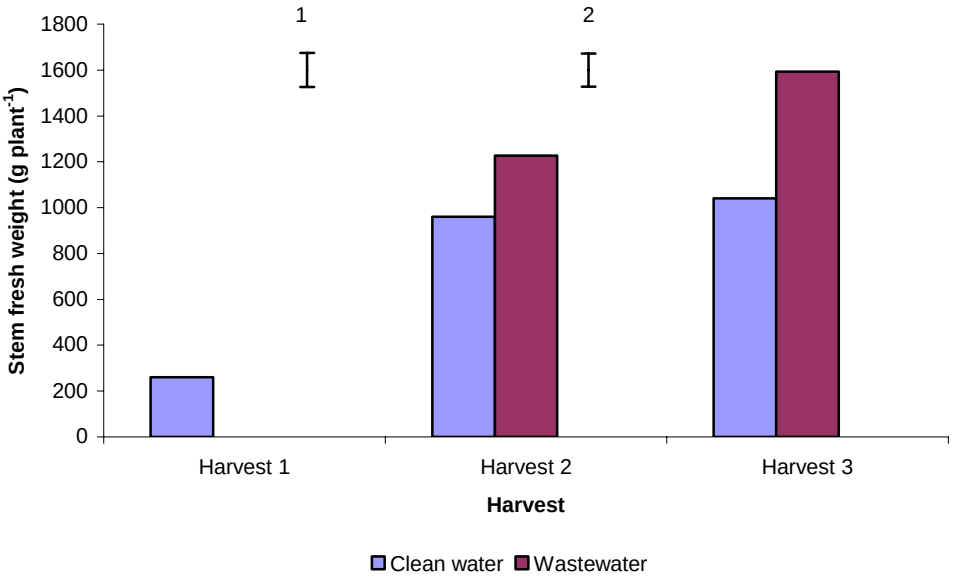


Figure 4.23. Influence of irrigation treatment on stem fresh weight plant^{-1} averaged over all species for Harvests 1, 2 and 3 (August 2005, July 2006 and November 2006). Vertical bars numbered 1 and 2 show SED values for comparing irrigation treatments and changes with time.

4.3.9 Stem dry weight

Stem dry weight plant^{-1} increased between Harvests 1 and 3 and was consistently greater in plants irrigated with wastewater ($P<0.05$; Fig. 4.24). The values were similar in *B. vulgaris* and *B. nutans* and *c.* 34 % greater than in *D. giganteus* (Fig. 4.24). A significant irrigation treatment*time interaction was detected ($P<0.05$), as shown by the much greater increase in stem dry weight plant^{-1} between Harvests 2 and 3 in plants irrigated with wastewater compared to those receiving clean water (Fig. 4.25).

4.3.10 Shoot fresh and dry weight

Total shoot fresh weight plant^{-1} increased greatly between Harvests 1 to 2 in all species and both irrigation treatments, but this was more pronounced in the wastewater treatment for all species ($P<0.05$ Fig. 4.26). Smaller increases occurred between Harvests 2 and 3. A significant treatment*time interaction ($P<0.05$) was apparent as shoot fresh weight plant^{-1} was greatest in *B.*

vulgaris at Harvest 2 but in *B. nutans* at Harvest 3. Total shoot dry weight exhibited a similar pattern (Fig. 4.27).

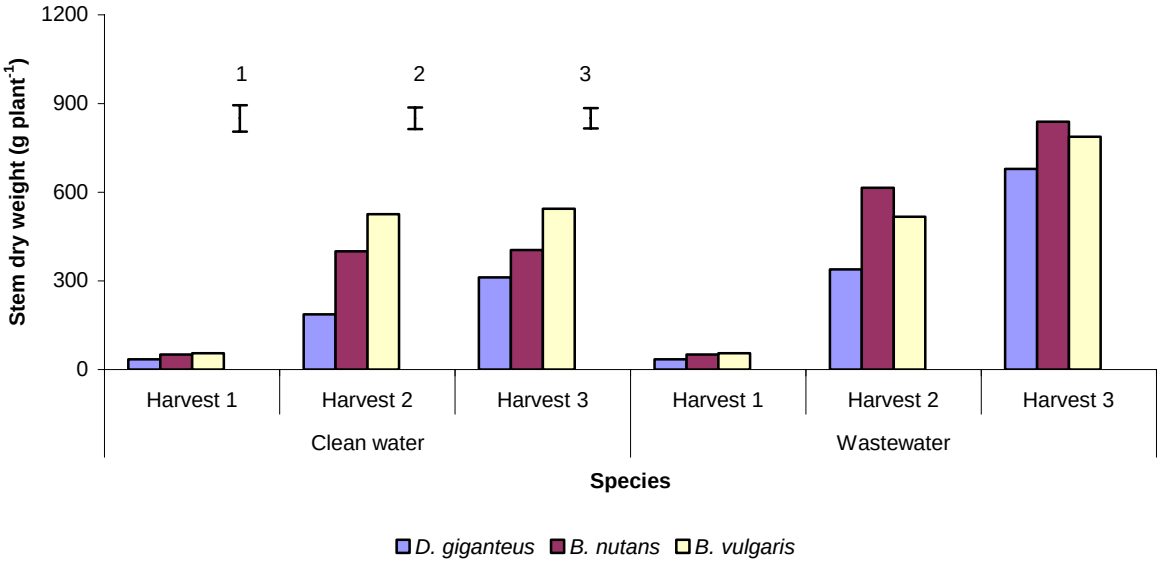


Figure 4.24. Mean stem dry weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

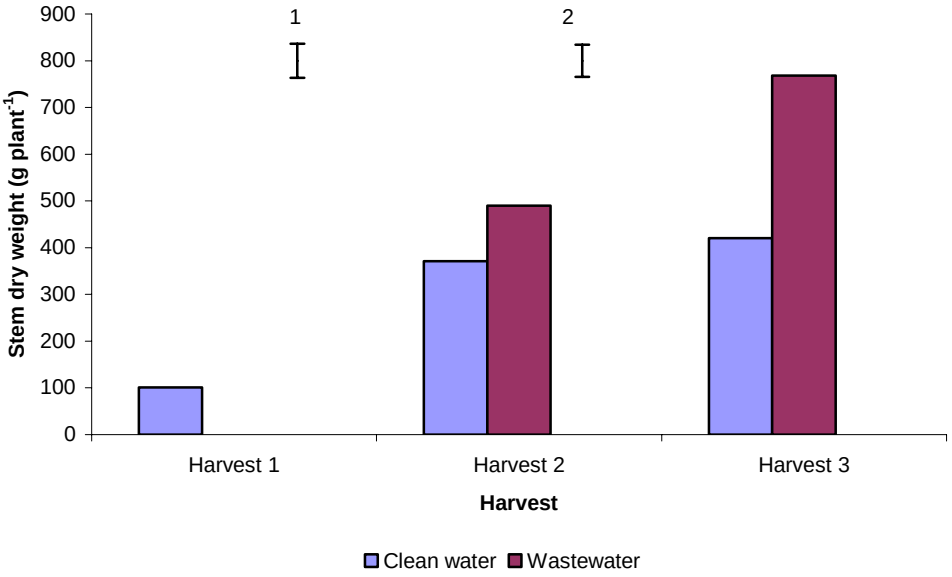


Figure 4.25. Influence of irrigation treatment on stem dry weight plant^{-1} averaged over all species for Harvests 1, 2 and 3 (August 2005, July 2006 and November 2006). Vertical bars numbered 1 and 2 show SED values for comparing irrigation treatments and changes with time.

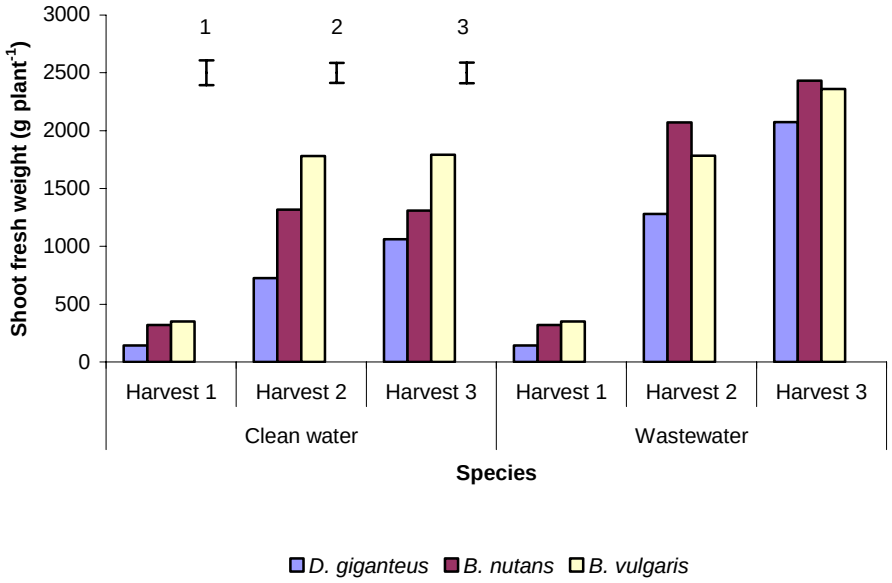


Figure 4.26. Mean shoot fresh weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

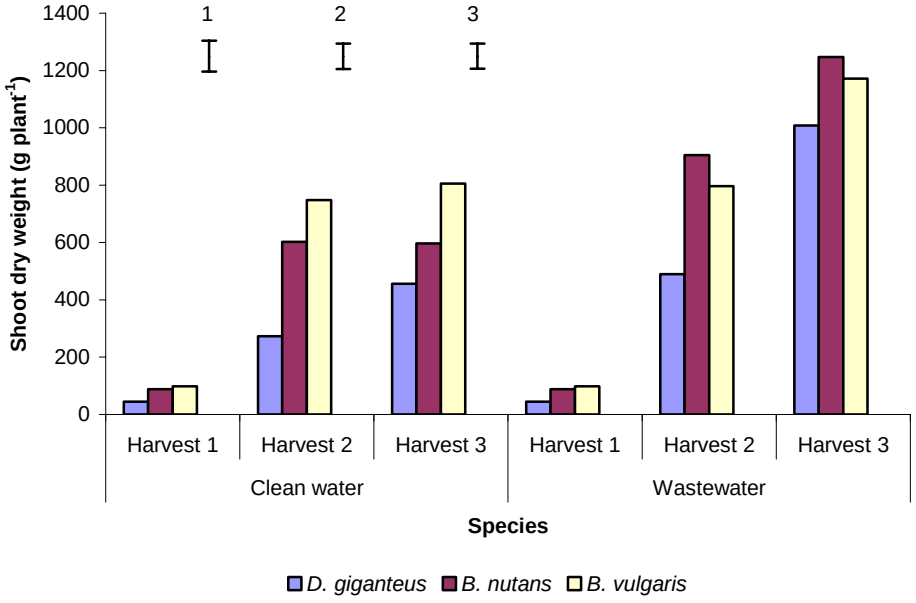


Figure 4.27. Mean shoot dry weight plant^{-1} for three bamboo species irrigated with wastewater or clean water and harvested 1, 9 and 15 months after transplanting to the field experiment (Harvests 1, 2 and 3). Vertical bars numbered 1, 2 and 3 respectively show SED values for comparing species, irrigation treatments and time of harvest.

4.4 Experiment 2

Plant height, branch number plant^{-1}, individual leaf area and green leaf area ratio (proportion of total leaf area which is green) differed between species ($P<0.001$), but not between irrigation treatments (Figs. 4.28-4.31). Plant height was slightly greater in *B. vulgaris* than in the other species in all irrigation treatments (Fig. 4.28) and branch number plant^{-1} was substantially greater than in the other species in all irrigation treatments ($P<0.01$; Fig. 4.29). By contrast, individual leaf area was much lower in *B. vulgaris* than in the other species in all irrigation treatments ($P<0.05$; Fig. 4.30). Green leaf area was significantly reduced in the domestic wastewater treatment of *B. nutans*, but not in the other irrigation treatments ($P<0.05$; Fig. 4.31).

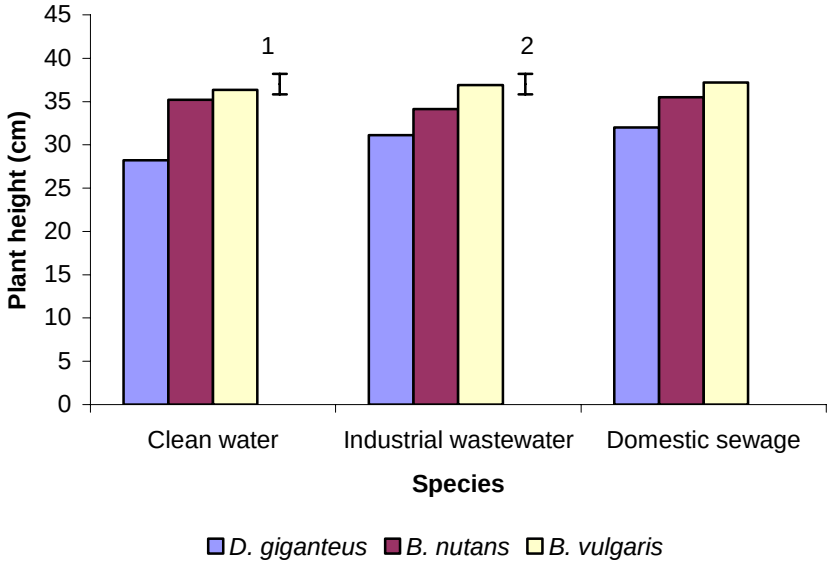


Figure 4.28. Plant height for three bamboo species receiving three irrigation treatments. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

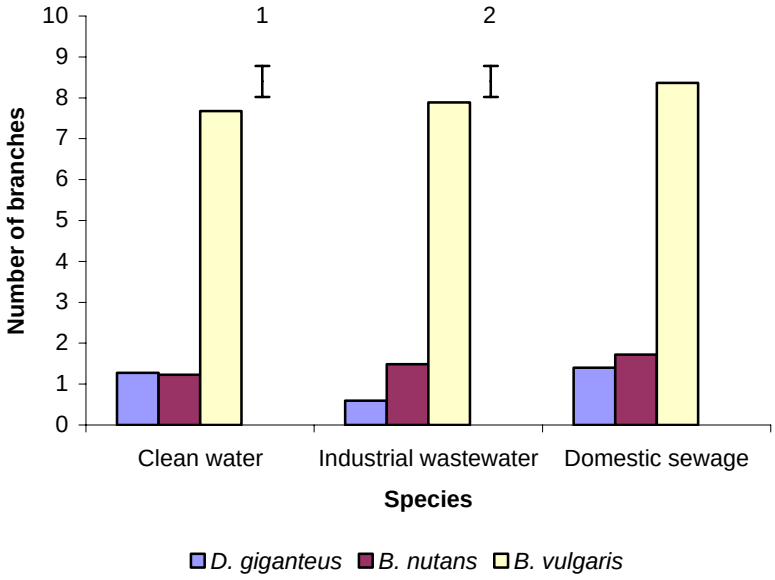


Figure 4.29. Branch number plant^{-1} for three bamboo species receiving three irrigation treatments. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

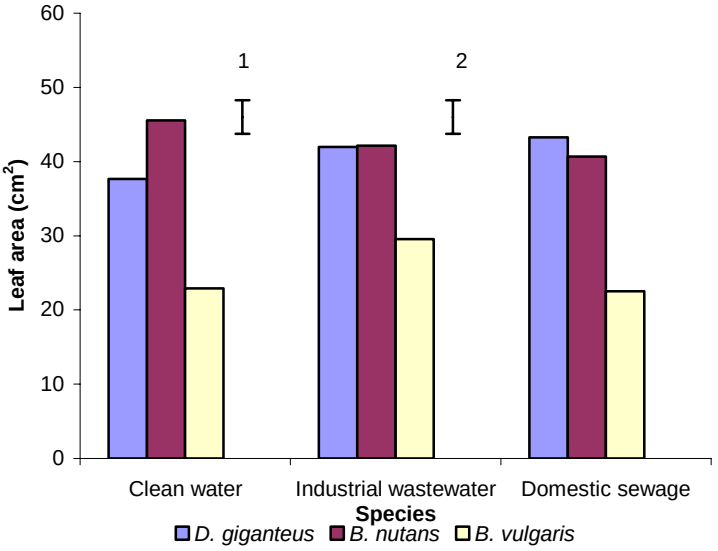


Figure 4.30. Individual leaf area for three bamboo species receiving three irrigation treatments. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

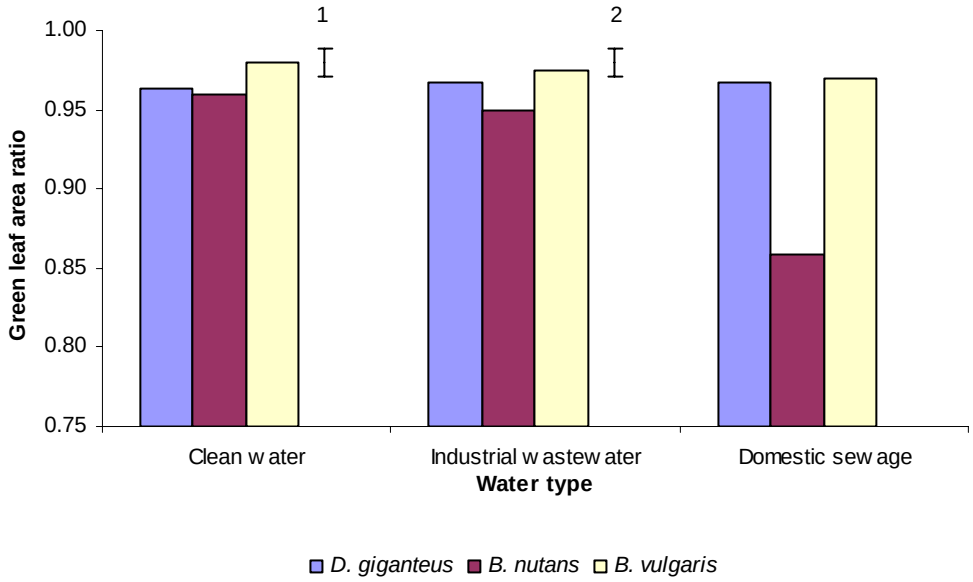


Figure 4.31. Green leaf area ratio plant^{-1} for the three bamboo species receiving three irrigation treatments. Vertical bars numbered 1 and 2 respectively show SED values for comparing species and irrigation treatments.

Table 4.1. Correlation coefficients for the relationship between branch number and selected climatic variables. * denotes significance at P<0.05).

Correlation between branch number and climatic variables						
	Water type	SD	Rain	Radiation	Max. temp.	Mean temp.
D. giganteus	Clean water	-0.34	0.17	-0.41	-0.34	-0.17
D. giganteus	Wastewater	**-0.57***	0.33	**-0.48***	**-0.51***	-0.25
B. nutans	Clean water	-0.27	0.26	-0.22	-0.17	0.03
B. nutans	Wastewater	-0.23	-0.05	-0.35	-0.25	-0.19
B. vulgaris	Clean water	-0.26	0.09	-0.3	-0.23	-0.09
B. vulgaris	Wastewater	-0.16	0.09	-0.22	-0.13	-0.05

Table 4.2. Correlation coefficients for the relationship between plant height and selected climatic variables. No significant effects were detected.

Correlation between plant height and climatic variables						
	Water type	SD	Rain	Radiation	Max. temp.	Mean temp.
D. giganteus	Clean water	-0.48	0.13	-0.49	-0.48	-0.32
D. giganteus	Wastewater	-0.40	0.20	-0.39	-0.35	-0.18
B. nutans	Clean water	-0.32	0.11	-0.21	-0.25	-0.11
B. nutans	Wastewater	-0.38	0.18	-0.30	-0.30	-0.16
B. vulgaris	Clean water	-0.27	0.20	-0.19	-0.16	-0.01
B. vulgaris	Wastewater	-0.41	0.21	-0.36	-0.35	-0.18

Table 4.3. Correlation coefficients for the relationship between leaf area and selected climatic variables. No significant effects were detected.

Correlation between leaf area and climatic variables						
	Water type	SD	Rain	Radiation	Max. temp.	Mean temp.
D. giganteus	Clean water	-0.09	-0.11	-0.18	-0.14	-0.15
D. giganteus	Wastewater	-0.33	0.07	-0.40	-0.50	-0.45
B. nutans	Clean water	-0.37	-0.25	-0.33	-0.52	-0.49
B. nutans	Wastewater	-0.36	0.29	-0.31	-0.37	-0.27
B. vulgaris	Clean water	0.25	-0.69	-0.07	-0.07	-0.42
B. vulgaris	Wastewater	-0.39	0.15	-0.34	-0.34	-0.14

4.5 Discussion

4.5.1 Influence of species on growth

No significant difference in collar diameter was detected between species at any time during Experiment 1, perhaps due to its relatively short 15 month duration. Although bamboo culms rapidly increase in height, their lateral expansion occurs over an extended period (Banik, 1988); the study period may therefore have been too short for any appreciable increase in girth to occur. There were also no significant species*irrigation treatment or species*irrigation treatment*time interactions, although a species*time interaction was detected, indicating that responses to clean and wastewater irrigation varied between species during the study period (*cf*. Figs. 4.9, 4.10 & 4.11). This interaction occurred because *B*. *vulgaris* and *B*. *nutans* initially had smaller collar diameters than *D*. *giganteus* but recovered to regain parity partway through the experimental period (*cf*. Figs 4.10 & 4.11), perhaps because biomass accumulation was higher in the former species. Although an above-ground productivity of 47 t ha^{-1} yr^{-1} has been reported for *Bambusa bambos* (Shanmughavel and Francis, 1996; Scurlock *et al*., 2000), previous literature concerning the dynamics and productivity of natural bamboo stands is almost non-existent.

Bamboos possess a wide range of physiological characteristics (Scurlock *et al*., 2000) and also appear to exhibit differential responses to irrigation with wastewater according to results from the

present study. The increase in branch number observed when *B*. *nutans* and *B*. *vulgaris* were irrigated with wastewater (Figs. 4.15 & 4.16) is consistent with previous reports that irrigation with wastewater may improve tree growth (Singh and Bhati, 2003). Moreover, although these two species had smaller leaves than *D*. *giganteus*, their greater branch number gave them a larger total leaf area. Thus, leaf area also varied significantly between species, being much greater in *D*. *giganteus* than in *B*. *vulgaris* and *B*. *nutans*, and increased with time (Figs. 4.14 & 4.15). Mean leaf area was generally between 57-63 cm^2 $plant^{-1}$ except in November 2005 and February and March 2006, when the values decreased to *c*. 49 cm^2 $plant^{-1}$. The decline in February and March 2006 may have resulted from the prevailing dry conditions between December and March. Leaf area ranged between 58-72 cm^2 $plant^{-1}$ between May and November 2006.

A significant water*time interaction was apparent for leaf area as this was greater in plants irrigated with wastewater than in those receiving clean water between September 2005 and April 2006, whereas the converse applied in May and July 2006 (Figs. 4.12 & 4.13). The significant species*time interaction occurred because leaf area in *D*. *giganteus* was initially more than double that in *B*. *vulgaris* and *B*. *nutans* (Fig. 4.14). A significant species*irrigation treatment*time interaction was also apparent as the values for *D*. *giganteus* were almost three times greater than those for the other two species examined when irrigated with wastewater, whereas the difference between species was much smaller when clean water was applied (Figs. 4.12 & 4.14).

As noted previously, *B*. *nutans* and *B*. *vulgaris* produced more branches and leaves than *D*. *giganteus* and so had a greater leaf area $plant^{-1}$; branch number in *B. nutans* was *c.* 2.5 times greater than in *D. giganteus* (Fig. 4.16). The greater biomass and higher transpiration rates of large plants compared to small plants enhances their suitability for phytoremediation (Westphal and Isebrands, 2001). A fundamental requirement for phytoremediation is the uptake of pollutant elements from the soil, which in turn is closely linked to transpiration (Bolan *et al*., 2005). The

larger transpiring surface areas of *B. nutans* and *B. vulgaris* may therefore enhance their suitability for phytoremediation (Hinchman *et al*., 1996; Environment Canada, 2001).

4.5.2 Influence of irrigation treatments and climatic conditions

Irrigation with wastewater increased shoot fresh and dry weight by 30-40 % compared to plants irrigated with clean water (Figs. 4.26 & 4.27), consistent with observations of a 37 % yield increase in crops irrigated with wastewater (Shende, 1985; Scott *et al*., 2000; Scott *et al*., 2004). Increases in growth of 80-186 % have also been reported for forests irrigated with wastewater (Marilyn *et al*., 1997). In the present study, the increase in height was slightly greater when plants were irrigated with wastewater as opposed to clean water, although the difference was not significant (Figs. 4.8 & 4.9). Irrigation with wastewater has been previously been shown to increase leaf area, biomass production and shoot:root ratio in trees (Guo and Sims, 2000; Thomas *et al*., 2007).

The complex correlation between plant physiological processes and climatic variables is illustrated by the correlation coefficients shown in Table 4.1. The wastewater treatment of *D. giganteus* exhibited the only significant correlation ($P<0.05$) between branch number and SD, solar radiation and maximum temperature; no significant correlations were found for *B. nutans* or *B. vulgaris*, or for plants receiving clean water (Table 4.1). Significant negative correlations between the flowering of trees and rainfall have been reported (Sundarapandian *et al*., 2005). However, no significant correlation between branch number and rainfall or mean temperature was detected for any of the species and treatments examined in the present study, although correlations between branch number and dry weight and water stress have been reported for soybean (Frederick *et al*., 2001); temperature has been shown to influence the ability of trees to recover nutrients from

wastewater (CSIRO, 1995). The data presented in Table 4.1 suggest that the selected climatic variables had little influence on branch number in the three bamboo species examined. The plant height in *D. giganteus* exhibited a closer correlation with radiation, SD and maximum temperature than in *B. vulgaris* and *B. nutans* (Table 4.2), although this was not significant. All three species exhibited non-significant negative correlations for these variables, with correlation coefficients ranging between 0.26-0.44. No significant correlation between plant height and rainfall or mean temperature was found for any of the species and treatments examined. Similarly, no significant correlation between leaf area and the selected climatic variables was detected (Table 4.3), although many showed weak negative relationships, an observation consistent with the absence of any significant correlation between leaf area and rainfall for 42 tree species in India (Sundarapandian *et al*., 2005).

While leaf area apparently exhibited a weak negative correlation with rainfall in plants irrigated with clean water and a positive correlation in plants supplied with wastewater, these differences were not significant. The observation that leaf area plant^{-1} was greater in the wastewater than in the clean water treatment for all species ($P<0.05$; Fig. 4.13) is consistent with findings that leaf area in eucalyptus trees irrigated with effluent was almost double that of control trees (Pereira and Chaves, 1993; White *et al*., 1994; Chaiprapata and Sdoodee, 2007). Such an increase in leaf number and area would be expected to increase both photosynthesis and biomass (Guo, 1998).

The observation that irrigation with wastewater increased plant height, branch number, leaf area plant^{-1} and biomass production in all species relative to plants receiving clean water is consistent with previous reports (Sopper, 1980; White *et al*., 1994). Similar improvements in growth were observed when tomatoes were irrigated with wastewater of varying strengths (Al-Lahham *et al*., 2002), probably due to its relatively high concentration of essential nutrients. Kiziloglu *et al*., in 2007, also reported that wastewater increased the yield and N, P, K, Fe, Mn, Zn, Cu, B and Mo

concentrations in cabbage plants. The wastewater used in the present experiment contained 6.5 mg kg^{-1} Zn, 536 mg kg^{-1} K, 84 μg L^{-1} P and 1.6 % N, while the clean water was almost nutrient-free (*cf*. Section 8.3.4). The daily irrigation would have led to an accumulation of nutrients in the soil and hence the plants, potentially improving growth. As the movement of nutrients from soil to plant, and the elemental content of bamboo rhizomes were not analysed, it was not possible to make any firm conclusions regarding nutrient accumulation.

Perhaps because the bamboo plants were grown under irrigation, and rainwater was excluded from the tanks in which they were grown, their growth characteristics were poorly correlated with rainfall conditions. Possible reasons why growth was poorly correlated with climatic conditions may be that, although wastewater may increase productivity by improving water and nutrient supplies, it may also reduce growth by inducing salinisation and toxic effects, increasing the leaching of nutrients and reducing soil oxygen levels (Johns and McConchie, 1994). The wastewater used in Experiment 1 contained 873 mg L^{-1} Na (Table 7.1) or 1.4 dS/m, and irrigation with it would be expected to cause a 10% reduction in yields in many crops (Evans, 2006). As the mechanisms underlying responses to salt stress are not yet fully understood, it is unclear whether decreases in transpiration induced by salinity are the cause or the consequence of reduced growth (Shalhevet, 1994). If increases in metabolism or decreases in photosynthesis associated with osmoregulation or ion toxicity are responsible for reducing growth, decreases in transpiration are a secondary effect, whereas reductions in transpiration may be the cause of the reduced growth if decreases in osmotic potential in the soil reduce water uptake; in other words, salinity may induce a form of drought stress (Karlberg, 2002). It was observed that a new flush of leaves was produced following the onset of the rains, although this may have been associated with the decline in temperature during the rainy season, as has been reported in a study of 42 tree species in India (Sundarapandian *et al*., 2005). *D. giganteus*, which has much broader leaves than the other species

examined, appeared to be most sensitive to climatic variables, although the reason for this is unclear as *B*. *vulgaris* and *B*. *nutans* both had much greater total leaf areas because of their greater branch numbers.

The results for plant height, branch number, leaf area and green leaf area ratio in Experiment 2 revealed significant differences between species ($P<0.001$), but not between irrigation treatments (Figs. 4.28-4.31), possibly because the experiment duration was too short to elicit their full effects. Irrespective of irrigation treatment, plants of *B*. *vulgaris* were taller and had a greater branch number and green leaf area ratio than the other species examined ($P<0.05$). However, individual leaf area was smaller in *B*. *vulgaris* than in *B*. *nutans* and *D*. *giganteus*, which might reduce any potential competitive advantage over the other two species; measurements of total leaf area plant^{-1} would have provided a more reliable basis for comparison. Root growth in the relatively young plants examined in Experiment 2 might not have reached a point where the roots were absorbing sufficient quantities of nutrients and water to promote detectable differences in above-ground growth. Uptake of water and nutrients would also have been limited by the sparse canopy development in these relatively young plants, a potentially important factor as Maoyi, in 1998, reported a strong physiological integration between culms and rhizomes in bamboo. The quantity of resources devoted to below- and above-ground development during the early stages of bamboo growth depends on growth stage and inter-specific variation (Stern *et al*., 1999), and may reflect changing growth strategies or ecological factors which influence population dynamics at different growth stages. This, in turn, may have influenced inter-specific responses to the three irrigation treatments applied as growth in bamboo initially favours rhizome development. As far as phytoremediation is concerned, larger and more well established plants might be expected to give better results.

In both Experiments 1 and 2, the concentrations of nutrients and heavy metals supplied in the irrigation water were well below those expected to adversely affect growth in bamboo (Tables 7.1, 7.6 and 7.11). A much longer period of irrigation would have been necessary for significant accumulation of nutrients and heavy metals in the soil and consequently in the shoot tissues.

CHAPTER 5

SOIL MOISTURE BALANCE

5.1 Introduction

When irrigation is applied, it is essential to ensure that sufficient, but not excessive, soil moisture is available to support plant growth (Greenland *et al*., 1986). Inadequate and excessive soil moisture may both induce stress conditions and adversely affect plant growth (Madakadze and Kwaramba, 2004). Plant water status depends on the balance between the absorption and transpiration of water and the two processes are intimately interlinked. Excess soil moisture is likely to induce oxygen deficiency within the rooting zone, reducing the absorption of water and hence transpiration and leading to consequences resembling those of inadequate soil moisture, adversely affecting nutrient uptake and root growth (Lombardini, 2006). When insufficient water is available, normal plant functions are perturbed and plants gradually wilt, cease growth and eventually die if the stress is not alleviated. The great majority of the water absorbed is not permanently retained within plants, but instead is transpired to the atmosphere. The soil environment, plant physiological characteristics and atmospheric conditions interact to determine the rates of absorption and transpiration (Qiang *et al*., 2004). In the present study, soil moisture content was routinely determined to ensure the experimental plants received sufficient water to avoid compromising the primary experimental objectives.

5.2 Materials and methods

All plants received 7 L d^{-1} of tap water or wastewater from the University sewage treatment ponds (*cf*. Section 2.2.3). Access tubes for the PR2 Soil Moisture Probe (Delta T Devices, Cambridge, UK) which was used to estimate soil moisture content were installed in selected pots in both irrigation treatments of all bamboo species before imposing these treatments (*cf*. Section 2.2.9).

The regular measurements made during the observation period involved inserting the PR2 Probe into each access tube in turn and recording percentage soil moisture content for each measurement depth; full details are given in Section 2.2.9.

5.3 Results

5.3.1 Soil moisture meter calibration

The procedure for calibrating the soil moisture meter was described in Section 2.2.10. Moisture meter readings were taken daily for 10 d in 100 L tanks containing forest soil (andosol) which had been saturated with water; excess water was allowed to drain from the base, which was raised above ground level, as noted previously. The correlation between meter readings and soil core measurements was significant ($P<0.05$; Table 5.1; Fig.5.1). The high moisture contents recorded are normal for andosols.

Table 5.1. Relationship between moisture meter readings and volumetric soil moisture content.

Depth (cm)	V*	Moisture metre
20	37.0	41.4
20	36.1	35.9
20	38.7	39.3
20	36.4	42.3
20	37.1	36.1
20	39.8	41.5
60	39.4	98.3
60	40.0	86.6
60	39.3	88.2
60	38.9	100.0
60	39.7	87.6
60	37.6	87.6

* Volumetric soil moisture
Correlation: 0.67* ($P<0.05$)

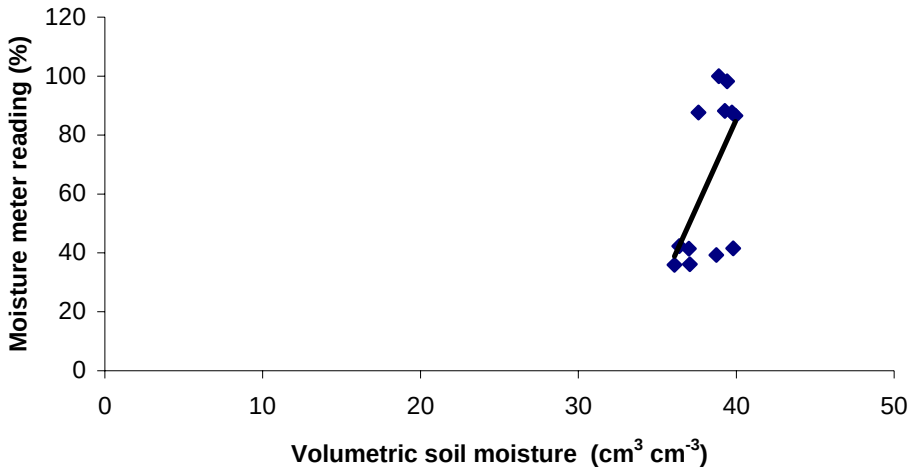


Figure 5.1. Calibration of soil moisture meter values.

5.3.2 Treatment and species effects on volumetric soil moisture content

The observation that volumetric soil moisture content showed no significant variation with time between May and November 2006 in all treatments (Figs. 5.2, 5.3) suggests that soil water availability remained unchanged during this period.

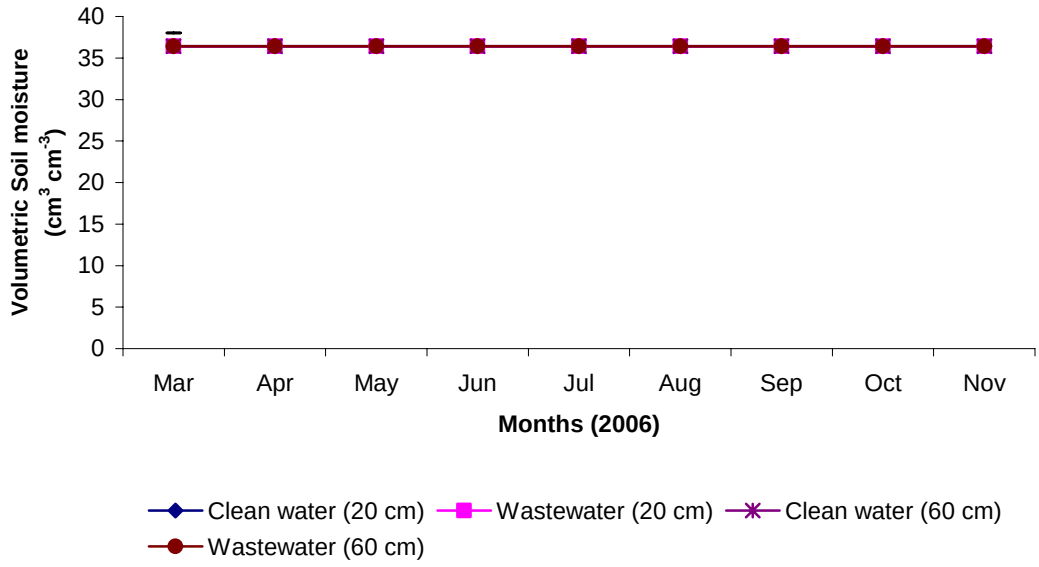


Figure 5.2. Timecourses for soil volumetric water content for the clean and wastewater treatments water at depths of 20 and 60 cm between March and November 2006. Values are the means for all three bamboo species. The vertical bar shows the SED value for comparing irrigation treatments.

There was a small, but significant, difference in volumetric water content (VWC) between the 20 and 60 cm soil depths (P<0.001; Fig. 5.2), which ranged between 32.8 and 32.9 cm^3 cm^{-3}. Differences in VWC between the clean and wastewater treatments were not significant between March and November 2006 (P<0.963), whereas those between the 20 cm and 60 cm depths were significant (P<0.001); values ranged between 35 and 37 cm^3 cm^{-3} (Fig. 5.3). Soil moisture content differed significantly between species between March and November 2006 (P<0.001; Fig. 5.5), being lowest in *B. vulgaris* and greatest in *D. giganteus*. The values for VWC measured at a depth of 20 cm were lower than at 60 cm for all species (P<0.001); this difference was greatest in *D. giganteus*, followed by *B. nutans* (Fig. 5.6).

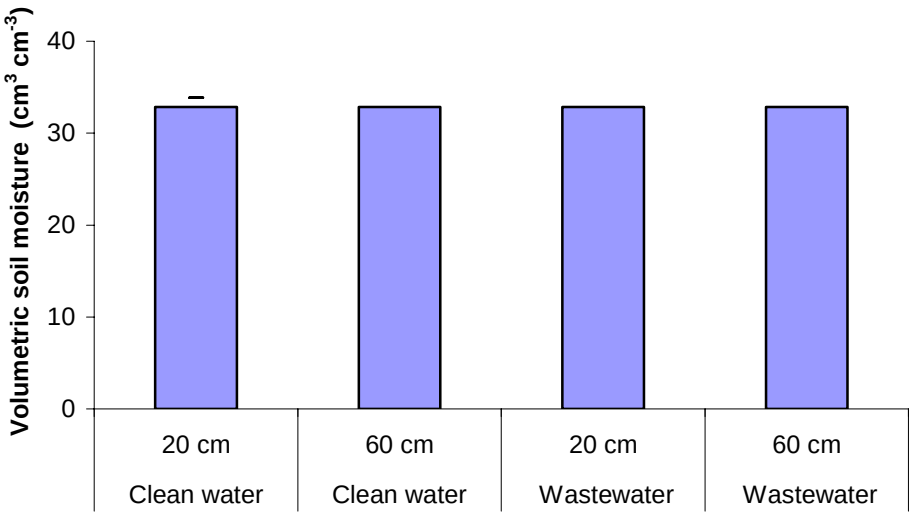


Figure 5.3. Volumetric soil water content for clean and wastewater treatments at depths of 20 and 60 cm between May and September 2006. The vertical bar shows the SED value for comparing measurement depths.

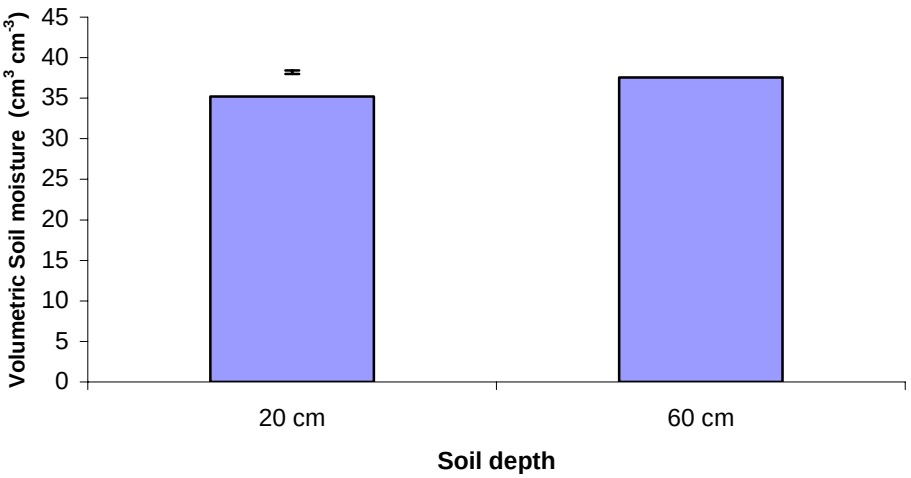


Figure 5.4. Mean volumetric water content at soil depths of 20 and 60 cm between March and November 2006. The vertical bar shows the SED value for comparing measurement depths.

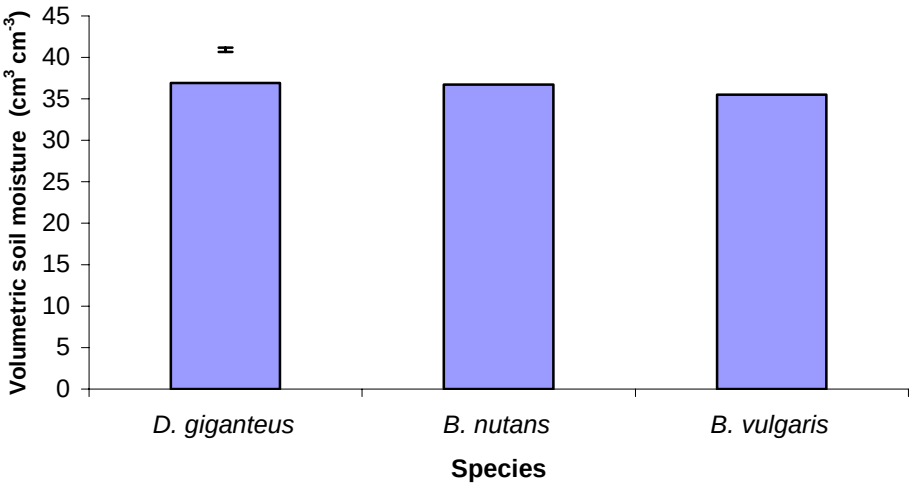


Figure 5.5. Mean volumetric water content for three bamboo species between March and November 2006. The vertical bar shows the SED value for comparing species.

VWC showed small but significant differences ($P<0.001$) between irrigation treatments and soil depths (Fig. 5.6), with lower values being recorded for clean water at a depth of 20 cm and higher values at 60 cm compared to soil irrigated with wastewater.

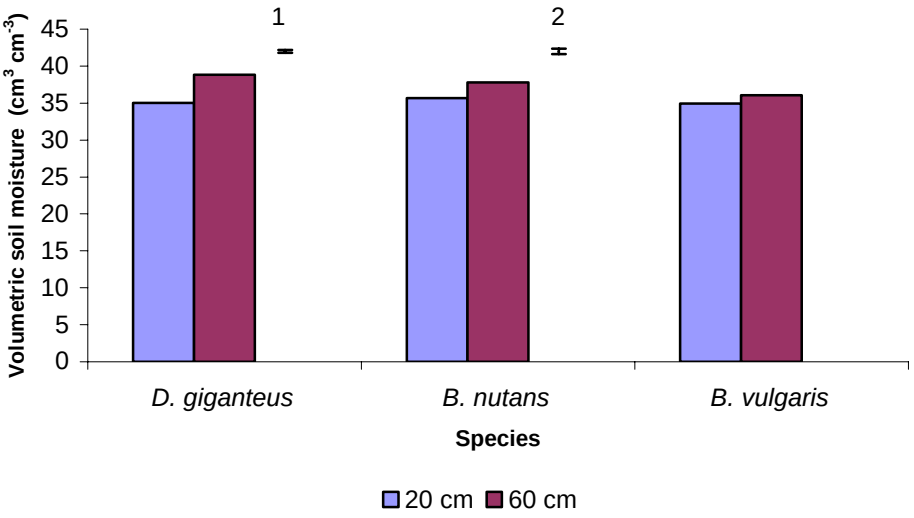


Figure 5.6. Mean volumetric water content for the three bamboo species at two soil depths (20 and 60 cm) between March and November 2006. The vertical bars numbered 1 and 2 show the SED values for comparing species and soil depths.

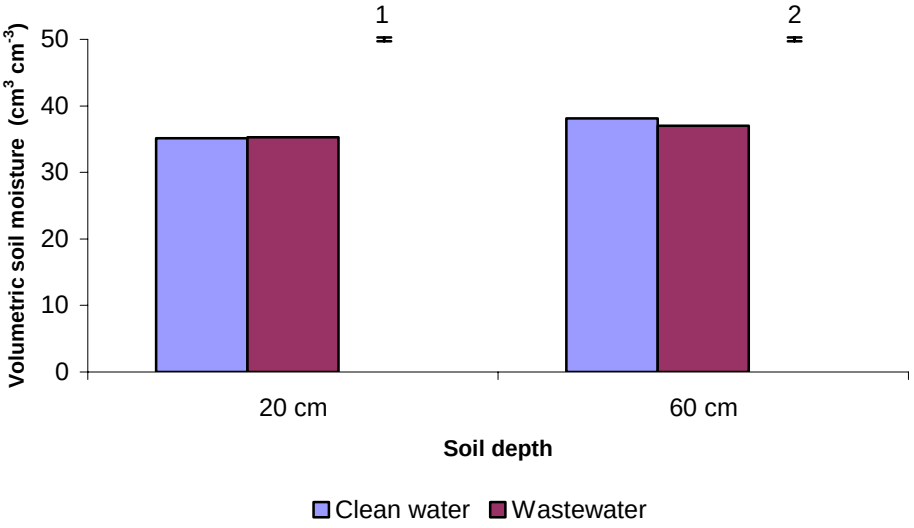


Figure 5.7. Mean volumetric water content for both irrigation treatments at two soil depths (20 cm and 60 cm) between March and November 2006. The vertical bars numbered 1 and 2 show the SED values for comparing irrigation treatments and soil depths.

5.4 Discussion and conclusions

Soil moisture is predominantly held in the pores between soil particles, but may also be held as thin films around individual soil particles in the form of hygroscopic water, which is too firmly held to be available for uptake by plants (Okalebo *et al*., 2002). Andosols, which include forest

soils, are highly organic, and can hold more than their own dry weight in moisture content (Sombroek *et al*., 1982). In the present study, there was also the possibility that standing water might collect at the base of the tanks due to impeded drainage, causing the soil within the tanks to become saturated. The pits in which the tanks were installed were lined with polythene sheeting to prevent entry of water from the water table, but this might also have prevented the drainage of excess water. Accumulation of water at the bottom of the tanks might therefore have occurred during rainy periods when transpiration and hence absorption were low.

It is important to use reliable methods to confirm that water supplies to plants are neither insufficient nor excessive as either may obscure the effects of the primary treatments being examined (Fares and Alva, 2000). Proper calibration and use of the soil moisture meter (Masahiro and Masharu, 2002) is vital in such experiments to avoid drought stressing the plants or creating waterlogged conditions. This is especially important in pot experiments where water status changes more rapidly than under field conditions (Earl, 2003). The measurements of soil moisture content made for calibration purposes would have been more representative of low soil moisture conditions if the drying period had extended over more than 10 days and had taken account of the seasonal variation in weather conditions as July, when these measurements were made, is also the coldest month in Juja.

The bamboo species which produced a greater leaf biomass (*B. vulgaris and B. nutans*) also exhibited a correspondingly lower soil moisture content (Fig. 5.6). It is likely that their greater leaf area enabled these species to transpire more rapidly than *D. giganteus*, which had broader but fewer leaves. The measurements of VMC showed no significant variation with time, but differed significantly between soil horizons. Thus, VMC increased with soil depth, possibly reflecting the effects of surface evaporation, drainage and uptake of water from the surface horizons by the roots

of bamboo. There was also no significant difference in VMC between the clean and wastewater treatments, suggesting that the plants in both treatments received similar quantities of water.

CHAPTER 6

GAS EXCHANGE AND SPAD MEASUREMENTS

6.1 Introduction

Gas exchange by plants is influenced by many factors, both internal and external, and is conditioned by biotic and abiotic factors such as drought and disease (Valladares *et al*., 2007). The prevailing atmospheric conditions, including solar radiation, wind, temperature and saturation deficit, determine the potential rate of gas exchange, although the actual rate is strongly dependent on stomatal conductance, which in turn depends on stomatal density and aperture. Plant nutritional status and environmental conditions such as soil water content, aeration and pH are important factors influencing stomatal opening and water flow through plants (Bingham, 2001). These and other phenomena are important in the regulation of CO_2 and O_2 exchange.

6.2 Materials and methods

In Experiment 1, *Dendrocalamus giganteus*, *Bambusa vulgaris* and *B*. *nutans* were grown in 100 litre tanks in a factorial design with an initial population of 75 plants (*cf*. Section 2.1.4). Five plants of each species were harvested in August 2005 immediately before the irrigation treatments began to establish base-line data for key growth variables. The remaining 60 plants were grown until July 2006, when Harvest 2 was made. Harvest 3 was carried out in November 2006. Between Harvests 1 and 3, half of the plants were watered with tap water, while the remainder were irrigated with wastewater from the University sewage treatment ponds (*cf*. Section 2.1.4). Air temperature, relative humidity, solar radiation and rainfall were measured daily using standard meteorological equipment (*cf*. Section 2.1.2). In Experiment 2, 351 plants were arranged in three blocks each containing 39 plants, replicated three times. The plants were *c*. two months old and 30 cm tall when planted out (*cf*. Section 2.2.3).

6.3 Results

6.3.1 Experiment 1

The results obtained were sub-divided into three separate datasets for the period prior to Harvest 2 between January-June 2006, then from August-October 2006, and finally from July-November 2006. The first two datasets involved measurements of one mature leaf for all plants while the final dataset included measurements for leaves located in the lower, middle and upper portion of the canopy.

6.3.1.1 Species

Stomatal conductance differed between species, irrigation treatment and leaf position in the canopy between January and June ($P<0.05$; Figs. 6.1 & 6.2) and significant species*irrigation water*time interactions was found between January-June 2006 ($P<0.05$; Fig. 6.2) and August-November 2006 (Fig 6.3; $P<0.017$); the values for the wastewater treatment of *B. vulgaris* and *B. nutans* were particularly high in March 2006 (Fig. 6.2).

Although the differences in transpiration rate between species, water and leaf position were not significant between July-November 2006 (Fig. 6.4; $P<0.07$), a significant species* irrigation water *time interaction was detected ($P<0.05$; Fig. 6.5) as the values for the wastewater treatment of *B. vulgaris* and *B. nutans* were high in March 2006. Transpiration rate decreased with time ($P<0.001$) in all species and both irrigation treatments after April 2006. Transpiration rate also exhibited a significant species*time* irrigation water interaction ($P<0.05$; Figs. 6.4, 6.5 & 6.6) because the values for the clean water treatment of *G. giganteus* and wastewater treatment of *B. nutans* declined rapidly between August and October 2006, whereas those for the other treatments

exhibited smaller or no responses; no further change was observed between October and November 2006 (Fig. 6.5).

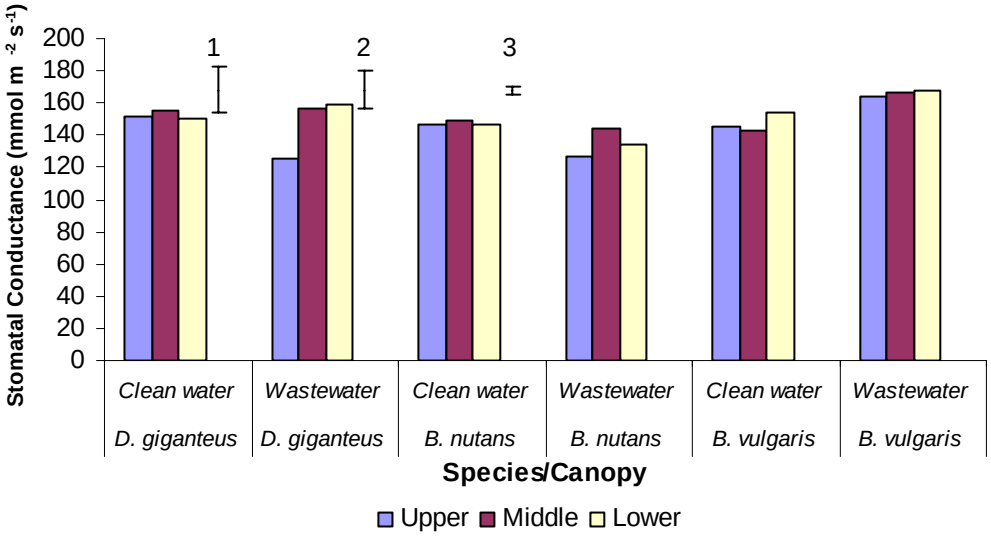


Figure 6.1. Mean stomatal conductance between January-June 2006 for the upper, middle and lower leaves of all species and two irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and canopy levels. n=5.

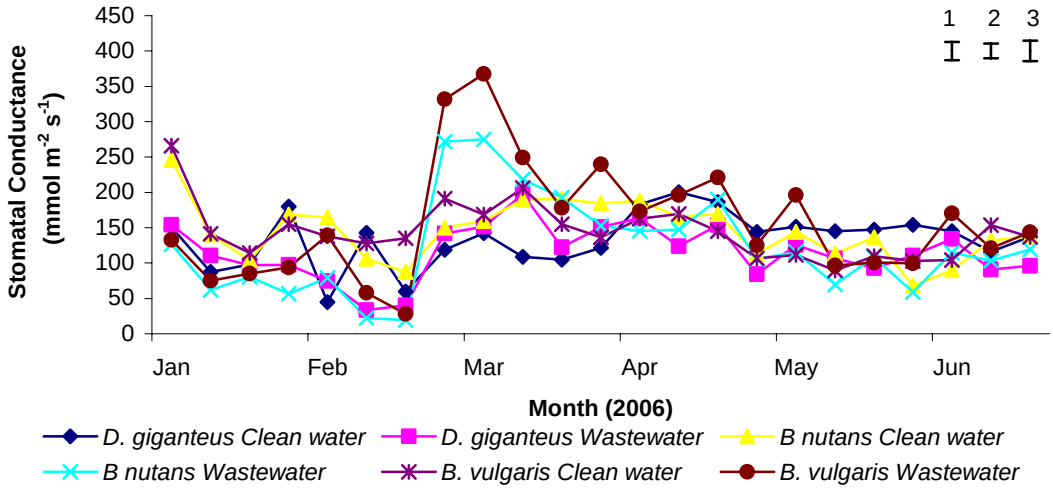


Figure 6.2. Timecourses of stomatal conductance measured in the upper canopy for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

As might be expected, the observed trend for transpiration reflected that for stomatal conductance (Fig. 6.5). The gentle decrease in transpiration rate between August and November 2006

resembled that for stomatal conductance (Fig. 6.3) and coincided with the cooling associated with the start of the short rains in October (Fig. 6.6).

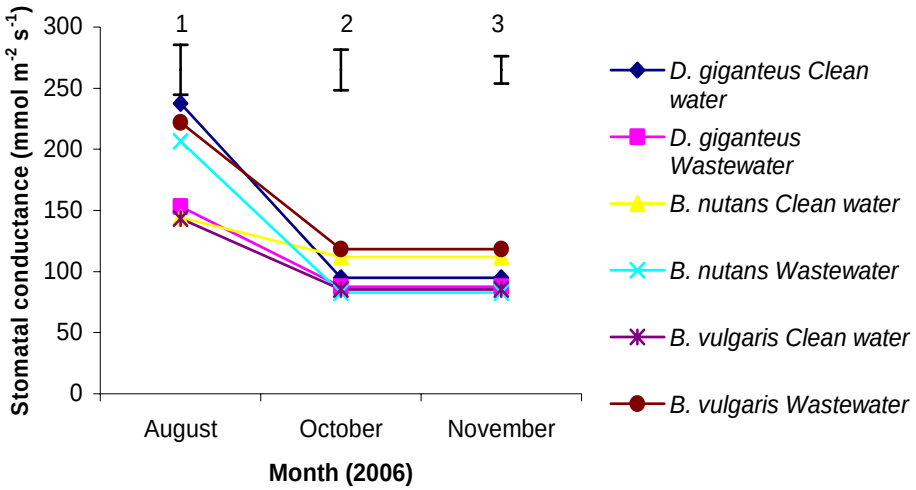


Figure 6.3. Timecourses of stomatal conductance measured in the upper canopy for all species and irrigation treatments between August and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

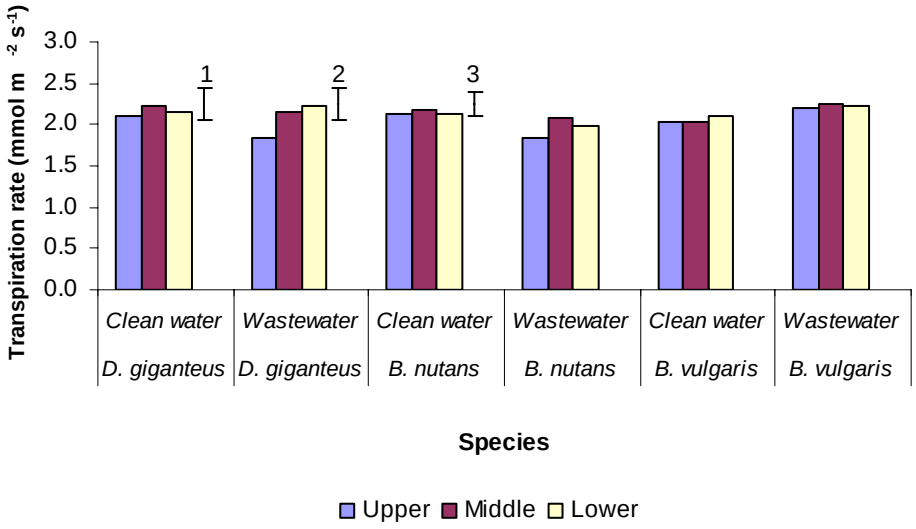


Figure 6.4. Mean transpiration rate throughout the July to November observation period for the upper, middle and lower leaves of all species and both irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and leaf position. n=5.

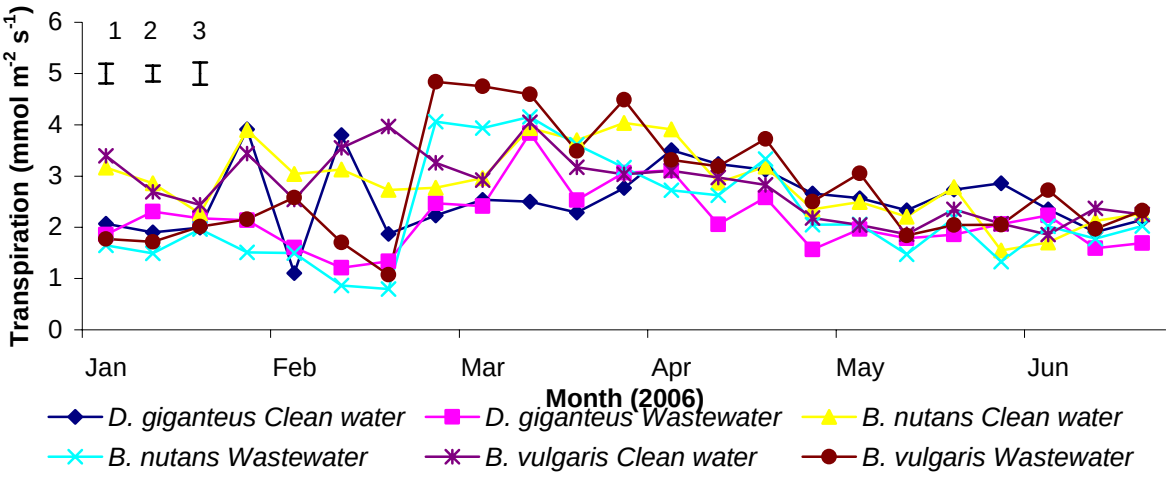


Figure 6.5. Timecourses of transpiration rate measured in the upper canopy for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and times. n=5.

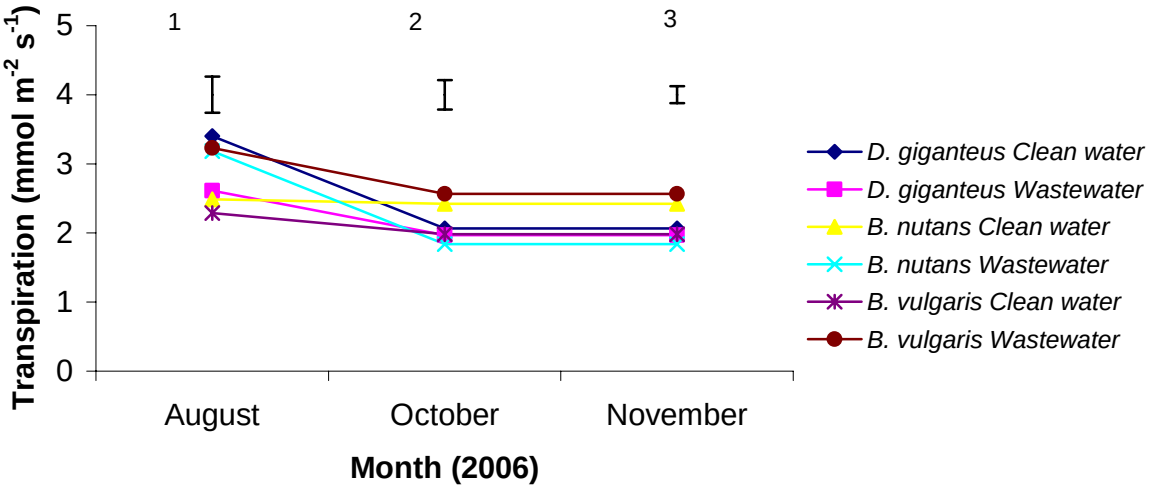


Figure 6.6. Mean transpiration rates measured in the upper canopy in August, October and November 2006 for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and times. n=5.

6.3.1.2 Impact of irrigation treatment

Measurements of the rate of net photosynthesis for the lower, middle and upper, leaves of all species revealed a significant species*time interaction between July and November 2006 ($P<0.05$; Fig. 6.7). No significant species or irrigation effects were detected (Fig. 6.8), although the values were consistently higher in the wastewater treatment. Among plants irrigated with wastewater, *D.*

giganteus exhibited the highest photosynthetic rates, followed by *B. vulgaris*. Wide fluctuations were observed for all species both before and after the onset of rainfall (October-November) and when temperature began to rise after a relatively cool period in July (Fig. 6.7).

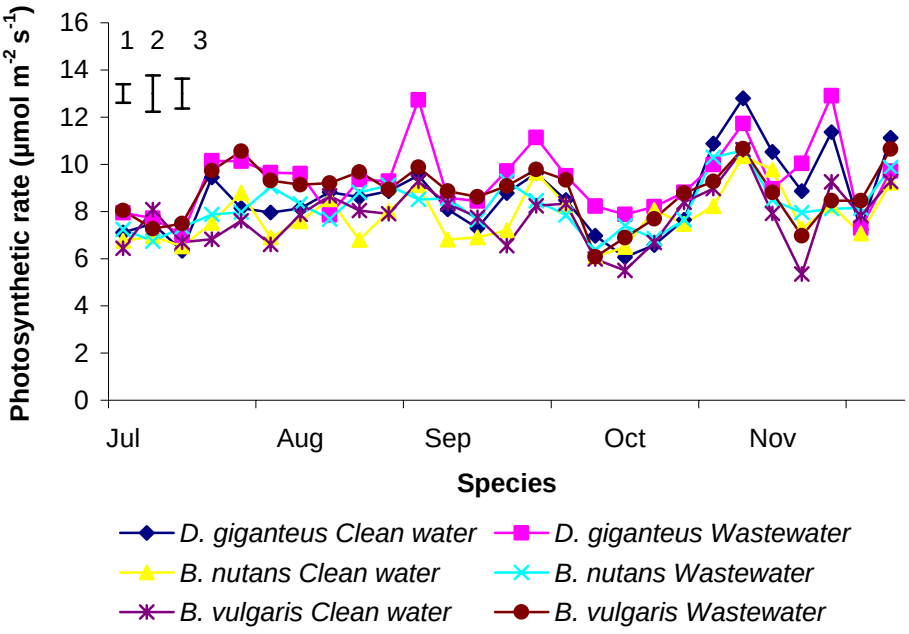


Figure 6.7. Seasonal timecourses of net photosynthetic rate for all species and irrigation treatments between July and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

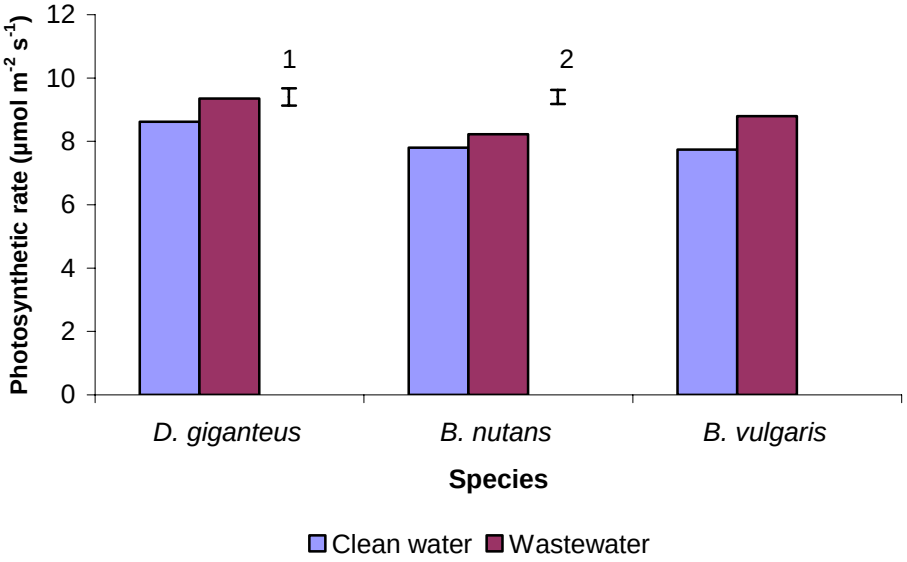


Figure 6.8. Mean net photosynthetic rate for the lower, middle and upper leaf positions for all species and irrigation treatments between July and November 2006. Vertical bars 1 and 2 show SED values for comparing species and irrigation treatments. n=5.

Although the mean values for net photosynthesis averaged over both irrigation treatments were initially similar in all species, they were generally greatest in *D. giganteus* between September and November (Fig. 6.9; P<0.05) and lowest in *B. vulgaris* during the latter stages of the observation period. The values varied during the observation period (P<0.05) and increased slightly with time. The influence of species and irrigation treatment on photosynthetic rate was not significant between August and November 2006 (Fig. 6.10), although the values appeared to be slightly higher in *B. vulgaris* than in the other two species in October and November 2006. Irrigation treatment affected photosynthetic rate (P<0.05; Fig. 6.11) as the values for plants receiving wastewater were greater than for those receiving clean water.

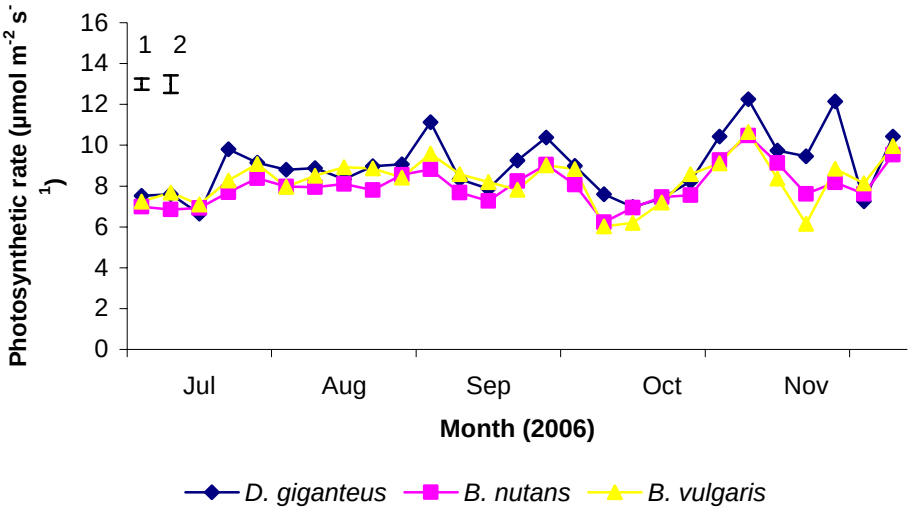


Figure 6.9. Timecourses of net photosynthetic rate for the lower, middle and upper leaf positions for all species between July and November 2006; values for each species are the means for both irrigation treatments. Vertical bars 1 and 2 show SED values for comparing species and time. n=5.

6.3.1.3 Effect of leaf position on gas exchange parameters

Net photosynthetic rate was higher in both irrigation treatments of *D. giganteus* than in *B. nutans* and *B. vulgaris*, for which the values were similar, although the differences were not significant (Fig. 6.12; P<0.33). Net photosynthetic rate was invariably lowest in leaves from the upper canopy (P<0.05). A significant species*leaf position interaction was apparent (P<0.05; Fig. 6.13)

as the increase in net photosynthesis between the youngest and oldest leaves was most pronounced in *D. giganteus*. Net photosynthetic rate was generally lowest in the upper leaves ($P<0.001$) and comparable for the middle and lower leaves (Fig. 6.14). In contrast to transpiration rate, net photosynthetic rate showed no species*irrigation water*time interaction.

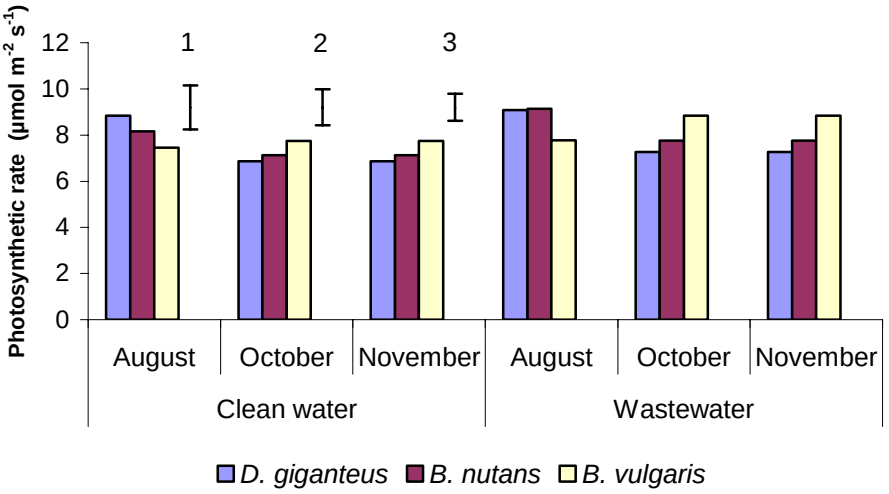


Figure 6.10. Mean photosynthetic rates for the upper canopy of all species and irrigation treatments in August, October and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

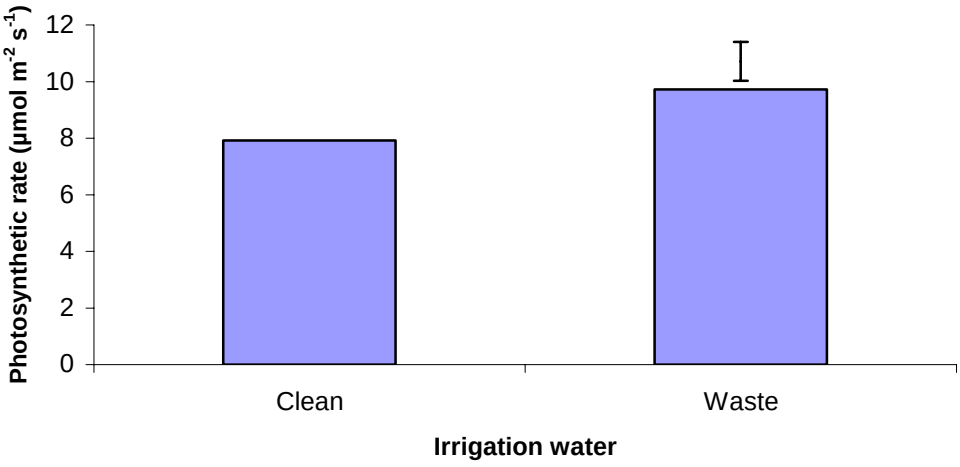


Figure 6.11. Influence of irrigation treatment on mean net photosynthetic rate for all three species examined. Vertical bar shows the SED value for comparing irrigation treatments. n=5.

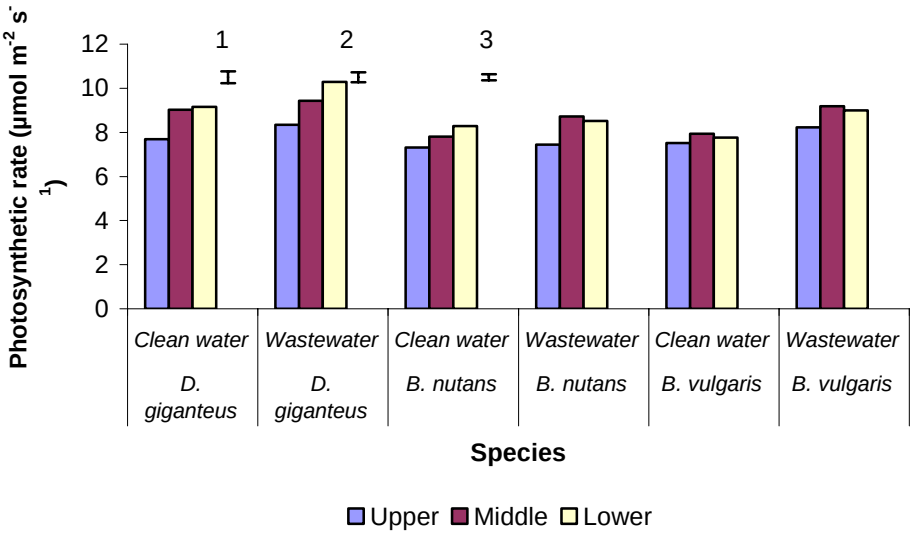


Figure 6.12. Influence of species, irrigation treatment and leaf position on mean net photosynthetic rate between July and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation type and leaf position. n=5.

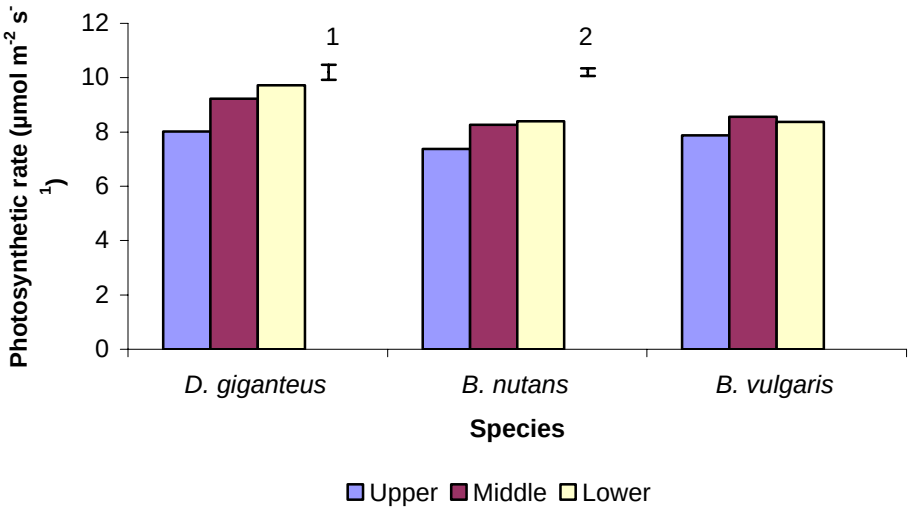


Figure 6.13. Mean net photosynthetic rates for the lower, middle and upper leaves of all species averaged over both irrigation treatments for the period between July 2006 and November 2006. Vertical bars 1 and 2 show SED values for comparing species and leaf position. n=5.

The measurements of stomatal conductance for the lower, middle and upper leaves of individual plants revealed a significant species*water*time interaction between July and November 2006 ($P<0.001$; Fig. 6.15). The values are means for three leaves at each leaf position within the canopy, in contrast to the period between January-June 2006 (Fig. 6.1), when measurements were

made for a single leaf per plant. Wide fluctuations in conductance were apparent between June and September 2006 as temperature increased, before declining to November for all species; the values were greatest in the wastewater treatment of *B. vulgaris* and *D. giganteus*. The measurements between July and November 2006 revealed significant effects of irrigation treatment and leaf position (P<0.01; Fig. 6.16), with the younger leaves having higher stomatal conductances.

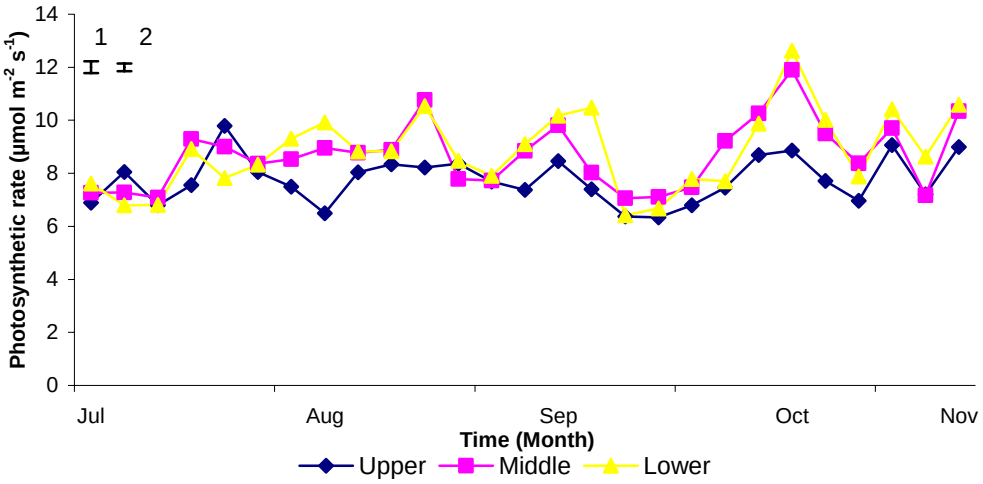


Figure 6.14. Timecourses of net photosynthetic rate for the lower, middle and upper leaves averaged over all species and irrigation treatments between July and November 2006. Vertical bars 1 and 2 show SED values for comparing leaf positions and time. n=5.

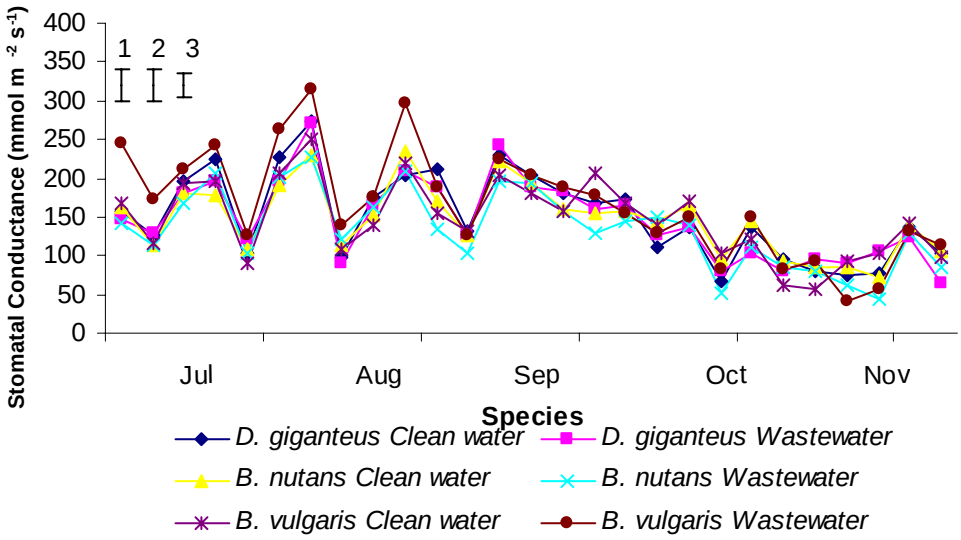


Figure 6.15. Timecourses of stomatal conductance for all species and irrigation treatments between July 2006 and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

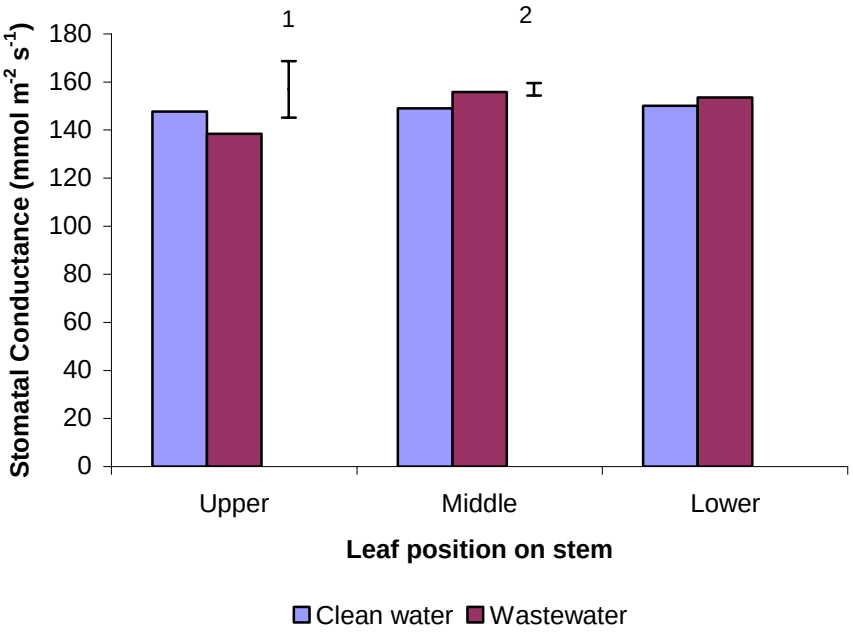


Figure 6.16. Mean stomatal conductances for leaves in the lower, middle and upper canopy positions for all species and irrigation treatments between July and November 2006. Vertical bars 1 and 2 show SED values for comparing leaf position and irrigation treatment. n=5.

A significant species*leaf position interaction was found for stomatal conductance ($P<0.05$; Fig. 6.17) between July 2006 and November 2006 because the values for *D. giganteus* and *B. nutans* were greater in the middle leaves than in the lower and upper leaves, whereas the opposite applied for *B. vulgaris* Stomatal conductance also showed a significant species*water*leaf position interaction ($P<0.05$; Fig. 6.18) as the values were generally slightly smaller for lower leaves and greater for the upper leaves. Values were generally lowest in the upper leaves of *B. nutans*. Stomatal conductance was higher in the clean water treatment of *B. nutans* than in the wastewater treatment, whereas the opposite applied for *B. vulgaris* and *D. giganteus*, except for the upper leaves of *D. giganteus*, whose behaviour resembled *B. nutans* (Fig. 6.18).

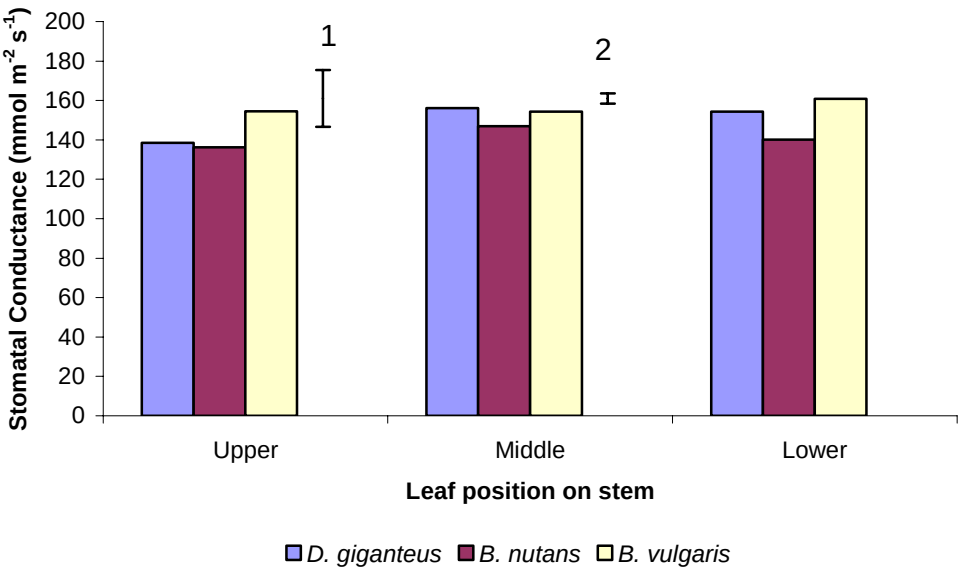


Figure 6.17. Influence of leaf position on mean stomatal conductance for both irrigation treatments between July 2006 and November 2006 for all species. Vertical bars 1 and 2 show SED values for comparing species and leaf position. n=5.

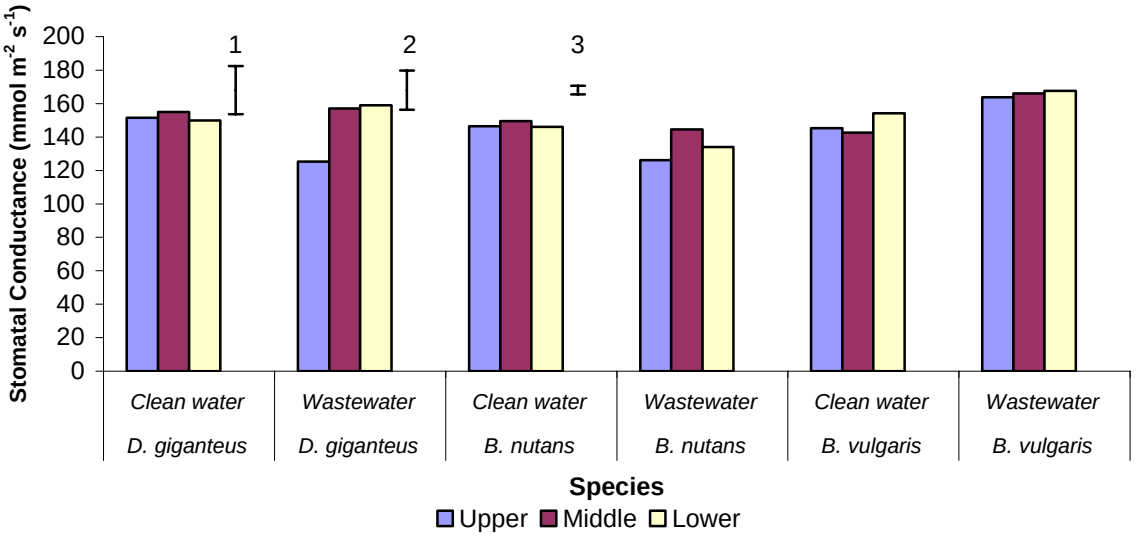


Figure 6.18. Influence of species and irrigation treatment on stomatal conductance. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and leaf positions. n=5.

The three bamboo species examined did not respond uniformly to the irrigation treatments between July and November 2006 (Fig. 6.19); thus, transpiration was greatest in the wastewater treatment of *B. vulgaris* between July and mid-November, followed by the clean and wastewater treatments of *D. giganteus* and finally the wastewater treatment of *B. nutans*. Rate of transpiration

rate varied greatly between June and October 2006, with values ranging between 1.5 and 3 mmol m^{-2} s^{-1} in all species, reflecting day-to-day variation in the prevailing weather conditions; thus, transpiration rate remained relatively high between July and early October, when a brief trough was followed by an increase when the short rains began. A significant species*irrigation treatment*time interaction was apparent (P<0.001; Fig. 6.19). Analysis of the influence of leaf position on transpiration rate revealed that the values were greatest for the middle leaves, followed successively by the lower and upper leaves (P<0.001; Fig. 6.20) in all species.

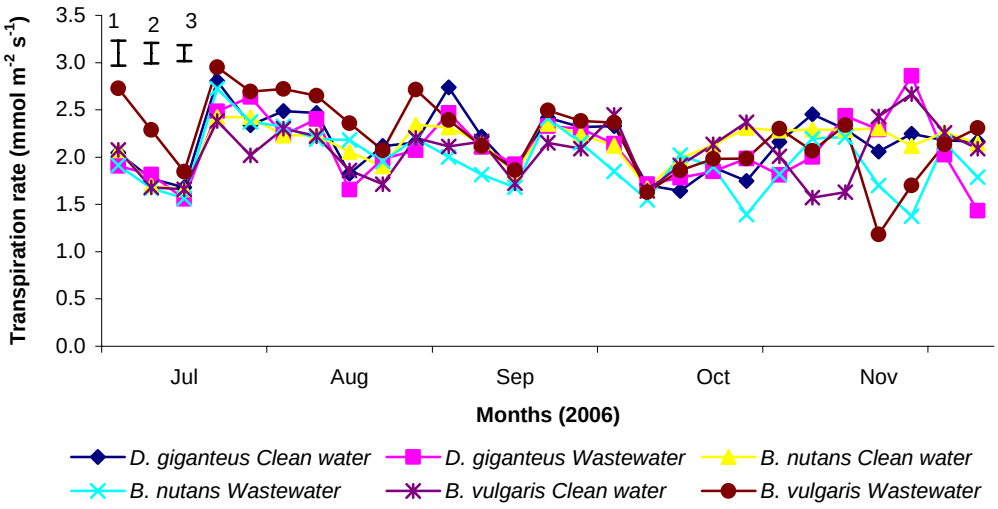


Figure 6.19. Timecourses of transpiration rate for the lower, middle and upper leaves in all species and irrigation treatments between July 2006 and November 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

6.3.1.4 Instantaneous transpiration efficiency

Instantaneous transpiration efficiency (ITE) was calculated by dividing the values for net photosynthetic rate by the corresponding values for transpiration rate (Robredoa *et al*., 2007). Figures 6.21-6.24 show the timecourses for ITE between July and November 2006 for all species, irrigation treatments and leaf positions. During this period, the trends for ITE duplicated those for transpiration, being relatively unchanged between July and October, but with wide fluctuations in November following the onset of the rains (Figs. 6.21 & 6.22). There was no consistent variation

between different leaf positions within the canopy (Fig. 6.23). Similar patterns for photosynthetic rate were observed for species and irrigation type, species and leaf position, and leaf position during this period (Figs. 6.7, 6.9 & 6.14).

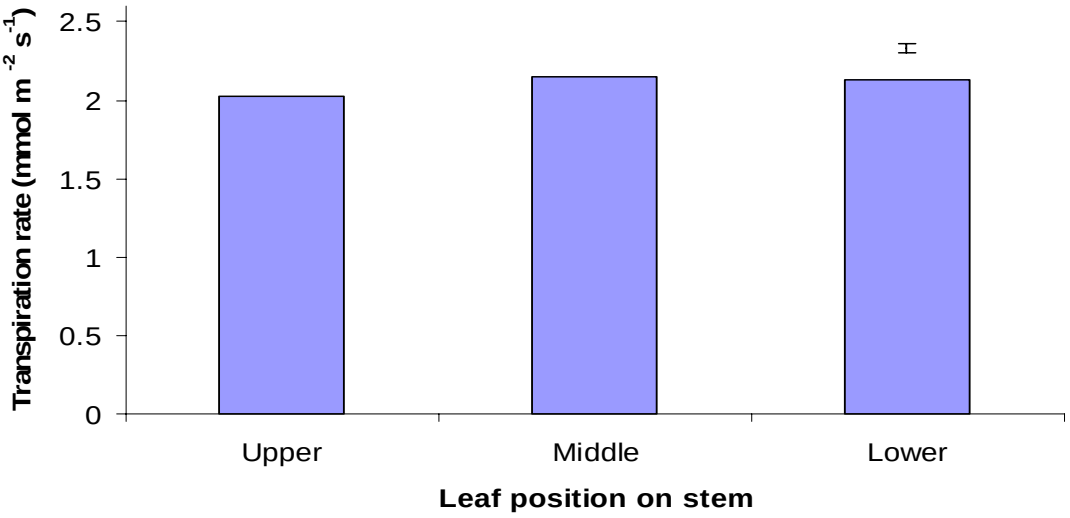


Figure 6.20. Mean transpiration rates for each leaf position averaged over all species and irrigation treatments between July 2006 and November 2006. Vertical bar shows SED value for comparing leaf positions. n=5.

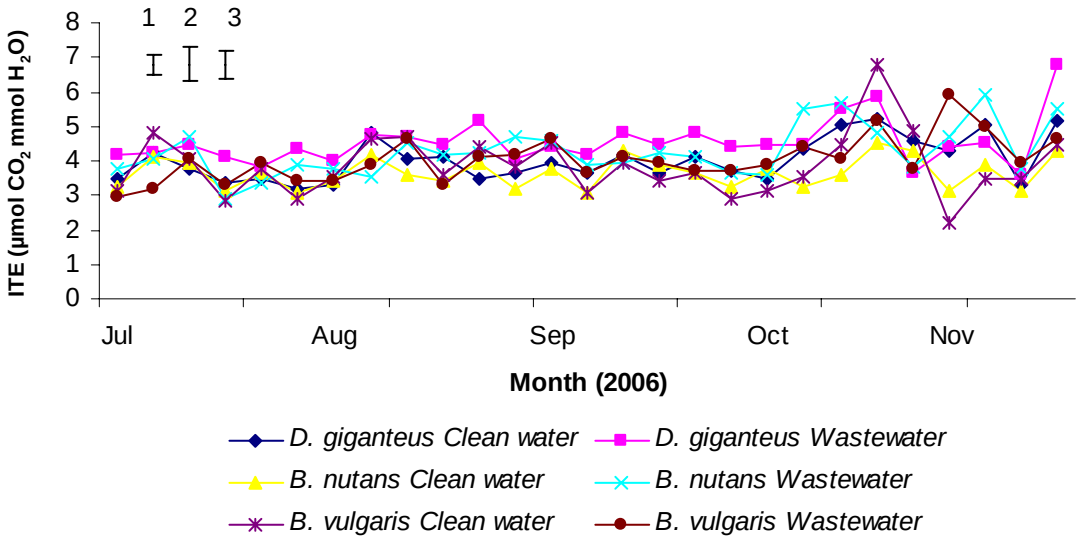


Figure 6.21. Timecourses of ITE for all species and irrigation treatments averaged for lower, middle and upper leaf positions between July and December 2006. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatment and time. n=5.

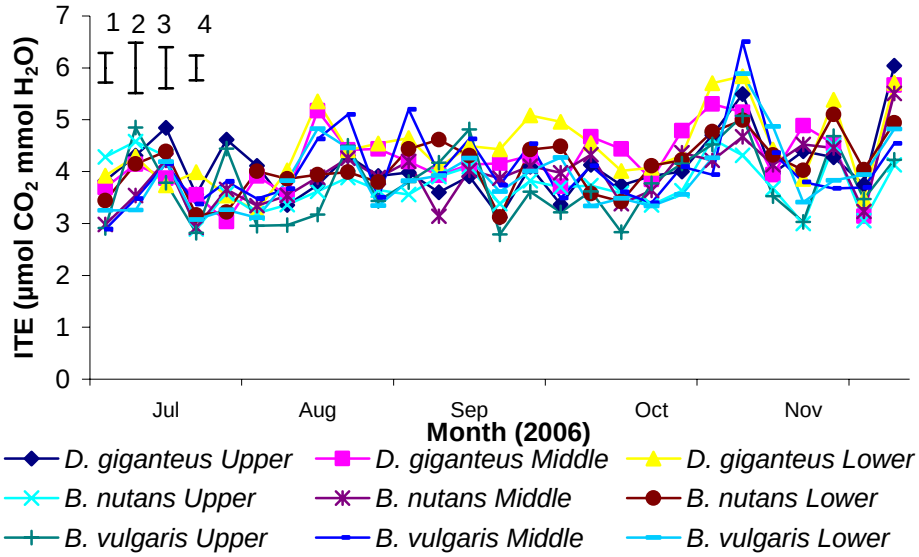


Figure 6.22. Timecourses of ITE for the lower, middle and upper leaf positions averaged over species and irrigation treatments between July and December 2006. Vertical bars 1, 2, 3 and 4 show SED values for comparing species, irrigation treatment, time and leaf position. n=5.

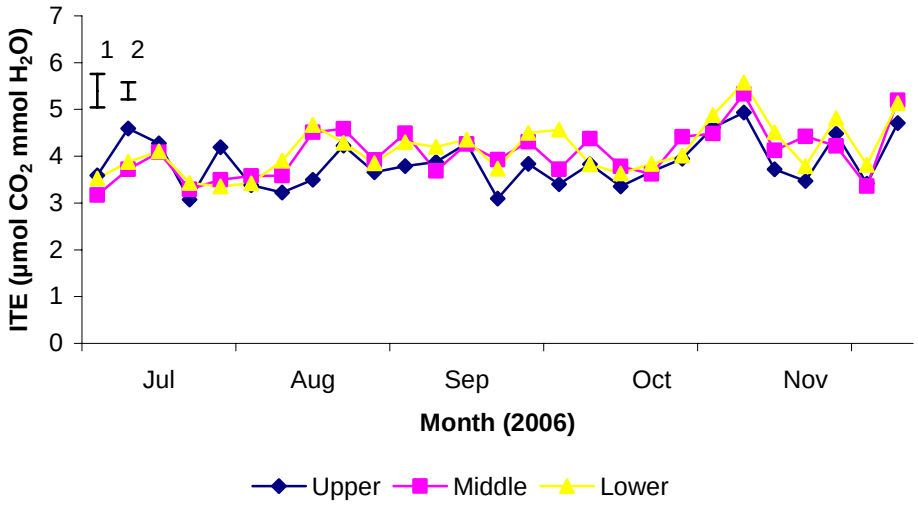


Figure 6.23. Mean timecourses of ITE for the lower, middle and upper leaf positions averaged over all species and irrigation treatments between July and December 2006. Vertical bars 1 and 2 show SED values for comparing measurements for time and different leaf positions. n=5.

Figure 6.24 shows the diurnal timecourses for all species and irrigation treatments. ITE values were lower in the wastewater treatment of *B. vulgaris* and *B. nutans*, whereas the converse applied

for *D. giganteus*; values ranged between 2.8 and 5 µmol CO_2 mmol H_2O^{-1}. ITE increased between 0800-0900 h and then fluctuated for the rest of the diurnal period; there was no strong diurnal trend.

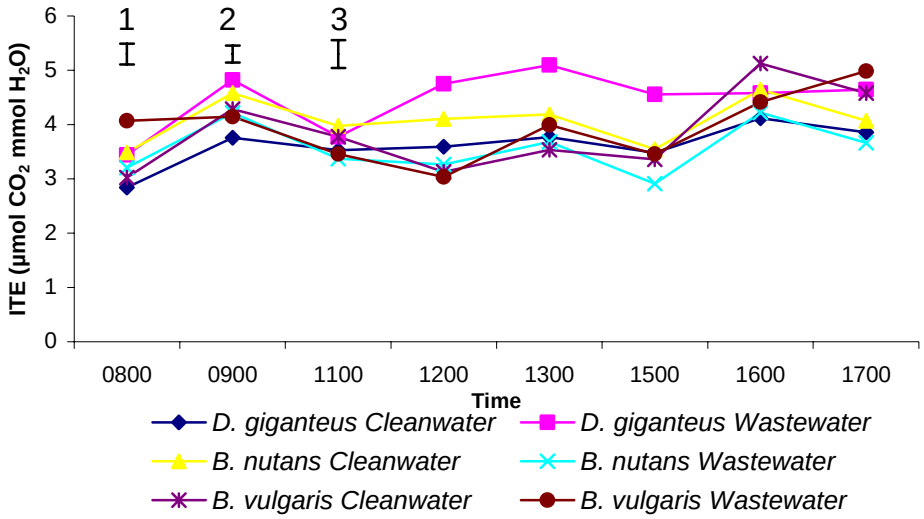


Figure 6.24. Mean diurnal timecourses of ITE for all species and irrigation treatments between 0800-1700 h. Vertical bars 1, 2, and 3 are the SED values for comparing species, irrigation treatment and time. n=5.

The relationships between net photosynthetic (*A*) and transpiration rates (*E*) between June and November 2006 exhibited significant positive correlations for all species (P<0.01; Fig. 6.25); the values are means for the both irrigation treatments and all three leaf positions. The values for *A* at specific levels of *E* were higher (P<0.01) in *D. giganteus* than in *B. nutans* and *B. vulgaris*, particularly at higher transpiration rates; the relationship for *B. nutans* and *B. vulgaris* were closely comparable, as indicated by the similar slopes of the fitted regressions.

6.3.1.5 Influence of external factors on gas exchange

Climatic conditions

Gas exchange was correlated with specific climatic variables between January-June 2006 to test for potential causal relationships (Table 6.1; Quero *et al*., 2006). The correlation coefficient for

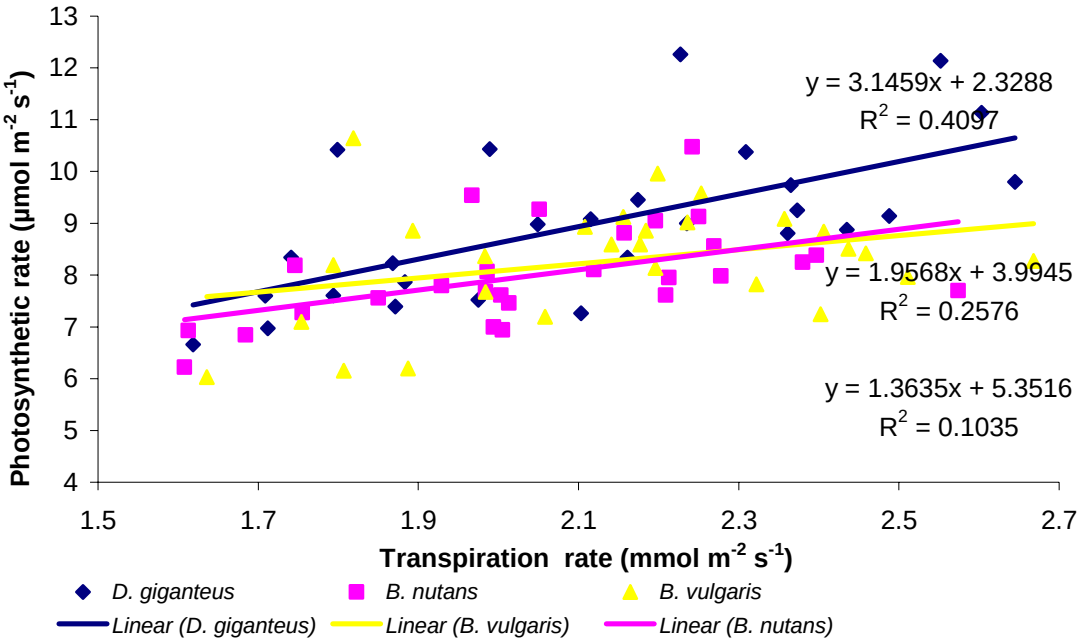


Figure 6.25. Relationship between net photosynthetic and transpiration rates for all three species averaged over both irrigation treatments and all three leaf positions between June and November, 2006.

the relation between stomatal conductance and mean air temperature ranged from 0.30 in *D. giganteus* to 0.52 in *B. vulgaris* ($P<0.05$), while that for minimum air temperature ranged from 0.56 in *B. nutans* to 0.60 in *B. vulgaris* ($P<0.01$). Stomatal conductance was significantly correlated with relative humidity in *D. giganteus* ($P<0.05$).

Transpiration rate showed strong correlations with mean and minimum air temperature in all species (0.56-0.77; $P<0.01$) and a weaker correlation with maximum temperature in *B. nutans* and *B. vulgaris* (0.31-0.52; $P<0.05$). The correlations between transpiration and solar radiation, relative humidity and SD were extremely weak. Photosynthetic rate showed no significant correlation with any of the climatic parameters examined, although the correlations with mean and maximum temperature were slightly stronger (0.28-0.38) than those for minimum temperature (0.11-0.21).

Table 6.1. Correlation (Pearson's r) of mean values for gas exchange parameters and selected climatic variables for all species and irrigation treatments between January and June 2006. * and ** denote significance at P<0.05 and P<0.01 respectively. n=5.

Correlation between gas exchange and climatic parameters			
Stomatal conductance			
Factor	*D. giganteus*	*B. nutans*	*B. vulgaris*
Temp. (max)	-0.17	0.14	0.14
Temp. (mean)	0.30	0.47*	0.52*
Temp. (min)	0.59**	0.56**	0.6**
Radiation	-0.23	-0.02	0.00
RH (1200 h)	0.44*	0.09	0.08
SD	-0.35	0.01	0.02
Transpiration rate			
Temp. (max)	0.31	0.42*	0.52*
Temp. (mean)	0.57**	0.65**	0.77**
Temp. (min)	0.59**	0.56**	0.66**
Radiation	0.07	0.17	0.24
RH (1200 h)	0.12	-0.06	-0.13
SD	0.08	0.24	0.33
Photosynthetic rate			
Temp. (max)	0.28	0.32	0.33
Temp. (mean)	0.32	0.28	0.31
Temp. (min)	0.21	0.11	0.14
Radiation	0.05	0.13	0.15
RH (1200 h)	-0.27	-0.29	-0.29
SD	0.27	0.30	0.32

6.3.1.6 Timecourses for SPAD values

SPAD values were higher in the wastewater treatments than in the clean water treatments, being greatest in *B. vulgaris* followed successively by *D. giganteus* and *B. nutans* (P<0.001; Fig. 6.26). Due to the decrease in SPAD values in all treatments between January-April 2006, the effect of time was significant but no significant species*time, irrigation treatment*time or species* irrigation treatment *time interactions were detected. The differences in SPAD values between species were not significant (Figs. 6.26-6.28). The mean SPAD values for both irrigation treatments of all species showed no significant variation between species (Fig. 6.27) but declined gradually with time (P<0.05; Fig. 6.28). When averaged over all species and irrigation treatments, significant changes with time were apparent (P<0.05; Fig. 6.29); thus, the initial mean

SPAD value in January 2006 of 40.7 declined until late March 2006, before stabilising at *c*. 34 units for the remainder of the observation period.

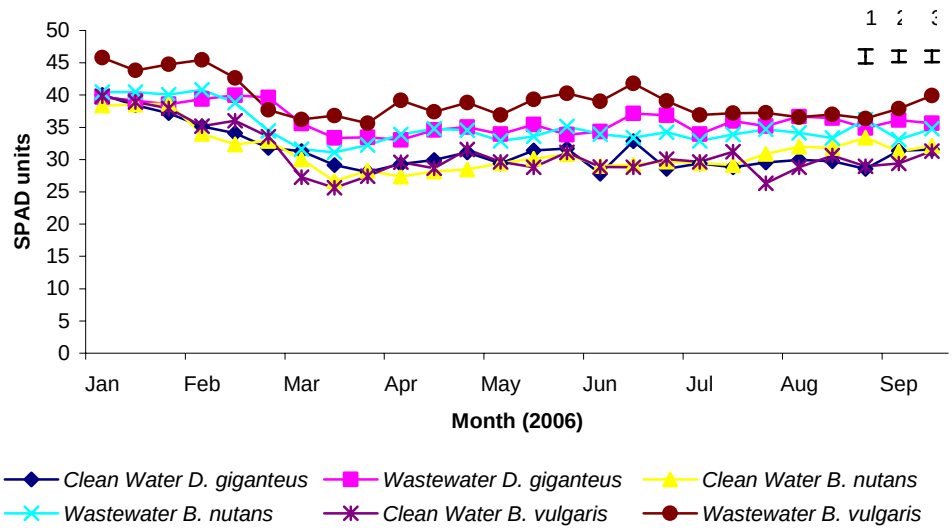


Figure 6.26. Timecourses of SPAD values for all species and irrigation treatments. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=5.

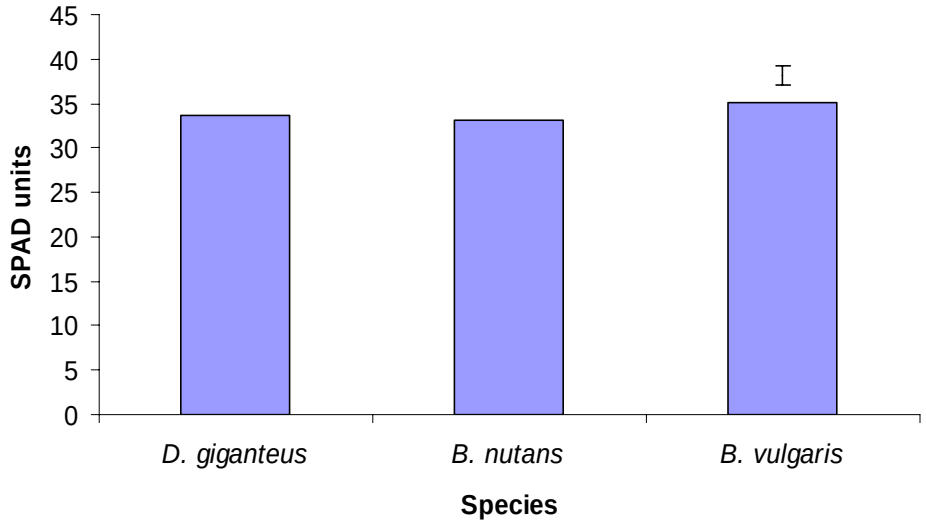


Figure 6.27. Mean SPAD values for all species averaged over both irrigation treatments between February and September 2006. Vertical bar show the SED value for comparing species. n=5.

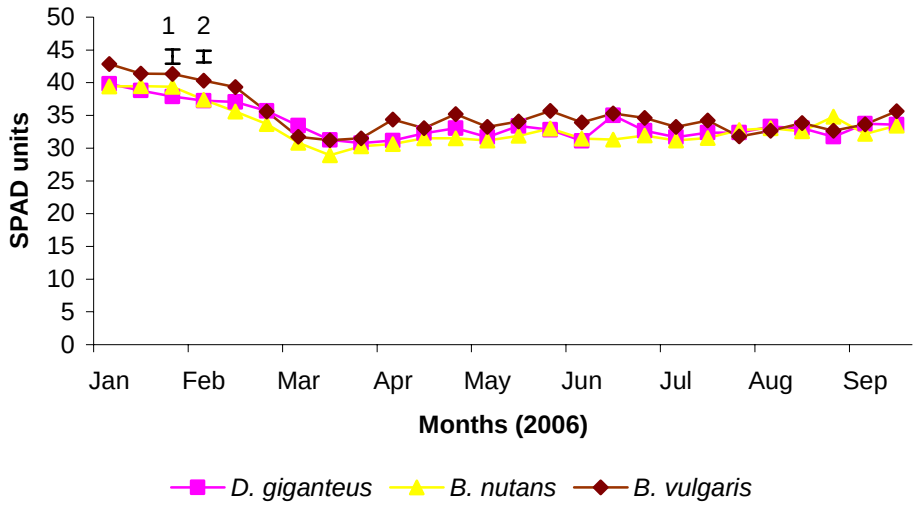


Figure 6.28. Seasonal timecourses for SPAD values for all species averaged over both irrigation treatments between January and September 2006. Vertical bars 1 and 2 show SED values comparing species and time. n=5.

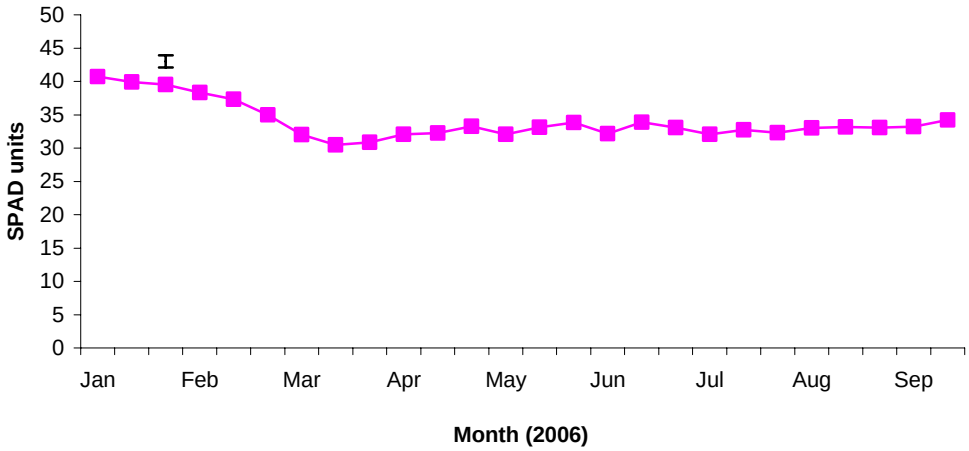


Figure 6.29. Seasonal timecourses for mean SPAD values for both irrigation treatments of all species between February and September 2006. Vertical bar shows the SED value for comparing changes with time. n=5.

6.3.1.7 Influence of species and irrigation treatment on SPAD values

The SPAD values showed no significant variation between the three species examined and no species*irrigation treatment interaction (Fig. 6.26). SPAD values were greater in plants irrigated with wastewater than in those receiving clean water (P<0.001; Fig. 6.26), and the values for the wastewater treatment of *B. vulgaris* were always higher than for the other two species. The

difference between the wastewater treatment of *B. vulgaris* and the equivalent treatments of *B. nutans* and *D. giganteus* was greater than that between the wastewater and clean water treatments.

6.3.1.8 Relationship between SPAD and IRGA values

Somewhat surprisingly in view of the fact that SPAD values provide an indirect measure of foliar chlorophyll content, net photosynthesis decreased with increasing SPAD values in all species during the period between January-September 2006, although these relationships were not significant (Fig. 6.30; Table 6.2); the decrease was smallest in the wastewater treatment of *B. vulgaris*.

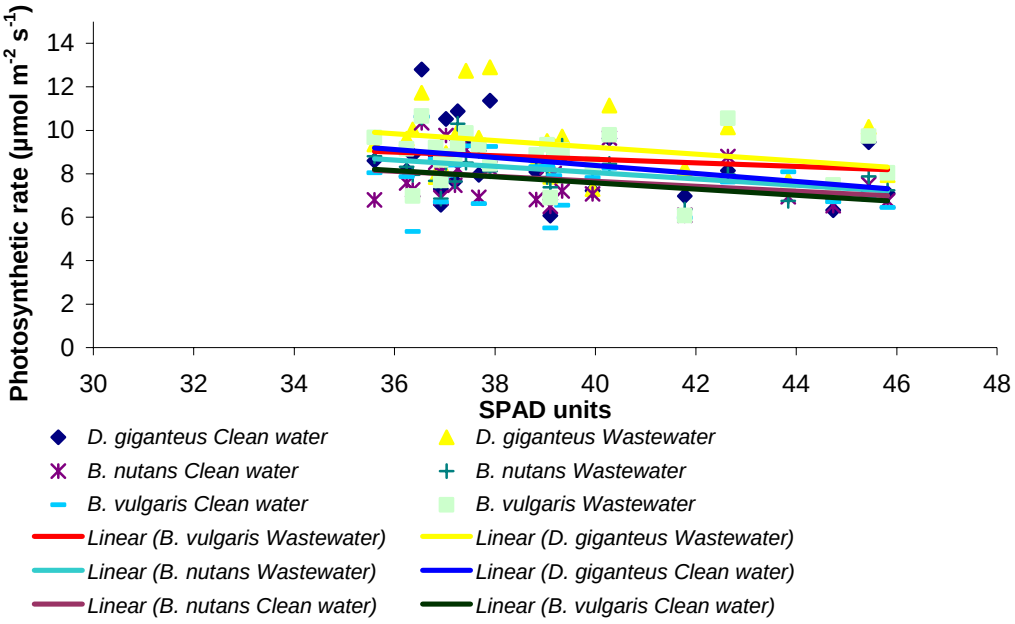


Figure 6.30. Relationship between net photosynthetic rate and SPAD values for all species and irrigation treatments between January and September 2006. n=5.

Pearson's *r* was used to test for correlations between gas exchange variables and SPAD values. No significant correlations were detected (Table 6.2), although *B. vulgaris* exhibited the highest correlation coefficient for photosynthetic rate (0.35), followed by *D. giganteus* (0.29). Weak negative correlations between stomatal conductance and SPAD values were apparent for all species.

Table 6.2. Correlation (Pearson's r) between stomatal conductance, photosynthetic and transpiration rates and SPAD values between January and June 2006. n=5.

Correlation between gas exchange parameter and SPAD values			
Species	Stomatal conductance	Net photosynthesis	Transpiration
D. giganteus	-0.31	0.29	0.13
B. nutans	-0.25	0.07	-0.01
B. vulgaris	-0.11	0.35	0.17

6.3.2 Experiment 2

6.3.2.1 Effect of species on gas exchange

No significant species or species*irrigation interaction was detected for net photosynthetic rate (Table 6.3). Similarly, no significant effects of species, irrigation treatment or species*irrigation treatment interaction were found for stomatal conductance or transpiration rate.

Table 6.3. F values for photosynthetic rate, stomatal conductance and transpiration rate between December 2006 and February 2007. ns and * denote no significant effect and significance at P<0.05. n=5.

Source of Variation	*Net photosynthesis* F	*Stomatal conductance* F	*Transpiration* F
Species	0.255 ns	0.153 ns	0.141 ns
Irrigation	0.014*	0.054 ns	0.079 ns
Species*Irrigation	0.078 ns	0.90 ns	0.856 ns

6.3.2.2 Effect of irrigation type on gas exchange

Of the three irrigation sources used, photosynthetic rate was greatest for plants irrigated with domestic wastewater, with the exception of *B. vulgaris*, for which values for the industrial wastewater treatment were highest, followed successively by the domestic and clean water treatments (P<0.05; Fig. 6.31). *D. giganteus* plants irrigated with domestic wastewater had higher photosynthetic rates than their counterparts irrigated with clean water or industrial wastewater. Of the three species, *B. vulgaris* showed the lowest sensitivity to irrigation treatment.

6.3.2.3 Relationship between photosynthesis and transpiration rates

Figure 6.32a-c shows the relationship between net photosynthetic and transpiration rate for measurements made between December 2006 and March 2007 for all species and irrigation treatments.

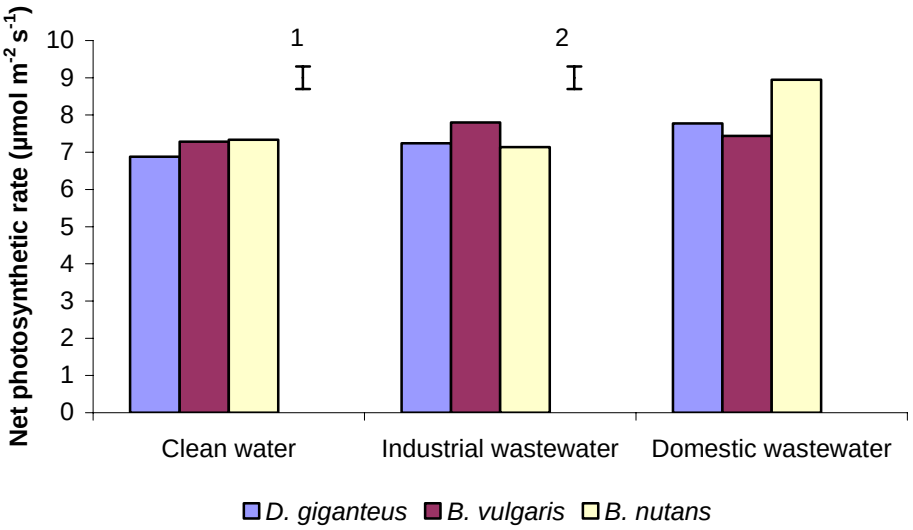


Figure 6.31. Influence of species and irrigation treatment on mean net photosynthetic rate between December 2006 and March 2007. Bars 1 and 2 show SED values for comparing species and irrigation treatments. n = 27.

The domestic wastewater treatment of *B. vulgaris* was the only one in which net photosynthetic rate appeared to decrease as transpiration rate increased (Fig. 6.32c), whereas a significant increase in photosynthesis ($P<0.05$) with transpiration occurred in all other treatments. ITE values were greater ($P<0.014$) in the domestic wastewater treatments than in the industrial wastewater and clean water treatments (Fig. 6.33), with the clean water treatments of *B. vulgaris* and *D. giganteus* having lower ITEs than the other irrigation treatments. ITE ranged between 3-8 µmol CO_2 mmol H_2O^{-1}.

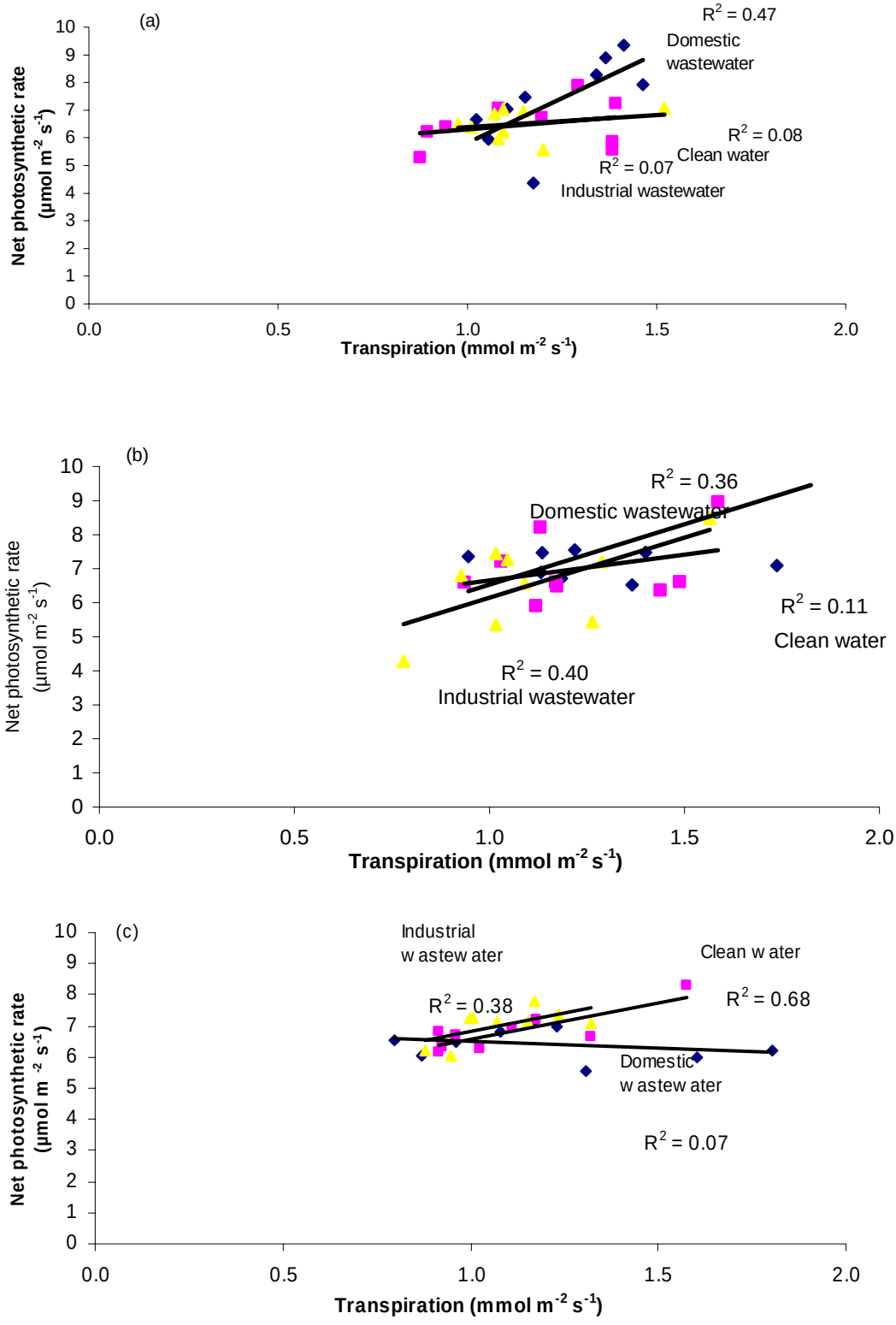


Figure 6.32. Relationship between net photosynthetic and transpiration rates for all irrigation treatments between December 2006 and March 2007 for (a) *D. giganteus* (b) *B. nutans* and (c) *B. vulgaris*.

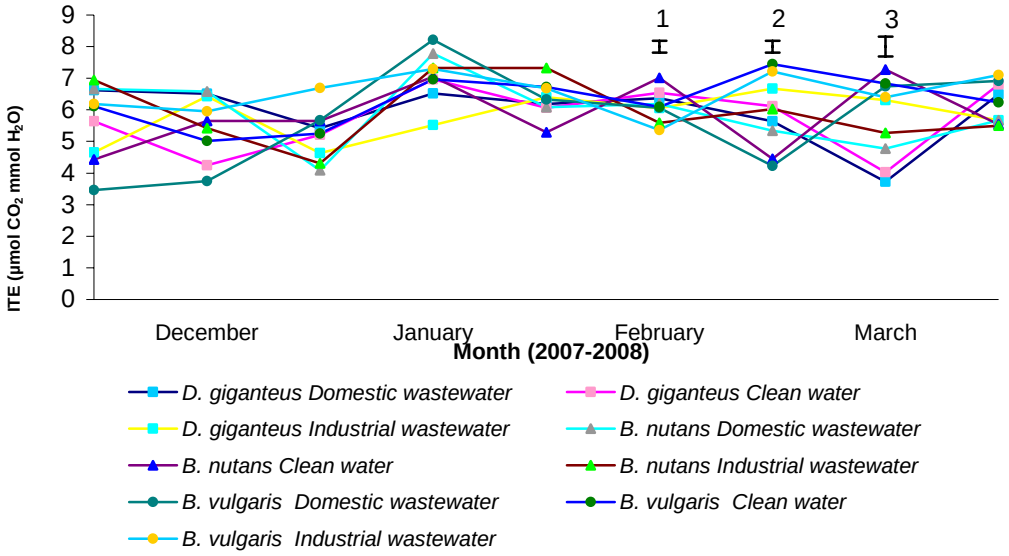


Figure 6.33. Mean timecourses of ITE for all species and irrigation treatments between December 2006 and March 2007. Vertical bars 1, 2 and 3 show SED values for comparing species, irrigation treatments and time. n=27.

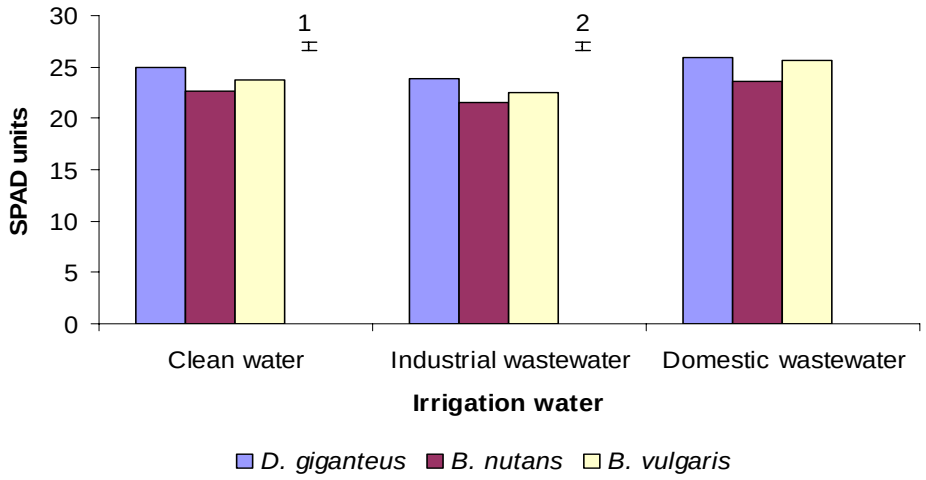


Figure 6.34. Influence of species and irrigation treatment on mean SPAD values between December 2006 and March 2007. Bars 1 and 2 show SED values for comparing species and irrigation treatments. n = 27.

6.3.2.5 Influence of irrigation treatment on SPAD values

Irrigation treatment affected SPAD values, which were greatest in the domestic wastewater treatment, followed by the clean and industrial wastewater treatments ($P<0.001$; Fig. 6.36); no significant species*irrigation treatment interaction was detected (Fig. 6.34).

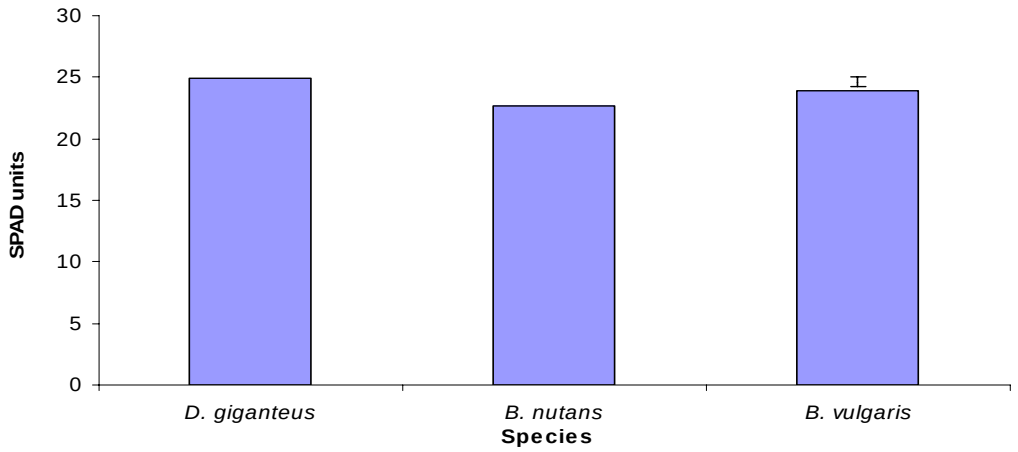


Figure 6.35. Mean SPAD values for healthy young leaves at the top of the canopy averaged over all irrigation treatments for each species between December 2006 and March 2007. Vertical bar shows the SED value for comparing species. n = 27.

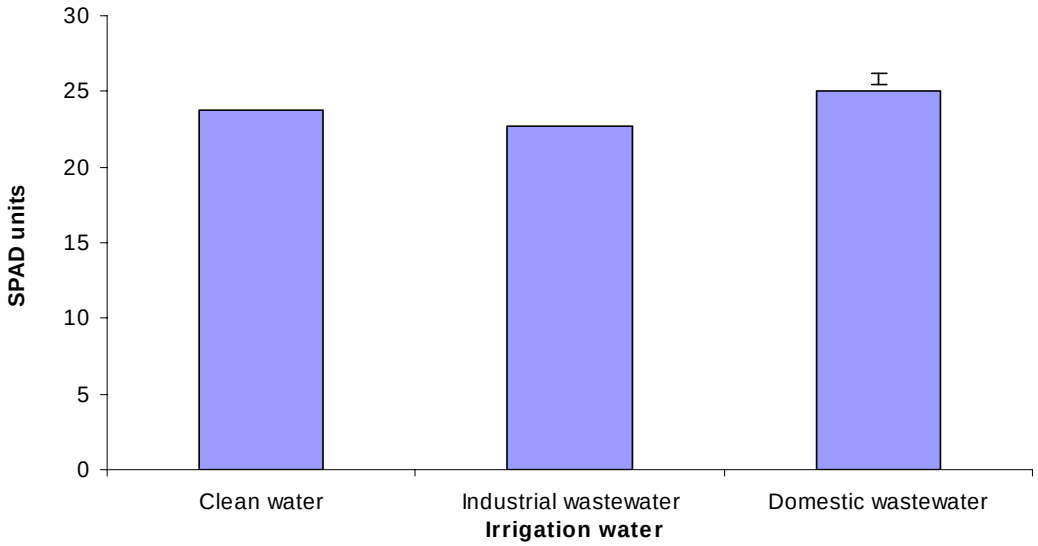


Figure 6.36. Mean SPAD values for healthy young leaves at the top of the canopy averaged over all species for each irrigation treatment between December 2006 and March 2007. Vertical bar shows the SED value for comparing species. n = 27.

6.3.2.6 Temporal changes in the relationship between IRGA and SPAD values

The relationships between net photosynthetic rate and SPAD values for all species and irrigation treatments showed no consistent change with time (Table 6.4) as the correlation coefficients differed between sampling dates, irrigation treatments and species during a period when there was a gradual change from rainy to dry weather conditions. The results for *D. giganteus* revealed significant positive correlations between photosynthetic rate and SPAD values for plants irrigated with clean or industrial sewage on two of the five observation dates, whereas plants of the same species receiving domestic wastewater showed no detectable correlation. *B. nutans* irrigated with clean or domestic wastewater and *B. vulgaris* irrigated with industrial waste both showed significant correlations ($P<0.05$) on one occasion. *B. nutans* irrigated with industrial wastewater, *B. vulgaris* irrigated with clean water and *D. giganteus* irrigated with industrial sewage or clean water, showed significant correlations on two occasions ($P<0.01$). *B. vulgaris* irrigated with wastewater showed significant correlations ($P<0.05$ or $P<0.01$) on four of the five measurement dates.

Table 6.4. Correlation coefficients for the relationships between net photosynthetic rate and SPAD values on five dates for all species and irrigation treatments. * and ** denote significance at P<0.05 and P<0.01 respectively. n = 27.

Correlations between SPAD Values and Photosynthetic Rate						
Species	Water type	01-Nov-06	09-Feb-07	21-Feb-07	02-Mar-07	08-Mar-07
D. giganteus	Clean water	-0.19	0.49**	-0.01	0.39**	-0.41
D. giganteus	Domestic sewage	-0.16	-0.24	0.04	-0.02	0.20
D. giganteus	Industrial sewage	0.29**	-0.07	-0.55	0.16	0.36**
B. vulgaris	Clean water	0.17	0.12	0.65**	0.10	0.39**
B. vulgaris	Domestic sewage	0.72**	0.22*	0.45**	-0.64	0.23*
B. vulgaris	Industrial sewage	-0.47	0.04	0.19	0.13	0.45**
B. nutans	Clean water	-0.01	0.46**	-0.28	-0.45	-0.16
B. nutans	Domestic sewage	-0.12	-0.06	0.6**	-0.16	0.10
B. nutans	Industrial sewage	0.15	0.23**	0.67**	-0.58	-0.58

6.4 Discussion

6.4.1 Gas exchange: Experiment 1

Stomatal conductance exhibited a relatively strong correlation with mean and minimum air temperature (0.30-0.52; Table 6.1). Vitale *et al*., in 2007 suggested that the close relationship between stomatal conductance and environment variables may allow the former to be predicted in a quantitative manner. It has also been observed that stomatal conductance is dynamic and under the control of many environmental variables, complicating its measurement and interpretation (Soar *et al*., 2004; Soar *et al*, 2006); it is also known that environmental variables influence plant physiology greatly, thereby regulating transpiration (Sanchez-Carrilo *et al*., 2001). Transpiration rate in the present study was correlated with temperature (0.57-0.77; $P<0.01$) but exhibited much weaker correlations with incident solar radiation (0.07-0.24), relative humidity (-0.06-0.12) and SD (0.08-0.33). Net photosynthesis exhibited mild positive correlations with air temperature (0.31-0.32) and SD (0.27-0.32) but a weak correlation with radiation (0.05-0.15; Table 6.1).

Mulkey *et al*., (1991), observed that stomatal conductance in *Psychotria limonensis* (*Rubiaceae*) was high when plants were irrigated, but decreased in unirrigated plants during the dry season. As the plants in the present study were watered daily, stomatal conductance was not expected to differ greatly between treatments. However, stomatal conductance was lower in the wastewater treatment of *B. vulgaris* than in the clean water treatment until March 2006, when the values increased sharply and became higher than in the clean water treatment until the end of the observation period (Fig. 6.2). The differences between treatments and change with time were significant ($P<0.05$). Stomatal conductance in the clean water treatment of *B. vulgaris* showed relatively little variation during the experimental period. At Juja, where the experiment was carried out, March coincides with the advent of rainfall and decreasing temperatures, which might be expected to induce decreases in all of the gas exchange variables examined.

By contrast, stomatal conductance was invariably lower in plants of *D*. *giganteus* and *B*. *nutans* irrigated with wastewater than in those receiving clean water except briefly in April (Fig. 6.2). Paranychianakis *et al*., in 2004 noted that stomatal conductance was reduced when Soultanina grapevines were irrigated with wastewater, an effect attributed to the formation of water deficits resulting from salt accumulation; they concluded that no specific threshold is required for this effect to occur. The variation in stomatal conductance in the wastewater treatment of *B*. *nutans* was much greater than in the clean water treatment. The peak in stomatal conductance observed in all treatments in March and April 2006 may have resulted from intermittent heavy rain interspersed with periods of hot sunshine. Substantial variation in stomatal conductance was apparent between July and September 2006 as air temperature began to increase. It is instructive to note that measurements of stomatal conductance in *Arabidopsis* are prone to substantial variation as each leaf may modify its stomatal behaviour independently of others (Cañamero *et al*., 2006). In the present study, leaves located higher in the canopy exhibited greater stomatal conductances than those deeper in the canopy (Figs. 6.15-6.18).

During the 23 week observation period, transpiration rate ranged between 1 and 5 mmol m^{-2} s^{-1} in all treatments. These values compare well with that of 2.3 mmol m^{-2} s^{-1} reported for bamboo (*P*. *pubescens*) in Australia (Kleinhenz and Midmore, 2002), and those reported by Muthuri *et al*., (2009) for *Grevillea robusta* and *Alnus acuminata* species but lower than those of *Paulownia fortunei* (3-7 mmol m^{-2} s^{-1}) in the same study in semi arid Kenya. Transpiration rate was initially lower in the wastewater than in the clean water treatment of *B*. *vulgaris* before March 2006, but subsequently became higher in the wastewater treatment. Conversely, transpiration rate in *D*. *giganteus* was higher in the clean water treatment except during March. Sunflower has previously been shown to exhibit a similar trend as transpiration rate was greater in plants irrigated with tap

water than in those irrigated with wastewater from a detergent and oil factory (Gadallah, 1995). Transpiration rate in *B*. *nutans* was substantially greater in the clean water than in the wastewater treatment before March but then became similar in both treatments (Fig. 6.5). The effect of the April rains and prevailing low temperatures was therefore to reduce the treatment effects, as observed in previous studies in which rainfall and cloudy conditions during the mid-summer period were associated with decreased transpiration in bamboo (CSIRO, 1996; Kleinhenz and Midmore, 2002).

During the period between August and November 2006, mean daily temperature increased to 22 ^{o}C following the colder period between May and July when mean temperatures were 20 and 18 ^{o}C, respectively (Fig. 4.1). The rainy season also occurred between October and November, when mean relative humidity (1200 h) increased from *c*. 45 to 70 % (Figs. 4.1 & 4.3). These environmental changes may have contributed to the general reduction in stomatal conductance and transpiration rate (Monteith, 1993) observed for all species and both irrigation treatments (Fig. 6.3 & 6.6). Photosynthetic rate was greater in plants irrigated with wastewater than in those receiving clean water (Fig. 6.11). Previous studies have shown that the higher organic and nutrient content of wastewater increased photosynthetic rate in *Populus* and *Hardwickia binata* (Harrington *et al*., 1997; Paliwal *et al*, 1998; Coyle and Coleman, 2005; Janssen *et al*, 2005).

The diurnal timecourses showed that instantaneous transpiration efficiency (ITE) increased between 0800 and 0900 h before fluctuating for the rest of the day (Fig. 6.24). No significant variation between species or irrigation treatments was detected. Changes in the intensity of solar radiation are the primary driving force for stomatal opening and closure (Dodd *et al*., 1996), causing them to open in the morning and close in the afternoon or evening. Thus, ITE initially increased as increasing solar radiation promoted net photosynthesis until net photosynthesis and

transpiration became balanced as increased radiation and temperature impacted positively on photosynthesis, but negatively on stomatal conductance (Brugnoli and Björkman, 1992; Gu *et al*, 2005; Karlberg *et al*, 2006; Kaipiainen and Pelkonen, 2007). The diurnal pattern of variation in ITE showed little difference between bamboo species, contrary to previous reports for *Bambusa multiplex, Sasa palmata, Phyllostachys vivax* and *Phyllostachys aureosulcata* under temperate conditions in Germany (Sauerbeck *et al*., 2000). A possible explanation for the limited diurnal variation in ITE in the present study may be that this variable is strongly correlated with solar radiation in the tropics. Thus, stomata may remain open from mid-morning, when photosynthesis reaches a maximum, until late afternoon under conditions of favourable water supplies, so providing little variation in ITE. By contrast, midday depression of stomatal conductance and net photosynthesis has been reported for bamboo (Xu and Shen, 1997) associated with at least partial stomatal closure (Farquhar and Sharkey, 1982). A similar midday depression was not found in the present study, probably because the soil was maintained near field capacity in all treatments. Nevertheless, photosynthesis and stomatal conductance did not peak at midday as might have been expected from the observed diurnal trends for temperature and radiation. ITE values decreased successively from the lower canopy through the middle canopy to the upper canopy between July-November 2006 (Figs. 6.22 & 6.23).

ITE values were higher in plants irrigated with wastewater than in those receiving clean water between July and November 2006 (Fig. 6.21). Water use efficiency (WUE) often increases during periods of mild to moderate water stress as photosynthesis is less strongly affected by reductions in stomatal conductance than transpiration (Brugnoli and Björkman, 1992; Gu *et al*, 2005; Karlberg *et al*, 2006; Kaipiainen and Pelkonen, 2007). The richer mineral and organic constitution of wastewater (Table 7.1) may have increased net photosynthesis in this treatment, while plants irrigated with wastewater may have experienced water stress as Na concentration was 873 mg L^{-1}

(Table 7.1), sufficient to cause a 10 % reduction in yield (Evans, 2006) as mean daily temperature increased and stomatal conductance decreased, without affecting net photosynthetic rate (Gu *et al*., 2005; Kaipiainen and Pelkonen, 2007), causing ITE to be greater than their clean water counterparts. This might also explain why net photosynthesis was greatest in plants irrigated with wastewater for all species, particularly *D*. *giganteus* (Figs. 6.9, 6.10, 6.12 and 6.13).

Photosynthetic rate increased gradually with time in all species, particularly *D*. *giganteus* (Figs. 6.7 and 6.10), an effect which may be explained by the general increase in temperature during the observation period (Gratani *et al*., 2008). The values for *B*. *nutans* and *B*. *vulgaris* were indistinguishable, exchanging their relative rankings between July and November 2006 (Figs. 6.7 & 6.9). The observation that the leaves of *D*. *giganteus* were deeper green in colour and had higher SPAD values than the other species provides a likely explanation for its higher photosynthetic rates as SPAD values have been shown to provide reliable estimates of leaf N and chlorophyll concentration (Schroder *et al*., 2000). Net photosynthesis was greatest for leaves located towards the base of the stem and decreased with height (Figs. 6.13 & 6.14), contrary to observations for cassava (El-Sharkawy and De Tafur, 2007).

SPAD measurements have been found to be closely correlated with chlorophyll and N concentrations (Debaeke *et al*., 2006). The influence of irrigation treatment and time on SPAD values was significant ($P<0.001$) and significant species*irrigation treatment*time, species*irrigation treatment and species*time interactions were detected ($P<0.05$; Figs. 6.26-28). Environmental conditions are known to influence photosynthetic rate; for example, Kaipiainen and Pelkonen, in 2007, reported that individual leaves of willow reacted to their immediate environment rather than bulk atmospheric conditions. The significant species*leaf position interaction occurred because photosynthetic rate was higher in the middle leaves of *B*. *vulgaris*

than in the lower leaves. The leaves of *B*. *giganteus* were fewer, broader and deep green, in contrast to *B*. *vulgaris* and *B*. *nutans*, in which they were more numerous, narrow and light green to yellow in colour.

The mean SPAD value for plants of all species irrigated with wastewater was 36.8, compared to 31.2 for plants receiving clean water. Although this apparently large difference was not significant (Fig. 6.26), SPAD values ranging between 30 and 45 have been reported for *P*. *pubescens* in Northern Australia (Zhu *et al*., 2008). The relatively narrow range of SPAD values recorded in the present study may reflect the fact that environmental conditions were relatively uniform and measurements were made only for healthy young leaves. Although a substantial decline in SPAD values occurred during the early stages of the observation period, they subsequently stabilised and remained relatively constant (Figs. 6.26 & 6.28). Only the effects of irrigation treatment and time were significant for SPAD values (Fig. 6.28); the species* irrigation treatment*time interaction was not significant. No significant correlations between gas exchange variables and SPAD values were found (Table 6.2), contrary to previous findings for peanut (Sheshshayee *et al*., 2006).

Stomatal conductance was significantly affected by leaf position in the canopy and time, and significant species*irrigation treatment*time, irrigation treatment*leaf position, species*leaf position, species*irrigation treatment*leaf position and irrigation treatment*time interactions were detected ($P<0.05$; Figs. 6.16-6.18). For the species*canopy level interaction, *B*. *vulgaris* exhibited the highest stomatal conductances for all leaf positions, followed successively by *D*. *giganteus* and *B*. *nutans*. For the irrigation treatment*leaf position interaction, plants irrigated with wastewater exhibited the highest stomatal conductances in the middle and lower canopy, whereas the values for plants receiving clean water were greatest in the upper canopy (Figs. 6.19 & 6.20). Stomatal conductance declined steadily between July and November 2006, with wide fluctuations being

observed in July and September (Fig. 6.15). During this period, the various treatments frequently interchanged their relative rankings to produce the observed species*irrigation treatment*time interaction, although the general trend was for the values to decrease with time (Fig. 6.15). Stomatal conductance was greatest in the middle leaves of plants receiving clean water for all species except *B. vulgaris* (Figs. 6.16- 6.18).

Transpiration rate was greatest in the wastewater treatment of *B. vulgaris* between July and September 2006, but was subsequently greatest in the wastewater treatment of *D. giganteus*. A significant species*time*irrigation interaction was detected ($P<0.001$; Fig. 6.19). Transpiration rate was greater for leaves in the middle of the canopy than in the upper and lower canopy (Fig. 6.20), perhaps because the upper leaves were still developing, while those at the base of the canopy were less exposed to prevailing atmospheric conditions which influence transpiration. This observation is broadly consistent with the values for net photosynthetic rate, which was lowest for the upper leaves but was comparable for the middle and lower leaves (Figs. 6.12 & 6.13).

6.4.2 Gas exchange: Experiment 2

The plants used in this experiment were relatively young (two months) and approximately 30 cm tall. No significant treatment effects on gas exchange were detected, except for that of irrigation treatment on net photosynthetic rate. The absence of significant effects may reflect the short duration of experiment and the carry-over effect of nutrients from the fertile soil in which they were initially grown; thus, the irrigation treatments in Experiment 2 were applied to plants in their original pots and soil, whereas the plants used in Experiment 1 were re-potted in forest soil. Net photosynthetic rate in Experiment 2 was greatest in the domestic wastewater treatment of *D. giganteus* and *B. nutans* (Fig. 6.29) and the industrial wastewater treatment of *B. vulgaris* (Fig.

6.30a-c). The plants in Experiment 2 exhibited a wider ITE range of 3-8 μmol CO_2 mmol H_2O^{-1}, compared to 3-6 μmol CO_2 mmol H_2O^{-1} in Experiment 1. High water use efficiencies have been reported previously for bamboo genotypes in Germany (Sauerbeck *et al*., 2000).

SPAD values were greatest for plants irrigated with domestic wastewater, followed successively by the clean and industrial wastewater treatments ($P<0.001$; Fig. 6.33). Although the effect of irrigation treatment and species was significant (Figs. 6.33 & 6.34), there was no interaction; thus SPAD values were greatest in *D*. *giganteus* followed successively by *B*. *vulgaris* and *B*. *nutans* ($P<0.001$; Fig. 6.34). Mean values for all species were greatest in the domestic wastewater treatment (Fig. 6.35) and were generally lower than in Experiment 1, probably because the plants in Experiment 2 were younger and growing more rapidly than those of Experiment 1.

The closeness of the correlation between net photosynthetic rate and SPAD values varied greatly between sampling dates; thus significant positive correlations obtained on specific dates ($P<0.001$) might be followed by negative correlations at the next sampling date, possibly reflecting physiological changes within bamboo plants caused by water or osmotic stress or varying environmental conditions (Table 6.3). This observation emphasises the difficulty of drawing conclusions from such correlations, especially when based on datasets collected on a limited number of occasions.

6.5 Conclusions

Experiment 1 extended over a 15 month period, allowing gas exchange to be measured during both the rainy and dry seasons. However, as the IRGA could not be used when it was raining or the leaves were wet, the data presented here were obtained under reasonably uniform conditions, despite seasonal change in climatic conditions. Changes in irradiance, temperature and SD affect

gas exchange as transpiration rate increased during periods of high SD and decreased during periods of low SD (Vose *et al*., 2003). The influence of irrigation treatment on gas exchange parameters for the three bamboo species examined appears to be complex and affected by a range of environmental factors. Although photosynthetic rate was higher in plants irrigated with wastewater than in those receiving clean water, effects on stomatal conductance and transpiration rate were less obvious, perhaps due to effects of wastewater composition on the osmotic status of plants, which might have been compounded by variation in temperature. The responses to environmental conditions exhibited by plants in both irrigation treatments appear to be complex and cannot be explained solely by differences in the composition of the irrigation water.

Experiment 2 lasted less than four months, during which heavy rainfall was experienced. This may have had a diluting effect on the irrigation treatments and also interrupted the gas exchange data collection. The duration of the experiment might also have been insufficient for detectable responses to the irrigation treatments to develop, as the early growth of bamboo is directed primarily towards roots and rhizomes rather than the shoots. Nevertheless, an effect of irrigation treatment was apparent as domestic wastewater enhanced photosynthetic rate, followed successively by the industrial wastewater and clean water treatments, suggesting that domestic wastewater had short term advantages over clean water. All other gas exchange parameters showed no significant effect, perhaps due to the short duration of the experiment. Calculations of ITE and correlations between gas exchange parameters and SPAD values revealed no major differences. The SPAD values obtained in the present experiment are comparable to those of 30-45 reported for *P. pubescens* in Australia (Zhu *et al*., 2008), but are lower than those reported for *Eucalyptus grandis*, *Cordia africana* and *Grevillea robusta*, also C3 species, in a previous study at Juja (Kuya, 2008); which ranged from 10-50.

CHAPTER 7
ELEMENTAL ANALYSIS

7.1 Introduction

Elemental analysis of the clean and wastewater irrigation supplies, the soil on which plants were grown and the plant tissues produced was essential to determine the flow of elements from the irrigation water through the soil to the bamboo plants. The hypothesis that bamboo species may differ in their ability to accumulate elements contained in the irrigation water was tested, as well as the influence of the two irrigation sources used in Experiment 1 and three sources used in Experiment 2 on the performance of the three bamboo species examined. Determination of elemental composition involved analysis of all irrigation sources, soil and plants. Such analyses may be qualitative to determine which elements are present or quantitative to determine the concentration of each element; the latter approach was adopted in the present study.

Parameswaran (1999) reported that the concentrations of all nutrients except Na and Fe were greater in shoots than in tubers following irrigation of *Helianthus tuberosus* L. with wastewater in Australia, while Kiziloglu *et al*. (2007) found that irrigation with wastewater for one year significantly affected chemical properties in the 0-30 cm soil horizon, nutrient concentrations within plants, increased salinity, organic matter content and exchangeable Na, K, Ca, Mg, plant-available P and trace element concentrations and decreased soil pH, while increasing yield and N, P, K, Fe, Mn, Zn, Cu, B and Mo concentrations in cabbage. Tani and Barrington (2005) observed that the concentrations of Cu and Zn in wastewater were beneficial rather than detrimental to buckwheat. Kiziloglu *et al*. (2007) concluded that irrigation with wastewater increased macronutrient concentrations and enhanced micronutrient concentrations in plants with the exception of Ca and Mg.

The reliability of chemical analysis of samples is critically dependent on sample collection and preparation procedures. Several stages are required to obtain results which accurately represent the sampled material. Preparation of solid, as opposed to liquid, samples requires appropriate digestion procedures and apparatus of sufficiently high quality to resist the corrosive effect of inorganic acids. The procedures employed usually allow the results to be guaranteed using certified reference materials. Organisations such as the United States Environmental Protection Agency (USEPA) have developed sample preparation and validation procedures which have been adopted as standard protocols by many laboratories (Jacobsen and Lorbeer, 1998).

Soil analysis procedures for nitrogen (N) include analysis of Kjeldahl digests by titration, steam distillation and colorimetric or auto-analyser methodologies, while phosphorus (P) concentration may be determined using sodium acetate, Bray, sodium bicarbonate (Olsen), ammonium bicarbonate-DTPA (Diethylene triamine Pentaacetic Acid), Morgan extracting solutions or water-based methods (Ryan *et al*., 2001). Colorimetric methods include ascorbic acid and Fiske-Subbarrow tests using spectrophotometetric, auto-analyser or inductively coupled plasma mass spectrophotometry (ICPMS) approaches. Potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) are normally determined by atomic absorption spectrophotometry (AAS) or ICPMS. Total sulphur (S) may be determined by ICPMS following nitric-perchloric digestion or infrared and high-temperature combustion furnace analysers. Inorganic-S (SO_4-S) is determined by ICPMS, turbidimetric or gravimetric methods following ammonium acetate, water and monocalcium phosphate extraction (Ryan *et al*., 2001). Zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), boron (B) and molybdenum (Mo) are determined using DTPA and ammonium bicarbonate-DTPA extracting solutions or by AAS and ICPMS. B may also be determined colorimetrically using hot water extracts. Organic matter (OM) and total carbon (C)

concentrations may be determined following combustion, while the Walkley-Black method and loss on ignition (LOI) are also popular methods (Jacobsen and Lorbeer, 1998; Ryan *et al*., 2001).

Plant nitrogen (N) concentration may be determined using sulphuric acid digests with Kjeldahl catalyst addition and titration or auto-analyser techniques. P, K, Ca, Mg, Zn, Mn, Cu, Fe, B and Mo concentrations in plant tissues may be analysed following preparation by wet ashing using a nitric-perchloric acid mixture or dry ashing by emission, AAS or ICPMS spectrophotometry. N and P may also be determined colorimetrically. Techniques for determining B concentration include colorimetric analysis (Jacobsen and Lorbeer, 1998). Methodologies for determining sulphur (S) concentration in plants include digestion using nitric-perchloric acid mixtures or dry ashing with the addition of magnesium nitrate followed by analysis using infrared, ICPMS or turbidimetric methods (Ryan *et al*., 2001).

For water analysis, suspended solids are determined gravimetrically. Adjusted sodium adsorption ratios (SARs) were calculated from measurements of Ca, Mg, K and Na concentrations determined by AAS or ICPMS; trace element concentrations were also determined using AAS or ICPMS (Jacobsen and Lorbeer, 1998; Ryan *et al*., 2001).

7.2 Objectives

These were to: (i) establish the uptake of nutrients and trace elements by the shoots of three bamboo species irrigated with clean or wastewater on the basis of harvests made at the beginning (H1), middle (H2) and end (H3) of the Experiment 1 and at the end of Experiment 2; (ii) determine changes in the elemental composition of the soil during the experimental period in Experiment 1 and; (iii) quantify differences in elemental composition between irrigation sources (domestic, industrial, and clean water) used in Experiments 1 and 2.

7.3 Analysis

7.3.1 Atomic Absorption Spectrophotometry and ICPMS

All samples collected at H1 in Experiment 1 were analysed by atomic absorption spectrophotometry (AAS) to provide baseline values before the irrigation treatments began. Procedures for sample preparation and analysis are described in Section 2.1.17. Inductively coupled mass spectroscopy (ICPMS) was used to analyse soil, water and plant samples collected at Harvests 2 and 3 in Experiment 1 (Section 2.1.16). The ICPMS was not available when the samples for H1 in Experiment 1 were analysed. All samples from Experiment 2 were analysed by ICPMS.

7.4 Results

7.4.1 Experiment 1

K, P, Cu, Zn, Ni, Na Cd and Pb were analysed using AAS, while N, K, Ca, Mg, Mn, Fe, Na, Mn, Co, Mo, Ni, Cu, Zn, Al, Ti, V, Cr, As, Se, Rb, Sr, Ag, Cd, Sn, Sb, Cs, Ba, Lu, Au, Pb, Bi, Th, U and 51V were analysed by ICPMS. Phosphate (P), total organic carbon content and loss on ignition (LOI) were also determined (*cf*. Sections 2.9 and 2.10).

7.4.2 Water and soil characteristics

Table 7.1 shows baseline values for soil and water characteristics in the clean and wastewater treatments at the start of Experiment 1 (H1). The wastewater contained high concentrations of Na

Table 7.1. Concentrations of elements in the irrigation water and soil at Harvest 1 in Experiment 1.

Constituent	Soil	Clean water	Wastewater	
Units	mg kg^{-1}	mg L^{-1}	mg L^{-1}	Pr
Na	120.1	0.0	873.3	<0.001
K	812.3	0.0	536.7	<0.001
P	5.5	0.0	8.4	<0.001
Cu	13.2	0.0	0.1	<0.001
Zn	198.6	0.1	6.5	<0.001
Pb	16.1	0.1	0.0	<0.001
Ni	15.1	0.0	0.2	<0.001
Cd	0.0	0.0	0.1	<0.001
TOC*	-	0	5.8	<0.001
TC	-	0	37.9	<0.001
IC	-	0	32.1	<0.001
Organic matter	30400	0.0	-	<0.001
N	-	0.0	1600	<0.001

*** TOC = Total Organic Carbon; TC = Total Carbon; IC = Inorganic Carbon. - indicates that measurements were not made.**

and K, and Na concentration in the wastewater was over six times greater than in the soil ($P<0.001$; Table 7.1). P concentration in the wastewater was also higher than in the soil, or clean water ($P<0.001$; Table 7.1). TOC provides an estimate of the natural organic matter in water and was much greater in the wastewater supply than in the clean water in which TOC was undetectably low ($P<0.001$; Table 7.1). Compared to the indicative safe levels for wastewater (Pettygrove and Asano, 1985), the elemental compositions of all three irrigation sources were not toxic.

7.4.3 Analysis of plant samples at Harvest 1

Because of their small size, the oven-dried samples of leaf, stem and root tissues were combined for each species at Harvest 1 for analysis of N, P, K, Cu, Ni, Zn, Na, and Pb concentrations (Fig. 7.1). Trace metals were not present in toxic concentrations. N, P, Cu, Na, and Zn concentrations differed between species and organs ($P<0.001$), whereas K concentration differed between organs ($P<0.05$) but not between species (Fig. 7.1). Ni and Pb concentrations differed between species and organs ($P<0.01$; Fig. 7.1g & h).

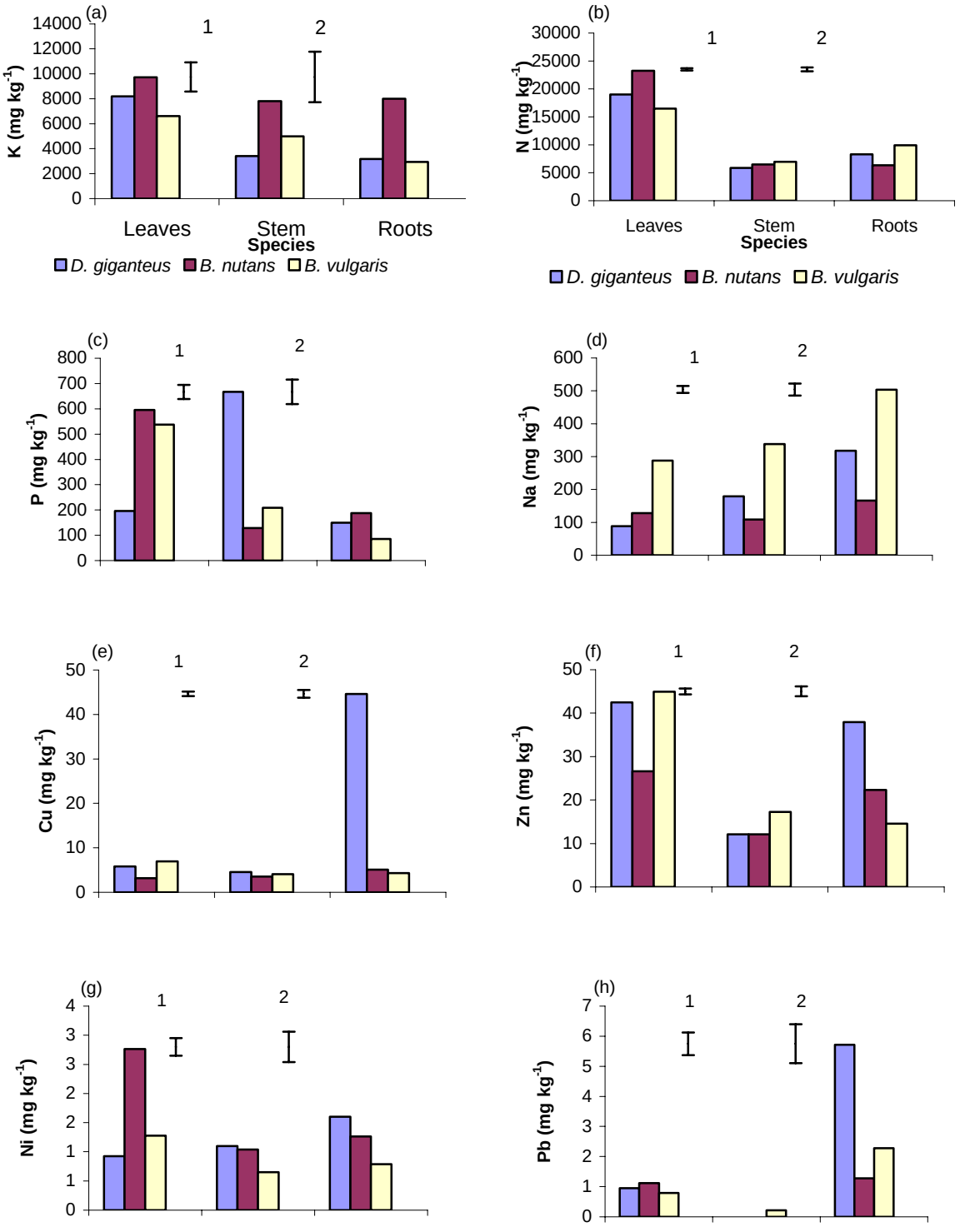


Figure 7.1. Elemental concentrations of (a) K, (b) N, (c) P, (d) Na, (e) Cu, (f) Zn, (g) Ni, and (h) Pb in the leaves, stems and roots of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1 and 2 show SED values for comparing species and organs.

7.4.4 Leaf analysis at Harvests 2 and 3

Figure 7.2 shows elemental concentrations in the leaves of all three species for both irrigation treatments in Experiment 1. Mg concentration was greatest in *B. vulgaris* and lowest in *D. giganteous* for both irrigation treatments and harvests ($P<0.001$; Fig. 7.2a), and increased with time ($P<0.001$). Zn concentration was generally greater in *D. giganteus* than in *B. vulgaris* and *B. nutans*, particularly at H2 ($P<0.5$; Fig. 7.2b). No consistent trend was observed for Cu, although the variation with time was significant ($P<0.01$; Fig. 7.2c). Mo concentration varied between irrigation treatments and with time ($P<0.01$ & $P<0.001$; Fig. 7.2d). Ni concentration was lower in the wastewater than in the clean water treatment ($P<0.01$; Fig.7.2e), while Al concentration did not differ between species or irrigation treatments, although the effect of time was significant ($P<0.001$; Fig. 7.2f). As concentration varied with time and was greatest in the wastewater treatment at H2 ($P<0.01$; Fig. 7.2g), and Cd concentration was lower, sometimes by a substantial margin, at H3 than at H2 ($P<0.001$; Fig. 7.2h).

7.4.5 Stem analysis at Harvests 2 and 3

Elemental analysis of the entire stem showed that Mg concentration was generally greater in the wastewater than in the clean water treatment for all species ($P<0.05$; Fig. 7.3a). Zn concentration was greater in the wastewater treatment than in the clean water treatment of *B. nutans* and *B. vulgaris*, whereas the opposite was true for *D. giganteus*; the values were lower at H3 than at H2 ($P<0.001$; Fig. 7.3b). Cu concentration was greatest in both irrigation treatments of *D. giganteus* at H2 but no consistent species effects were apparent at H3; the influence of time was significant ($P<0.001$; Fig. 7.3c). Ni and As concentrations did not differ significantly between species or irrigation treatments (Figs. 7.3d & g). Al concentration was greater in the clean water treatment of all species at both harvests ($P<0.05$; Fig. 7.3f), whereas the reverse applied for Cd concentration ($P<0.001$; Fig. 7.3e).

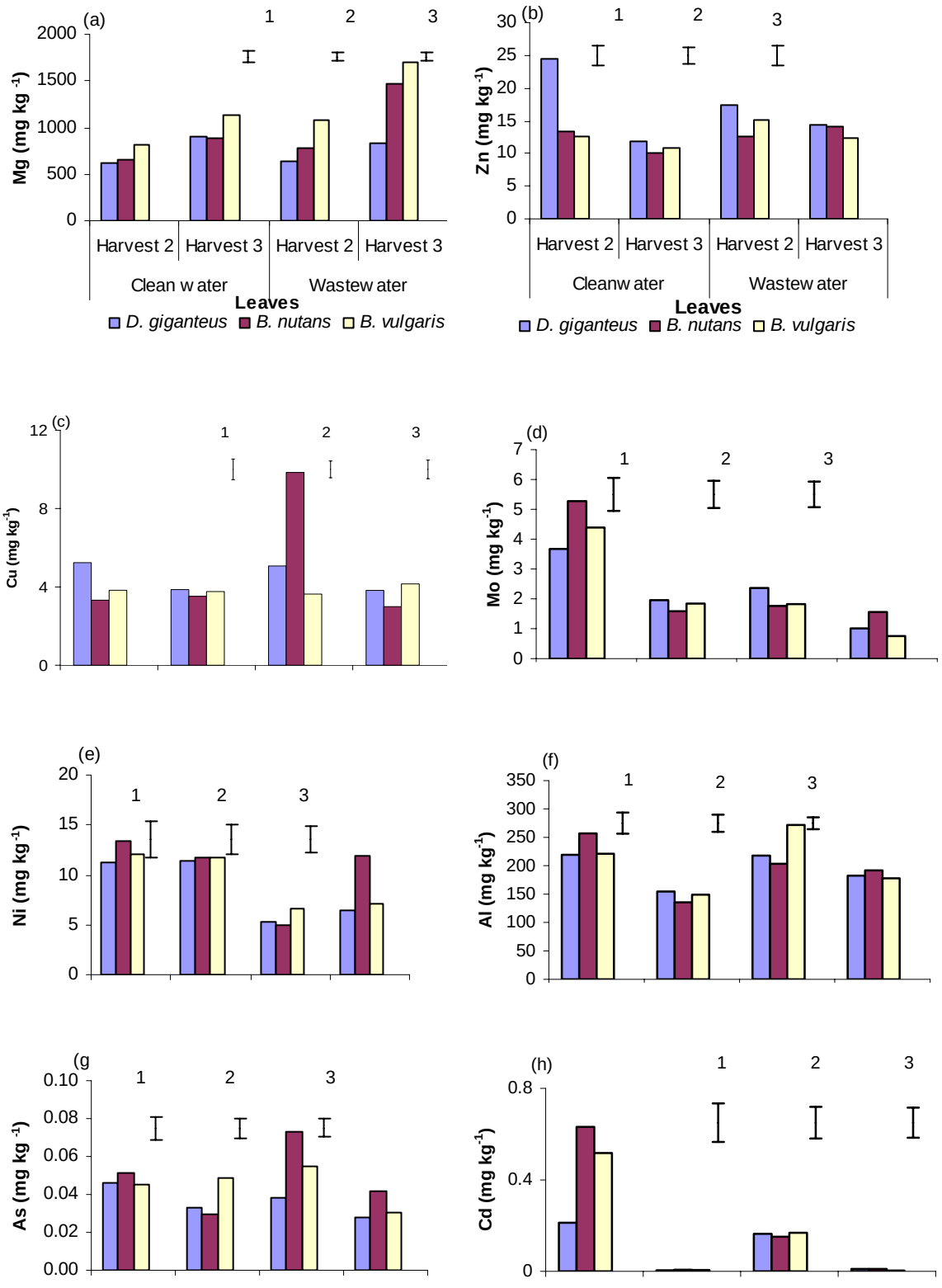


Figure 7.2. Elemental concentrations of (a) Mg (b) Zn, (c) Cu, (d) Mo, (e) Ni, (f) Al, (g) As,and (h) Cd concentrations in the leaves of *D. giganteus*, *B. nutans* and *B. vulgaris* at Harvests 2 and 3. Vertical bars 1, 2 and 3 represent the SED values for comparing species, irrigation treatments and harvests.

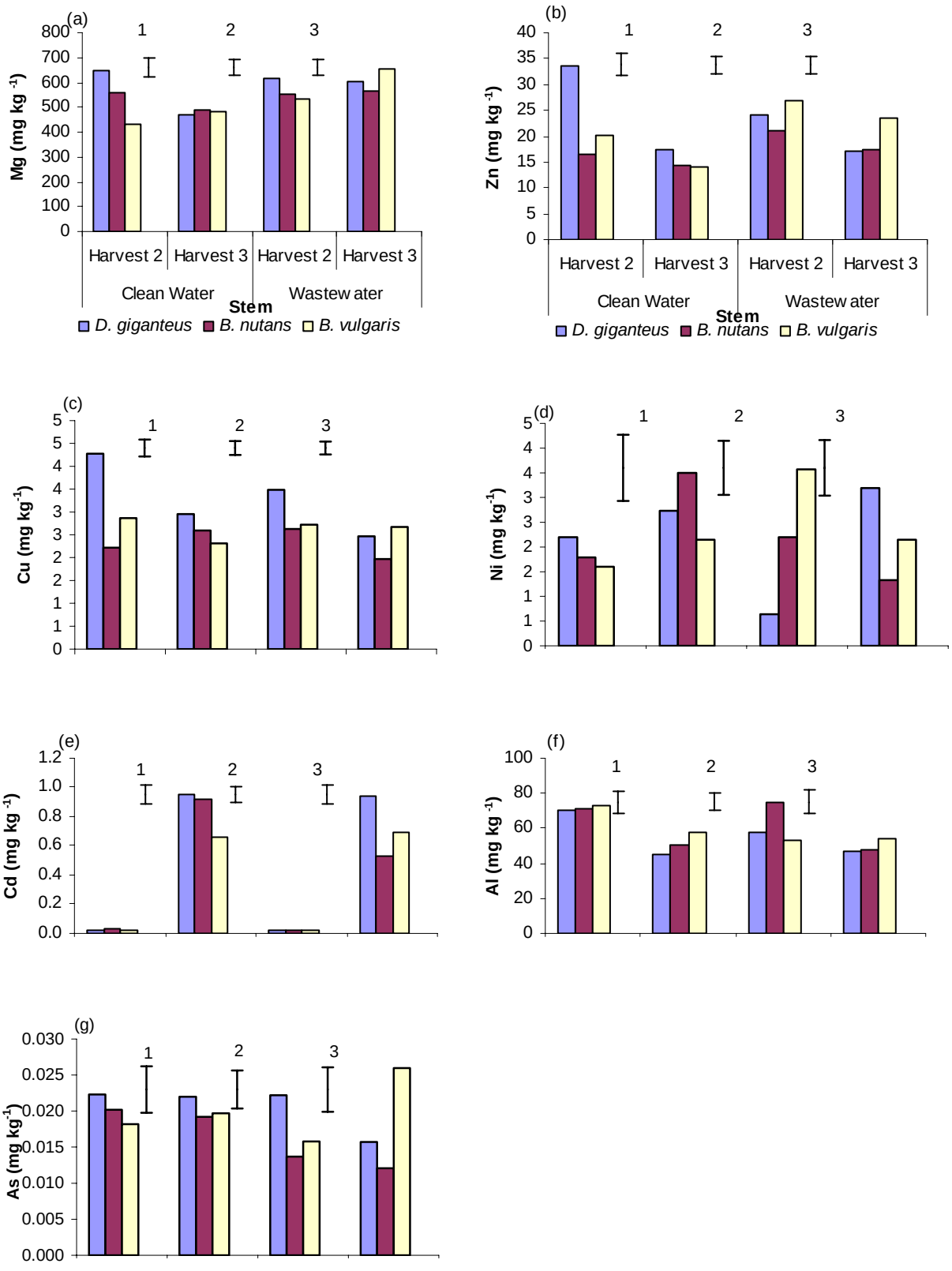


Figure 7.3. Effect of harvest date and irrigation treatment on Mg, Zn, Cu, Ni, Cd, Al, and As concentrations in the stems of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1, 2 and 3 represent SED values for comparing species, irrigation treatments and harvests.

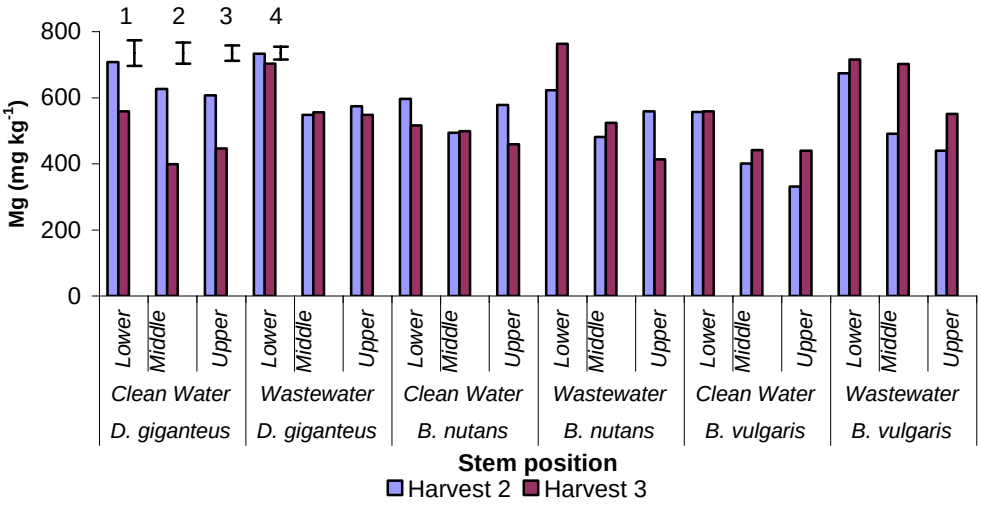
7.4.6 Analysis of upper, middle and lower stem segments at Harvests 2 and 3

Analysis of the upper, middle and lower stem segments revealed significant variation in elemental concentration. Mg concentration was greater ($P<0.05$; Fig. 7.4a & b) in plants irrigated with wastewater than in those receiving clean water, and significant differences were also detected for stem position ($P<0.001$) and the species*time ($P<0.001$) and irrigation treatment*time interactions ($P<0.01$). Mo concentration was much greater at H3 than at H2 ($P<0.001$; Fig. 7.5a & b), but the differences between irrigation treatments, species and stem positions were not significant. Mg and Mn concentrations, averaged over all species and both irrigation treatments, were greatest in the lower stem, followed successively by the middle and upper stem segments ($P<0.001$; Table 7.2). Mn concentration was higher in the lower than in the middle and upper stem segments ($P<0.001$; Fig.7.6a) but no significant differences were found for species, irrigation treatment and time. Zn concentration was greater in the lower stem in both irrigation treatments ($P<0.001$; Fig. 7.6b) and significant effects of species ($P<0.05$) and time ($P<0.001$) were detected, including a significant species*time interaction ($P<0.01$). At both harvests, the stems of plants irrigated with wastewater contained higher concentrations of Al than those receiving clean water ($P<0.05$; Fig. 7.7), and there was a significant irrigation treatment*time interaction ($P<0.01$). Al concentration in the stems of plants in the clean water treatment was higher at H2 than at H3, although the difference was not significant.

Table 7.2. Mean Al and Mn concentrations in the upper, middle and lower stem segments averaged over all three bamboo species and both irrigation treatments.

	Upper stem	Middle stem	Lower stem	Pr.	*SED*
Mg	495.8	513.7	642.1	<0.001	23.28
Mn	57.2	74.2	105.2	<0.001	5.04
Zn	17.73	18.38	25.46	<0.001	1.452

(a)



(b)

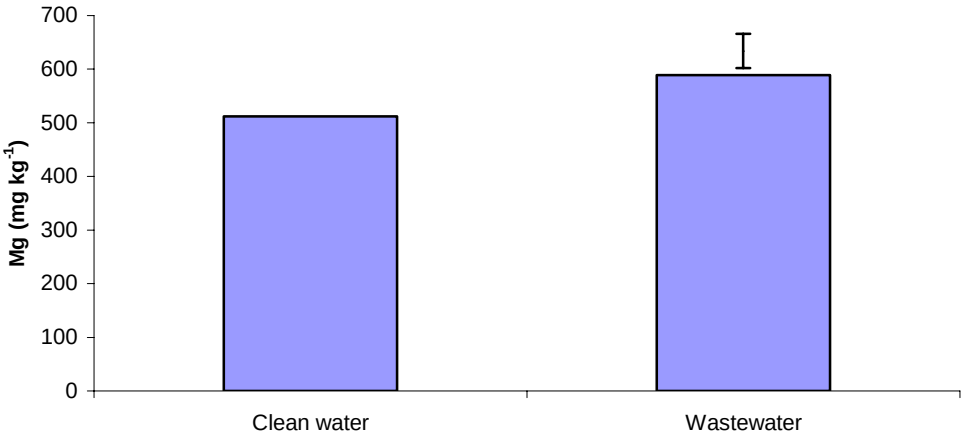
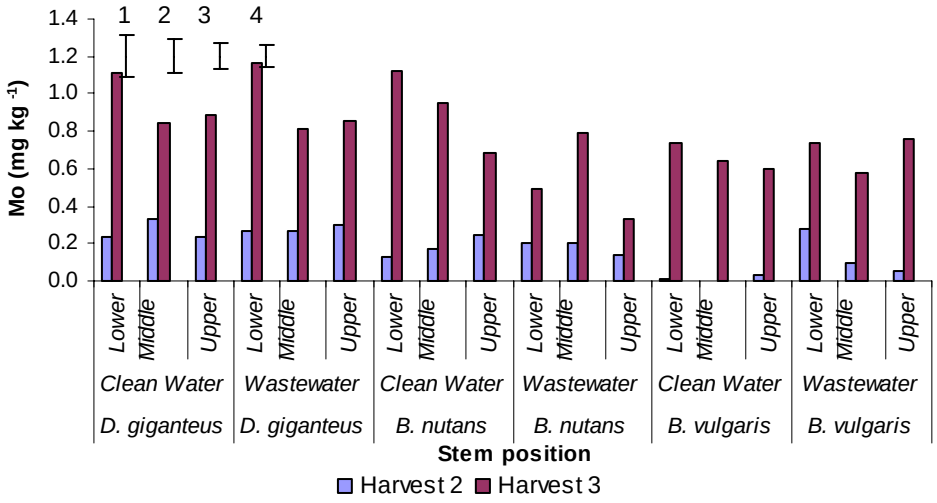


Figure 7.4. (a) Influence of species, stem position, irrigation treatment and harvest date on Mg concentration in the stems of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1, 2, 3 and 4 represent SED values for comparing species, stem positions, irrigation treatments and time. (b) Mean Mg concentration for all stem segments averaged for all species and both harvests. Vertical bar represents the SED value for comparing irrigation treatments.

(a)



(b)

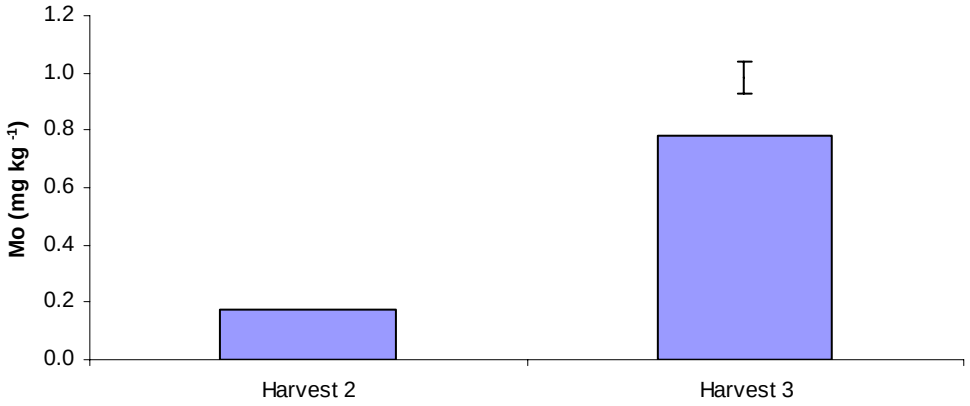


Figure 7.5. (a) Influence of species, stem position, irrigation treatment and harvest date on Mo concentration in the stems of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1, 2, 3 and 4 represent SED values for comparing species, stem position, irrigation treatment and time. (b) Influence of time on Mo concentration in the stem averaged for all species and both irrigation treatments. Vertical bar represents SED value for comparing harvest dates.

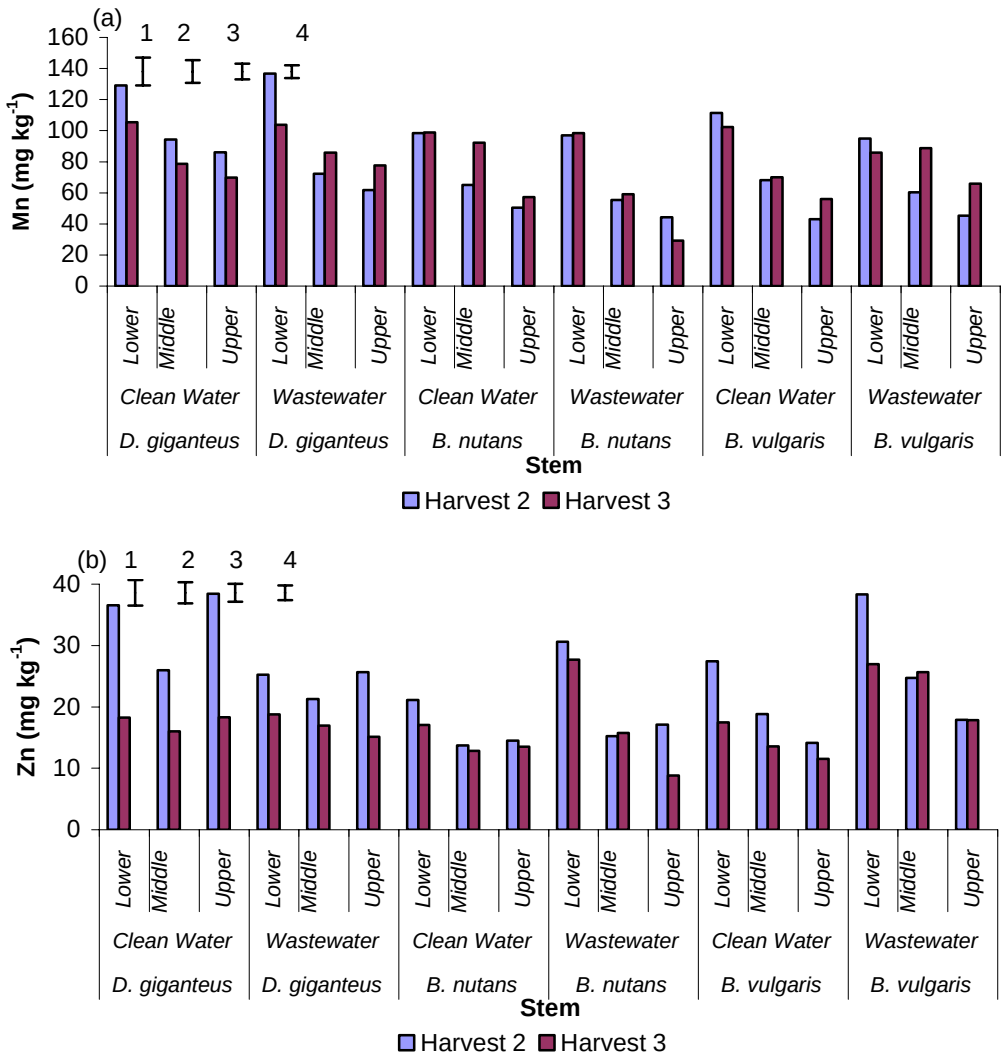


Figure 7.6. Influence of stem position and irrigation treatment on (a) Mn and (b) Zn concentration in *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1, 2, 3 and 4 represent SED values for comparing species, irrigation treatments, stem position and time.

Al and Zn concentrations in the stem were higher at H2 than at H3 for *D. giganteus* and *B. nutans*, and for Zn in *B. vulgaris* ($P<0.001$; Fig. 7.8a; $P<0.001$; Fig. 7.8b). Al concentration in the stems of *B. vulgaris* was lower at H2 than at H3. Ni concentration tended to decrease from the upper to the lower stem at H2, whereas the reverse applied at H3 (Fig. 7.9a & b), although these trends were not significant; however, a significant stem position*time interaction was detected (Fig. 7.9a & b; $P<0.01$). Zn concentration was higher in the wastewater treatment of *B. nutans* and *B. vulgaris*, whereas the opposite applied for *D. giganteus* ($P<0.05$; Fig. 7.10).

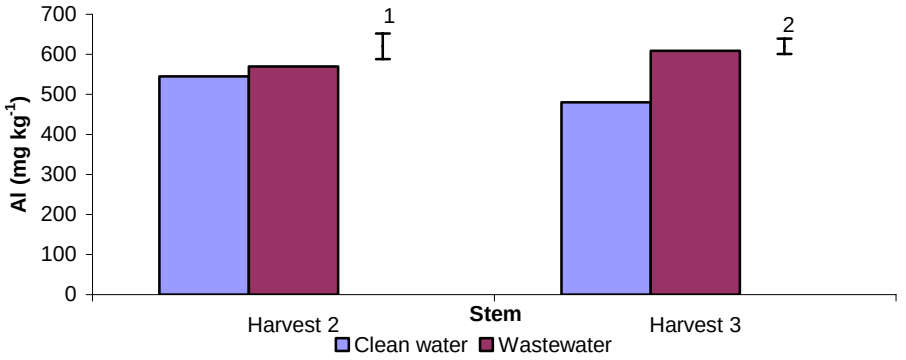


Figure 7.7. Influence of irrigation treatment and harvest date on Al concentration in the stem averaged over all three species and both harvest dates. Vertical bars 1 and 2 represent SED values for comparing irrigation treatments and harvest dates.

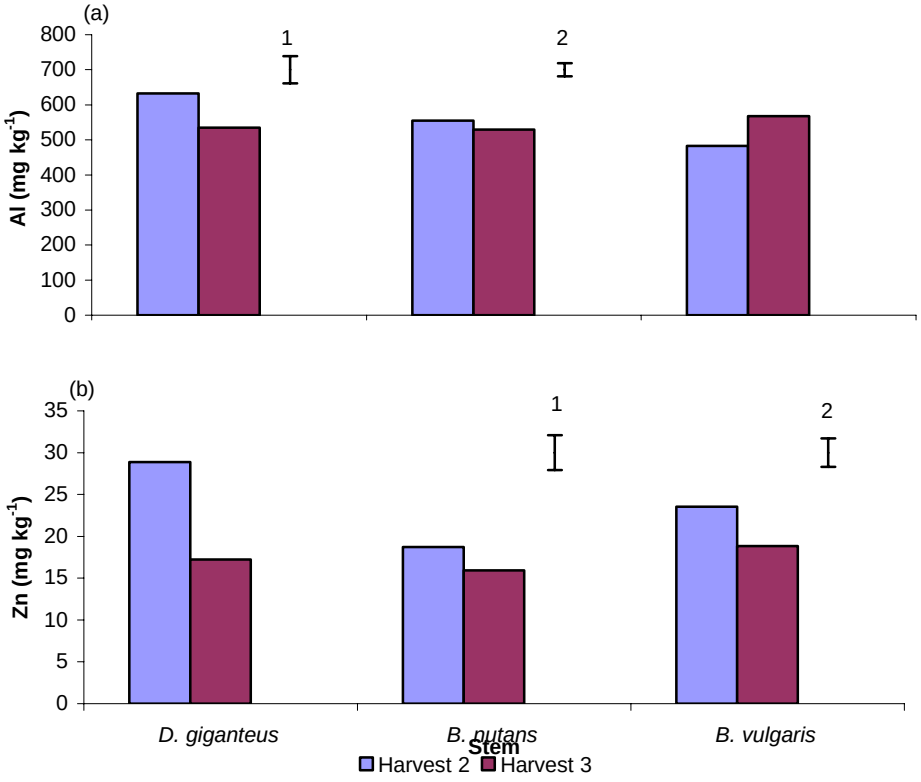
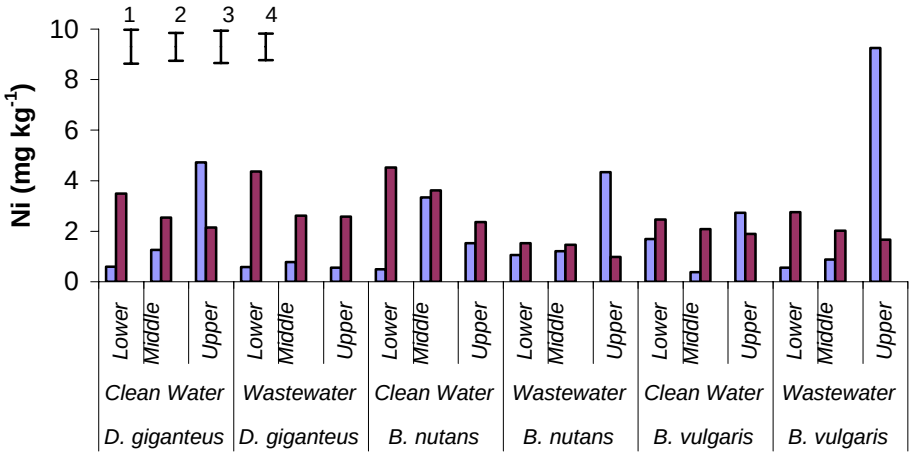


Figure 7.8. Influence of species and harvest date on (a) mean Al and (b) Zn concentrations for three bamboo species averaged for both irrigation treatments. Vertical bars 1 and 2 represent SED values for comparing species and harvest dates.

(a)



(b)

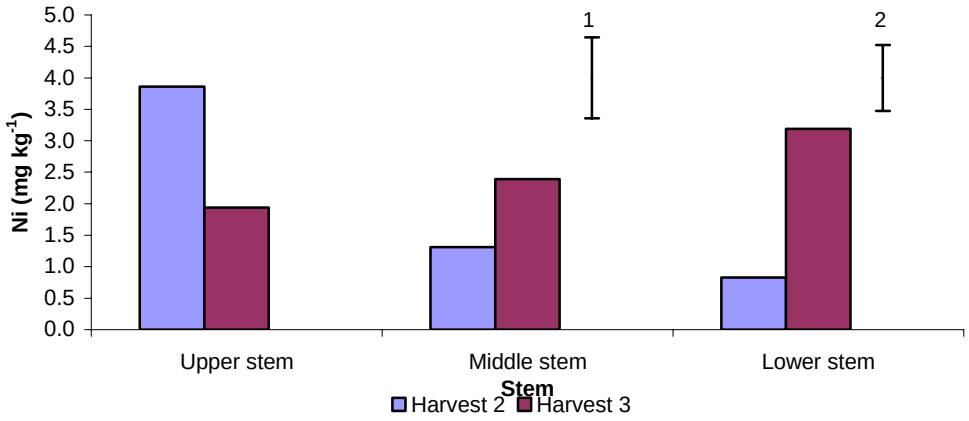


Figure 7.9. (a) Influence of stem position and irrigation treatment on Ni concentration in the stems of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1, 2, 3 and 4 represent SED values for comparing species, irrigation treatments, harvest dates and stem positions. (b) Influence of stem position on Ni concentration averaged over all species and both irrigation treatments. Vertical bars 1 and 2 represent SED values for comparing stem positions and harvest dates.

7.4.7 Soil analysis at Harvests 2 and 3

Somewhat surprisingly, soil Cd concentration was greater in the clean than in the wastewater treatment ($P<0.001$; Fig 7.11), perhaps because Cd uptake by plants was greater in the latter. Soil Cd concentration was greater under *D. giganteus* than in the treatments containing *B. nutans* and *B. vulgaris* ($P<0.01$), and was greater at H3 than at H2 ($P<0.001$; Fig. 7.11).

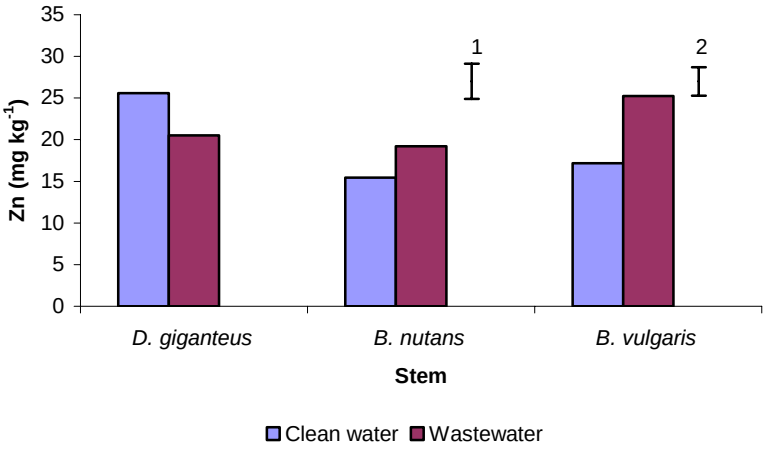


Figure 7.10. Influence of species and irrigation treatment on Zn concentration in the stems of *D. giganteus*, *B. nutans* and *B. vulgaris*. Vertical bars 1 and 2 represent SED values for comparing species and irrigation treatments.

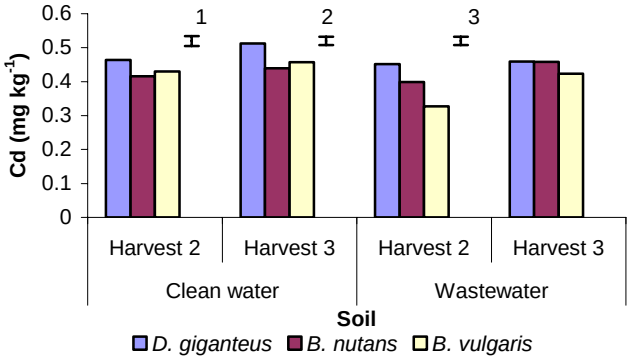


Figure 7.11. Influence of species, irrigation treatment and time on soil Cd concentration. Vertical bars 1, 2 and 3 represent SED values for comparing differences between species, irrigation treatments and time.

Soil Mg concentration averaged over both irrigation treatments was greater under *D. giganteus* than in the corresponding treatments of *B. nutans* and *B. vulgaris* ($P<0.001$; Table 7.3). Mean soil Mg concentration averaged over all species and irrigation treatments was greater at H3 than at H2 ($P<0.01$; Table 7.5). Mean soil As concentration for both irrigation treatments was greatest under *B. vulgaris* ($P<0.001$; Table 7.3), and was also greater in the wastewater than in the clean water treatment ($P<0.05$; Table 7.4).

Table 7.3. Cd, Mg and As concentrations (mg kg^{-1}) for soil planted with *D. giganteus*, *B. nutans* or *B. vulgaris* and irrigated with clean or wastewater; values are means for both irrigation treatments.

Element	*D. giganteus*	*B. nutans*	*B. vulgaris*	*Pr.*	*SED*
Cd	0.472	0.428	0.409	<0.001	0.0146
Mg	1940	1794	1554	<0.001	63.8
As	9.73	9.04	10.91	<0.001	0.442

Table 7.4. Cd and As concentrations (mg kg^{-1}) for soil planted with *D. giganteus*, *B. nutans* or *B. vulgaris* and irrigated with clean or wastewater; values are means for all species.

Element	Clean water	Wastewater	*Pr.*	*SED*
Cd	0.453	0.431	<0.01	0.0119
As	9.47	10.32	<0.05	0.361

Table 7.5. Cd and Mg concentrations (mg kg^{-1}) at Harvests 2 (July 2006) and 3 (November 2006) for soil planted with *D. giganteus*, *B. nutans* or *B. vulgaris* and irrigated with clean or wastewater; values are means for all species and irrigation treatments.

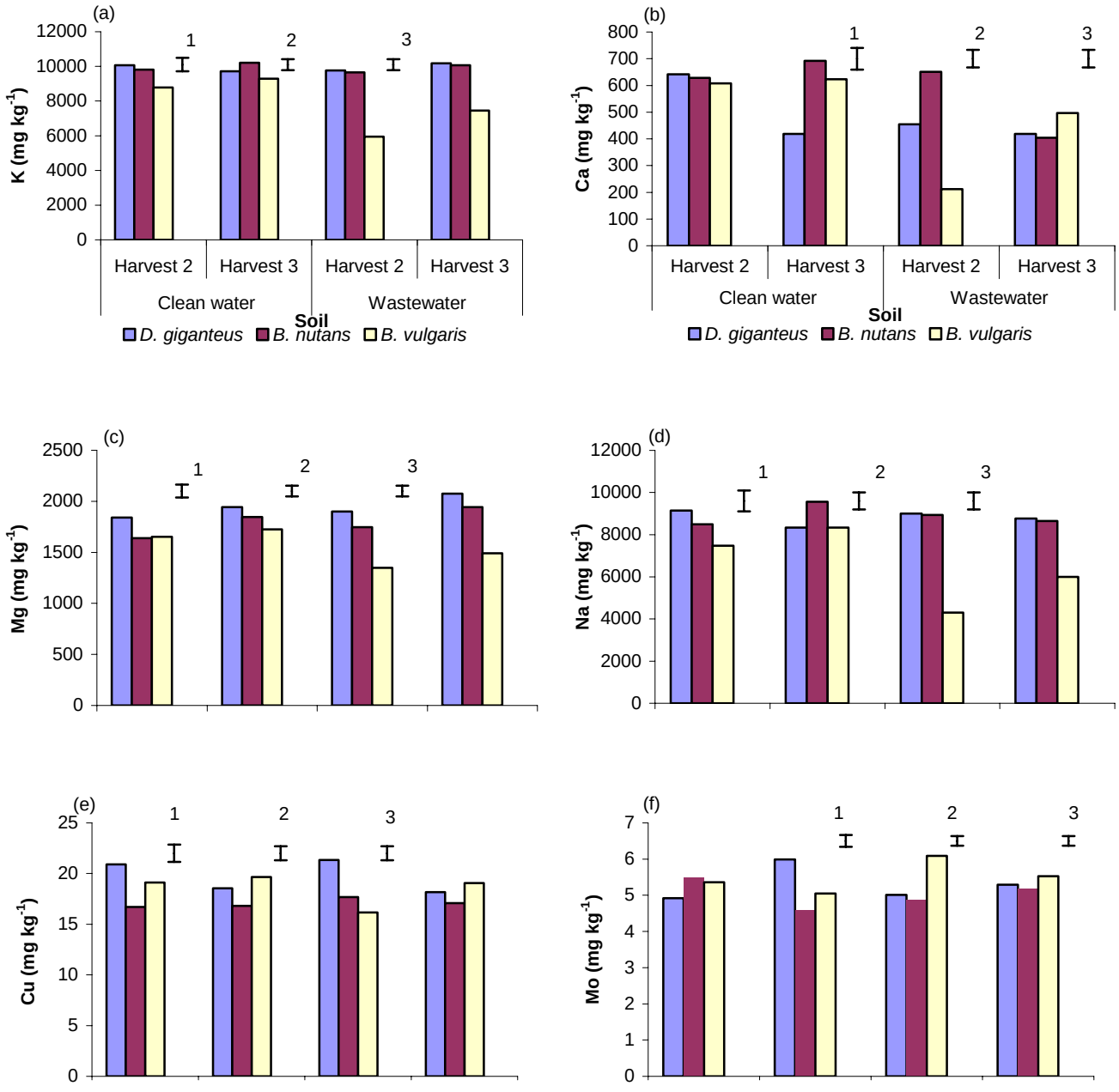
Element	Harvest 2	Harvest 3	*Pr.*	*SED*
Cd	0.414	0.458	0.001	0.0119
Mg	1688	1838	0.009	52.1

Soil K concentration was influenced by both species and irrigation treatment (P<0.001 and P<0.01 respectively; Fig. 7.12a), although the effect of time was not significant, and was lower under *B. vulgaris* than for the other two species at both harvests and irrigation treatments. Ca concentration differed between species and irrigation treatment and there were significant species*time and species*water*time interactions (P<0.05, P<0.001, P<0.05, P<0.01 respectively; Fig. 7.12b), but the effect of time was not significant. Mg concentration differed between species and harvests (P<0.001 and P<0.05 respectively; Fig. 7.12c) but the effect of irrigation treatment was not significant; however, a species*water interaction was apparent (P<0.05). Na concentration in the soil differed between species and irrigation treatments, and a significant species*water interaction was detected (P<0.001, P<0.05 and P<0.05 respectively; Fig. 7.12d). Although soil Cu concentration was higher under *D. giganteus* than the other species at H2, the value was greatest

under *B*. *vulgaris* in both irrigation treatments at H3 ($P<0.05$; Fig. 7.12e), although the influence of irrigation treatment and time were not significant. The wastewater treatment of *B*. *vulgaris* exhibited the highest soil concentration for Mo at both harvests ($P<0.05$; Fig. 7.12f); the influence of irrigation treatment and time was not significant, although species*time, species*water and species*water*time interactions were detected. Soil Fe concentration differed between species, being greatest in the wastewater treatment of *B*. *vulgaris* at H2, and between irrigation treatments ($P<0.001$ and $P<0.05$, respectively; Fig. 7.12g), but effect of time was not significant; however, there was a significant species*water interaction ($P<0.05$). Soil Zn concentration was lowest under *B*. *vulgaris* and was greater in the clean water treatment of all species ($P<0.001$ and $P<0.05$, respectively; Fig. 7.12h), although the effect of time was not significant; however, a significant species*water interaction was detected ($P<0.016$). Ni concentration in the soil was greatest in the *B*. *vulgaris* wastewater treatment at H2 and lowest in the clean water treatment of *B*. *nutans* at H3 ($P<0.05$); Ni concentration was greatest in the wastewater treatment across all species ($P<0.01$) and, while the effect of time was not significant, a significant species*water*time interaction was detected ($P<0.05$; Fig. 7.12i). Al concentration in the soil differed between species and irrigation treatments ($P<0.001$; Fig. 7.12j), but the effect of time was not significant; significant species*irrigation treatment and species*irrigation treatment*time interactions were detected ($P>0.01$ and $P<0.05$ respectively). Soil As was highest under *B*. *vulgaris* and was greater in the wastewater treatment ($P<0.001$ and $P<0.05$ respectively; Fig. 7.12k), but the effect of time was not significant.

Soil K and Zn concentrations were lowest under *B*. *vulgaris* ($P<0.001$; Fig. 7.13a & c), while the differences between *D*. *giganteus* and *B*. *nutans* were small in both irrigation treatments. Soil Fe concentration showed little difference between *D*. *giganteus* and *B*. *nutans* in both irrigation

treatments, whereas the value for the wastewater treatment of *B. vulgaris* was much higher than those for the other two species (P<0.001; Fig. 7.13b).



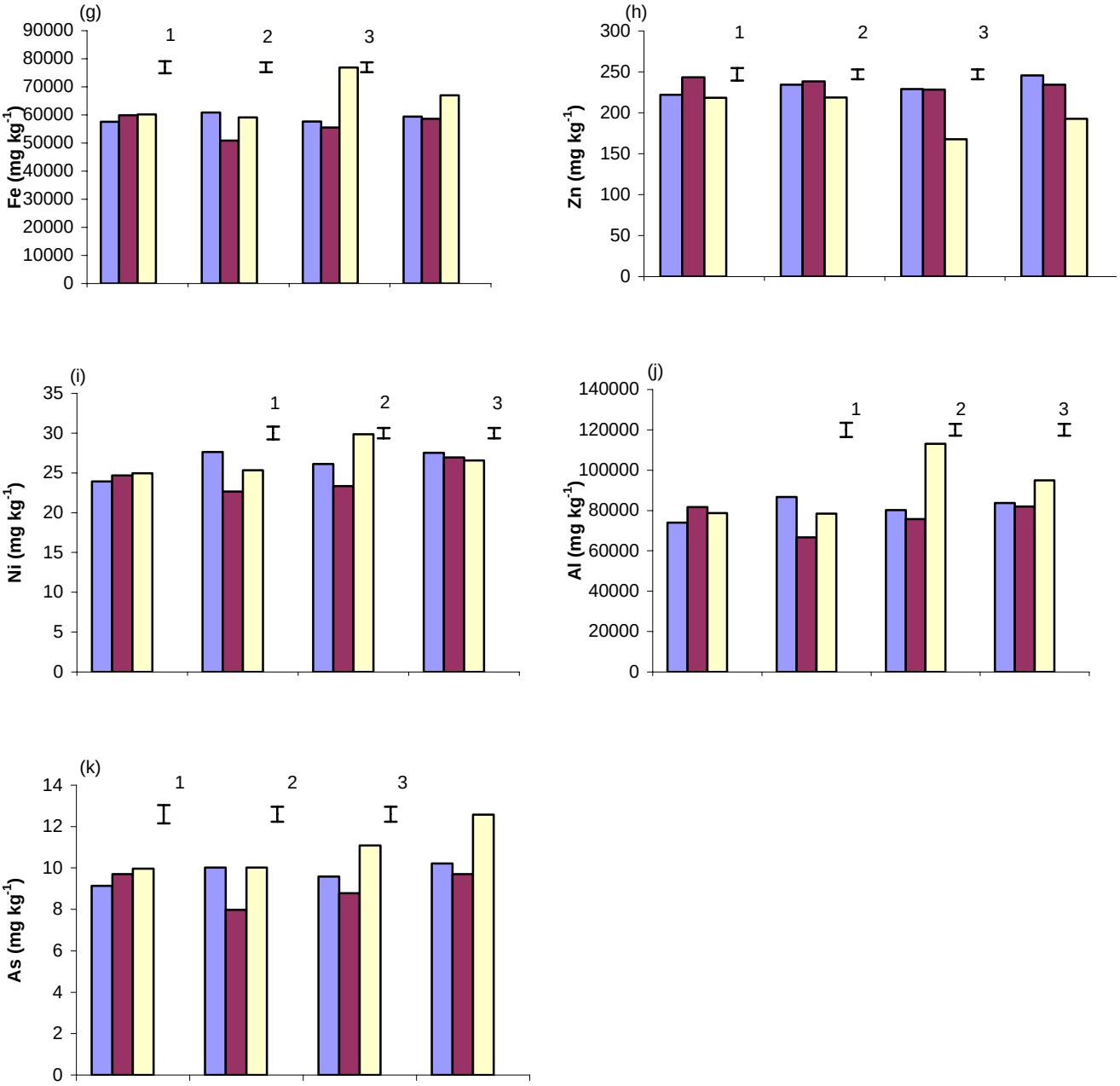


Figure 7.12. Influence of irrigation treatment and harvest date on soil concentrations of (a) K, (b) Ca, (c) Mg, (d) Na, (e) Cu, (f) Mo, (g) Fe, (h) Zn, (i) Ni, (j) Al and (k) As; values are means for all three bamboo species. Vertical bars numbered 1, 2 and 3 represent SED values for comparing species, irrigation treatments and harvests.

7.5 Experiment 2

The domestic wastewater (JKU) contained lower concentrations of all elements except Mo than the two other wastewater sources (Table 7.6). The BIDCO industrial wastewater contained much higher concentrations of Pb, Mg, K, Fe, Zn and As than the other irrigation sources, while the industrial wastewater from KEL had the greatest concentrations of Al, Ca and Mn ($P<0.001$ for all elements except Pb and Mo ($P<0.01$; Table 7.6). The clean irrigation water had the lowest concentrations of all elements tested.

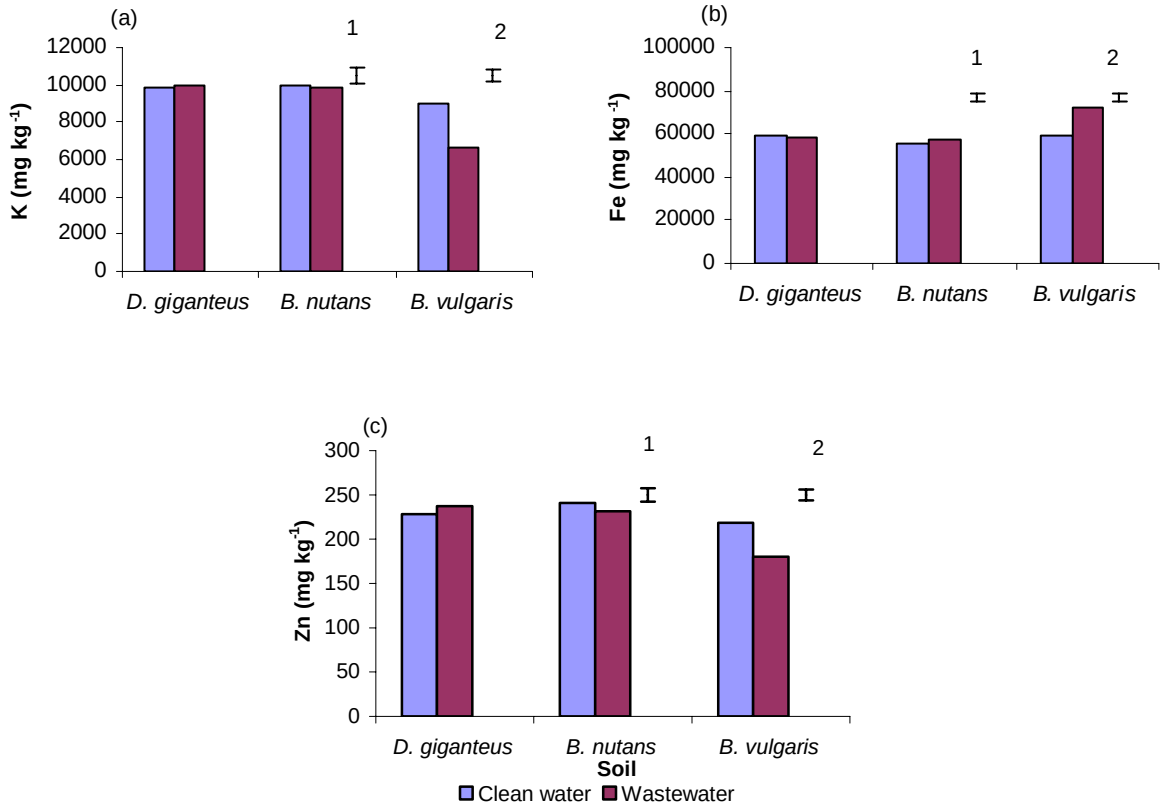


Figure 7.13. Influence of species and irrigation treatment on soil concentrations of (a) K, (b) Fe and (c) Zn; values are means for H1 and H2. Vertical bars 1 and 2 represent SED values for comparing species and irrigation treatments.

7.5.1 Soil analysis

Only the three elements shown in Figure 7.14 exhibited significant differences associated with species or irrigation treatment. K, Mg, Mo, Mn, Cu, Zn Ni, Pb, Cd and Al concentrations in the soil showed no significant difference with regard to species or irrigation treatment (Table 7.7).

Table 7.6. Concentrations of Mg, K, Ca, Mn, Fe, Zn, Mo, Al, Pb and As (mg L^{-1}) in the irrigation sources used in Experiment 2. BIDCO and KEL represent industrial wastewater sources, while JKU represents domestic wastewater sourced from the university wastewater ponds. The clean water was a drinking water control.

	Clean Water	BIDCO	JKU	KEL	Pr	*SED*
Mg	0.00	21.16	2.93	17.19	<.001	0.110
K	0.00	55.09	16.48	27.89	<.001	0.139
Ca	0.00	9.10	2.83	25.54	<.001	0.190
Mn	0.00	1.45	1.04	11.29	<.001	0.129
Fe	0.00	25.08	0.12	0.07	<.001	0.213
Zn	0.58	0.42	0.06	0.14	<.001	0.004
Mo	0.00	0.00	0.00	0.00	0.004	0.000
Al	0.00	1.23	0.10	1.77	<.001	0.007
Pb	0.07	0.00	0.00	0.00	0.002	0.000
As	0.00	0.00	0.00	0.00	0.001	0.000

Fe and As concentrations were greatest in soil irrigated with industrial wastewater, followed by that receiving clean water and finally domestic wastewater ($P<0.05$; Table 7.8; Fig. 7.14a & c).

Table 7.7. Mean soil concentrations of K, Mg, Mo, Mn, Cu, Zn, Ni, Pb, Cd, and Al (mg kg^{-1}) after being grown with three bamboo species and irrigation with clean water, industrial wastewater (BIDCO, then KEL) or domestic wastewater.

	D. giganteus	*B. nutans*	*B. vulgaris*	Pr.	*SED*
K	11918	12866	12902	0.161	576.60
Mg	1899	2055	2007	0.131	78.20
Mo	13	13	13	0.077	0.30
Mn	5173	5751	6233	0.099	486.40
Cu	21	26	25	0.065	2.34
Zn	330	355	331	0.258	17.42
Ni	50	51	50	0.718	1.63
Pb	80	97	93	0.124	8.67
Cd	1	1	1	0.572	0.08
Al	192825	183854	185534	0.176	5052.90

Table 7.8. Mean soil concentrations of Fe and As (mg kg^{-1}) for all three bamboo species following irrigation with clean water, industrial wastewater (BIDCO then KEL) or domestic wastewater.

	Clean water	Industrial wastewater	Domestic wastewater	*Pr.*	*SED*
Fe	157693	162991	153002	P<0.05	3306.5
As	20.5	20.9	20	P<0.05	0.3

Mean values for soil Ca concentration showed significant differences between species ($P<0.05$; Fig.7.14a); in *B. nutans* the highest values were found in the industrial and domestic wastewater treatments, whereas in *B. vulgaris* Ca concentration was highest in the clean and domestic

wastewater treatments (P<0.05). Values were similar for all irrigation treatments of *D. giganteus*. When averaged over all three irrigation treatments, soil Ca concentration was higher under *B. nutans* and *B. vulgaris* than under *D. giganteus* (P<0.05; Fig. 7.15). Soil Fe concentration was similar in the clean water treatment of all species but differed between the industrial and domestic wastewater treatments (P<0.05; Fig. 7.14b). Arsenic concentration did not differ significantly between species, although small differences between irrigation treatments were apparent (P<0.05; Fig. 7.14c).

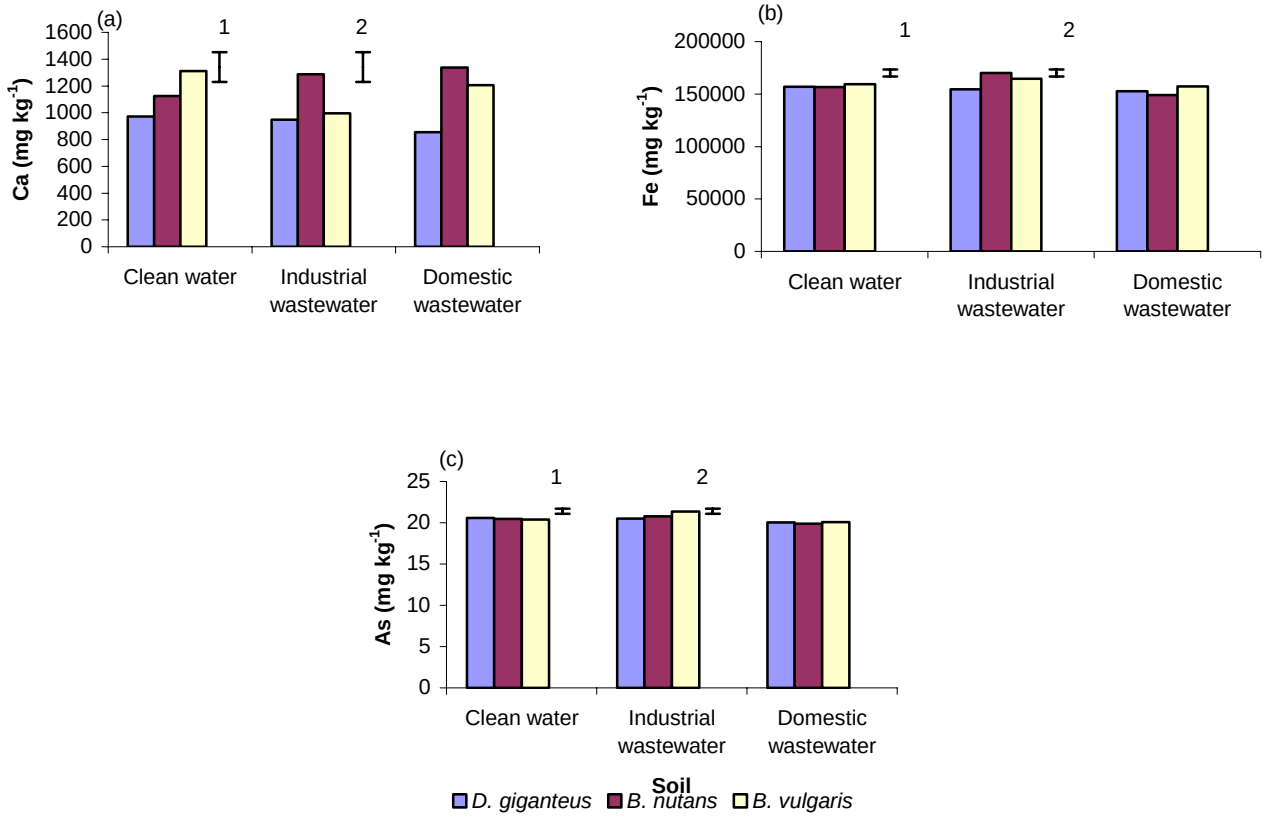


Figure 7.14. Influence of species and irrigation treatment on the concentrations of (a) Ca, (b) Fe, and (c) As in soil planted with *D. giganteus*, *B. nutans* or *B. vulgaris* and irrigated with three sources of water. Vertical bars 1 and 2 show SED values for comparing species and irrigation treatments.

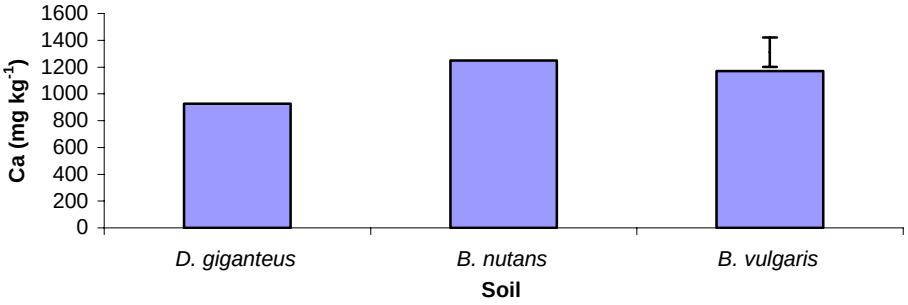


Figure 7.15. Concentrations of Ca in soil planted with *D. giganteus*, *B. nutans* or *B. vulgaris* and irrigated with clean water, domestic wastewater or industrial wastewater. Vertical bar represents the SED value for comparing species.

7.5.2 Plant tissue samples

Only the five elements presented in Figure 7.16 exhibited significant effects of species or irrigation treatment on tissue concentration; K, Fe, Ni, Al, Cd, Pb and As (Table 7.9) showed no significant influence of either species or irrigation treatment. When averaged over all irrigation treatments, Mg concentration was found to be higher in the shoots of *D. giganteus* and *B. nutans* f than in *B. vulgaris* ($P<0.001$; Table 7.9). Ca concentration was lowest in *D. giganteus* and highest in *B. nutans* and *B. vulgaris* in between, while Zn concentration was greatest in *B. vulgaris* and comparable in *B. nutans* and *D. giganteus* ($P<0.01$ and $P<0.001$ respectively; Table 7.9). Plants irrigated with clean water contained higher concentrations of Mo and Mn than in the other irrigation treatments; shoot Mo concentration was lowest in the domestic wastewater treatment, while Mn concentration was lowest in industrial wastewater treatment ($P<0.05$; Table 7.10). Mg concentration was highest in *D. giganteus* for all irrigation treatments and lowest in *B. nutans,* and the industrial wastewater treatment had the lowest concentrations of Mg in comparison to the clean water and domestic wastewater treatments ($P<0.001$; Fig. 7.16a). Ca concentration was highest in *B. vulgaris* for all irrigation treatments, followed successively by *B. nutans* and *D. giganteus*, and the domestic wastewater treatment had the lowest concentration of Ca compared to the clean water and industrial wastewater treatments ($P<0.01$; Fig. 7.16b). Zn concentration was greatest in *B.*

nutans for all irrigation treatments, followed by *D. giganteus*, and *B. vulgaris,* while the *B. nutans* industrial wastewater treatment had the lowest concentration of Zn compared to the other two irrigation treatments, though the clean water and industrial wastewater Zn concentrations for *D. giganteus* and *B. vulgaris* did not differ very much (P<0.001; Fig. 7.16c).

Table 7.9. Concentrations of Mg, Ca, K, Cu, Zn, Fe, Al, Cd, Pb, Ni and As (mg kg^{-1}) in the shoots of *D. giganteus, B. nutans* and *B. vulgaris* following irrigation with clean water, industrial wastewater or domestic wastewater; values are means for all irrigation treatments.

	D. giganteus	*B. nutans*	*B. vulgaris*	Pr.	*SED*
Mg	69.00	56.20	51.60	<.001	3.960
Ca	18.24	23.95	21.71	0.007	1.768
K	893.00	876.00	893.00	0.912	44.900
Cu	0.20	0.19	0.32	<.001	0.021
Zn	1.08	0.95	1.93	<.001	0.136
Fe	25.70	22.50	17.50	0.09	3.720
Ni	0.78	0.33	1.75	0.55	1.315
Al	24.00	21.90	16.20	0.159	4.170
Cd	0.00	0.00	0.00	0.302	0.000
Pb	0.04	0.04	0.22	0.164	0.111
As	0.00	0.00	0.00	0.148	0.001

Table 7.10. Concentrations of Mn and Mo (mg kg^{-1}) following irrigation with clean water, industrial wastewater or domestic wastewater; values are means for all three bamboo species.

	Clean water	Industrial wastewater	Domestic wastewater	*Pr.*	*SED*
Mn	7.1	6.2	4.6	<0.05	0.8
Mo	0.052	0.039	0.048	<0.05	0.005

Mo concentration was greater in the clean and domestic wastewater treatments of *B. nutans* than in the other two species examined, while *D. giganteus* had the highest Mo concentration of all species in the domestic wastewater treatment, and the industrial wastewater treatment for all the three species had the lowest concentrations of Mo when compared to the clean water and domestic wastewater treatments (P<0.05; Fig. 7.16d). Mn concentration was generally greatest in *B. vulgaris,* and the clean water treatment had the highest concentrations of Mn across all the species, followed by the industrial wastewater treatment, with the domestic wastewater irrigated plants having the lowest concentrations of Mn (P<0.05; Fig. 7.16e).

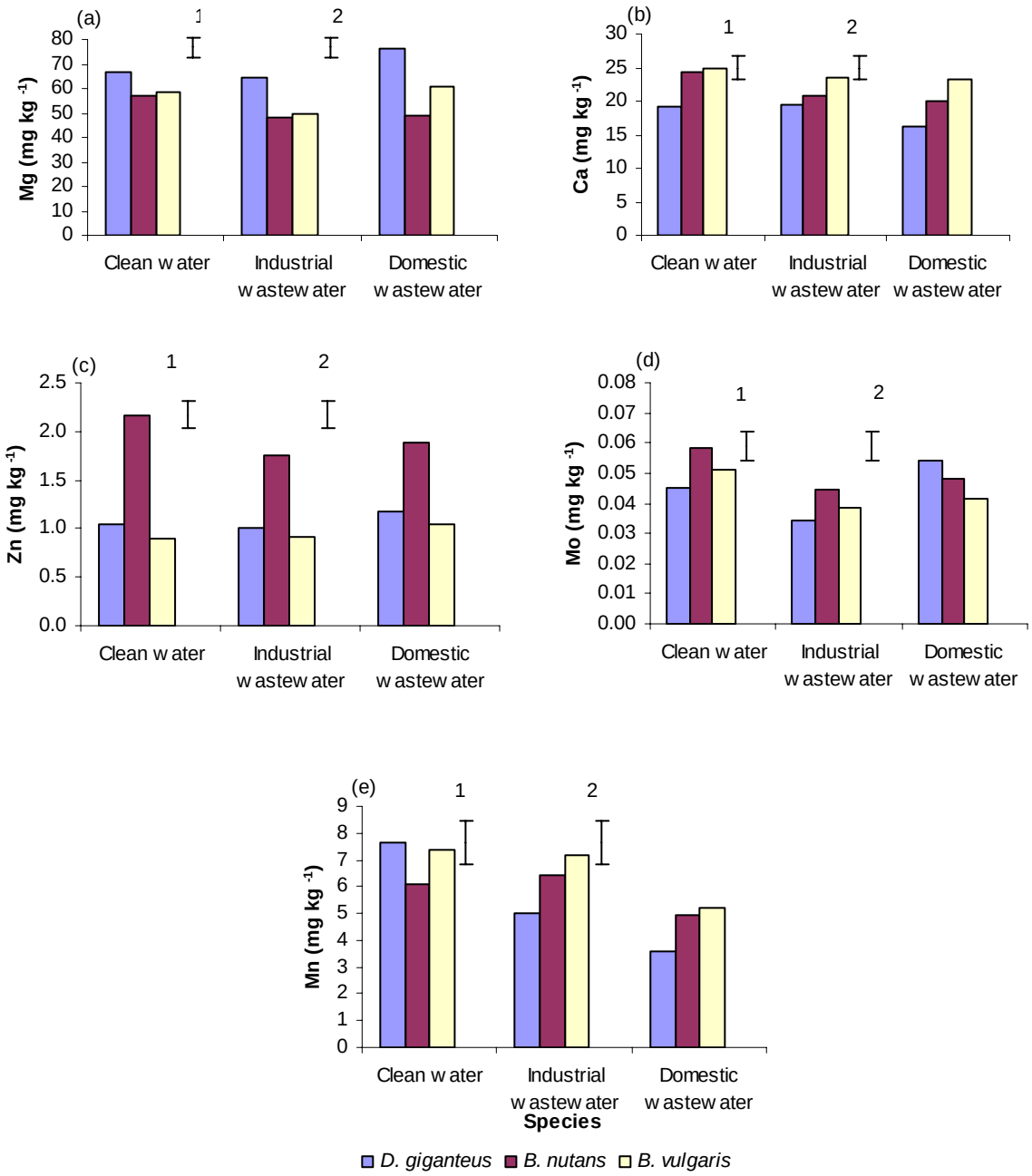


Figure 7.16. Influence of species and irrigation treatment on concentrations of (a) Mg, (b) Ca, (c) Zn, (d) Mo and (e) Mn in the shoots of *D. giganteus*, *B. nutans* and *B. vulgaris* following irrigation with clean water, industrial wastewater or domestic wastewater. Vertical bars 1 and 2 represent SED values for comparing species and irrigation treatments.

7.6 Discussion

Comparison of the elemental composition of the wastewater used in Experiment 1 (Table 7.1) with supplies from JKU, BIDCO and KEL used in Experiment 2 (Table 7.6) reveals that they complied with the minimum safe levels reported by Pettygrove and Asano (1985) and did not contain toxic concentrations of any element. Soil analysis at the start of Experiment 1 (H1) revealed that individual elements did not approach toxic concentrations (Pescod, 1992) and organic matter content was low (Table 7.1). The wastewater source used in Experiment 1 contained high concentrations of Na (873 mg L^{-1}), which has been reported to be sufficient to reduce yield 10 % (Evans, 2006), and K; these were expected to influence soil elemental composition as the experiment progressed. The clean water showed negligible elemental concentrations compared to wastewater in both Experiments 1 & 2 (Tables 7.1 and 7.6). The highest concentrations reported by Bosire (2007) for wastewater sourced in Nairobi was 0.67 mg Pb L^{-1}, compared to 0.0002 mg Pb L^{-1} in the KEL water supply used in the Experiment 2, 0.1 mg Cd L^{-1} compared to 0.009 mg Cd L^{-1}, 0.1 mg Cu L^{-1} compared to 0.015 mg Cu L^{-1} and 6.5 mg Zn L^{-1} compared to 0.008 mg Zn L^{-1} for the wastewater sample analysed at H1 in Experiment 1 and 0.42 mg L^{-1} for the BIDCO water supply in Experiment 2 (Tables 7.1 & 7.6).

Long term irrigation with wastewater has been shown to affect soil composition (Waly *et al*., 1987; Russell, 1988; Falkiner, 1997; Guo and Sims, 2003). Na and K improve plant productivity when supplies are within the optimum concentration range, but excessively high Na concentrations may pose problems associated with salinity, porosity and soil permeability (Pettygrove and Asano, 1985; FAO, 1992). The wastewater analysed at H1 contained almost 1000 mg Na L^{-1} (Table. 7.1), which is relatively high, while the soil initially contained *c*. 200 mg Na kg^{-1}. High concentrations of Na in the soil would be expected to affect the mobility of Ca^{++} (Grattan, 1999; Orcutt and Nilsen, 2000) and are injurious to root tissues (Jacoby, 1994).

At H1, N concentration in the leaves was greatest in *B*. *nutans*, followed successively by *D*. *giganteus* and *B*. *vulgaris* ($P<0.001$; Fig. 7.1b). K concentration was also greatest in the leaves, stems and roots of *B*. *nutans*. Cu concentration was greatest in the roots of *D*. *giganteus*, followed successively by *B*. *nutans* and *B*. *vulgaris*. Ni, Cd and Pb concentrations within the plant tissues were negligible in all treatments. In a previous study, Singh *et al*. (2003) reported that N, P, Cu, Fe, Mn and Zn concentrations were all greater in seedlings irrigated with municipal effluent than in those receiving clean water. This was not observed in the present study (Fig. 7.1 & 7.2), perhaps because the substantial amounts of litter produced by bamboo was not included in the analysis and elemental accumulation in the below-ground organs was not determined.

The elemental analyses carried out at harvests H1, H2 and H3 showed that Zn concentration in plant tissues declined with time and Pb and Ni concentrations were invariably negligible. The decline in Zn concentration may have occurred because the increase in biomass as the bamboo plants grew larger diluted elemental concentrations, or because supplies in the wastewater were negligible. A possible reason why Cd concentration was higher in soil irrigated with clean water than in soil receiving wastewater may be that Cd uptake was greater in the latter treatment because the improved supply of essential nutrients increased plant growth. Maximum safe Cd concentrations in irrigation water for long and short term use generally range between 0.01 and 0.5 mg L^{-1} respectively, although concentrations of 0.1 mg L^{-1} may be toxic to some species (Pettygrove and Asano, 1985; McGuire *et al*., 2003). Cd concentrations in the wastewater used and plant material analysed were well below these limits, although the values were greater in *D*. *giganteus* than in *B*. *nutans* and *B*. *vulgaris*. The longer period of irrigation may explain why soil Cd concentration was greater at H3 than at H2.

The soils used in Experiments 1 and 2 had high concentrations of Fe (up to 150000 mg kg^{-1} and 75000 mg kg^{-1} respectively; Fig. 7.14b, Table 7.8, Fig. 7.12g) and Al (192825 mg kg^{-1} and 110000 mg kg^{-1}), compared to values of 13300 mg Fe kg^{-1} and 2450 mg Al kg^{-1} reported for savannah soils in Nigeria (Agbenin, 2003). Soil Mg concentration was greatest under *D. giganteus*, followed by *B. nutans* and *B. vulgaris* ($P<0.001$; Table 7.3); As concentration was greatest under *B. vulgaris* and showed no significant difference between *D. giganteus* and *B. nutans*. Soil Mg concentration was greatest at H3 ($P<0.05$; Table 7.5), while As concentration was greatest in soil irrigated with wastewater ($P<0.05$; Table 7.4), probably due to accumulation following repeated application of wastewater. The toxicity to plants of As present in irrigation water varies greatly (Pettygrove and Asano, 1985), ranging between 0.1 mg L^{-1} for long term use and 2 mg L^{-1} for short term use; the concentration of 0.08 mg kg^{-1} observed in leaves in the present study (Fig. 7.2g) is therefore well below toxic levels. Ca and Al concentrations in the soil varied greatly between species and irrigation treatments and with time ($P<0.001$; Fig. 7.12b & j). Soil Mo and Ni concentrations also showed significant variation between irrigation treatments and species and with time, although the extent of the variation was smaller ($P<0.05$; Fig. 7.12f & i). Soil K, Fe and Zn concentrations varied between irrigation treatments only under *B. vulgaris* but differed between species for all three elements ($P<0.001$; Fig. 7.12a, g & h). Soil planted with *D. giganteus* or *B. vulgaris* showed significant variation in Cu concentration between species and with time ($P<0.05$; Fig. 7.12e). Long term accumulation of Zn^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+} may cause soil toxicity, adversely affecting plant growth (Gadallah, 1995).

Cd, Al, Zn and As concentrations within leaves were lower at H3 than at H2 (Fig. 7.2h, f, b, & g). Although there was no apparent explanation for this decline, which was significant in all treatments, this may have resulted from the redistribution of assimilates within the actively growing plants. The lowest (H3) and highest (H2) Al concentrations for leaves of bamboo

irrigated with wastewater in the present study (150 and 250 mg kg^{-1} respectively) are both higher than the accumulation of 44-144 mg Al kg^{-1} over a six month period reported by Kleinhenz and Midmore (2002). The plants at H3 were larger and had more leaves than at H2, perhaps causing a dilution of elemental concentration within the plant if uptake could not match biomass accumulation. The leaves of plants irrigated with wastewater also contained lower concentrations of Ni than in the clean water treatment. Leaf Zn concentration was greatest in *D. giganteus* and did not differ significantly between *B. vulgaris* and *B. nutans*. Cd and Mo concentrations in the leaves were much lower at H3 than at H2 ($P<0.05$; Fig. 7.2h & d). Differences in Cd concentration between species were significant at H2 ($P<0.001$; Table 7.2h). Mg concentration in the leaves was higher at H3 than at H2 and greatest in *B. vulgaris*, followed by *B. nutans* ($P<0.001$; Fig. 7.2a). Cu concentration in the leaves showed no clear pattern of variation between species or with time, ranging between 4 and 10 mg kg^{-1}, although *B. nutans* showed a sharp increase in concentration in the wastewater treatment at H2. Cu concentrations were lower than reported by Kleinhenz and Midmore (2002).

While Cd concentration in the stem was higher at H3 than at H2 ($P<0.001$; Fig 7.3e), Al, Cu and Zn concentrations were marginally lower ($P<0.05$, $P<0.001$ and $P<0.001$ respectively; Fig. 7.3f, c & b); similar trends applied for the leaves ($P<0.001$, $P<0.01$ and $P<0.05$ respectively; Fig. 7.2f, c, & b). Mg concentration in the stems averaged over all species was higher in the wastewater than in the clean water treatment, while the corresponding values for Zn and Cu were greatest in *D. giganteus* and lowest in *B. nutans* ($P<0.05$ for Mg, $P<0.001$ for Zn and Cu; Fig. 7.3a, b & c). Zn concentration in the stems of *D. giganteus* was lower in the wastewater than in the clean water treatment, whereas the converse applied for *B. nutans* and *B. vulgaris*; the values were higher at H2 than at H3. Seasonal variation in environmental factors such as precipitation, solar radiation, air temperature and vapour pressure deficit which influence transpiration rate and elemental uptake

may also influence the impact of irrigation with effluent on soil responses and plant growth (Tzanakakis *et al*., 2007).

In addition to determining mean elemental concentrations for the whole stem, concentrations for the upper, middle and lower stem segments were determined at H2 and H3. Al concentration was greater in the lower stem segments in the wastewater treatment at both harvests ($P<0.001$; Fig. 7.4a), but Mn and Zn concentrations were greater at H2 than at H3 ($P<0.001$; Fig. 7.6a & b); the converse applied for Mo ($P<0.001$; Fig. 7.5a). Al, Mn and Zn concentrations were greatest in the lower stem segment, suggesting selective deposition in the older tissue, or greater accumulation due to age. Ni concentration was also highest in the lower stem segment at H3, but was greatest in the upper stem at H2 ($P<0.01$; Fig. 7.9a &b). Hopmans and Clerehan (2006) reported a similar differential distribution of mineral nutrients at different levels in the canopy of *Pinus radiata*. Zn concentration was greatest in the wastewater treatment of *B*. *vulgaris* and the clean water treatment of *D*. *giganteus* ($P<0.001$; Fig. 7.6b).

In Experiment 2, three sources of irrigation water were used, industrial wastewater collected from the BIDCO and KEL industrial premises in Thika Town, 12 km from the experimental site, domestic wastewater from the JKUAT collection ponds, and clean tap water as a control. Pb, Mg, K, Fe, Zn and As concentrations were highest in the BIDCO wastewater supply, whereas Al, Ca, and Mn concentrations were greatest in the KEL water supply ($P<0.001$ for all elements except Pb ($P<0.01$; Table 7.6). Wastewater from JKUAT contained the highest concentration of Mo and lower concentrations of all other elements than the BIDCO and KEL supplies ($P<0.01$; Table 7.6). B and Mo are trace elements which are essential plant nutrients, but may become phytotoxic at high concentrations (Bouwer and Idelovitch, 1987). The differing composition of the irrigation sources was expected to induce differences in the soil and plant samples analysed, even though the

duration of the experiment was only three months. For example, Ojonoma and Nnennaya (2007) documented alterations in the physico-chemical constitution of soil following treatment with palm oil effluent. However, comparison of the concentrations of elements in the three wastewater sources used in Experiment 2 (Table 7.6) with the recommended maximum concentrations shown in Table 7.11 shows that no elements were present at concentrations sufficient to be phytotoxic either in the short or long term.

Table 7.11. Indicative safe levels for wastewater constituents (after Pettygrove and Asano, 1985).

	Long-Term Use (mg L^{-1})	Short-Term Use (mg L^{-1})	
Zinc	2	10	Toxic to many plants at widely varying concentrations
Molybdenum	0.01	0.05	Non-toxic to plants at normal concentrations in soil and water
Manganese	0.2	10	Toxic to a number of crops at few-tenths to a few mg/l in acidic soils.
Lead	5	10	Can inhibit plant cell growth at very high concentrations.
Iron	5	20	Not toxic to plants in aerated soils.
Cadmium	0.01	0.5	Toxic to beans, beets, and turnips at concentrations as low as 0.1 mg/l in nutrient solution
Arsenic	0.1	2	Toxicity to plants varies widely, ranging from 12 mg L^{-1} for Sudan grass to less than 0.05 mg L^{-1} for rice.
Aluminum	5	20	Can cause nonproductiveness in acid soils.

Fe and As concentrations in the soil were greater when industrial water was applied than in the clean and domestic wastewater treatments ($P<0.05$; Table 7.8). Soil planted with *B*. *nutans* had a greater Ca concentration than soil planted with the other two species ($P<0.05$; Fig. 7.14a). The entire shoots of plants harvested in Experiment 2 were used for elemental analysis as the plants were too small to analyse the leaves and stems separately. Mg concentration was greatest in *D*. *giganteus*, while Ca and Zn concentrations were greatest in *B*. *nutans* and *B*. *vulgaris*, respectively, reinforcing the view that individual species and populations may differ in their capacity to extract nutrients from soil, and that this may be an adaptive trait (Boyd and Martens, 1998). Mo and Mn concentrations were greatest in plants irrigated with clean water ($P<0.05$; Table7.10).

7.7 Conclusions

As time progressed, it was expected that, as in previous studies (Guo and Sims, 2000), irrigation with water of differing elemental composition would affect the concentrations of these elements in

the rhizosphere. It was anticipated that changes in the elemental composition of soil associated with the various irrigation treatments would be complicated by the possibility that the three species examined might differ in their ability to extract and accumulate specific elements. For example, Boyd and Martens (1998) noted that populations of *Thlaspi montanum* var. *montanum* differed in their ability to extract certain elements. These effects were expected to be more pronounced in Experiment 1 than in Experiment 2 because of its longer duration. As the plants increased in size, their effect on the fixed volume of soil in the 100 L pots would also be expected to increase. As the species examined differed in growth rate, their influence on soil elemental composition would also be expected to vary. Consistent with these expectations, soil K, Mg, Ca, Fe, Mo, Zn, Ni, As and Al concentrations all showed significant variation with species, irrigation treatment and time.

The lower concentrations of Zn, Cd, Al and As in the leaves at H3 relative to H2 may reflect the inability of the now larger plants to access sufficient quantities these elements, assuming that soil supplies were being depleted more rapidly than they were replenished by the very low concentrations present even in the wastewater supplies. The decrease in elemental concentrations between H2 and H3 in the leaves also occurred in the stems, and the explanation may be similar. Zn, Al and Ni concentrations were greater in the lower stem segment in all species, perhaps indicating preferential transport and storage from the upper parts of the shoot or longer and greater accumulation resulting from increasing age.

In Experiment 2, Fe and As concentrations were greatest in soil irrigated with industrial water, as was to be expected as this contained higher concentrations of these elements than the domestic sewage effluent; Al-Nakshabandi *et al*. (1997) made a similar observation for eggplants irrigated with wastewater in Jordan. There were clear differences in elemental composition between the BIDCO, KEL and JKU water supplies which would have differentially affected the elemental

constitution of soil and plant tissues. Although the experiment continued for only three months, the three species examined differed significantly in their uptake of Mg (highest in *D*. *giganteus*), Ca (highest in *B*. *nutans*) and Zn (highest in *B*. *vulgaris*).

CHAPTER 8

FINAL DISCUSSION AND CONCLUSIONS

Erratic and unpredictable weather conditions and natural disasters have made sustainable environmental management a central theme in social, economic and scientific fora throughout the world. As the global population grows and industrialisation occurs in developing countries, the environment is subjected to massive pollution inputs with potentially dire consequences for plant, animal and human life. Concern has been raised about global warming, which has been attributed to human activities, and rapidly expanding human populations in parts of the world which produce insufficient food to support them, so creating additional avenues for environmental degradation. Major cities in developing countries are crisis centres with regard to the environment as populations seek a means of survival. The present study aimed to establish the impact of irrigation with wastewater on the growth, gas exchange and productivity of three bamboo species and assess their suitability for solving wastewater disposal problems while providing alternative non-timber woody products with multiple end-user applications.

8.1 Growth characteristics

The significant growth in height with time in all species ($P<0.05$; Figs. 4.6, 4.7, & 4.8) was most pronounced during the first few weeks after shoots emerged from the ground as marked increases occurred on a daily basis, a characteristic feature of bamboo (Soderstrom, 1981), although differences between species were never significant. Collar diameter in newly emerged bamboo shoots is typically relatively large initially but declines during the ensuing weeks (Wu *et al*., 2005), a pattern which was also apparent in the present study (Fig. 4.9). As for plant height, collar diameter did not differ significantly between species, although the values were initially greater in plants irrigated with wastewater than those receiving clean water ($P<0.05$; Fig. 4.9).

Increased growth has been reported in groundnut following irrigation with wastewater (Saravanamoorthy and Kumari, 2007). In the present study, branch number plant^{-1} increased with time in both irrigation treatments of all species in Experiment 1 (Figs. 4.15 & 4.16), varied between species, and showed significant species*time, irrigation treatment*time and species*irrigation treatment*time interactions ($P<0.05$). Values were greater for plants irrigated with wastewater than for those receiving clean water; this effect was greatest for *B. nutans*, followed by *B. vulgaris*, while *D. giganteus* showed little response. The leaves of *D. giganteus* were broader than in the other species examined and individual leaf area was greater in the wastewater than in the clean water treatment ($P<0.05$; Figs. 4.13 & 4.14). Mean individual leaf area varied between species and irrigation treatments ($P<0.05$). Significant increases in leaf area ratio and leaf area index were also observed in *Arachis hypogaea* L. following irrigation with wastewater (Saravanamoorthy and Kumari, 2007).

8.1.1 Biomass accumulation

The productivity of bamboo differs from that of tree species in degree only when grown in the same ecoclimatic region (Kleinhenz and Midmore, 2001). In view of the superior yields obtained, irrigation with wastewater is preferred to the use of clean river water in the Indian subcontinent (Bradford *et al*., 2003). This potentially beneficial effect of wastewater was confirmed by the significant irrigation*treatment* time interaction for leaf dry weight as the increase between Harvests 2 and 3 was greater in plants irrigated with wastewater than in those receiving clean water ($P<0.05$; Fig. 4.19). This may be attributed to the greater concentration of nutrients in wastewater relative to clean water (Bradford *et al*., 2003). If the experiment had continued for longer than nine months, the differences might possibly have become even greater. As might be expected, a similar pattern was apparent for leaf fresh weight plant^{-1} (Fig. 4.17). Leaf dry weight

plant^{-1} in *B*. *vulgaris* and *B*. *nutans* was much greater than in *D*. *giganteus* irrespective of irrigation treatment (P<0.05; Fig. 4.19). *D*. *giganteus* had fewer leaves which were much broader than in the other two species. Leaf dry weight was substantially greater in the wastewater treatment than in plants receiving clean water (P<0.05).

Stem dry weight plant^{-1} in *B*. *vulgaris* and *B*. *nutans* was much greater than for *D*. *giganteus* irrespective of irrigation treatment (P<0.05; Fig. 4.24), again demonstrating the greater vigour of those species relative to *D*. *giganteus*, at least for the relatively young plants examined here. The increases in stem fresh and dry weight between Harvests 2 and 3 were similar to those for leaf fresh and dry weight for all species and both irrigation treatments; differences between irrigation treatments was smallest for *B*. *vulgaris* (Figs. 4.21 & 4.24), as reported for crops irrigated with wastewater in India and Pakistan (Bradford *et al*., 2003).

8.2 Gas exchange and SPAD values

SPAD values and gas exchange parameters exhibited contrasting correlations with environmental factors between January and June 2006. Correlations for air temperature (maximum, mean and minimum) ranged between 0.59 and -0.17, while those for solar radiation (-0.23 to 0.00), relative humidity (0.44 to 0.08) and saturation deficit (-0.35 to 0.02) also showed substantial variation (Table 6.1). Transpiration rate showed a high correlation with temperature (0.57 to 0.77), whereas the correlations between photosynthetic rate and temperature, SD, RH and radiation were all below 0.33. Non-significant correlations between gas exchange and environmental variables have also been reported for wheat (Fotovat *et al*., 2007). No significant differences in photosynthetic rate were detected between species between July and November 2006 (P<0.52; Fig. 4.13), although a species*time interaction was observed at the canopy level (P<0.05; Fig. 4.12). Stomatal conductance showed a species*irrigation treatment*time interaction between January and

June 2006 ($P<0.001$; Fig. 4.17) and between July and November 2006 (Fig. 4.1). Stomatal conductance is known to be closely correlated with leaf water potential (Bañuls and Primo-Millo, 1995; Paranychianakis *et al*., 2006). The photosynthetic rate did not differ significantly between species between August and November 2006, although transpiration rate and stomatal conductance showed significant species*irrigation treatment*time interactions ($P<0.05$), reflecting observations that gas exchange is strongly influenced by plant water status (Scheuermann, 1991). Stomatal closure is known to respond to salinity (Munns *et al*., 2002), which was sufficiently high in the present study (Table 8.1) to adversely affect plant growth (Evans, 2006). Photosynthetic rate was greater for plants irrigated with wastewater than in those receiving clean water ($P<0.05$; Fig. 4.6).

Instantaneous transpiration efficiency (ITE) was similar in *B*. *vulgaris* and *B*. *nutans* and lower than in *D*. *giganteus* throughout the experimental period ($P<0.05$; Fig. 4.7). Apart from having fewer and broader leaves than the other two species, the leaves of *D*. *giganteus* were thicker and deeper green, perhaps conferring a more rapid net photosynthetic rate, while the greater leaf thickness may have helped to reduce transpiration rate. ITE values vary between species and locations (FAO, 2007) and were higher in plants irrigated with wastewater than in those receiving clean water (Figs. 6.21, 6.24 & 6.33), in agreement with observations that transpiration rate was lower in plants irrigated with wastewater (Figs 6.5, 6.6 & 6.19). Successful phytoremediation requires transpiration of sufficient contaminated water to extract worthwhile quantities of pollutant from the soil (Vose *et al*., 2003). The greater transpiration efficiency of crops irrigated with wastewater may be beneficial as, in addition to using a resource that may otherwise pose environmental problems (Bradford *et al*., 2003), they are also using the water more effectively than would be the case with clean water.

The measurements of net photosynthesis for leaves of different age and position in the canopy revealed significant species*time and species*leaf position interactions as photosynthetic rate was greater in the lower leaves than in the upper leaves (Fig. 4.23). A similar pattern was found for stomatal conductance and transpiration rate (Figs. 4.19-22). Significant species*irrigation treatment*time and irrigation treatment *leaf position interactions were apparent for stomatal conductance, while a species*irrigation treatment*time interaction was found for transpiration rate as values for the middle and lower leaves were greater than for the upper leaves. Contrasting results for stomatal conductance have been reported for *Leandra lacunosa*, although transpiration rates were reported to be comparable (Damascos *et al*., 2005).

SPAD values initially decreased at the start of the nine month experimental period in Experiment 1 before becoming relatively constant (Figs. 6.26, 6.28 & 6.29). No significant differences between species were apparent, although the values tended to be higher for plants irrigated with wastewater than in those receiving clean water (Fig. 4.25). Correlations between gas exchange parameters and SPAD values were not significant (Table 4.2), consistent with findings for sugarcane (Silva *et al*., 2007), suggesting that factors other than chlorophyll content are important in determining gas exchange by plants, possibly including solar radiation, windspeed, air temperature, SD, plant water status and rhizospheric conditions. Silva *et al*. (2007) also recorded modest negative correlations between environmental and physiological parameters and SPAD values. Significant correlations between plant development, SPAD values and ITE after correction for seasonal differences in vapour pressure deficit (VPD) have been reported for groundnut (Sheshshayee *et al*., 2006), providing the first evidence of a significant positive relationship between chlorophyll concentration and ITE.

In Experiment 2, in which the plants were younger than in Experiment 1 and industrial wastewater provided an additional treatment, no significant differences between species or species*irrigation

treatment interactions were detected for photosynthetic and transpiration rates or stomatal conductance. Of the three irrigation sources used, photosynthetic rate was greatest in the domestic wastewater treatment, followed successively by industrial wastewater and clean water ($P<0.05$; Fig. 4.29). These results are not surprising due to the short duration of the experiment as any toxic effects of industrial wastewater may require a longer period to induce damage.

As observed in Experiment 1, ITE was greater in *D. giganteus* than in *B. vulgaris* and *B. nutans* in Experiment 2 ($P<0.05$; Fig. 4.21). Photosynthetic rate was lower ($P<0.05$) in the wastewater treatment of *B. vulgaris* than in plants irrigated with clean water even though transpiration rate was greater. The other treatments showed a slight increase in net photosynthetic rate as transpiration increased (Fig. 4.30). As C3 species such as bamboo have a lower water use efficiency than C4 species (FAO, 2007), their superior water pumping capabilities may make them an attractive proposition for decontaminating wastewater. In China, the maximum annual transpiration rate of bamboo has been estimated at 33 million L ha^{-1} (Kleinhenz *et al*., 2003).

SPAD values were greatest in *D. giganteus* (Fig. 4.22), followed successively by *B. vulgaris* and *B. nutans*, and were highest in the domestic wastewater treatment, followed by the clean and industrial wastewater treatments. As noted previously, no consistent relationships between the values for the various gas exchange parameters and SPAD values were detected and the changes with time showed no clear pattern. In wheat, the relation between SPAD values and drought stress was significant, although the correlation between ITE and SPAD values was not (Fotovat *et al*., 2007). However, had the duration of Experiment 2 had been extended, significant treatment effects may have developed as nutritional benefits or toxicities associated with wastewater treatment developed.

8.3 Elemental analysis

Nutrient uptake by plants is site-specific and depends on local factors affecting potential growth, transpiration rate and water uptake (Sharma, 2006). Water, soil and plant samples were analysed for over 40 elements by AAS and ICP-MS (Chapter 2); however, with the exception of Na, elemental concentrations were not sufficiently high to reach their thresholds for toxicity (Table 7.1). For example, the highest recorded Pb concentration of 0.003 mg L^{-1} for wastewater from BIDCO, Thika (Table 7.6) was 43 times lower than the lowest value (Nairobi Dam) reported by Bosire (2007) for five sites in Nairobi; the other sites examined by Bosire (2007) were Siranga, Soweto, Lindi and Gatwekera. Although Zn concentrations in wastewater from the KEL and BIDCO sources were higher than reported by Bosire (2007), it is important to note that the soil used in the present study was exposed to contaminated water for relatively short periods (nine months in Experiment 1 and four months in Experiment 2) compared to the sites in Nairobi examined by Bosire (2007) which had been irrigated with wastewater for many years. Thus, soil elemental concentrations reported by Bosire (2007) ranged from 875.8-1032.5 mg kg^{-1} for Zn and 34.8-38.5 mg kg^{-1} for Cu, compared to 250 mg Zn kg^{-1} and 20 mg Cu kg^{-1} for Harvest 3 in the present study (Fig. 7.12e). Moreover, the soils used by Bosire (2007) were continuously supplied with wastewater, unlike the present study in which fixed quantities of water were applied on a daily basis. This difference in experimental design may explain the contrasting results from the two studies. Values for the initial concentrations of P, K, Cu, Ni, Cd, Pb, Zn, Na and soil organic matter content were determined for soil samples collected at the start of the experiment in the present study.

The soil used in both experiments was collected from a single source and was therefore initially uniform in composition. Any changes in elemental composition during the experimental period must therefore have resulted from a combination of inputs from irrigation water or uptake by

bamboo plants growing on the soil. Although the soil in all pots received identical quantities of water irrespective of irrigation treatment, elemental concentrations differed significantly at Harvests 2 and 3 between initially identical soil planted with different bamboo species. This may have resulted from differential uptake by the various species examined associated with their differing requirements or growth rates. *B. vulgaris* extracted greater quantities of Na, Mg, K and Zn than the other species ($P<0.001$ for all three elements; Fig. 7.12a, c, d, & h), while *B. nutans* was most effective in extracting Mo and Cu ($P<0.026$ & $P<0.017$ respectively; Fig. 7.12 f & e). It is worth noting that bamboo stores substantial quantities of resources in its rhizomes (Kochhar, 1998; Liese, 1998), although this aspect was not examined in the present study. Shanmughavel and Francis (2001) reported that complete harvest of the above-ground biomass of bamboo six years after planting removed 2341 kg N ha^{-1}, 22 kg P ha^{-1}, 2653 kg K ha^{-1}, 1211 kg Ca ha^{-1} and 1356 kg Mg ha^{-1}, compared to 227 kg N ha^{-1}, 7.3 kg P ha^{-1}, 181 kg K ha^{-1}, 284 kg Ca ha^{-1} and 38.9 kg Mg ha^{-1} after four years by acacia. These results suggest that bamboo is the superior phytoextractor.

Soil elemental concentrations were generally higher under *D. giganteus*, which grew more slowly than *B. vulgaris* and *B. nutans*, providing a possible explanation for the slower depletion of soil nutrient supplies. Although irrigation with wastewater increased soil As and Al concentrations compared to soil irrigated with clean water ($P<0.001$ for both elements; Fig. 12j & k), this was not true for Ca ($P< 0.001$; Fig. 7.12b). At Harvest 3, soil concentrations of K, Mg, Na, and Zn were lower under *B. vulgaris* than under the other species examined ($P<0.001$ for all four elements; Fig. 7.12a, c, d, and h), continuing a trend initially seen at Harvest 2. *B. nutans* reduced soil Ni concentration to a greater extent than the other two species ($P<0.022$; Fig. 7.12i); soil Cd and Mg concentrations were lowest under *B. vulgaris*, whereas As concentration was greatest in this treatment ($P< 0.001$ for all three elements; Table 7.3 & 7.4). These disparities suggest that there

may have been genotypic differences in extraction capacity as growth was comparable in *B. vulgaris* and *B. nutans*. At Harvest 3, Ca and K concentrations were greater in soil irrigated with clean water than in that receiving wastewater ($P<0.001$ for both elements; Fig. 7.12a & b). As the clean water contained negligible concentrations of these elements, the explanation for this treatment effect may be that uptake of these elements by plants from soil irrigated with wastewater was enhanced by the presence of other elements, or that the greater transpiration rates of plants irrigated with wastewater (Figs. 6.5 & 6.6) favoured elemental uptake (Toni and Barrington, 2005). The presence of Ca is known to mitigate the possible effects on soil pH associated with the presence of other trace elements in the irrigation water (Toni and Barrington, 2005). Soil irrigated with clean water showed little variation in Fe and Al concentrations between species, whereas the corresponding values for soil irrigated with wastewater were lower under *B. nutans*, perhaps reflecting greater extraction by this species ($P<0.001$ for both elements; Fig. 7.12g & i). In the wastewater treatment, soil Ca concentration was lowest under *B. vulgaris* and *B. nutans* ($P<0.018$; Fig. 7.12b).

While soil in the wastewater treatment analysed after Harvest 3 would have received additional elemental supplies in the form of irrigation water, extraction by plants grown on these soils is likely to have increased as they grew larger, developed a larger root system and extracted increasing quantities of nutrients and other elements. Soil Mo and Mg concentrations were higher at Harvest 3 than at Harvest 2 ($P<0.0.01$ and $P< 0.026$ respectively; Fig. 7.12 c & f), although the values for other elements did not differ significantly. Soil K, Na, and Zn concentrations were lower under *B. vulgaris* than under the other bamboo species for which soil concentrations were almost identical $P<0.001$ for all three elements; Fig. 7.12a, b & h). High concentrations of Na, Ca and K affect nutrient availability, competitive ion uptake and transport and/or partitioning of ions, creating nutrient imbalances within the plant (Shannon, 1987; Grattan and Grieve, 1999; Orcutt

and Nilsen, 2000). Indeed, Na concentration in the wastewater used in the present experiment was sufficiently high (873 mg L^{-1}; Table 7.1) to limit plant growth (Evans, 2006) and would have been expected to inhibit transpiration by inducing saline soil conditions (Gadallah, 1995), although this was not the case in the present study. In Jordan, Al-Nakshabandi *et al.* (1997) observed that despite problems with salinisation, irrigation with wastewater has a positive effect on plant growth. Susceptibility to salinity varies between species (Bernstein, 1980; Maas, 1987; Francois and Maas, 1994) and depends on growth stage (Maas, 1986). Fe concentration was greatest under *B*. *vulgaris* ($P< 0.001$; Fig. 7.12g), while soil Cu concentration was lowest under *B*. *vulgaris* and *B*. *nutans* at Harvest 2 ($P<0.017$; Fig. 7.12e) but was greatest under *B*. *vulgaris* at Harvest 3.

The fact that soil Cd concentration was higher in the clean water than in the wastewater irrigation treatment ($P<0.01$; Table 7.5) could have been due to contamination as the high sensitivity of ICP-MS may have resulted in the detection of Cd inadvertently added to the sample following contamination from dust in the air or residues left after washing glassware during preparation. Analysis of the clean water used for irrigation revealed no detectable concentration of Cd. Arsenic was in lower concentration in soil irrigated with clean water than in soil irrigated with wastewater ($P<0.001$; Fig. 7.12k).

There appears to have been a close correspondence between the quantities of elements added to the soil by irrigation and those extracted by plants as the analyses carried out at Harvests 2 and 3 showed little change for most elements (Fig. 7.12a-k). Although bamboo is considered to be an excellent candidate for cleaning up wastewater due to its strong water pumping characteristics (Janssen, 1991; Liese, 2003), it is important to note that its rhizomes, the primary storage organ (McClure, 1966; Liese, 1998), were not analysed in the present study. In the initial elemental analysis of shoot material (N, Cu, Ni, Cd, Pb, Zn, K, Na and P) performed at the start of the

experiment, no significant presence of heavy metals was observed within the plant tissues. Grifferty and Barrington (2000) and Salah and Barrington (2006) reported that concentrations within irrigation water and transpiration rate are important in determining shoot Cd and Zn concentrations in wheat. Soil N concentration was initially low at the start of the experiment although organic matter, an important source of N, was 30,400 mg kg^{-1} (Table 7.1). Guo and Sims (2000) observed that soil irrigated with wastewater had a higher N concentration (5360 mg kg^{-1}) than that irrigated with clean water (5180 mg kg^{-1}).

Although Zn and Cu concentrations were higher in the stem tissue of *D. giganteus* than in the other species examined ($P<0.001$ for both elements; Fig. 7.3b & c), this must be balanced by the fact that the greater foliar biomass of the other two species would tend to offset their lower tissue concentrations. It has been reported that the elevated concentrations of Zn and Cu in wastewater may be beneficial rather than toxic to buckwheat plants, and that Cu has an antagonistic effect on Zn absorption which may decrease tissue Zn concentrations over longer time periods (Tani and Barrington, 2005). Recorded Cu and Zn concentrations within the stem (*c*. 3 and 20 g kg^{-1} respectively; Fig.3c & b) were well below the maximum recommended levels for plants (40 and 150 g kg^{-1} respectively); concentrations ranging between 43-34 mg Cu kg^{-1} and 56-64 Zn mg kg^{-1} have been reported for buckwheat irrigated with water containing elevated concentrations of Cu and Zn (Tani and Barrington, 2005). In the present study, the stems of plants irrigated with clean or wastewater showed no difference in elemental concentration except for Mg, for which the value was greater in plants irrigated with wastewater ($P<0.025$; Fig. 7.3a). Mg, Fe and Cu concentrations in the stems of *D. giganteus* were greater than in the other species examined, although differences in biomass between species would again influence total uptake. Ni concentration was greater in the upper portion of the stems, while Mg concentration was greater in the lower stem segment. The Cu and Zn concentrations within the stem of 4 and 10 mg kg^{-1} in the

present study (Fig. 7.3c & b) were much lower than those reported by Bosire (2007) of 30-214, and 77-234 mg kg^{-1}, respectively. Stem Mg, Zn and Al concentrations were greater in plants irrigated with wastewater than in those receiving clean water, and were higher in the lower stem than in the upper stem (P0.025, P<0.001 and P<0.02 respectively; Fig3a, b and f). Singh and Bhati (2003) reported similar findings for Cu, Mn, Zn, N, P and Fe concentrations in Eucalyptus seedlings irrigated with municipal effluent.

Al and Zn concentrations in the stem were greater at Harvest 2 than at Harvest 3, perhaps due to a 'dilution effect' as the plants grew larger (P<0.001for both elements; Fig.7.8a & b). Zn, Cd, and Ni concentrations in *D. giganteus* were greater in the clean than in the wastewater treatment (P<0.001 for Zn and Cd, and P<0.958 for Ni; Fig. 7.3b, e and d), whereas the reverse applied for Mg, whose concentration was greatest in *B. vulgaris* (P<0.001; Fig7.3a). At Harvest 3, as at Harvest 2, leaf Ni and Mo concentrations were greater in plants irrigated with clean water than in those receiving wastewater, whereas leaf Al and Zn concentrations were higher in plants treated with wastewater. Mo uptake may be inhibited by high soil sulphate concentrations, as is the case for soils irrigated with wastewater (Howarth and Stewart, 1992; Grattan and Grieve, 1999), perhaps explaining why Mo concentration was higher in plants receiving clean water. Leaf Ni concentration was greatest in *B. nutans*, while Mg concentration was greatest in the wastewater treatments of *B. nutans* and *B. vulgaris*. Leaves have a relatively short lifespan and it has been reported that *c*. 60% of the N and P present in pot-grown *Eucalyptus globulus* seedlings was in the leaves (Pereira, 1989; Guo and Sims, 2000). When the rate of leaf death approaches that of new leaf production as a result of salt accumulation within the plant tissues, the supply of assimilates and growth regulators to growing leaves decreases sharply, further reducing growth (Bernstein, 1980; Catlin *et al*., 1993; Boland *et al*., 1993). Concentrations of Cu, and Zn in the leaves of bamboo in the present study were 9 and 24 mg kg^{-1}, respectively (Fig 7.2c & b), again generally

much lower than the corresponding values of 239, and 43-191 mg kg^{-1} reported by Bosire (2007) for plants grown on contaminated soils in Nairobi.

In contrast to Experiment 1, the plants in Experiment 2 were relatively young (two months old at the start of the experiment) and the experimental duration was short. It is therefore likely that these relatively young plants would have been actively allocating a large proportion of their photosynthetic assimilates to support rhizome growth (McClure, 1966), with the result that effects on the soil and plant tissues would have been less profound than in longer experiments using more established plants. Soil irrigated with industrial wastewater contained the highest Fe and As concentrations ($P<0.05$; Table 7.8), followed by the clean water and domestic water treatments. Soil Ca concentration was least under *D. giganteus* ($P<0.013$; Fig. 7.14a), reflecting its greater ability to extract elements from the soil compared to the other two species. In addition to daily addition of elements as a result of irrigation, their distribution within the soil and plants is influenced by complex interactions between environmental factors which affect transpiration, and also by antagonistic interactions between specific soil nutrients (Pettygrove and Asano, 1985; Tani and Barrington, 2005).

Above-ground Mg concentration was lowest in *B. vulgaris*, while above-ground Zn concentration was lowest in *D. giganteus* and *B. nutans* ($P<0.001$; Table 7.9). Mo and Mn concentrations were greater in plants irrigated with clean water than in those receiving wastewater ($P<0.049$ & $P<0.012$, respectively; Table 7.10); above-ground Mo concentration was lowest in plants irrigated with industrial wastewater, whereas Mn concentration was lowest in the domestic wastewater treatment ($P<0.001$ & $P<0.012$ respectively; Fig. 7.16d & e). A significant irrigation treatment*species interaction was apparent for green leaf area ratio (GLAR) as the values for *B. vulgaris* and *D. giganteus* were relatively high in all irrigation treatments (Fig. 4.31), whereas the

value for the sewage water treatment of *B. nutans* was substantially lower than in the clean and industrial wastewater treatments. As noted previously, the plants may not have been exposed to wastewater for sufficiently long in Experiment 2 to induce salt-related effects on leaves (Bernstein, 1980; Catlin *et al*., 1993; Boland *et al*., 1993). Heavy rainfall in December and January 2006 would also have had a diluting influence on elemental concentrations within the soil. Wastewater from the industrial sources (BIDCO and KEL) did not contain unusually high concentrations of trace elements, although Pb, Mg, K, Fe, Zn and As concentrations were greater in the BIDCO water supply ($P<0.01$ for Pb and $P<0.001$ for other elements; Table 7.6), whereas Al, Ca and Mn concentrations were greater in the KEL industrial wastewater ($P<0.001$; Table 7.6). However, concentrations were well within statutory limits (Table 7.14; Pettygrove and Asano, 1985).

8.4 Impact of research on wastewater disposal in densely populated areas

Results from the present study should enable urban councils in developing countries to structure their wastewater disposal facilities so that biomass production becomes an integral part of the system, providing a cost-effective, clean and productive way of disposing of wastewater. Incorporating the urban poor in these systems in an informed and educated way would ensure that risks such as disease associated with wastewater handling are eliminated. It would be possible to estimate the area of bamboo required to address local pollution problems and facilitate planning of such systems depending on the indigenous human population, wastewater supplies and other local or regional factors such as weather conditions.

8.5 Summary of major results and conclusions

At maturity, *D. giganteus* is the largest bamboo species (Scurlock, 2000), although *B. vulgaris* and *B. nutans* produced more leaves, branches and biomass in the present study, potentially making them better candidates for wastewater treatment. Clearly, the choice of plant material will play a major role in the success or failure of wastewater treatment gardens. The industrial wastewater used in the present study was found not to contain harmful substances at concentrations likely to induce phytotoxicity (Table 7.11). Wastewater pollution in urban environments may therefore be a function of the quantity of wastewater applied rather than how severely it is contaminated. Water passing through dumpsites is more likely to contain high heavy metal concetrations than industrial effluent (Palela, 2008), although this depends on the type of material and effluent involved. The influence of weather conditions on plant water use and the associated uptake of nutrients and potentially toxic trace elements needs to be carefully considered before establishing wastewater treatment gardens.

8.5 Recommendations for future work

Further experiments to assess the potential impact of urban wastewater on plant growth are required as the wastewater used in the present study contained substantially lower concentrations of trace elements than anticipated. Moreover, solutions to the negative effects of wastewater reuse need to be found through further research. In India, for example, crops irrigated with untreated wastewater had lower yields than those supplied with primary or secondary effluent (Friedel *et al*., 2000; EPA, 2004). Additionally, the quantity of wastewater applied, distribution and mode of application, species selection, nutrient dynamics, impact of salts on the soil and plant growth rates are all factors to be considered (Murni Po *et al*., 2003) in addition to long-term investigations to determine the extent of salt accumulation and water deficits which individual species can tolerate. The possible impact of future research on wastewater handling in densely populated areas also

needs to be understood in order to estimate the area and plant populations needed to process sufficient wastewater for human use. Further research is also required regarding the uptake of nutrients by roots, rhizosphere responses to irrigation with wastewater and the use of agroforestry practices in wastewater irrigation. Such studies need to be carried out in conjunction with research regarding plant water relations and nutrient uptake to provide a detailed understanding of the factors which determine the transport of water and dissolved substances from the rhizosphere through the plant to the atmosphere. Especially in Africa, information concerning the distribution of bamboo plantations at the homestead and landscape levels is incomplete, necessitating further research. Simple methods for determining production are needed to exploit bamboo effectively; a simple productivity relationship linking biomass production to prevailing environmental conditions is needed. Also required are studies to elucidate the impact of irrigation with wastewater on CO_2 assimilation, leaf area development, biomass accumulation and carbon sequestration over extended periods.

The suitability of agroforestry for the safe use of wastewater for irrigation and issues regarding the acceptability of the products also requires further study to establish the risks involved and the nature of any problems associated with such products being mixed with a country's traditionally produced farm exports. Information regarding the distribution and productivity of bamboo in Africa is currently incomplete and likely to underestimate the use of these species as it lacks details for homesteads and plantations. More extensive and complete mapping of bamboo populations, including the species used is essential. Straightforward methods for determining biomass production which link bamboo biomass with climatic conditions are needed by growers. The methods previously used were adapted from forest systems and are not necessarily suited for bamboo. Further research is required to collect the data needed to establish such relationships.

Longer term studies of the potential of bamboo to remove pollutants from wastewater are required as most studies of wastewater reuse have involved forest species or short-season horticultural crops, whose growth differs from bamboo. Any study of the suitability of bamboo for wastewater reuse would be incomplete unless it is conducted over a sufficiently long period to cover all of its growth phases. Such studies should be carried out alongside tree species for comparative purposes so that conclusions on CO_2 sequestration and biomass production by bamboo may be obtained.

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APPENDIX 1: Bamboo World Genera

1.1 Bamboo world genera

A conspectus of bamboo genera is presented below (Li, 1999):

I. **Subtribe Arthrostylidiinae:** 1. Actinocladum, 2. Alvimia, 3. Apoclada, 4. Arthrostylidium, 5. Athroostachys, 6. Atractantha, 7. Aulonemia (Matudacalamus), 8. Colanthelia, 9. Elytrostachys, 10. Glaziophyton, 11. Merostachys, 12. Myriocladus, 13. Rhipidocladum.
II. **Subtribe Arundinariinae:** 14. Acidosasa, 15. Ampelocalamus, 16. Arundinaria, 17. Chimonocalamus, 18. Drepanostachyum (Himalayacalamus), 19. Fargesia (Borinda, Yushania), 20. Ferrocalamus, 21. Gaoligongshania, 22. Gelidocalamus, 23. Indocalamus, 24. Oligostachyum, 25. Pseudosasa, 26. Sasa, 27. Thamnocalamus
III. **Subtribe Bambusinae:** 28. Bambusa (Dendrocalamopsis), 29. Bonia (Monocladus), 30. Dendrocalamus (Klemachloa, Oreobambos, Oxynanthera, Sinocalamus), 31. Gigantochloa, 32. Dinochloa, 33. Holttumochloa, 34. Kinabaluchloa (Maclurochloa, Soejatmia), 35. Melocalamus, 36. Sphaerobambos, 37. Thyrsostachys
IV. **Subtribe Chusqueinae:** 38. Chusquea, 39. Nerolepis
V. **Subtribe Guaduinae:** 40. Criciuma, 41. Eremocaulon, 42. Guadua, 43. Olmeca, 44. Otatea
VI. **Subtribe Melocanninae:** 45. Cephalostachyum, 46. Davidsea, 47. Leptocanna, 48. Melocanna, 49. Neohouzeaua, 50. Ochlandra, 51. Pseudostachyum, 52. Schizostachyum, 53. Teinostachyum
VII. **Subtribe Nastinae:** 54. Decaryochloa, 55. Greslania, 56. Hickelia, 57. Hitchcockella 58. Nastus, 59. Perrierbambus
VIII. **Subtribe Racemobambodinae:** 60. Racemobambos (Neomicrocalamus, Vietnamosasa)
IX. **Subtribe Shibataeinae:** 61. Chimonobambusa, 62. Indosasa, 63. Phyllostachys, 64. Qiongzhuea, 65. Semiarundianria (Brachystachyum), 66. Shibataea, 67. Sinobambusa, 68. Temburongia

Table 1.1 Geographical distribution of bamboo sub-tribes, genera and species (Li, 1999).

Geographical area	Sub-tribes	Genera	Species	Chromosome Number
Asia	6	44	*c.* 600	46, 48, 64, 72
Africa	2	3	5 (endemic)	72
Madagascar	2	6	20 (endemic)	
Australia	2	2	3	
Pacific	2	2	4	
America	4	21	*c.* 400	40, 46, 48
Total	9	68	*c.* 1000	

1.2 Botanical description: *Dendrocalamus giganteus* (Clayton, 2006 onwards)

Habit: Perennial; caespitose. Rhizomes are short and pachymorph. Culms erect, 20-30 m in length and 20-30 cm in diameter, woody; culm internodes terete, thin-walled and 30-45 cm long. Lateral branches numerous and dendroid with one dominant branch which is thinner than the stem. Culm-sheaths are deciduous, coriaceous, purple, pubescent, hairy throughout; dark brown hairs; auriculate; glabrous on shoulders. Culm sheath ligule is 6-12 mm high, ciliate and dentate. Culm sheath blade is lanceolate and spreading. 5-15 leaves per branch. Ligule is an eciliate membrane,

3 mm long and erose. Leaf blade base has a brief petiole-like connection to sheath. Leaf blades are lanceolate, 15-45 cm long, 30-60 mm wide. Leaf blade venation has distinct cross veins; leaf blade margins are scabrous.
Inflorescence: Synflorescence is bractiferous and arranged in stellate clusters at the nodes; inflorescences are 20-25 mm long, dense and have glumaceous subtending bracts with axillary buds at base of spikelet; prophyllate below lateral spikelets and leafless between clusters.
Fertile spikelets: The fertile spikelets are sessile and comprise 4-8 florets without rhachilla extensions. Spikelets are lanceolate, laterally compressed, 12-15 mm long, 3-4 mm wide and break up at maturity, disarticulating below each fertile floret. Rhachilla internodes are suppressed between florets.
Glumes: The glumes persistent, dissimilar and shorter than the spikelets. The lower glume is orbicular, 0.7-0.8 times the length of the upper glume, chartaceous lacks keels and has 13 veins. The lower glume has lateral veins with cross veins and its apex is acute. The upper glume is oblate, 8-13 mm long, of similar length to the adjacent fertile lemma, is chartaceous, lacks keels and has 15 veins. The upper glume has lateral veins with cross veins and its apex is acute.
Florets: The fertile florets increase in size upwards. The lemma is oblate, 8-13 mm long, chartaceous, lacks a keel and has 25 veins. The lemma has lateral veins with cross veins, its margins are ciliate and its apex is acute. The palea is 0.9 times the length of the lemma, chartaceous, has six veins and has two keels with the exception the uppermost which lack keels. The palea keels are ciliate.
Flowers: Lodicules are absent. There are six anthers, 7-10 mm in length; the anther tip is apiculate. One stigma. Ovary umbonate and pubescent all over.
Fruit: Caryopsis has adherent pericarp; 7-8 mm long and hairy at the apex.
Distribution Africa: western Indian ocean. Asia-temperate: China and eastern Asia. Asia-tropical: India, Indo-China, and Malaysia.

1.3 Botanical description: *Bambusa vulgaris* (Clayton *et al*., 2006)
Habit: Perennial; caespitose. Rhizomes are short and pachymorph. Culms are geniculately ascending, 15-20 m in length, 4-10 cm in diameter, woody and lack nodal roots. Culm internodes are terete and thin-walled. Lateral branches are dendroid. Bud complement is one and branch complement is three or several in a clump; one branch is dominant and thinner than the stem. Culm sheaths are deciduous, hispid, with dark brown hairs, are auriculate and ciliate on the shoulders. The culm sheath ligule is 5-8 mm in length. The culm sheath blade is ovate, 5-15 cm long, pubescent and acute. There are 8-9 leaves per branch. Leaf sheaths are pubescent and the oral hairs are ciliate. Leaf sheath auricles are falcate. Ligule is an eciliate membrane. The collar has an external ligule. The base of the leaf blade is adly rounded with a brief petiole-like connection to sheath. Leaf blades are lanceolate, 15-30 cm long and 18-45 mm wide. Leaf blade margins are scabrous; the leaf blade apex is acuminate and hardened.
Inflorescence: Synflorescence is bractiferous, clustered at the nodes in untidy dense tufts, 1-3 cm long with spathaceous subtending bracts, axillary buds at the base of the spikelet; prophyllate below lateral spikelets and leafy between clusters.
Fertile spikelets: Spikelets comprise 4-12 fertile florets with diminished florets at the apex. Spikelets are oblong, laterally compressed, 10-20 mm long and break up at maturity, disarticulating below each fertile floret. Rhachilla internodes definite.
Glumes: Glumes are persistent, similar to or shorter than spikelets. The lower glume isovate, 0.7-0.8 times the length of upper glume, coriaceous and without keels. The upper glume is ovate, 0.5 times the length of the adjacent fertile lemma, coriaceous and lacks keels.
Florets: Fertile lemma is ovate, 9-11 mm long without a keel and has 11-15 veins. The margins of the lemma are ciliate and hairy above and its apex is acute. The palea is oblong, is the same length

as the lemma and has six veins; its keels are wingless and ciliate. The sterile apical florets resemble fertile florets but are underdeveloped.
Flowers: these have three membranous and ciliate lodicules. The six anthers are 5 mm in length. There are 2-3 stigmata and the ovary is umbonate.
Fruit: Caryopsis with adherent pericarp.
Distribution: Africa: Macaronesia, west tropical, west-central tropical and western Indian ocean. Asia-temperate: China and eastern Asia. Asia-tropical: India, Indo-China, Malaysia and the north Indian ocean. Australasia: Australia. Pacific: southwestern, south-central, northwestern, and north-central. North America: Mexico. South America: Mesoamericana, Caribbean, northern South America, western South America and Brazil.

1.4 Botanical description: *Bambusa nutans* (Clayton *et al*., 2006)
The growth habit of *Bambusa nutans* is perennial and caespitose. Rhizomes are short and pachymorphous. Culms are erect, 6-12 m long, 4-7 cm in diameter, woody and produce aerial roots from the nodes. Culm internodes are terete, thick walled, 35-45 cm long and mid-green in colour. Culm nodes are glabrous or pubescent. Lateral branches are dendroid. Culm sheaths are 15-23 cm long, pubescent, with appressed black hairs and are truncate at the apex, auriculate and setose on their shoulders. The culm sheath ligule is 2.5-5 mm long and dentate. The culm sheath blade is triangular, 15-23 cm long, pubescent and acute. The leaves are cauline, while the leaf sheaths are striately veined and pubescent; their oral hairs are setose and their auricles are falcate. The ligule is an obtuse eciliate membrane. The collar has external ligule while the base of the leaf-blade has a brief petiole-like connection to sheath which is 0.3-0.5 cm long. The leaf blades are lanceolate, 15-30 cm long, 25-35 mm wide and glandular with a conspicuous midrib. The leaf blade has 14-20 secondary veins and its surface is glabrous or puberulous with abaxial hairs. The margins of the leaf blade are scabrous and its apex is acuminate and antrorsely scabrous.
Inflorescence: The synflorescence is bractiferous and clustered at the nodes in untidy tufts with spathaceous subtending bracts and axillary buds at the base of the spikelets which are prophyllate below lateral spikelets.
Fertile spikelets: Spikelets comprise 3-5 fertile florets with diminished florets at the apex. Spikelets are lanceolate, subterete, 17-25 mm long and break up at maturity, disarticulating below each fertile floret. Rhachilla internodes definite, clavate, pilose and hairy at the tip.
Glumes: Glumes several with 2-3 empty glumes.
Florets: Fertile lemma ovate, 10 mm long, without a keel; the inner surface is pubescent. The apex of the lemma isacute and mucronate. Palea keels are ciliate. Sterile apical florets resemble fertile florets but are underdeveloped.
Flowers: These contain three membranous, veined and ciliate lodicules. There are 6-7 anthers with apiculate tips and 2-3 sparsely hairy stigmata. The ovary is umbonate and pubescent at its apex.
Fruit: The caryopsis has an adherent pericarp, oblong and hairy at its apex.
Distribution: Asia-tropical: India and Indo-China.

APPENDIX 2: Wastewater Constitution

Table 2.1. Chemical constituents of concern for wastewater reuse (adapted from (Pettygrove and Asano, 1985).

Constituent	Measured Parameters	Reasons for Concern
Suspended Solids	Suspended solids, volatile components and absorbed on particulates	Organic contaminants and heavy metals may be absorbed on particulates. Suspended matter can shield microorganisms from disinfectants. Excessive suspended solids cause plugging in irrigation systems
Biodegradable Organics	Biochemical oxygen demand, chemical oxygen demand, total organic carbon	Aesthetic and nuisance problems. Organics provide substrates for microorganisms, adversely affect disinfection processes, render water unsuitable for some industrial or other uses, consume oxygen, and may induce acute or chronic effects if reclaimed water is used
Nutrients	Nitrogen, Phosphorus, Potassium	Nitrogen, phosphorus, and potassium are essential plant nutrients and their presence normally enhances the value of the water for irrigation
Stable Organics	Specific compounds (e.g. pesticides, chlorinated hydrocarbons)	Some organics resist conventional wastewater treatment methods and are toxic to the environment; their presence may limit the suitability of reclaimed water for irrigation or other uses
Hydrogen Ion Concentration	pH	The pH of wastewater affects disinfection, coagulation, metal solubility and the alkalinity of soils. Normal range in municipal wastewater is 6.5-8.5, but industrial waste may significantly alter pH
Heavy Metals	Specific elements (e.g. Cd, Zn, Ni, and Hg)	Some heavy metals accumulate in the environment and are toxic to plants and animals. Their presence may limit the suitability of reclaimed water for irrigation or other uses
Dissolved Inorganics	Total dissolved solids, electrical conductivity, specific elements (e.g. Na, Ca, Mg, Cl, and B)	Excessive salinity may damage some crops. Specific inorganic ions may accumulate to toxic concentrations (e.g. Cl, Na, B) elements for some crop species
Residual Chlorine	Free and combined chlorine	Excessive concentrations of available chlorine (>0.05 Cl mg L^{-1}) may cause leaf-tip burn and other injury in sensitive species. However, most chlorine in reclaimed water is in a combined form, which does not damage crops

Table 2.2 Recommended limits for constituents in wastewater used for irrigation (from (Pettygrove and Asano, 1985).

Constituent	Long-Term Use (mg L^{-1})	ShortTerm Use (mg L^{-1})	Remarks
Aluminum	5.0	20.0	Reduces productivity in highly acidic soils, but precipitation in soil pH range 5.5-8.0 eliminates toxicity
Arsenic	0.10	2.0	Toxicity varies from <0.05 mg L^{-1} in rice to 12 mg L^{-1} in Sudan grass
Beryllium	0.10	0.5	Toxicity varies from 0.5 mg L^{-1} in bush beans to 5 mg L^{-1} in kale
Boron	0.75	2.0	Essential plant nutrient, with optimum yield being obtained at a few-tenths of 1 mg L^{-1} in nutrient solutions. Toxic to sensitive species (e.g. citrus) at 1 mg L^{-1}. Quantities in reclaimed water usually sufficient to correct soil deficiencies. Most grasses are tolerant to concentrations between 2.0-10 mg L^{-1}
Cadmium	0.01	0.5	Toxic to beans, beet and turnip at concentrations as low as 0.1 mg L^{-1} in nutrient solution. Conservative limits recommended
Chromium	0.1	1.0	Not an essential plant nutrient. Conservative limits recommended due to lack of knowledge on toxicity
Cobalt	0.05	5.0	Toxic to tomato at 0.1 mg L^{-1} in nutrient solution. Tends to be inactivated by neutral and alkaline soils
Copper	0.2	5.0	Toxic to many species at 0.1-1.0 mg L^{-1} in nutrient solution
Fluoride	1.0	15.0	Not an essential plant nutrient. Inactivated in neutral and alkaline soils
Iron	5.0	20.0	Not toxic to plants in aerobic soils but can contribute to soil acidification and loss of essential phosphorus and molybdenum
Lead	5.0	10.0	Can inhibit cell growth at high concentrations
Lithium	2.5	2.5	Tolerated by most crops at concentrations <5 mg L^{-1}; mobile in soil. Toxic to citrus at low concentrations; recommended limit is 0.075 mg L^{-1}
Manganese	0.2	10.0	Toxic to several crop species at few-tenths of 1 mg L^{-1} in acidic soils
Molybdenum	0.01	0.05	Essential nutrient which is not toxic to plants at normal concentrations in soil and water. May be toxic to livestock if forage is grown on soils with high available molybdenum concentrations
Nickel	0.2	2.0	Toxic to some plants at concentrations between 0.5-1.0 mg L^{-1}; toxicity reduced under neutral or alkaline pH

Selenium	0.02	0.02	Toxic to plants at low concentrations and to livestock if forage is growno soils with low selenium concentration
Tin, Tungsten Titanium	---	---	Effectively excluded by plants; specific tolerance levels unknown
Vanadium	0.1	1.0	Toxic to many plants at relatively low concentrations
Zinc	2.0	10.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH 6 or above and in fine-textured or organic soils
Constituent	**Recommended Limit**		**Remarks**
pH	6.0		Effects of soil pH on plant growth are usually indirect (e.g. by affecting heavy metal toxicity)
TDS	500-2000 mg L^{-1}		1000 mg L^{-1} TDS in irrigation water may affect sensitive species; at 1000-2000 mg L^{-1}, TDS may affect many crops and careful management practices should be followed. Above 2000 mg L^{-1}, water can be used regularly only for tolerant species on porous soils
Free Chlorine Residual	<1 mg L^{-1}		Concentrations >5 mg L^{-1} cause severe damage to most species and some sensitive species may be damaged at concentrations as low as 0.05 mg L^{-1}

Table 2.3 Threshold concentrations of trace elements for crop production (from Pescod, 1992).

	Element	Recommended maximum concentration (mg L^{-1})	Remarks
Al	Aluminium	5.0	May reduce productivity in acid soils (pH <5.5), but is precipitated in alkaline soils (pH >7.0), preventing toxicity
As	Arsenic	0.10	Toxicity ranges from <0.05 mg L^{-1} in rice to 12 mg L^{-1} in Sudan grass
Be	Beryllium	0.10	Toxicity ranges from 0.5 mg L^{-1} in bush beans to 5 mg L^{-1} in kale
Cd	Cadmium	0.01	Toxic to beans, beet and turnip at concentrations of 0.1 mg L^{-1}. Conservative limits recommended due to its potential to accumulate in soil and plants to concentrations harmful to humans
Co	Cobalt	0.05	Toxic to tomato at 0.1 mg L^{-1} in nutrient solution; tends to be inactivated in neutral and alkaline soils
Cr	Chromium	0.10	Not an essential growth element; conservative limits advised due to lack of knowledge on its phytotoxicity
Cu	Copper	0.20	Toxic at concentrations of 0.1-1.0 mg L^{-1} in nutrient solution
F	Fluoride	1.0	Inactivated in neutral and alkaline soils
Fe	Iron	5.0	Not phytotoxic in aerobic soils but can contribute to acidification and reduced availability of phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings
Li	Lithium	2.5	Tolerated by most crops at concentrations ≤5 mg L^{-1}; mobile in soil. Toxic to citrus at low concentrations (<0.075 mg L^{-1}). Acts similarly to boron
Mn	Manganese	0.20	Toxic to several crops at few-tenths of 1 mg L^{-1}; toxicity most common in acid soils
Mo	Molybdenum	0.01	Not phytotoxic at normal concentrations in soil and water but can be toxic to livestock if forage is grown on soils with high available molybdenum concentrations
Ni	Nickel	0.20	Phytotoxic to various species at 0.5-1.0 mg L^{-1}; toxicity reduced at neutral or alkaline pH
Pd	Lead	5.0	May inhibit cell growth at very high concentrations
Se	Selenium	0.02	Phytotoxic at concentrations as low as 0.025 mg L^{-1} and to livestock if forage is grown on soils with relatively high levels of added selenium. Essential element for animals at very low concentrations

Ti	Titanium	-	Effectively excluded by plants; specific tolerance unknown
C	Carbon	0.10	Phytotoxic to many species at relatively low concentrations
Zn	Zinc	2.0	Phytotoxic to many species at widely varying concentrations; toxicity is reduced at pH >6.0 or in fine textured or organic soils

APPENDIX 3: Characteristics of Raw Sewage Used in Irrigation

Table 3.1. Chemical characteristics and input of nutrients from irrigation with raw sewage (from Parameswaran, 1999).

Parameter input[b]	Mean	Nutrient (kg/ha)
Colour (Pt/Co)	117.0	–
pH	6.8	–
E. coli ('000000) (no./100 ml)	13.0	–
MBAS	4.5	–
Suspended solids	417.0	–
BOD 5	481.0	–
TDS	1035.0	–
TOC	328.0	–
Nitrite-N	< 0.1	0.6
Nitrate-N	0.2	1.0
Ammonia-N	28.8	158.0
Organic-N	19.9	109.0
(Total N)	(49.0)	(268.0)
Total P	12.0	66.0
Cu	0.2	1.0
Cr	0.1	0.6
Cd	< 0.01	< 0.1
Fe	2.3	12.6
Pb	0.1	0.6
Hg	< 0.01	< 0.1
Zn	0.2	1.0
Mn	0.2	1.0
Ni	0.1	0.6
Bo	5.0	27.4
Mo	< 0.1	0.6
Co	< 0.1	0.6

[a] Concentrations in mg/l unless otherwise indicated.
[b] Based on total quantity of wastewater applied.

APPENDIX 4: Global Assessment of Bamboo Resources

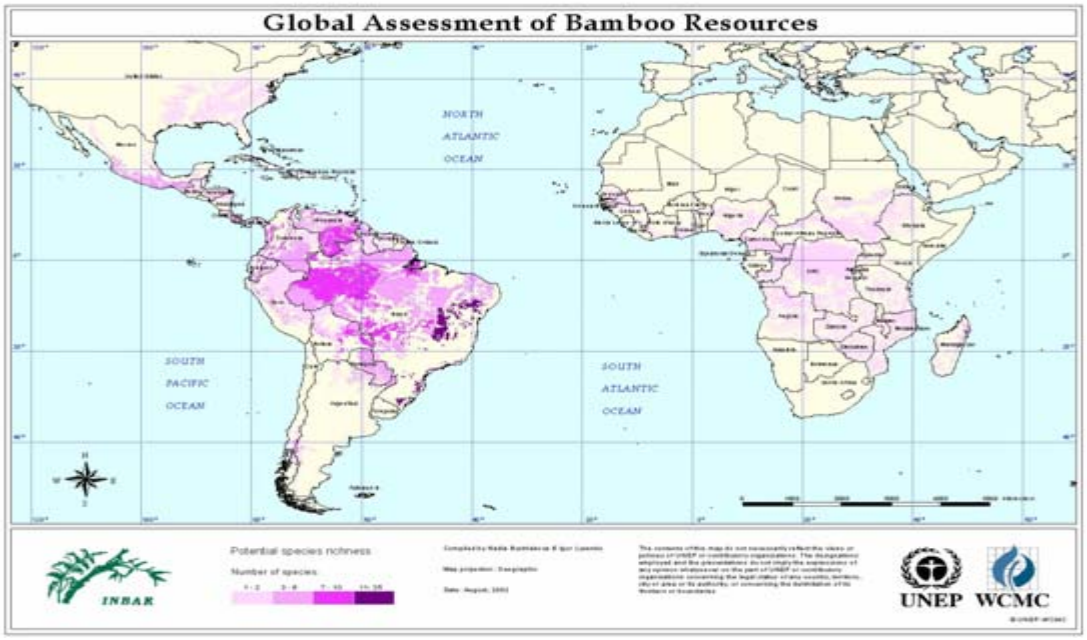

Figure 4.1. Regions of the world where bamboo species have been documented, showing species richness in Africa, South and Central America.

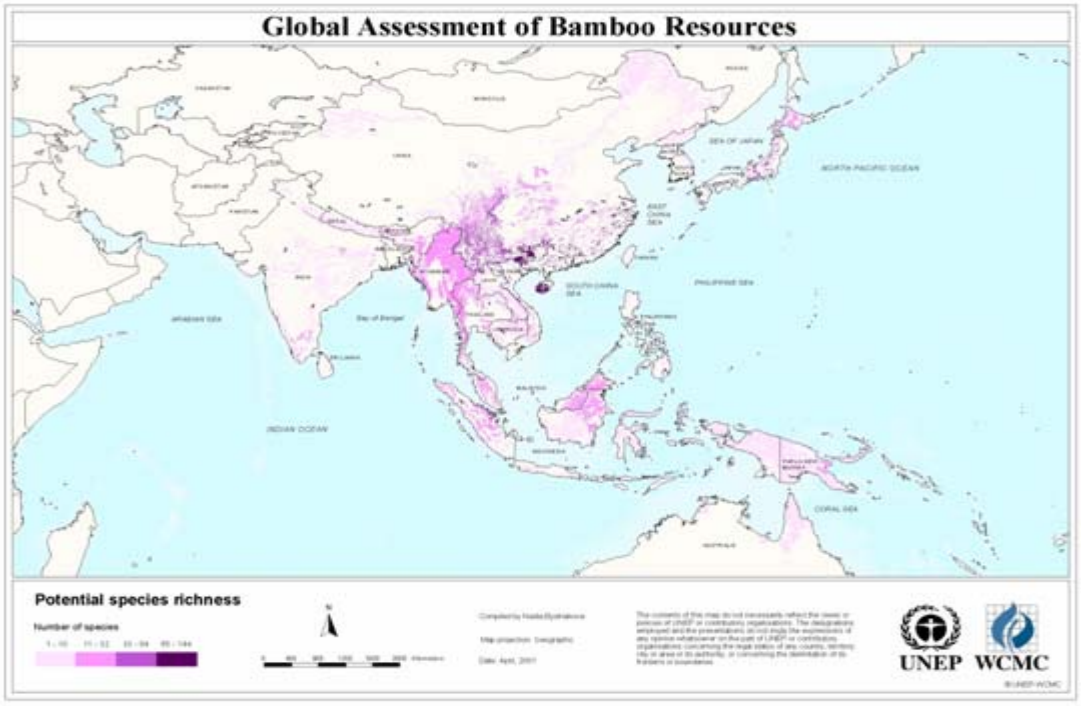

Figure 4.2. Regions of the world where bamboo species have been documented, showing species richness. South-East Asia (from United Nations Environment Programme, World Conservation Monitoring Centre).