Münir Öztürk · Muhammad Ashraf
Ahmet Aksoy · M. S. A. Ahmad
Khalid Rehman Hakeem *Editors*

Plants, Pollutants and Remediation

Springer

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Editors

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Springer

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Foreword

Centuries ago, nature was dominant and human interference was negligible. Gradually population and industrialization increased, resulting in pollution of natural resources. Pollution is a glocal (global and local) problem. Of late, considerable efforts have been put in for decontaminating the polluted substrates such as air, water, and soil. In this regard, the subject of phytoremediation gained global momentum and has grown phenomenally.

Nature's cure using plant resources (phytoremediation) is a sustainable solution for environmental decontamination. As of now, about 25,000 articles have been published on various aspects of using biological resources for environmental cleanup starting with only 11 in 1989. The use of plants for the remediation of surface soils polluted and contaminated with toxic heavy metals is well established. The plant-based technologies are applicable to inorganic and organic contaminants and pollutants. A wide variety of technologies using plants and microbes to remediate or decontaminate soils, groundwaters, surface waters, or sediments, including air, are currently researched in various laboratories all over the world. These technologies have become attractive alternatives to conventional cleanup technologies due to relatively low capital costs and the inherently aesthetic nature. Biodiversity is the raw material for bioremediation and is an invaluable toolbox for wider application in the realm of geoenvironment and human health protection.

The industrial revolution, a feather in the cap of human civilization, has unwittingly rendered thousands of hectares of land tainted with the toxic by-products of many industries such as mining, batteries, and paints. Conventional remediation, which involves the physical removal and burial of contaminated soils, is neither feasible nor affordable. The growing awareness of the existence of a number of metal-accumulating plant species, called hyperaccumulators, that are endemic to metalliferous soils and can accumulate and tolerate high levels of heavy metals in the shoot is a major factor in the growing interest in phytoremediation. This technology, which uses plants with their extensive root systems and efficient uptake of a wide variety of molecules, offers a low-input affordable alternative to conventional remediation. The identification of several metal hyperaccumulator plant species

demonstrates that the genetic potential exists for successful phytoremediation of contaminated soils.

Although extremely effective at accumulating metals, naturally occurring hyperaccumulators are less than ideal for phytoremediation due to their slow growth rate and low-to-the-ground rosette architecture, which makes them difficult to harvest. The transfer of these hyperaccumulating properties from the hyperaccumulators into a high-biomass-producing plant has been suggested as a potential avenue for making phytoremediation a commercial technology. Transgenic plants are used effectively for the remediation of soils containing a number of different xenobiotic contaminants. Progress in this area, however, is hindered by a lack of understanding of the basic physiological mechanisms involved in uptake into roots and translocation to aboveground tissues.

Recent research has focused on understanding the native molecular and physiological mechanisms of how plants remove pollutants from soils to aid in the creation of transgenic varieties optimized for soil remediation. Attempts made to cross these hyperaccumulators with their larger fast-growing relatives to produce desirable hybrids are not yet successful. To effectively accumulate a metal, a plant must be able to efficiently absorb, translocate through the xylem, unload into the shoot tissues, and finally sequester the metal into vacuoles. Many workers have speculated that the ability to hyperaccumulate metals could be the result of a broader change in the regulation of a response pathway. To be truly effective, plants used for the phytoremediation of metals need to be able to extract the toxic element from the soil and accumulate it in their aboveground tissues which can then be harvested and either composted or ashed to retrieve the extracted metals.

Soil, water, and air are the important natural resources that must be clean. Unfortunately, natural resources are polluted globally. Rapid industrialization and extraction of a large quantity of natural resources, including indiscriminate extraction of groundwater have resulted in environmental contamination and pollution. Large amounts of toxic wastes have been and are still dispersed in thousands of sites spread across the globe, resulting in varying degrees of contamination and pollution. Thus, every one of us is getting exposed to contamination from past and present industrial practices and emissions in natural resources (air, water, and soil) even in the most remote regions. The risk to human and environmental health is rising, and there is evidence that this cocktail of pollutants is a contributor to the global epidemic of cancers and other degenerative diseases. The challenge is to develop innovative and cost-effective solutions to decontaminate polluted environments from inorganic as well as organic pollutants.

Soil contamination with organics and inorganics is growing as a perennial problem all over the world. Its association with human health makes it a topic of more concern. Therefore, there is a need for research to evolve approaches and strategies for promoting sustainable technologies for environmental management which includes bioremediation. Currently the “gentle soil remediation options (GRO)” and the emerging “phytomanagement” practices highlight the use of bio-/phyto-/rhizoremediation-borne biomass as feedstock for “biorefinery” and ecosystem services.

In recent years, the number of studies evaluating GRO at a field level has been steeply on the rise. Most of the papers published are lab-scale and hydroponic experiments. This has received inadequate support from policy and decision makers who believed that phytoremediation is a temporary solution of transferring the pollutants and contaminants from one place to another. Often, scientists and academia are also subscribed to this feeling. Regulators have expressed apprehensions about phytoremediation due to the lack of contemporary knowledge of environmental sustainability. Thus, it is generally believed that pollution prevention by plants through phytoremediation strategy and approach is a temporary solution. Further, how to dispose of the contaminated photomaps is a puzzling question posed by environmental managers and regulators.

The move from greenhouse to field conditions requires incorporating agronomical and ecological knowledge into the remediation process. Agronomic practices such as crop selection, crop rotations/intercropping, planting density, fertilization, irrigation schemes (including chelator-supplemented water), bioaugmentation with microbial inoculants, and weed, pest, and herbivory management can be modified so as to suit both the characteristics of the contaminated soils and to meet the requirements of effective phytoremediating crops.

GRO can bring beneficial ecosystem services (e.g., habitat, C-storage, soil erosion, temperature regulation, etc.) and can also provide valuable sources of renewable biomass for the bio-based economy (e.g., bioenergy, biocatalysis and platform molecules for green chemicals, and ecomaterials). Harvested biomass can be burned/chemically converted for the energy sector and the recovery of accumulated metals (phytoextraction) or for the production of biomass suitable for the biorefinery industry (other GRO, e.g., phytostabilization). Some GRO-borne biomasses can be used as ecomaterials, notably in combination with plastics/biocomposites including geopolymers. Many economies are dependent on the supply of raw materials and trading values of metals such as copper, nickel, and zinc have been steadily on the rise. Metal-rich plant biomass has been used as an alternative to nonrenewable mineral materials to produce Lewis acid catalysts. GRO can offer a means of metal extraction, recovery, or recycling. For TE-contaminated soils, GRO are based on practices which decrease the labile ("bioavailable") pool and/or total contents of TE in the soil and include (in situ) contaminant stabilization ("inactivation") and plant-based (generally termed "phytoremediation") options. Phytoextraction aims to remove TEs from soils through their uptake and accumulation in plant parts that are removed by harvest. Here, bioavailable contaminant stripping (BCS) targets in particular the labile TE pool in the soil. Phytoextraction (or phytomining) can be carried out on metal-contaminated soils as well as low-grade ores or naturally metal-rich (serpentine) soils that cannot be economically utilized by traditional mining technology. Aided phytostabilization aims to establish a vegetation cover and progressively promote (in situ) inactivation of metal(loid)s by combining the use of TE-excluding plants and soil amendments. Although this technology does not lead to a cleanup of the soil, by altering TE speciation and mobility, it moderates potential negative environmental impacts and pollutant linkages.

I fervently believe that the chapters included in this book will contribute towards a broader understanding of pollution prevention by plants and the remediation strategies and approaches.

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Prof. Dr. M.N.V. Prasad

Preface

The hazards of environmental degradation transcend borders and are fully highlighted by different organizations. Environmental pollution is one of the most important and urgent problems faced by the global society, a problem which does not respect traditional political or geographical boundaries. Several steps have been taken by several organizations to look at the critical environmental and developmental challenges, arising from unprecedented pressures on the environment of planet Earth.

Nearly two decades have passed after the launch of Our Common Future, which defined sustainable development as a blueprint to address our environmental and developmental challenges. An evaluation of such issues is a social imperative of our time. We are experiencing rapid environmental change all around us, and many more problems like water shortages, land degradation, and biodiversity loss are on the horizon.

The problems related to the water resources are likely to grow wider. This will affect economic and social development as well as environmental sustainability. In order to overcome water scarcity, integrated water resource management will be of crucial importance. This will also be important for international peace and security and eradication of global poverty together with future developmental goals. The countries on individual basis will not be in a position to protect our environment. We on this planet are badly in need of a more coherent system of international environmental governance. We must move forward rapidly for the sake of current and future generations towards the global response to these challenges.

In the light of the statements given above, this book is being published at a time when there is a need for the pace of environmental degradation with a new sense of realism. The unprecedented environmental changes we face today are highlighted here. The book contains 19 chapters, which provide an overview of global social and economic trends, as well as the human dimensions of these changes. It highlights the challenges of environmental change, an outlook for the future, and policy options to address present and emerging environmental issues.

Environmental pollution endangers our biodiversity on one side and human health on the other. These pollutants, although generated in megacities and industrial

areas, affect rural areas equally well through transport and dispersal. The relative distance from the pollutants in no way guarantees a lack of impact on our environment. Although several pollution control measures have been adopted, even then rapid development continues to produce significant impacts on global environmental quality. Many phytotoxic compounds, heavy metals, pesticides, and acidic precipitation highly affect our biodiversity. Many bioindicator species are used effectively to assess pollutant impacts; however, the knowledge and experience of the researchers is critical for an accurate evaluation.

Each chapter in this book includes information representing a compilation of material and references by internationally recognized experts. The main contribution is to provide a broad-based reference for pollutants in relation to our plant life. The need for producing this book was felt because environmental study is one of the most important and integral parts of life sciences. It describes the different components of the environment and their influence on plant as well as animal diversity. The degradation of the environment and its effects on our health have attained great importance in this branch of science. A global awareness on environment has been generated.

The editors have thus spent efforts to put together the fundamentals of existing knowledge in environmental perspectives and their remediation. Attempts have been made to include latest information available in this field. The environmental issues have been reviewed and efforts for protection as well as remediation outlined in different chapters. We were encouraged to undertake this editorial effort by the participation of a large number of scientists from all over the world together with Nobel laureates and other leading scientists. We express gratitude to all these participants who joined us. We hope this volume will be useful to all researchers as well as others concerned with our environment.

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The foundation for editing this volume was laid during the “International Conference on Plants and Pollutants” held at Erciyes University. Our actively working colleagues from different parts of the world were kind enough to collaborate with us. We therefore take this opportunity to thank the contributors for their patience, full cooperation, and support.

The conference was sponsored by the IDB (Islamic Development Bank), Rectorate of Erciyes University (Rector Prof. Dr. Fahrettin Keleştemur), Provincial Grand Mayor Municipality of Kayseri (Mr. Mehmet Özhaseki), Trades Union of Kayseri (Mr. Hasan Ali Kilci), and Kayseri Directorate of the Ministry of Environment and Forests–Turkey. Our special thanks are due to them for the support and encouragement.

The motivation from the Nobel laureates Prof. Dr. Yuvan T Lee (Taiwan) and Prof. Dr. Ferid Murad (USA) as well as UNESCO laureate Prof. Dr. Atta-ur-Rehman (FRS) (Pakistan) inspired us greatly to work on this book. The editors would like to express their indebtedness and special thanks to all of them.

The success in the preparation of this volume depended largely on the encouragement from the Springer team who collaborated with us; therefore, our greatest appreciation goes to them.

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Visible Injury, CO_2 Assimilation and PSII Photochemistry of *Eucaliptus* Plants in Response to Boron Stress

Cristina Nali, Alessandra Francini, Elisa Pellegrini, Stefano Loppi, and Giacomo Lorenzini

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Abstract Boron is an essential element required for the normal growth of plants, but in high concentrations it is toxic, causing reduction of leaf area, induction of chlorotic and necrotic lesions in older leaves, delay in development and general inhibition of growth. To gain an insight into the role of photosynthetic mechanisms in the response to boron toxicity, physiological parameters were analyzed in seedlings of *Eucaliptus globulus* treated with 0.1 (control), 1 and 10 mg l^{-1} (excess) H_3BO_3 in nutrient solution during 12 weeks. After 42 days of treatment, plants grown in the excess of boron developed symptoms in the mature leaves, in form of marginal necrosis. At the end of treatment, CO_2 assimilation and stomatal conductance decreased (−71 % and −30 %, respectively, compared to control) when plants were supplied with 10 mg l^{-1} H_3BO_3; a reduction in growth (−30 % compared to control) and increase of B concentration in roots as a consequence of the treatment have been also observed.

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1 Introduction

Boron (B) is an essential element required for the normal growth of vascular plants. It is unique as a micronutrient in that the threshold between deficiency and toxicity is very narrow (Yau and Ryan 2008; Ozturk et al. 2010): it has long been known that the optimum B level for one species could be either toxic or insufficient for other species (Blevins and Lukaszewski 1998). The role of B in plant nutrition is little understood, which is surprising since on a molar basis the requirement for B is, at least for dicotyledons, higher than any other micronutrient (Marschner 1995); moreover, it has restricted mobility in many species and is freely mobile in others (Brown and Shelp 1997). B is implicated in three main processes: keeping cell wall structure, maintaining membrane function and supporting metabolic activities. However, in the absence of conclusive evidence, the primary role of B in plants remains elusive (Bolaños et al. 2004).

Boron toxicity is largely a local phenomenon, restricted to areas where soil or water supplies high amount of B (Aucejo et al. 1997). For this reason, worst cases of B toxicity occur in irrigated agricultural fields, re-vegetation projects and adjacent to industrial sites that emit B-laden aerosols (Sage et al. 1989). When the B concentration at root level is high, this element is accumulated in the leaf cell walls and may reach the cytoplasm, disturbing metabolism and resulting in the development of toxicity symptoms (Matoh 1997), generally in the older leaves in the form of marginal or interveinal chlorotic and/or necrotic lesions (Paull et al. 1992; Nable et al. 1997). As B concentrations in the roots remain relatively low compared to those in leaves even at very high levels of B supply (Nable et al. 1997), perhaps toxic concentrations do not occur in root tissues. The main concern is that B is mainly transported via the transpiration stream and B concentration typically decreases from older to younger leaves, and apical to basal leaf parts. Thus, stomatal movement may be affect B uptake behaviour. Increased stomatal resistance against the excessive B uptake was reported by several Authors (Alpaslan and Gunes 2001; Papadakis et al. 2004b; Gunes et al. 2006), no data being available on eucalypts.

Boron toxicity is an important disorder that causes negative physiological effects such as decreased leaf chlorophyll, inhibition of photosynthesis (Lovatt and Bates 1984), deposition of lignin and suberin (Ghanati et al. 2002), increased membrane leakiness. B excess inhibits photosynthesis by causing structural damage to thylakoids and thus decreasing CO_2 uptake. These effects disrupt photosynthetic transport of electrons, favoring a condition where molecular oxygen operates as an alternative acceptor for non-utilized electrons and light energy leading to generation of reactive oxygen species (ROS) (Molassiotis et al. 2006).

Although there are many reports in the literature relating to the development of leaf symptoms of B toxicity, the available information concerning the effects of B excess on CO_2 assimilation (Kamali and Childers 1967; Lovatt and Bates 1984; Sotiropoulos et al. 2002; Papadakis et al. 2004a; Han et al. 2009) and carbohydrate metabolism (Papadakis et al. 2004b; Cervilla et al. 2007) are scarce. Considering that in the eucalypts there is a certain sensitivity to B excess (Marcar et al. 1999; Poss et al. 1999) and the available information concerning the effects of B on photosynthesis, leaf anatomy and growth is scarce, we carried out this experiment in order to bridge some gaps.

2 Materials and Methods

2.1 Plant Material, Growth Conditions and Treatments

Uniform sized 1-year old *Eucaliptus globulus* were randomly assigned to 20-l pots (∅ 24 cm) filled with sand-vermiculite substrate (1:1, by vol.). Experiments were carried out in a greenhouse with natural daylight. During May-July, the minimum air temperature was 18 °C (night) and maximum 34 °C (day). Plants were irrigated with a modified Hoagland's solution (Hoagland and Arnon 1950). Three B concentrations were applied: 0.1 (control), 1 (B1) and 10 mg l^{-1} (B10, to induce B toxicity) as H_3BO_3. Each treatment solution was delivered to designated pots every 20 days (*ca.* 500 ml per pot). Daily irrigations were sufficiently frequent to avoid water stress.

2.2 Gas Exchange and Chlorophyll a *Analysis*

Measurements of leaf gas exchanges were carried out in mature leaves by an infrared gas-analyzer (CIRAS-1 PP-Systems) equipped with a Parkinson leaf chamber that controlled leaf temperature (25 °C), relative humidity (80 %), light (800 μmol m^{-2} s^{-1} PAR) and CO_2 concentration (350 ppm). Photosynthetic activity at saturation light level (A_{max}), stomatal conductance to water vapour (G_w) and apparent internal CO_2 concentration (C_i) were calculated according to the equations described in Von Caemmerer and Farquhar (1981) and related to one-sided leaf areas.

Modulated chlorophyll *a* fluorescence measurements were carried out with a PAM-2000 fluorometer (Walz) on dark-adapted leaves for 40 min using a dark leaf clip. Ground fluorescence, F_0 was determined using the measuring modulated light which was sufficiently low (<1 μmol m^{-2} s^{-1}) without inducing any significant variable fluorescence. The maximal fluorescence level, F_m, was determined by applying a saturating light pulse (0.8 s) at 8000 μmol m^{-2} s^{-1} in dark-adapted leaves; the variable fluorescence was calculated as $F_v = F_m - F_0$. The saturation pulse method was used for analysis of quenching components (qP and qN), as described by Schreiber et al. (1986).

Excitation pressure on PSII reflects the proportion of the primary stable quinone acceptor Q_A in the reduced state; it is calculated as $(1-qP)$. The actual quantum yield of PSII (Φ_{PSII}) was computed as $(F'_m - F_s)/F'_m$, where F_s is the steady-state fluorescence yield in the light-adapted state, as in Rohàček (2002). The apparent electron transport rate through PSII (ETR) was computed as $qP \times \Phi_{PSII} \times PFD \times 0.5 \times 0.84$ (Schreiber et al. 1986).

Chlorophylls were estimated non-destructively on intact parts of mature leaves with a SPAD meter (Minolta 502).

2.3 Boron Determination

After 12 weeks, plants were carefully removed from the pots and separated into leaves, stems and roots. All samples were washed with distilled water, then oven-dried at 60 °C for 4 days and weighed separately for dry mass determinations. The oven-dried samples were homogenised to a fine powder in a blender for subsequent analysis. About 300 mg of powder were mineralised with a 6:1 v:v mixture of ultrapure concentrated HNO_3 and H_2O_2 at 280 °C and a pressure of 0.55 MPa in a microwave digestion system (Milestone Ethos 900). Boron concentrations, expressed on a dry weight basis, were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer-Sciex Elan 6100). All analyses were carried out in triplicate in each of the three repeated experiments.

2.4 Statistical Analysis

Three repeated experiments were set up in a completely randomized design with seven replicate plants for each treatment. Data shown in tables and graphs represent the mean ± standard deviation. Analysis of variance (ANOVA) was applied in order to examine the effects of B treatment. Statistical analysis was conducted by using NCSS 2000 Statistical Analysis System software.

3 Results

Boron toxicity symptoms firstly appeared in leaves about 42 days after the beginning of the experiment only in B10 treatment. These symptoms occurred in the older leaves, as tip burn and marginal necrosis. However, it was observed that fresh (*data not shown*) and dry weight (DW) of these leaves were not significantly affected by B concentration in the nutrient solution (Fig. 1), while the roots growth decreased (−30 % compared to control). A significant increase of shoots DW (+21 %) was observed in plant treated with B1. Moreover, we observed that, the area between the

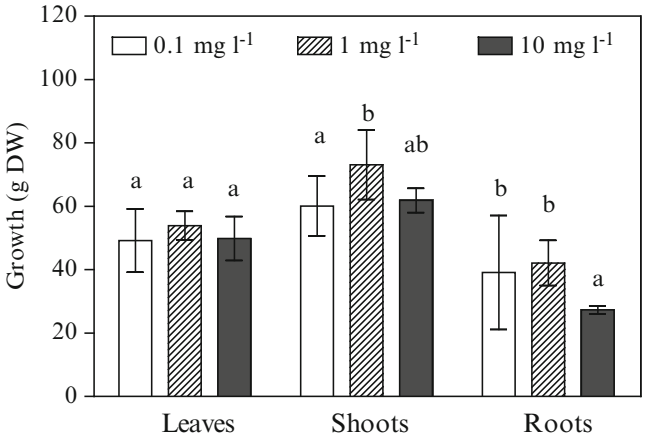

Fig. 1 Growth of leaves, shoots and roots of *Eucaliptus globulus* treated with 0.1 (control), 1 and 10 mg l^{-1} B. Values are means ± standard deviation. For each organ, different letters indicate values which statistically differ for $P \leq 0.05$

necrotic lesions remained green and apparently healthy: after 12 weeks of treatment total leaf chlorophyll ($a + b$) content was not changed by B supply (*data not shown*).

Boron concentration of all plant parts significantly increased as B concentrations in nutrient solution became higher, showing difference compared to control (Fig. 2). Higher B concentrations were found in leaves, while other organs had much lower contents, following the order leaves > stems > roots (Fig. 2). In particular, in the leaves, B concentration ranged in average between 29.4 (control) and 1624.4 mg kg^{-1} (B10).

After 12 weeks of treatment, gas exchange parameters are reported in Table 1. A_{max} of controls was higher than those of plants grown under B excess (−30 and −71 % in B1 and B10 plants, respectively). B stress induced a significantly decrease in G_w (−44 and −30 % in B1 and B10 plants, respectively) compared to control, as well as C_i that was reduced when B concentration became higher (−20 and −24 % in B1 and B10 plants, respectively).

The chlorophyll fluorescence parameters, F_v/F_m (which indicate the efficiency of excitation capture of PSII in the dark-adapted leaf) and F_v/F_0, significantly changed at the end of the experiment in leaves treated with the highest excess of B. This reduction corresponded to 4 % (F_v/F_m) and 19 % (F_v/F_0) (Table 2) and was essentially due to a concomitant decrease of F_0 and F_m. A significant increase of qN was observed in the B10 plants (+10 % compared to controls), while Φ_{PSII} decreased (−27 % when compared to controls). The reduction state of the primary stable quinone acceptor of PSII (Q_A) can be estimated as 1−qP: in leaves exposed to B10, the values increased (+11 % compared to control) (Table 2). A significant B-induced effect on ETR, whose decrease may be due to photoinhibition, was observed in B10 plants. The linear correlation between ETR and Ci showed positive slopes ($y = 5.92x - 930$, $R2 = 0.78$, $P = 0.019$). Moreover, the positive association between

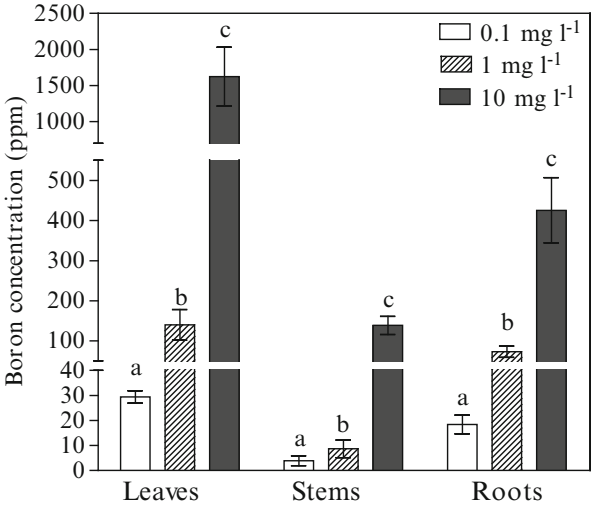

Fig. 2 Boron concentration in leaves, shoots and roots of *Eucaliptus globulus* treated with 0.1 (control), 1 and 10 mg l^{-1} B. Values are means ± standard deviation. For each organ, different letters indicate values which statistically differ for $P \leq 0.05$

Table 1 Effects of boron concentration in the nutrient solution on photosynthetic activity at saturation light level (A_{max}), stomatal conductance to water vapour (G_w) and apparent internal CO_2 concentration (C_i) of *Eucaliptus globulus*

B (mg l^{-1})	A_{max} (μmol CO_2 m^{-2} s^{-1})	G_w (mmol H_2O m^{-2} s^{-1})	C_i (ppm)
0.1	11.6 ± 0.72 a	256 ± 6.6 a	213 ± 18.8 a
1	8.2 ± 1.71 b	143 ± 11.7 b	171 ± 5.7 b
10	3.4 ± 0.30 c	179 ± 47.8 b	161 ± 14.1 b

Data (means ± standard deviation) were captured after 42 weeks. For each parameter, different letters indicate values which statistically differ for $P \leq 0.05$

ETR and A_{max} suggested that B induced effects were well established ($y = 9.81x + 28.67$, $R^2 = 0.96$; $P = 0.0004$).

Generally, at B1 concentration the parameters of chlorophyll *a* fluorescence did not change, with exception of $1 - qP$, Φ_{PSII} and ETR values (−35, +24 and +51 %, respectively, in comparison to controls).

4 Discussion

Critical values for B toxicity have been established in many crops and trees showing a lot of difference between species. This is extremely true for accumulation of B in leaves: these tissues normally accumulate about from 40 to 100 mg B kg^{-1}

Table 2 Effects of boron concentration in the nutrient solution on chlorophyll *a* fluorescence parameters (arbitrary units; mean ± standard deviation) of *Eucaliptus globulus* leaves

B (mg l^{-1})	F_0	F_m	F_v/F_m	F_v/F_0	1-qP	qN	Φ_{PSII}	ETR
0.1	0.131 ± 0.015a	0.799 ± 0.0057a	0.835 ± 0.0020b	5.086 ± 0.114b	0.369 ± 0.0047b	0.653 ± 0.0042a	0.404 ± 0.0073b	85 ± 0.011b
1	0.123 ± 0.021a	0.791 ± 0.0015a	0.842 ± 0.0015b	5.451 ± 0.099b	0.238 ± 0.0020c	0.662 ± 0.0025a	0.503 ± 0.0060c	128 ± 0.004c
10	0.171 ± 0.030b	0.872 ± 0.0318b	0.804 ± 0.0095a	4.097 ± 0.127a	0.408 ± 0.0147a	0.715 ± 0.0133b	0.295 ± 0.0050a	58 ± 0.024a

For each treatment, different letters indicate values which statistically differ for $P \leq 0.05$. Abbreviations: F_0 minimal fluorescence, F_m maximal fluorescence, F_v/F_m variable and maximal fluorescence ratio, *1-qP* reduction state of Q_A, *qN* total nonphotochemical quenching, Φ_{PSII} actual quantum yield of PSII, *ETR* apparent electron transport rate through PSII

DW. However, the leaves can contain 250 mg kg^{-1} DW, when B in the soil approaches toxic levels, increasing up to 700–1000 mg kg^{-1} DW in extreme condition of B toxicity (Nable et al. 1997). In our study, injury became evident after 42 days of treatment in B10 plants and continued to the end of exposure (12 weeks), when leaf B concentration exceeded 1624 ± 407 mg kg^{-1} DW. This value is in accordance with those (1033 ± 828 mg kg^{-1} DW) found in *Eucalyptus* leaf tissue sampled in San Joaquin Valley of California when plants showed B incipient injury (Poss et al. 1999).

It was observed that B concentrations of all plant parts increased, by increasing B concentration in the nutrient solution. This observation is in accordance with those reported by other Authors studying *Eucaliptus* species (Poss et al. 1999). Much higher B concentrations were found in the leaves than in the other vegetative parts: in B10 treatment, the leaves contained up to four times B than roots. These data are in agreement with those reported for other species, where B was accumulated in leaves and low concentrations was found in woody stems and roots (Papadakis et al. 2004a). As already reported by Eaton (1944), these results suggest that B was transported to the leaves via the transpiration stream and the remobilization of B in phloem from leaves to the other organs was limited. Generally, phloem immobility could be considered as an internal tolerance mechanism to B excess. Moreover, we observed that good vegetative growth may continue in young leaves suggesting that the old ones are able to maintain enough photosynthetic leaf area explaining as our data of chlorophyll content resulted unchanged. In an affected leaf, phloem immobility keeps B away from metabolic sites, retaining it in the leaf margins, where despite suffering leaf burn, plants are still able to maintain enough healthy photosynthetic leaf area. The adequate photosynthetic area as well as the termination of the experiment before leaf abscission due to B toxicity, might probably explain because the total fresh and dry weight of leaves was not significantly affected by B supply (Papadakis et al. 2004a).

Although the B concentration in roots was lower compared to those found in leaves, significant reduction of growth of this organ was observed. Few studies confirm our results, explaining that the effect of B toxicity in roots is associated with abnormal cell division at the meristem level (Cervilla et al. 2009) and might be a result of the formation of hypodermis and the progressive deposition of suberin in cortical cell walls (Ghanati et al. 2005).

Researches about photosynthetic gas exchange responses under B toxicity give very different results. Sotiropoulos et al. (2002) found that B toxicity in kiwifruit induced a significant decrease of the photosynthetic rate and a significant increase of the intercellular CO_2 concentration, whereas stomatal conductance remained unaffected. Papadakis et al. (2004a) in orange plants showed that intercellular CO_2 concentration was not significantly affected by the increased B concentration in the nutrient solution, while at the same time both photosynthetic activity and stomatal conductance significantly decreased. Leaf stomatal resistance indicates the degree

of stress in plants under adverse conditions and stomatal closure by reducing evaporation might play a restrictive role on the uptake of excessive B. Boron toxicity also may have damaged the ability of the stomata to open (Gunes et al. 1996). In the present study, the measured G_w showed the ability of *Eucalyptus* to close their stomata and to increase stomatal resistance under B excess. In Papadakis et al. (2003), higher stomatal resistance in B-tolerant *Citrus* genotype than that of B-sensitive *Citrus* genotype was reported.

Since PSII is believed to play an important role in the response of photosynthesis in higher plants under environmental stresses, the reduction of CO_2 assimilation by B excess should be reflected in the PSII behaviour. Our experiments have shown F_v/F_m decreased by increasing B concentration in the nutrient solution, as also observed by Papadakis et al. (2004a) in orange plants and by Guidi et al. (2009) in tomato leaves. Under non-stressed condition, C3-species had a theoretical value of F_v/F_m equal to 0.832 (Björkman and Demming 1987); this is true also for *E. globulus* and it is in agreement with other Authors (Rohàček 2002; Lee 2006). Its reduction means that treated plants were under stress conditions at the end of the experiments: molecular O_2 operates as an alternative acceptor for non-utilized electrons and light energy and, consequently, leads to generation of ROS.

The decrease of the F_v/F_0 is closely related to the structural damage of the thylakoid membranes that affect the photosynthetic transport of electrons (one of the probable reasons for the reduction of photosynthesis). Also the significant increase of F_0 observed suggests that this parameter is affected by environmental stresses that cause structural alterations in the pigment protein complexes of PSII or when the transfer from antennae to reaction centres is impeded (Bohlar-Nordenkampf et al. 1989). In addition, the increase in $1-qP$ and the decrease in F_v/F_m are known to be closely associated with photoinhibition (Ogren and Rosenqvist 1992). The higher level of $1-qP$ found in B10 concentration indicates that there was a greater excitation pressure on PSII centres and also suggests that a large proportion of PSII reaction centres are closed in severe B stressed leaves. The apparent electron transport rate through PSII was, in fact, reduced as effect of the photoinhibition.

Since Φ_{PSII} was significantly reduced, thus PSII reaction centres are unable to efficiently utilize the excitation energy which is dissipated as heat (Demmig-Adams et al. 1996). Highest B level induces an increase of photoprotective mechanism, qN showing higher values compared with controls. This indicates that thermal energy dissipation was activated trying to play a significant role in protecting plants from B excess.

These results clearly indicate that the B excess (10 mg l^{-1}) in *E. globulus*, even if in the presence of decrease of stomatal contuctance (and, thus, with reduced evaporation), leads to: (i) visible injury in old leaves; (ii) growth reduced in roots; (iii) increase in B concentration in all parts of plants following the order leaves > stems > roots; (iv) fall of photosynthetic activity, because of structural damage of the thylakoid membranes. Under these circumstances, *E. globulus* should be regarded as sensitive to B toxicity.

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Vanadium in the Environment and Its Bioremediation

Tatsuya Ueki

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Abstract Vanadium is an element with symbol V and atomic number 23. The vast majority of vanadium demand is from the steel industry, and the rest for titanium alloy and catalyst in chemical factory. Air pollution and water pollution by vanadium were recognized from early twentieth century. Increasing information on the toxicity and medicinal use enhanced the development of bioremediation of vanadium. In this chapter, the author would like to overview the history of pollution of vanadium, vanadium toxicity, bioaccumulation and bioremediation of vanadium.

Keywords Heavy metal • Bioremediation • Vanadium • Ascidians

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1 Introduction

Vanadium is an element with symbol V and atomic number 23. It is the 19th most abundant element in the earth's crust (0.015–0.016 %, 150–160 ppm) (Emsley 1998; WHO 2000). Metallic vanadium is not found in nature, but its compounds can be obtained as minerals such as vanadinite ($Pb_5(VO_4)_3Cl$) (Fig. 1), a lead vanadate ore from which vanadium was first discovered by a Mexican, Andrés Manuel del Río. In 1831, Nils Gabriel Sefström rediscovered this element and he called the element vanadium after Vanadis, an additional name of the Norse goddess Freyja, which represented beauty and fertility, because of beautifully colored chemical compounds of this element (Sefström 1831). Mine production including slag products increased year by year up to 75,000 tons in the world, about half of which is produced in China, followed by South Africa and Russia (Brown et al. 2014).

The vast majority (92 %) of vanadium demand is from the steel industry (Parles 2012). Vanadium is mainly used to produce high speed and high alloy tool steels. Vanadium is also used in the production of titanium alloys for aerospace and industrial purposes. Titanium alloys account for about 4 % of consumption in 2012 (Parles 2012). Vanadium pentoxide is used as a catalyst in sulfuric acid production and in the manufacture of ceramics. About 3 % of global vanadium consumption is in petrochemical, catalyst and pollution control applications as well as ceramic pigments, special glasses and other chemical industry applications.

In 2012, about 1 % of vanadium consumed was used in energy storage applications. Vanadium redox flow battery (Rychcik and Skyllas-Kazacos 1988) systems for grid energy storage applications and lithium battery systems incorporating vanadium for mobility applications are under development today with potential to have a significant impact on future vanadium demand (Parles 2012).

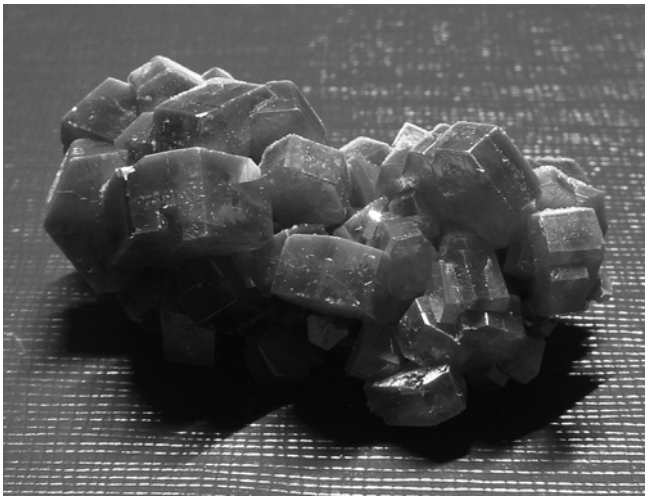

Fig. 1 Vanadinite, Mibladen Atlas Mountain, Morocco. *Dark orange color*

In this chapter, the author would like to overview the history of studies on pollution, toxicity, bioaccumulation and bioremediation of vanadium.

The readers may refer to a recent book on biological and biochemical aspects on vanadium edited by Dr. Michibata (2012). Bioinorganic and chemical topics can be found in a book by Dr. Rehder (2008).

2 Pollution of Vanadium

From early twentieth century, vanadium is regarded as a pollutant. Dutton was the first to describe vanadium poisoning, and produced a word "vanadiumism", which means a chronic intoxication caused by ingestion or absorption of some forms of vanadium, either industrially, medicinally, or accidentally (Dutton 1911). In his recognition, anemia is an early symptom, and the cough is a prominent and characteristic one. He also noted that some workers using vanadium are susceptible to tuberculosis. Anorexia, nausea and diarrhea indicated gastrointestinal involvement.

2.1 Air Pollution

Four principal oxides are known for vanadium: vanadium monooxide (VO), vanadium trioxide (V_2O_3), vanadium dioxide (VO_2) and vanadium pentoxide (V_2O_5), which ranges +2 to +5 oxidation states. Vanadium pentoxide dust is known to be one of hard metal irritants that affect the upper respiratory tract, producing tracheitis, bronchitis, pneumonia and pulmonary oedema (WHO 2014).

Experimental poisoning in animals indicated that accumulation does not occur and that acute and chronic symptoms are similar (Daniel and Lillie 1938). Studies in early 1900s on experimental administration of vanadium on animal models are well summarized in a review by Wyers (1946).

Stocks reported the relationship between atmospheric pollution in urban area and cancer, bronchitis and pneumonia (Stocks 1960). He especially noted the correlation between trace elements and lung cancer. Vanadium's action as respiratory irritant is significant.

Recent research on pollution of vanadium mainly focuses on the global movement of small particles. The United States of America and the European Union determined their own environmental baseline in 1971 and 1980, respectively, for PM10 and PM2.5. WHO first determined a guideline in Europe, and then extended it in 2005 as a global guideline (WHO 2005). In Japan, original guideline was first released in 1972, and the baseline for PM2.5 was determined in 2009.

Since vanadium is the major trace metal in fossil fuels (Filby and Branthaver 1987; Jacks 1976; Sundararaman et al. 1988), combustion of these materials provides an appreciable source of vanadium in the environment and can be a source for this heavy metal in particular materials in the air (Chen and Duce 1983; Duce and