Mitt. bad. Landesver. Naturkunde u. Naturschutz	N.F. 17	2	293-302	1999	Freiburg im Breisgau 23. September 1999

Juncus bulbosus as a pioneer species in acidic lignite mining lakes: Source of inorganic carbon assimilation and phosphorus uptake kinetics

by

ABAD CHABBI, Cottbus*

Zusammenfassung: Die Zwiebel-Binse (*Juncus bulbosus* L.) ist die Pionierpflanze in extrem sauren Tagebaurestseen des Lausitzer Braunkohlenreviers. Die pflanzenfeindlichen Bedingungen in den Restlöchern läßt auf das Vorhandensein von Anpassungsmechanismen schließen, die es der Pflanze erlauben, in diesem Milieu zu überleben und den Mangel an anorganischem Kohlenstoff sowie an Phosphor auszugleichen. Röntgenbeugungsanalysen zeigten, daß die Eisenplatten um die Wurzel hauptsächlich aus Goethit besteht, der in Anwesenheit von CO_2 gebildet wird. Die Rasterelektronenmikroskopie-Aufnahmen der Eisenplatten sind durch einen Freiraum zwischen Wurzel und Sandkörnern gekennzeichnet. Dieses ungewöhnliche Milieu wird von Mikroorganismen besiedelt. Chemische Analysen zeigten, daß die Eisenplatten Spuren mehrerer Wurzelexsudate enthalten. Dieses Ergebnis läßt auf eine Interaktion zwischen der mikrobiellen Komponente auf der Wurzeloberfläche und den Wurzelexsudaten unter den Eisenplatten (mineralfreier Raum) schließen. Dieser Mechanismus könnte für Kohlenstoff- und Phosphorkreisläufe in diesem Ökosystem von Bedeutung sein. Außerdem ist die Aufnahme dieser Elemente ein wichtiger Prozeß für die pH-Pufferungskapazität innerhalb der Eisenplatten.

Summary: Bulbous rush (*Juncus bulbosus* L.) initiates the plant colonization in acidic mining lakes in the Lusatian mining district. The extreme and hostile site conditions in mining lakes suggest the presence of adaptive mechanisms that enable bulbous rush to survive and overcome inorganic carbon stress and phosphorus deficiency in those ecosystems. Powder X-ray diffraction (XRD) showed that the iron oxide of the plaque is mainly goethite that has been developed in the presence of CO_2. Scanning electron microscopy shows that the iron plaques around the root are characterized by the presence of a mineral-free space between the root and the sand grains. This unusual microenvironment is inhabited by colonies of micro-organisms. Chemical analyses revealed that the iron plaque contains several trace concentrations of roots exudates. The results suggest that there are interactions of the microbial component embedded on the root surface and the root exudates beneath the iron plaque (i.e. micro-space). This pathway may be important for carbon and

* Author's address: Dr. ABAD CHABBI, Department of Soil Protection and Recultivation, Brandenburg Technical University of Cottbus, Faculty of Environmental Science, P.O. Box 101344, D-03013 Cottbus

phosphorus cycling in man-made ecosystems. Furthermore the assimilation of those elements by the plant is a significant process for pH buffering capacity inside the iron plaque.

Introduction

Acidic mining lakes resulting from coal mining operations in the eastern part of the Federal Republic of Germany have been regarded as environmental disasters. But for researchers, these man-made ecosystems offer a remarkable research opportunity (CHARLES 1998). In the Lusatian area alone, more than 100 lakes of various sizes contain water having a pH between 2.5 and 3.5 and concentrations of dissolved iron, manganese and aluminum at levels highly toxic or lethal to most plant species. Despite the extreme conditions, bulbous rush (*Juncus bulbosus* L.) was recognized as a pioneer species of the littoral areas of these lakes (PIETSCH 1979). The presence of an adaptive mechanism has been long speculated but remains an unresolved question. A recent study (CHABBI 1999) has shown that the plant responds to toxic compounds through a sophisticated ecophysiological adaptation. This report explores the effective interactions of root excreted compounds and microorganisms beneath an iron plaque formed on roots as key processes enabling the plant to avoid inorganic carbon limitation and phosphorus deficiency in the extreme environment of acidic mining lakes.

Material and Methods

Sites: Our investigations were carried out in Senftenberg See (lake SFB) and Koyne-Plessa (Lakes numbers 108 and 109) of the Lusatian mining district in the eastern part of the Federal Republic of Germany (54_{06} - 57_{06} & 54_{30} - 57_{06}; map references: 1/50 000, L 45-46 & L 45-48). These lakes are the final result of lignite mining that lasted for decades. The water levels fluctuate depending largely on ground water. The substrate is mainly Pleistocene-Sand with little Tertiary material (Senftenberg See) and Tertiary material rich in pyrite at Koyne-Plessa district. *Juncus bulbosus*, floating and submersed stands, is the dominant macrophyte species in the littorals of these lakes.

Plant material: On August 5, 1997, turgid and structurally intact living roots were collected at the sampling sites from both the sediment of acid lignite mine sediment rich in iron (roots with iron plaque formation) and the sediment of gravel pit poor in iron (control roots without iron plaque formation). Root collection were carefully carried out with a stainless steel shovel, keeping the root and soil intact. Afterwards the roots were placed in plastic bags, transported to the lab and stored overnight at 4° C. Root and soil were separated using de-ionised water. Root material with iron plaques was used for scanning electron microscopy and energy dispersive X-ray detector (SEM & EDX) investigations. The trace elements of root exudates in iron plaques were determined by chemical analysis.

Powder X-ray diffraction (XRD) of the rhizosphere of *Juncus bulbosus*: Roots with iron oxide plaque were quick-frozen, and freeze-dried. After 24 hours, the iron oxide around the roots (iron plaque) was separated from the roots and gently ground by hand. X-ray diffraction analyses of the powdered specimens were conducted using Co-Kα radiation as described by BIGHAM et al. (1990).

Scanning electron microscopy (SEM): For SEM, fresh roots segment with iron plaques (10 mm distance to apex) were fixed with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4° C overnight. After gently washing with buffer solution, segments were postfixed with 1% OsO_4 for 2 h, dehydrated with acetone and embedded in epoxy resin (SPURR 1969). After polymerization at 70° C for 24 h, specimens were cut into 4-mm sections, mounted on aluminum specimen mounts with epoxy resin. After gently grinding the specimens were polished and coated with carbon. The specimens were investigated with a SEM (Zeiss DSM 962) at 20 kV with a working distance of 25 mm using a backscattering electron detector and an energy dispersive X-ray detector (Oxford Instruments, Link ISIS).

Chemical analysis of trace elements of root exudates in iron plaque: For trace elements of root exudates, iron plaque was carefully separated from fresh root segment to expel iron plaque solution using a specially equipped cooling centrifuge, keeping sample temperature at 4° C, at 12 000 rev. min^{-1}, for 1 h. The amounts of exudates have been determined by ion chromatograph following the procedure described by SHEN et al. (1996) using a supported liquid membrane enrichment technique.

Results and Discussion

Juncus bulbosus was characterized as having an extensive plaque surrounding the roots (Fig. 1 b). The sediment oxygenation and subsequent increase of E_h results in oxidation of Fe^{2+} to Fe^{3+} which leads to the formation of iron oxide plaques (CHABBI 1999). Iron plaque formation is known to serve as a protective mechanism against the entry of phytotoxic levels of reduced elements into the root cells (CHABBI et al. 1998). In spite of the well-designed research reported on iron plaque formation, the specific nature of the processes involved in the protective mechanisms within species is not clear (MENDELSSOHN et al. 1995).

Fig. 1: Roots of *Juncus bulbosus* without iron plaque (white root) (A), and with iron plaque (B) clearly visible as an reddish brown precipitate; scale bar is 200 µm. The photo on the right (C) shows floating and submersed dense stand of *Juncus bulbosus* in lake SFB.

XRD indicated that the oxide deposit is rich in goethite (Fig. 2) which has an average crystal size (mean coherence length perpendicular to (110)) of only ca. 10 nm, as calculated from the corrected full width at half height of the (110) reflection using the Scherrer formula (SCHWERTMANN & FITZPATRICK 1977). This type consisting of very small goethite crystals form commonly from oxidation of Fe^{2+} in surface environments at ambient temperatures under circumneutral conditions (for a review see CORNELL & SCHWERTMANN 1996). CHEN, DIXON & TURNER (1980)

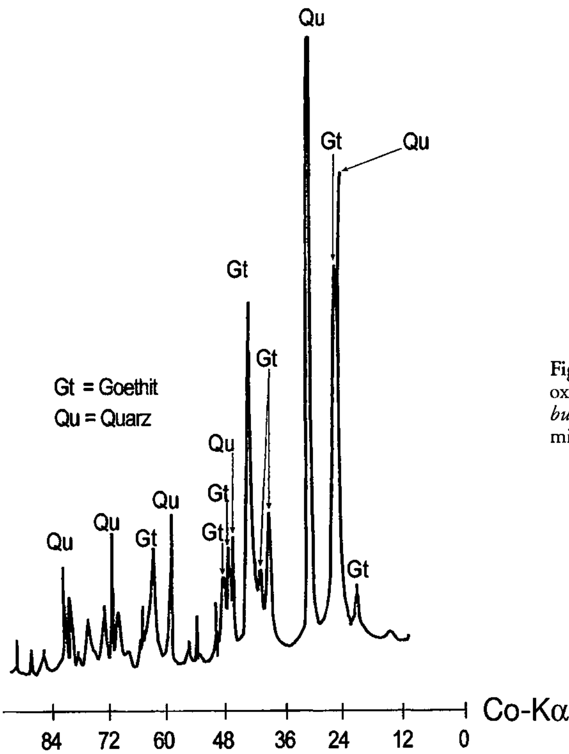

Fig. 2: X-ray diffraction pattern of oxidised roots channels from *Juncus bulbosus* growing in acid lignite mine sediment.

were the first to observe goethite in rice roots deposits. SCHWERTMANN (1959) and CARLSON & SCHWERTMANN (1990) demonstrated in laboratory experiments that CO_2 in the oxidation environment favored goethite (alpha-FeOOH) over lepidocrocite (gamma-FeOOH) formation. By thin-sectioning a root concretion (so-called pipe stem), SCHWERTMANN & FITZPATRICK (1977) showed that the inner part close to the root was rich in goethite, whereas lepidocrocite dominated in the outer part. From this I conclude that the release of CO_2 by the *Juncus* root/microorganisms has fostered goethite formation.

It is assumed that inorganic carbon is a limiting factor for primary production in extremely acidic lakes (GOLDMAN, OSWALD & JENKINS 1974). Moreover much evidence in the literature indicated that the rates of carbon uptake by roots of aquatic plants (SØNDERGAARD & SAND-JENSEN 1979) can be higher than uptake by their shoots particularly in acid lakes. The extreme acidity ($pH < 3.5$) combined with shallow acid lakes lead to a low concentration of the CO_2 (0.43 mg L-1, CHABBI unpubl. material) around the leaves of *Juncus bulbosus*. NIXDORF, WOLLMANN &

DENEKE (1998) reported that the concentration of DIC in the study lakes is very low and is below the level of detection (< 0.5 mg C L^{-1}). A subsequent study (KAPFER 1998) documented that DIC produced by microbial activities (e.g. sulfate reduction, denitrification, respiration) in the sediment is rapidly lost to the atmosphere. Published evidences however, demonstrated that inorganic carbon controls the growth dynamics of *Juncus bulbosus* and its competitive ability increases with increasing CO_2 in the system (ROELOFS, SCHURKES & SMITS 1984, WETZEL, BRAMMER & FORSBERG 1984; SVEDÄNG 1992). The question then is how *Juncus bulbosus* avoid inorganic carbon limitation in acidic mining lakes?

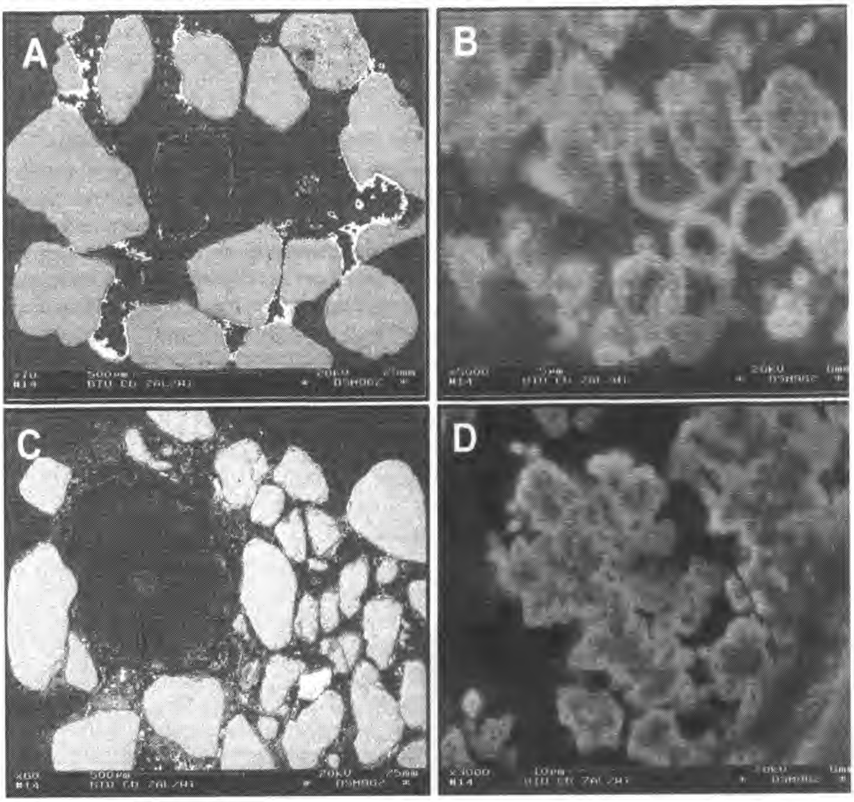

Fig. 3: Scanning electron microscopy of *Juncus bulbosus* root with iron plaque (10 mm distance to apex) taken from lake SFB (a) and lake n° 108 (c); visible micro-space between the root surface (centre) and the mineral component. Figures (b) and (d) represent the microbial component between the root surface and the mineral component with the red precipitate (in figures a and c).

Transverse sections of oxidized root channels show that the root is covered by quartz and iron oxide, mainly Goethite. Between the root and the sand grains exists a mineral-free space (Fig. 3 a, c) which is inhabited by colonies of micro-organisms (Fig. 3 b, d). The microbial component is quite interesting since observations show that the bacteria are true rhizobacteria attached to or embedded on the root surface beneath the iron plaque. This is in contrast to typical "iron associated bacteria" that one would expect to be literally coated with iron (BROCK & GUSTAFSON 1976).

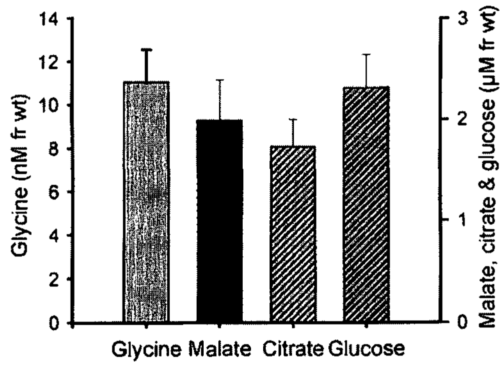

Fig. 4: Trace of root exudates extracted from iron plaque surrounding the roots of *Juncus bulbosus.*

The investigation of iron plaque (Fig. 4) revealed that this material contained several concentrations (nano- to micromolar) of malate, citrate, glucose and glycine which likely increased with plant stress (HALE et al. 1978; HALE & MOORE 1979; CURL & TRELOVE 1986) and thereby provided micro-organisms with available substrates for metabolism. These exudates may also function as complexing ligands (FRANCIS et al. 1992) for toxic metal immobility/and or in sequestering nutrients such as phosphorus for uptake by the plant (JONES et al. 1996). The feedback involving stress, quantity of exudates, and microbial establishment in this unusual rhizosphere environment may have beneficial effects that promote survival and plant growth in acidic mining lakes. The discovery of this unique microenvironment challenges current ideas about inorganic carbon assimilation and phosphorus uptake in such ecosystems. I believe that the micro-organisms likely metabolize root exudates to different extents and thereby cause an increase in carbon dioxide released in micro-space (Fig. 5). Furthermore the formation of iron plaque around root surfaces may prevent the loss of this inorganic carbon from the system. It is pos-

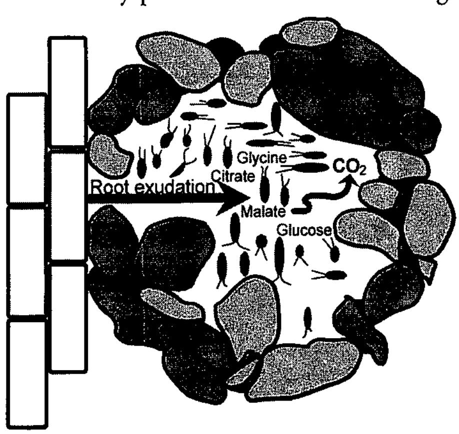

Fig. 5: Iron plaque formation, root exudates and microbial interactions in the micro-space.

sible that *Juncus bulbosus* conserves some of its carbon by exploiting the accessory CO_2 produced in the micro-space. This would accord with the finding of WETZEL, BRAMMER & FORSBERG (1984) who concluded that a substantial part (1/4 - 1/3) of the photosynthetically fixed CO_2 by *Juncus bulbosus* is derived from root uptake.

It is well known that ferrous iron oxidation and protons release from the roots generated acidification (AHMAD & NYE 1990, BEGG et al. 1994). These two sources of acidity can produce large changes in pH close to roots and may exacerbate the plant's problem in already acid sediments. Net CO2 assimilation may remove some of the acidity produced in the Fe^{2+} oxidation and in the cation-anion intake imbalance from the micro-space as follows:
$HCO_3^- + H^+ \rightarrow CO_2 + H_2O$ (BEGG et al. 1994). This reaction that may cause an increase in pH in the micro-space. It is suggested that *Juncus bulbosus* is not suffering from the acidity generated from the two processes mentioned above neither from the external pH. The dominate stands of *Juncus bulbosus* (Fig. 1) in acid mine lignite sediment suggest that the acidity and alkalinity generation in the micro-space may occur simultaneously/and or sequentially and that the plant fully controls these processes.

Several reports in the literature suggest that iron plaque on roots can influence phosphorus uptake by the affected plant due to the adsorbtion of phosphorus to iron-hydroxide (iron plaque) (Jaynes & Carpenter 1986, Wigand & Stevention 1997, Christensen & Wigand 1998). The high relationships found between solid phase inorganic phosphorus and iron plaque (Fig. 6) suggest that phosphorus was in a form less available for direct uptake. The tissue - phosphorus content of the

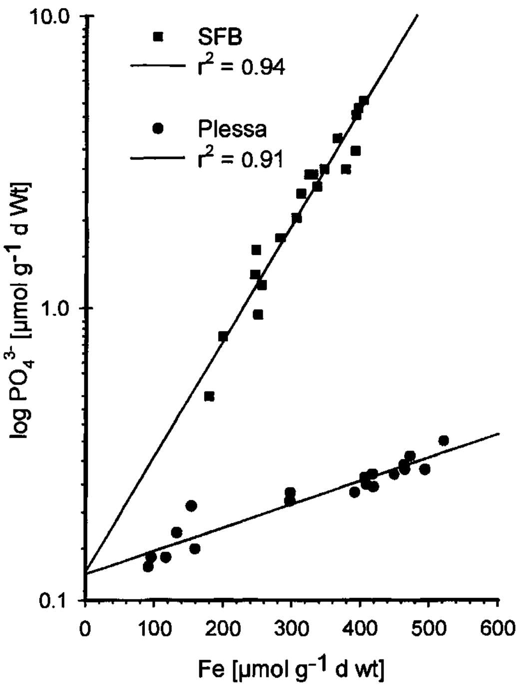

Fig. 6: Regression relationships of solid-phase inorganic phosphorus and iron plaque on the roots of *Juncus bulbosus* in two different vegetated sediments. Each value represents a single plant.

plant however, was at a level for optimal nutrition (0.02 & 0.03 mmol g^{-1} d wt; CHABBI et al. 1998) even though goethites typically exhibit a high capacity for phosphorus binding (SCHWERTMANN & TAYLOR 1977). The P status in plant tissue was further approved by using the model of VERHOEVEN et al. (1996) (Fig. 7) which

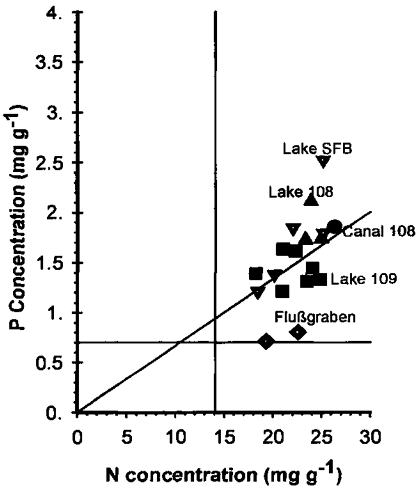

Fig. 7: Nitrogen (N) and phosphorus concentrations in the shoots of *Juncus bulbosus* (mg g^{-1} d wt). The horizontal and vertical lines indicate the critical P and N concentrations respectively. The line through the origin represents an N:P of 15 according to VERHOEVEN et al. 1996.

shows that most of our values are above the critical level for either N and P. The plant also does not support mycorrhizal associations that could mediate the process of solubilization of P bound to metals within the plaques. Bacterial metabolites, other than siderophores (i.e. phytochelators), however, are likely important in P solubilization and uptake patterns. The high P and K concentrations in the micro-space (Fig. 8) as shown by EDX support the hypothesis regarding P solubilization

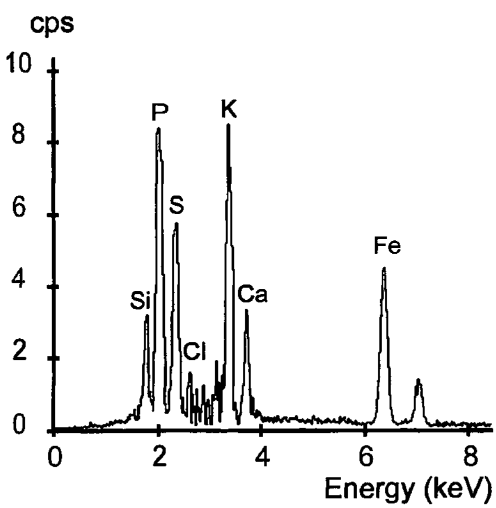

Fig. 8: Energy-dispersive X-ray analysis of the chemical characteristics in the micro-space.

and uptake kinetics inside the micro-space. It seems that iron plaque did not cause any decrease in the absorption of potassium and phosphorus as it has, on the contrary, been demonstrated in rice plants (YOSHIDA 1981, Benckiser et al. 1984) or in *Lobelia dortmanna* (CHRISTENSEN & WIGAND 1998). Likewise solubilization of phosphorus and its absorption by the plant may affect the internal charge balance of the plant (HEDLEY et al. 1982) causing excretion of HCO_3^- or organic ion anions (MCLAUGHLIN & JAMES 1991) which could raise solution pH. To my knowledge, evidence on the occurrence of P-solubilizing bacteria and their interaction with plaque in this unique extreme habitat has not been previously reported. It seems that the *Juncus* plant through the bacterial metabolites is able to increase the bioavailability of phosphorus with further implication on pH buffering capacity.

The specific micro-space beneath the iron plaque may foster a favorable zone for rhizobacteria establishment on root surfaces, nutrient uptake (e.g. phosphorus), and inorganic carbon source for plant metabolism. This processes that may could be not only important in carbon and phosphorus cycling in man made-ecosystems but also in pH buffering capacity inside the iron plaque. It is suggested that *Juncus bulbosus* growth in acid mine sediments is not a situation of directly living in and tolerating acidic milieu, rather the plant is capable of forming a favorable growth environment by achieving an alkaline micro-medium within an acidic milieu. The specific nature of these interactions may help to explain why *Juncus bulbosus* is a primary plant colonizer in acid lignite mining lakes in spite of extreme and unfavorable growth conditions.

Acknowledgements: I thank Prof. U. Schwertmann, TU Munich, for providing powder X-ray diffraction, Dr. W. Wiehe, Central Analytical Laboratory, TU Cottbus, for providing the SEM and TEM, Mrs N. Hendgen, R. Müller, and G. Franke, TU Cottbus, for technical assistance. I acknowledge the helpful and valuable comments of Prof. U. Schwertmann from TU Munich, Prof. Robert J. Kremer from the University of Missouri and two anonymous referees for their thorough reviews which improved the manuscript. This work was supported by the German Ministry of Research and Education (BMBF, FKZ 0339648), and LMBV GmbH.

References

AHMAD, A.R. & NYE, P.H. (1990): Couple diffusion and oxidation of ferrous iron in soils. I. Kinetics of oxidation of ferrous iron in soil suspension. – J. soil Sc., 41, 395-409.

BEGG, C.B.M., KIRK, G.J.D., MACKENZIE, A.F. & NEUE, H.U. (1994): Root-induced iron oxidation and pH changes in the lowland rice rhizosphere. – New Phytol., 128, 469-477.

BENCKISER, G., SANTIAGO, S., NEUE, H.U., WATANABE, I. & OTTOW, J.C.G. (1984): Effect of fertilisation on exudation, dehydrogenase activity, iron-reducing populations and Fe^{2+}. formation in the rhizosphere of rice (*Oryza sativa* L.) in relation to iron toxicity. – Plant & soil, 79, 305-316.

BIGHAM, J.M., SCHWERTMANN, U., CARLSON, L. & MURAD, E. (1990): A poorly crystallised oxyhydroxysulfate of iron formed by bacterial oxidation of Fe(II) in acid waters. – Geoch. & Cosmoch. Ac., 54, 2743-2758.

BROCK, T.D. & GUSTAFSON, J. (1976): Ferric iron reduction by sulphur-and iron-oxidising bacteria. – Appl. Environ. Microbiol., 32, 567-571.

CARLSON, L. & SCHWERTMANN, U. (1990): The effect of CO_2 and oxidation rate on the formation of goethite versus lepidocrocite from an Fe(II) system at pH 6 and 7. – Clay Mineral, 25, 65-71.

CHABBI, A., PIETSCH, W., WIEHE, W. & HÜTTL, R.F. (1998): *Juncus bulbosus* L.: Strategies of survival under extreme phytotoxic conditions in acid mine lakes in the Lusatian mining district, Germany. – Intern. J. Ecol. & Envir. Sc., 24, 271-292.

CHABBI, A. (1999): *Juncus bulbosus* as a pioneer species in acidic lignite mining lakes: Interactions mechanism and survival strategies. – New Phytol., (144/1, 133-142).

CHARLES, D. (1998): Wasteworld. – New Scientist, 157, 32-35.

CHEN, C.C., DIXON, J.B. & TURNER, F.T. (1980). Iron coatings on rice roots: mineralogy and quantity influencing factors. – Soil Sci. Soc. Am. J., 44, 635-639.

CHRISTENSEN, K.K. & WIGAND, C. (1998): Formation of root plaques and their influence on tissue phosphorus content in *Lobelia dortmanna*. – Aquat. Bot., 61, 111-122.

CORNELL, R.M. & SCHWERTMANN, U. (1996). The iron oxides. Structure, Properties, Reactions, occurrence and uses. – VCH publishers, New York, NY (USA).

CURL, E.A. & TRELOVE, B. (1986): The rhizosphere. – Adv. Ser. Agric. Sc., 15, 9-54.

FRANCIS, A.J., DODGE, C.J. & GILLOW, J.B. (1992): Biodegradation of metal citrate complexes and implications for toxic metal mobility. – Nature, 356, 140-142.

GOLDMAN, J.C., OSWALD, W.J. & JENKINS, D. (1974): The ktinetics of inorganic carbon-limited algal growth. – J. Water Pollut. Cont. Fed., 46(3), 554-574.

HALE, M.G., MOORE, L.D. & GRIFFIN, G.J. (1978): Roots exudates and exudation. – In: Interaction between non-pathogenic soil micro-organisms and plants (Eds.: Y.R. DOMMERGUES & S.V. KRUPA), p. 163-204. Elsevier Publishing Co., Amsterdam.

HALE, M.G. & MOORE, L.D. (1979): Factors affecting root exudation II,1970-1978. – Adv. Agron., 31, 93-124.

HEDLEY, M.J., NYE, P.H. & WHITE, R.E. (1982): Plant-induced pH changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. – New Phytol., 91, 31-34.

JAYNES, M.L. & CARPENTER, S.R. (1986): Effects of vascular and nonvascular macrophytes on sediment redox solute dynamics. – Ecol., 67, 875-882.

JONES, D.L., PRABOWO, A.M. &. KOCHIAN, L.V. (1996): Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: The effect of microorganisms on root exudation of malate under Al stress. – Plant & Soil, 182, 239-247.

KAPFER, M. (1998): Assessment of colonization and primary production of microphytobenthos in the littoral of acidic mining lakes in Lusatia, Germany. – Wat. Air & Soil Pollut., 108, 331-340.

MCLAUGHLIN, M.J. & JAMES, T.R. (1991): Effect of phosphorus supply to the surface roots of wheat on root extension and rhizosphere chemistry in an acidic subsoil. – Plant & Soil, 134, 73-82.

MENDELSSOHN, I.A., KLEISS B.A. & WAKELEY J.S. (1995): Factors controlling the formation of oxidized root channels: a review. – Wetlands, 15(1), 37-46.

NIXDORF, B., WOLLMANN, K. & DENKE, R. (1998): Ecological potentials for planctonic development and food web interactions in extremely acidic mining lakes in Lusatia. – In: Acidic Mining Lakes (Eds.: W. GELLER, H. KLAPPER & W. SALOMONS), Springer, p. 147-167.

PIETSCH, W. (1979): Zur hydrochemischen Situation der Tagebauseen des Lausitzer Braunkohlen-Reviers. – Arch. Naturschutz Landschaftsforsch. Berl., 19, 97-115.

ROELOFS, J.G.M. SCHUURKES, J.A.A.R. SMITS, A.J.M. (1984): Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. – Aquat. Bot., 18, 389-411.

SCHWERTMANN, U. (1959): Über die Synthese definierter Eisenoxyde unter verschiedenen Bedingungen. – Z. Anorg. Allg. Chemie., 298, 337-348.

SCHWERTMANN, U. & FITZPATRICK, R.W. (1977): Occurrence of lepidocrocite and its association with goethite in Natal soils. –Soil Sci. Soc. Am. J., 41, 1013-1018.

SCHWERTMANN, U. & TAYLOR, R.M. (1977): Iron oxides. – In: Minerals in soil environments (Eds.: B. DIXSON & S.B. WEEB), p. 145-179. – Soil Sci. Soc. Am. J., Madison, WI, USA

SHEN, Y., OBUSENG, V., GRÖNBERG, L. &. JÖNSSON, J.Å. (1996): Liquid membrane enrichment for the ion chromatographic determination of carboxylic acids in soil samples. – J. Chromatogr. A, 725, 189-197.

SØNDERGAARD, K. & SAND-JENSSEN, K. (1979): Carbon uptake by the leaves and roots of *Littorella uniflora* (L.) Ascher – Aquat. Bot., 6, 1-12.

SPURR, A.R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. – J. Ultrastr. Res., 26,31-43.

SVEDÄNG, M.U. (1992): Carbon dioxide as a factor regulating the growth dynamics of *Juncus bulbosus*. – Aquat. Bot., 42, 231-240.

VERHOEVEN, J.T.A., KOERSELMAN, W. & MEULEMAN, A.F.M. (1996): Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. - TREE, 11, 494-497.

WETZEL, R.G., BRAMMER, E.S., FORSBERG, C. (1984): Photosynthesis of submerged macrophytes in acidified lakes. I. Carbon fluxes and recycling of CO_2 by *Juncus bulbosus* L. – Aquat. Bot., 19, 329-342.

WIGAND, C., STEVENSON, J.C., CORWELL, J.C. (1997): Effects of different submersed macrophytes on sediment biogeochemistry. – Aquat. Bot., 56, 233-244.

WIGAND, C. & STEVENSON, J.C. (1997): Facilitation of phosphate assimilation by aquatic mycorrhizae of Vallisneria americana Michx. – Hydrobiol., 342/343, 35-41.

YOSHIDAS, S. (1981): Fundamentals of rice crop science. – International Rice Research Institute, Manila, Philippines.

(Received 19 April 1999)