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Comparison between Growth Responses of Autotrophic and Heterotrophic Populations of Lichen Photobiont *Trebouxia irregularis (Chlorophyta)* on Cu, Hg and Cd Chlorides Treatment

By

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With 7 Figures

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Summary

BAČKOR M., HUDÁK J. & BAČKOROVÁ M. 1998. Comparison between growth responses of autotrophic and heterotrophic populations of lichen photobiont *Trebouxia irregularis (Chlorophyta)* on Cu, Hg and Cd chlorides treatment. – Phyton (Horn, Austria) 38 (2): 239–250, 7 figures. – English with German summary.

Comparison between growth responses of autotrophic and heterotrophic populations of lichen photobiont *Trebouxia irregularis* (isolated from heavy metal tolerant population of lichen *Cladina mitis*) on Cu, Hg and Cd chlorides treatment were studied. Heavy metal content in the *C. mitis* thalli, relative toxicity of single metal chlorides, effect of the concentration, differences between toxicity to autotrophic and heterotrophic strains and effective concentrations are discussed.

Zusammenfassung

BAČKOR M., HUDÁK J. & BAČKOROVÁ M. 1998. Wirkungen der Schwermetallchloride (Cu, Hg und Cd) auf das Wachstum autotropher und heterotropher Popu-

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lationen des Photobionten *Trebouxia irregularis (Chlorophyta)* – ein Vergleich. – Phyton (Horn, Austria) 38 (2): 239–250, mit 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurde die Wirkung der Schwermetalle – Cu, Hg und Cd (als Chloride) auf den Wuchs auto- und heterotropher Populationen des Photobionten *Trebouxia irregularis* (isoliert von einer schwermetall-toleranten Population der Flechte *Cladina mitis*) untersucht. Der Schwermetall-Gehalt in Thalli von *C. mitis*, die relative Toxizität der einzelnen Metallchloride, der Einfluß der Konzentration und die unterschiedliche Empfindlichkeit der auto- und heterotrophen Stämme wird diskutiert.

Introduction

The lichens which are, in general, sensitive to different pollutants, play an important role in numerous studies focused on air pollution (for review see: AHMADJIAN 1993, GALUN & RONEN 1988, HAWKSWORTH 1990, NASH 1988, NASH & GRIES 1991, RICHARDSON 1988, 1991, SEAWARD 1989, TÜRK 1988).

The absorption, accumulation and toxicity of heavy metals in lichens are also well documented (AHMADJIAN 1993, BROWN 1991, GARTY 1993, NASH 1989, PURVIS & HALLS 1996, RICHARDSON 1995, SEAWARD & RICHARDSON 1989, TYLER 1989, TYLER & al. 1989). The main source of the heavy metals accumulation in lichens is air, however, in lichens growing on metalliferous rocks and derived soils from mine and smelter wastes (PURVIS & HALLS 1996) the substrate may also play an important role in their absorption.

Heavy metals affect both the mycobionts and photobionts associated in lichen thallus. Similarly, in non-lichenized algae (GENTER 1996) the changes in photosynthesis, respiration, chlorophyll content and in cyanolichens (e.g *Peltigera* sp.) nitrogen fixation are observed as a result of the air pollution and heavy metal influence (BOONPRAGOB & NASH 1991, DERUELLE & PETIT 1983, FIELDS 1988, GARTY & al. 1988, 1992, RICHARDSON & NIEBOER 1983, SHOWMAN 1972, VON ARB & al. 1990). However, only few investigations are given on poisoning of pollutants including heavy metals on isolated lichen bionts, although photobionts appear to be the key element in lichen sensitivity to air pollution (AHMADJIAN 1993). Effect of ozone on photobiont *Trebouxia* (from *Cladonia stellaris*) was observed by ROSENTRETTER & AHMADJIAN 1977. EWALD & SCHLEE 1983 showed the effect of sulfite on the proline content decrease in Trebouxia (influence on proline dehydrogenase as the last enzyme of proline synthesis). SCHLEE & al. 1984 studied the effect of sulphite on the activity of aminopeptidases in the lichen alga *Trebouxia* sp. Similarly, photobionts isolated from *Usnea* sp., *Hypogymnia physodes* and *Lecanora conizaeoides* (the latter lichen is known as very tolerant to air pollution) were sensitive to low concentrations of sulphite (HILL 1974). MARTI 1983, 1985 exposed cultured photobionts to aqueous solutions of sulfite, nitrite, sulfate and nitrate and

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shown that they were distinctly sensitive to sulfite. Nitrite and zinc were far less toxic, and it is interesting, sulfate and nitrate in a few cases increased the growth rates. NASH 1975 found that zinc and cadmium (at concentration 5×10^{-5} M) inhibited growth of two cultured photobionts (*Trebouxia gelatinosa, T. erici*).

Most species of Trebouxia can grow heterotrophically in the dark on organic media. Evidence for existence of heterotrophy in situ (in lichen thallus) comes from studies of Antarctic lichens where they are exposed to light for less than 2 months per year during the summer, and during the winter they grow up to 3 m under the snow (AHMADJIAN 1993).

Material and Methods

1. Isolation

Photobiont *Trebouxia irregularis* was isolated from lichen *Cladina mitis* (heavy metal tolerant population from copper ore depositions at Špania dolina, Central Slovakia) by both, centrifugation and micropipette methods (ASCASO 1980, AHMADJIAN 1993).

2. Influence of heavy metals

For cultivation, 1N Trebouxia medium (AHMADJIAN 1993) containing glucose (20g/l) and caseine (10g/l) was used. The final pH of the media was adjusted to 7. Medium (50 ml) was pipetted into Erlenmayer flasks and autoclaved. The chlorides of heavy metals (Cu, Cd, Hg) were added after the sterilisation of the media in final concentrations 30 μM and 300 μM (for Cu, Cd, Hg) and 3mM (for Cu).

Algae from culture were homogenised by gentle stirring on magnetic stirrer for 1 hour. Approximately 6×10^5 (final concentration $0{,}12 \times 10^5$ in 1 ml of cultivation medium) cells of photobiont was transferred to each flask as an inoculum. The presence and homogeneity of algae were examined microscopically. Growth of cultures was calculated using standard hemocytometer chamber every 5 days (up to 30 days) as number of cells per 1 ml of medium. Growth of heterotrophic cultures was calculated under green light.

3. Incubation

The autotrophic cultures were maintained at 25 °C under a 16-h photoperiod and 24 μM . m^{-2}. s^{-1} artificial irradiance. Heterotrophic cultures were kept in the dark at 25 °C.

Results and Discussion

1. Heavy metal content in lichen *Cladina mitis*

The mean content of metals in thallus of *Cladina mitis* was the following: 28,18 μg . g^{-1} for Cu, 0,88 μg . g^{-1} for Hg and 1,85 μg . g^{-1} for Cd (unpublished data). These concentrations are lower or higher in comparison with other studies (BARGAGLI & BARGHIGIANI 1991, GALUN & al. 1984, GARTY & AMMANN 1987, GARTY & al. 1986, NASH 1975, PAKARINEN 1985, RAO & al. 1977).

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2. Comparison between autotrophic and heterotrophic growth of control

Fig. 1 shows the differences between autotrophic (in the light) and heterotrophic (in the dark) growth of strains of *Trebouxia irregularis* on organic trebouxia medium. Although most species of *Trebouxia* can grow heterotrophically in the dark on organic media (AHMADJIAN 1993), our results suggest that great differences in quantity of the cells between autotrophic and heterotrophic populations (after 30 days of cultivation approximately 50 times more cells in the light cultivation) exist.

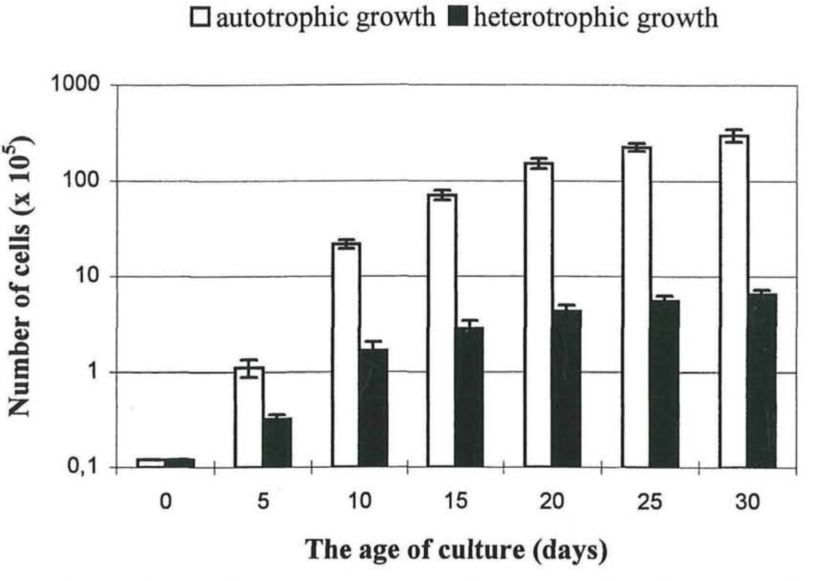

Fig. 1. The growth of Trebouxia irregularis calculated as number of cells for 1 ml of medium (x 10^5).

3. Influence of single metals on autotrophic growth

The figures 2–4 represent autotrophic growth of photobiont cultures after the Hg, Cd and Cu chloride treatment in different final concentrations (0,03 mM, 0,3 mM and for Cu 3 mM). After 30 day cultivation, the relative toxicity of metals within the given concentrations were as follows: for 0,03 mM: $Cu^{2+} << \text{control} \leq Cd^{2+} \leq Hg^{2+}$, for 0,3 mM: $\text{control} \leq Cu^{2+} < Cd^{2+} << Hg^{2+}$ and for 3 mM: $\text{control} << Cu^{2+}$. The concentrations used in our study are much more higher (for Hg, Cd) than the metal content in *C. mitis* thallus. For Cu due to its higher concentration in the thallus as well as chemical nature of substrate (dumps of metal ore depositions) three concentrations were used. The results suggest that only Cu at 0,03 mM concentration stimulates the growth of cultures.

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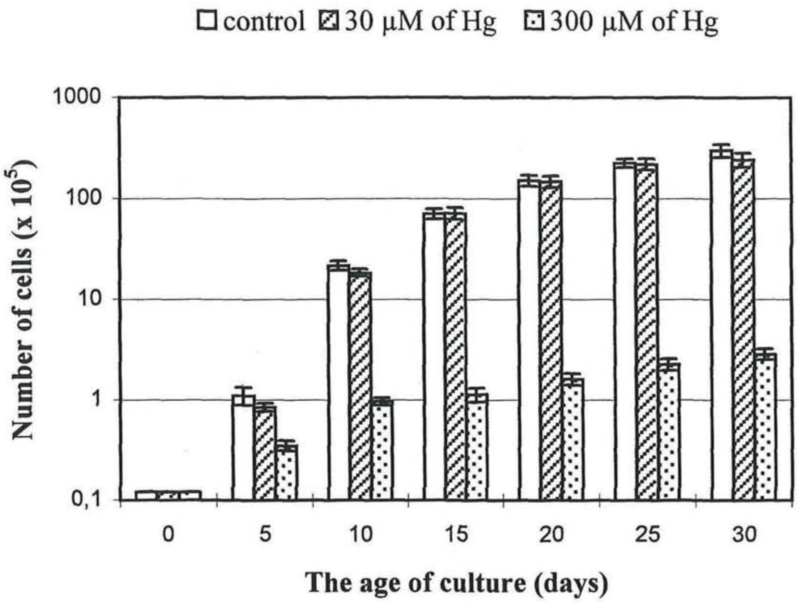

Fig. 2. The autotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

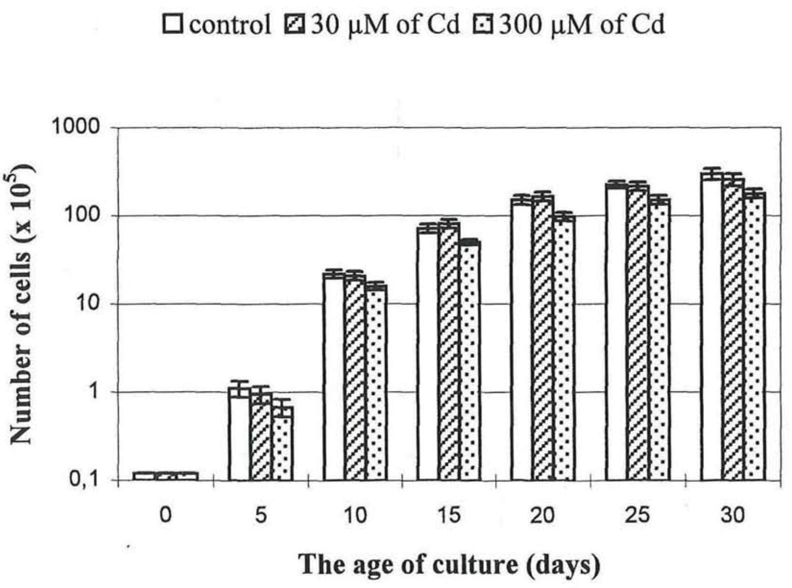

Fig. 3. The autotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

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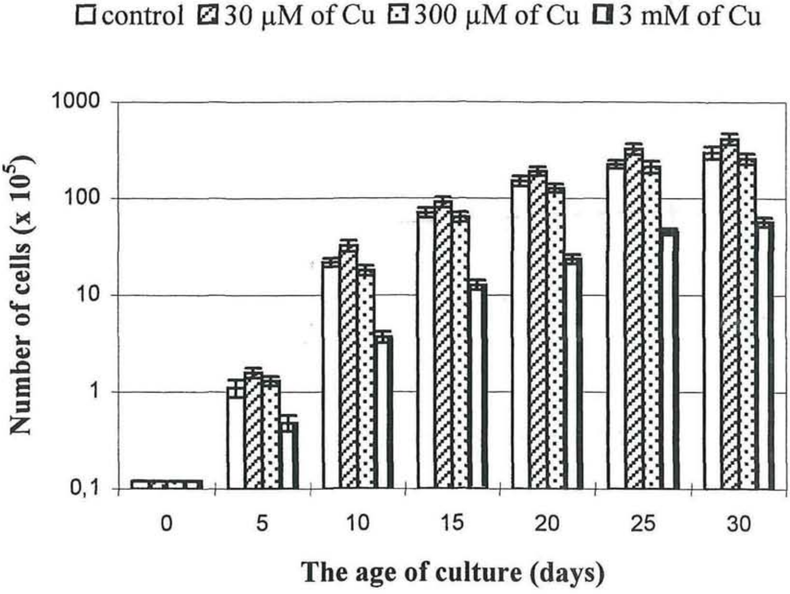

Fig. 4. The autotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

In our study we have used only chlorides of metals, and therefore a toxicity of different salts was not tested. LEE & al. 1993 observed no difference in toxicity between $CuCl_2$ and $CuSO_4$ in the growth studies of *Anacystis nidulans*. All metals, even those that are nutrients at low concentrations, are toxic to algae at high concentrations (RAI & al. 1981) and inhibition effect depends on concentrations of metals, where higher concentrations decrease growth of the cultures.

The effective concentrations (EC_{50}, what means that growth is inhibited for 50%) for 5 day or 30 day autotrophic cultivation are calculated as follows: for Cd 5 days EC_{50} = 0,9 mM and for 30 days EC_{50} = 0,88 mM, for Cu 5 days EC_{50} = 2,66 mM and for 30 days 1,6 mM, for Hg 5 days EC_{50} = 0,23 mM and for 30 days 0,11 mM growth inhibition. The results indicate that during 5 days the EC_{50} indexes are higher and result from biosorption, probably accumulation in cell wall components, which does not cause high toxicity.

4. Influence of single metals on heterotrophic growth

The figures 5–7 represent heterotrophic growth photobiont cultures after treatment of chlorides in different final concentrations, the same as for autotrophic growth. After 30 days cultivation the required sequences of metals toxicity were as follows: (from the best growth to less) for 0,03 mM

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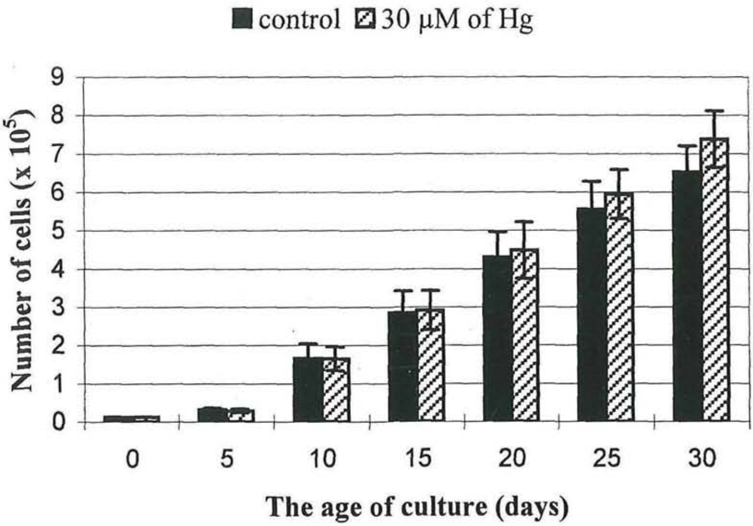

Fig. 5. The heterotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

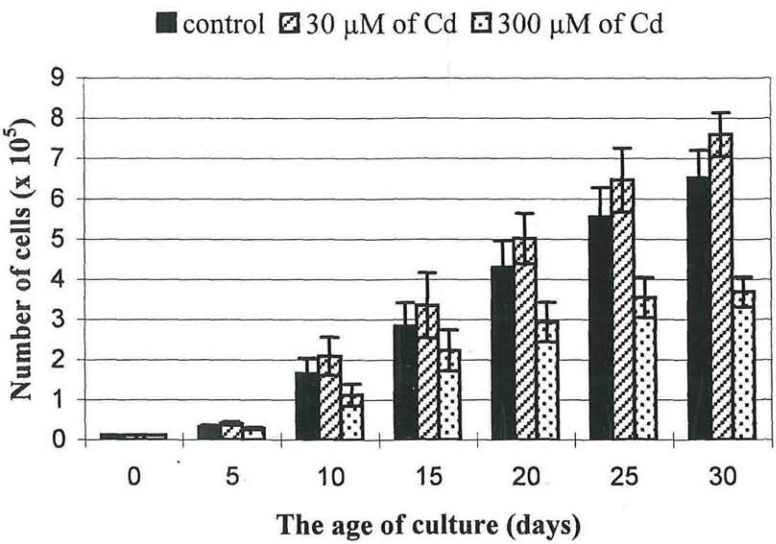

Fig. 6. The heterotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

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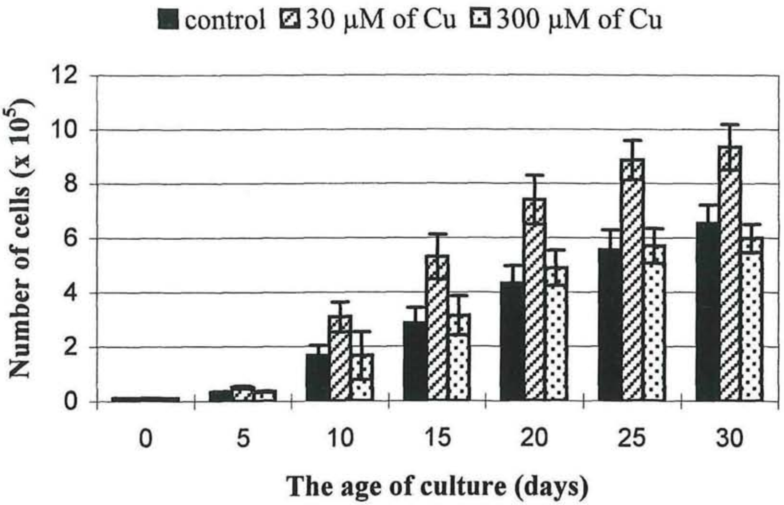

Fig. 7. The heterotrophic growth of Trebouxia irregularis cultures calculated as number of cells for 1 ml of medium (x 10^5).

are: $Cu^{2+} < Cd^{2+} \leq Hg^{2+} \leq$ control, for 0,3 mM are: control $\leq Cu^{2+} << Cd^{2+} <<< Hg^{2+}$. The results indicate, that probably exist different mechanisms of detoxications of metals in the light and dark. Similarly, the influence of single metals is different and depends on their final concentrations.

There are differences in toxicity of single chlorides of metals in medium between autotrophe and heterotrophe growth. There are two phases of heavy metals uptake (Rai & al. 1981). The metabolism independent phase (biosorption) involves the accumulation of inorganic chemicals to cell wall components. The metabolism dependent phase is often slower (lasting hours or days) and is inhibited by low temperatures, absence of energy sources (light) or metabolic inhibitors (Gadd 1988). Absence of energy sources (light in our case) is resulted in lower toxicity of metals in concentration 0,03 mM at heterotrophe growth. At 0,3 mM concentrations of metals the situation is different and metals are toxic in order: control $\leq Cu^{2+} << Cd^{2+} <<< Hg^{2+}$.

Following conclusions and explanations can be drawn from the above presented results. First, inorganic stressors almost always occurre as mixtures in nature (Genter 1996). Rai & al. 1981 found that the same algae may behave synergistically to one metal combination and antagonistically to another metal combination and, therefore different metal concentrations must be tested. Second, another important factor is metal concentration. Our results indicate relatively high tolerance to three metals at pH 7 (optimum for this strain) but pH which is an important factor of the

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heavy metal toxicity (PAWLIK & al. 1993, PETERSON & al. 1984) should also be tested. Third, toxicity of heavy metals depends on temperature (FLORENCE & al. 1994). Temperature used in our study (25 °C) is relatively high for *Trebouxia*. Algae at lower temperatures can tolerate higher concentrations of heavy metals. Fourth, relative toxicity of chemical mixtures can differ between clones of the same species (BRAEK & al. 1980). EDTA, and other organic substances, can bind to metals and reduce their toxicity (GENTER 1996).

Dumps of copper ore depositions in Špania dolina are relatively old and possibilities for genetic tolerance are an interesting question. TWISS & al. 1993 showed possibilities of the selection of a Cu-resistant strain from a Cu-sensitive strain of *Scenedesmus* by repeated culturing under sublethal concentrations of Cu at laboratory conditions. Probably, there are more mechanisms to tolerate heavy metals, but similar selection can occur in nature, in case of photobionts from heavy metal tolerant populations of lichens growing hundreds of years on heavy metal enriched substrates.

Acknowledgement

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Recensio

Flora of North America North of Mexico. Volume 3. *Magnoliophyta: Magnoliidae* and *Hamamelidae* 1997. Edited by Flora of North America Editorial Committee. – Lex. 8°, XXIV + 590 Seiten, 100 Abbildungen, 1470 Verbreitungskarten. – Oxford University Press, New York, Oxford. – £ 65,–. – ISBN 0-19-511246-6.

Der dritte Band der neuen Nord-Amerika-Flora enthält mit den Unterklassen *Magnoliidae* (angenehm bzw. praktisch, daß diese nicht weiter gesplittet sind) und *Hamamelididae* (sensu Cronquist) die Ordnungen *Magnoliales*, *Laurales*, *Piperales*, *Aristolochiales*, *Illiciales*, *Nymphaeales*, *Ranunculales*, *Papaverales*, *Hamamelidales*, *Urticales*, *Leitneriales*, *Juglandales*, *Myricales*, *Fagales* und *Casuarinales*. Dem Konzept der Flora entsprechend gibt es für alle Taxa, von der Familie an abwärts, ausführliche, sorgfältige Beschreibungen und Bestimmungsschlüssel. Auf den angenommenen Species-Namen mit Zitat folgt(-en) meist (ein) Volksname(n). Gegebenenfalls folgt ein Gefährdungshinweis. Ein E keinnzeichnet Arten, die nur im Gebiet der Flora vorkommen. Wichtige Synonyme sind angeführt. Nach der Beschreibung folgen Hinweise auf Blütezeit, Standort, Höhenverbreitung und geographische Verbreitung. Schließlich gibt es kurze Diskussionen nomenklatorisch-taxonomischer Fragen, von Variabilität, ökologischer Ansprüche, von Nutzung (Kultur, Heilpflanze), Unkrauteigenschaften oder anderes. Für jedes Taxon gibt es eine kleine, schematisierte Verbreitungskarte. Da der Band 741 Arten enthält und die Zahl der Karten im Verlagsprospekt mit 1470 angegeben wird, müssen außer den Typussippen zusätzlich noch 729 infraspezifische Taxa berücksichtigt sein. Welche Autoren welche Familie oder Gattungen bearbeitet haben, ist stets angegeben.

Bei der Erstellung des Konzeptes für die Flora, dürfte manche Inspiration von seiten der Flora Europaea eingeflossen sein, aber die Flora of North America ist in der Qualität weit über diese hinausgewachsen. Eine ganz exzellente Flora! Hoffentlich lassen die ausständigen Bände (die Flora ist auf 30 Bände angelegt) nicht zu lange auf sich warten; der Rezensent würde den Abschluß noch gerne erleben.

Der vorliegende Band hat auch dadurch erhebliche praktische Bedeutung, daß er viele vegetationsprägende Gehölze enthält, z.B. *Fagaceae* [alleine 90 *Quercus*-Arten, *Castanea* (3) mit ausführlicher Diskussion der Schäden durch *Cryphonectria parasitica*], *Betulaceae* s.l. [*Alnus* (5), *Betula* (18)], *Juglandaceae* [*Carya* (11), *Juglans* (6)], *Ulmaceae*, *Maclura*, *Platanus*, *Magnolia* u.a. Die größte behandelte Familie sind die *Ranunculaceae*.

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