

# Phytochemical characteristics

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## 1. PHYTOCHEMICAL CHARACTERISTICS

### 1.1. GENERAL CHEMICAL COMPOSITION AND INORGANIC COMPONENTS

#### 1.1.1. Water content

Lemnaceae contain between 86% and 97% water depending on growth conditions. The main variation lies between 91% and 96% (e.g. VERNADSKY and VINOGRADOFF 1931, KRZECZOWSKA et al. 1975, MUZTAR et al. 1978, AMADO et al. 1980, BONOMI et al. 1981, MONETTI and BASTELLI 1983). Favourable growth conditions (fast growth rate) result in a greater amount of water. On the other hand, resting fronds, especially turions, store a lot of starch. Therefore they have a much higher dry weight percentage than normally growing fronds. The water content in Lemnaceae fronds is lower at higher temperatures than at lower ones (MESTAYER et al. 1984). However, no distinct differences between different species have been observed. 8 species (2 species of each genus) cultivated under identical conditions varied only between 3.9% and 4.1% dry weight (AMADO et al. 1980). The differences between different species found by VERNADSKY and VINOGRADOFF (1931) must be attributed to different environmental conditions.

#### 1.1.2. Variation of mineral content

In table 1.1 the variations in mineral content for the different elements are listed. The results are not split up according to the species since the culture conditions in the different investigations varied considerably. In general, the variations of the mineral content between plants cultivated under different culture conditions are much greater than between different species. DYKYJOVA (1979) summarized the known results of measurements of mineral content of Lemnaceae and other water plants. Much more data have been published since. Some of the papers deal with Lemnaceae growing in waters charged with heavy metals from industrial wastes. For the abilities of Lemnaceae to accumulate heavy

Table 1.1. Variation of content of elements in % of the dry weight  
(according to many authors, see list in the text)

Ag	0.3 - 50 x 10 <sup>-6</sup>	Mg	0.04-2.8
Al	0.000-11.4	Mn	0.003-6.4
As	0.2-23.5 x 10 <sup>-3</sup>	Mo	0.2-0.4 x 10 <sup>-3</sup>
B	0.02-3.25	N	0.8-7.8
Ba	0.03-0.11	Na	0.03-1.3
Br	0.25-0.65 x 10 <sup>-2</sup>	Nb	0.2 x 10 <sup>-3</sup>
C	30.5-43.7	Ni	0.7 x 10 <sup>-4</sup> - 0.2
Ca	0.18-4.5	P	0.03-2.8
Cd	<0.1 x 10 <sup>-4</sup> - 6.7	Pb	0.2 x 10 <sup>-4</sup> - 0.2
Ce	0.2 x 10 <sup>-3</sup>	Pr	0.4 x 10 <sup>-4</sup>
Cl	0.08-4.29	Ra	traces
Co	0.9 x 10 <sup>-4</sup> - 1.1	Rb	0.0054
Cr	0.3-17.8 x 10 <sup>-3</sup>	S	0.33-7.0
Cs	0.4-50 x 10 <sup>-3</sup>	Sb	0.0015-0.012
Cu	0.2 x 10 <sup>-3</sup> - 3.2	Se	0.0018-0.12
F	0.2 x 10 <sup>-3</sup>	Si	0.41-5.35
Fe	0.007-3.2	Sn	0.2-3.6 x 10 <sup>-2</sup>
Ga	0.9 x 10 <sup>-4</sup>	Sr	0.008-0.11
H	5.4	Ti	0.0018-0.32
Hg	0.04-18 x 10 <sup>-4</sup>	V	0.3-10 x 10 <sup>-3</sup>
J	0.4-25 x 10 <sup>-4</sup>	Y	0.4 x 10 <sup>-4</sup>
K	0.03-7.0	Zn	0.004-0.14
La	0.9 x 10 <sup>-4</sup>	Zr	0.9 x 10 <sup>-4</sup>
Li	0.8-6 x 10 <sup>-3</sup>		

metals see chapter 2.5.3.3 and 3.5.3. A detailed enumeration of all literature indications as well as of the growth conditions and the species of Lemnaceae used was not possible for table 1.1 due to difficulties in making the many and complex data comparable. The results of the following publications have been incorporated within the data of table 1.1.:

AMADO et al. (1980), ALLENBY (1967, 1968, 1981), BAUMEISTER and ERNST (1978), BONOMI et al. (1981), BOYD (1968), CLARK et al. (1981), COWGILL (1970), CULLEY and EPPS (1973), CULLEY et al. (1978), DENTON (1966), DY-KYJOWA (1979), ERNST and MARQUENIE-VAN DER WERFF (1978), FANKHAUSER et al. (1976), GLANDON and McNABB (1978), GUTHRIE and CHERRY (1979a,b), HAKONSON and WHICKER (1975), HARVEY and FOX (1973), HUNTER (1976), HUTCHINSON and CZYRSKA (1975), KARPATI and POMOGYI (1979), KARPATI et al. (1985), KHAKIMOVA et al. (1971), KHAKIMOVA and GALKINA (1973), KOVACS et al. (1984), KRZECZOWSKA et al. (1975), LEDL et al. (1981), LIEBERT (1980b), MONETTI and BASTELLI (1983), MUDROCH and CAPOCIANCO (1979), MUZTAR et al. (1978), ORNES (1979), PEDKOVA and LUBIANOV (1969), PEVERLY (1985), PIISPANEN and LAEHDESMAEKI (1983), POLAR and KUECUEKCEZZAR (1986), REAY (1972), RIEMER and TOTH (1968), RODGERS et al. (1978), RUSOFF et al. (1980), SANKARAN (1972), SETO et al. (1979), SILVEY (1967), STRAUSS (1973, 1976), SUCKCHAROEN (1980), SUTTON and ORNES (1975), TAN (1970), THELLIER and LE GUIEL (1967a,b), TRIDECH et al. (1981), VAN DER WERFF (1981), VARENKO and CHUICO (1971), VARENKO and LUBJANOV (1973), VAVRUSKA (1966), VERNADSKI and VINOGRADOFF (1931), WEIMER and ARMSTRONG (1979), WENTSEL and BERRY (1975), WOLVERTON and McDONALD (1981).

The ratio of some elements in Lemnaceae is shown in table 1.2, it is also dependent on the environmental conditions (see chapter 1.1.5).

Table 1.2. Mineral ratios in Lemnaceae, according to many authors (see list in the text)

K/Na	0.67 - 37
K/Ca	1 - 2
Ca/Mg	0.05 - 20
C/N	6.7 - 8.6

### 1.1.3. Status of some elements within the Lemnaceae

Of the total amount of nitrogen within the frond of L. minor c. 12% are part of the water soluble amino acids and proteins. A very small amount (<1%) was identified as ammonium (BAUER et al. 1971). WEIMER and ARMSTRONG (1979) measured 3.8 mg P per g dry weight in Lemna sp. from Wisconsin. 13% of the phosphorus was organic. This organic P was bound to inositol to 37%. More than 70% of the total sulfur content of L. minor is incorporated into organic thio compounds, and more than 25% is present as free sulfate in the vacuoles (THOIRON et al. 1981). The iron is present in L. gibba in the ferric form (GOODMAN and DEKOCK 1982, GOODMAN et al. 1982). Most of the iron is antiferromagnetically-coupled at low temperatures. The most common compound found was ferritin, an iron-storage protein. Boron is found as borate in the cytoplasm and in the vacuoles of L. minor; also borate diester is present (THELLIER et al. 1979). SCHAUMANN et al. (1986) developed a method of analytical ion microscopy to demonstrate the distribution of nitrogen in L. gibba.

### 1.1.4. The mineral content of Lemnaceae in comparison with other water plants

According to DYKYJOVA (1979), a medium to great amount of most minerals is typical for Lemnaceae compared with other water and marsh plants (150 species tested). The content of the following elements was not, or rarely, reached in other plant families: N, P, Mn, Zn, Ni, Si, Ba, Rb, Pb, Ti, and B. The K/Ca ratio varying between 1 and 2 in Lemnaceae is very low for monocotyledons. In L. minor, the content of Ca, Na, and Zn is higher, the content of Fe lower than in Eichhornia (BONOMI et al. 1981). The accumulation of boron in L. minor is ten times higher than in Ceratophyllum demersum (GLANDON and McNABB 1978). VARENKO and CHUICO (1971) measured an especially great amount of Zn and Co in S. polyrrhiza compared with other water plants. Mn showed the second highest and Cu only a medium value. A very high accumulation of Cd, Zn, Cu, and Pb was observed by VAN DER WERFF and PRUIT (1982) in fronds of S. polyrrhiza and L. gibba. Only Elodea and Callitriche out of many investigated water plants showed similar or slightly higher content of these elements. A relatively low ratio of C/N for normal growing fronds of Lemnaceae seems

to be characteristic. The ratio is 7 in L. minor compared with 17 in Eichhornia (WOLVERTON and McDONALD 1981). This is explained by the fact that Lemnaceae have only thin cell walls and a very reduced vascular system (scarcely any sclerenchymatous tissue). STRAUSS (1973) pointed out the unusually high content of magnesium compared with Ca accumulated by L. minor. The Mg/Ca ratio was always above 1, under certain conditions it reached values up to 20. Measurements of other authors do not fully confirm these results. Mostly the Ca content was much higher (up to 20 times) than the Mg content (cf. COWGILL 1970, DYKYJOVA and KVET 1978, DYKYJOVA 1979). The content of inorganic anions is especially high in L. trisulca. It amounts to 7% S (in form of  $\text{SO}_4^{2-}$ ), 1.4%  $\text{Cl}^-$  (JANAUER 1982).

The great amount of phosphorus in Lemnaceae is explained by the ability to form inositolphosphates (see chapter 1.2.5) and condensed inorganic phosphates (BIELESKI 1968a). In several Lemnaceae species NIEMEYER (1975) and INHUELSEN and NIEMEYER (1975) demonstrated linear oligophosphates with two to seven phosphate residues, cyclic condensed metaphosphates (tri-, tetra-, penta- and hexa-metaphosphates) and high molecular condensed phosphates. The saturation of these poly-anions by cations is undoubtedly the basis of the unusual ability to accumulate heavy metals. The high accumulation of boron by Lemnaceae is explained by the ability to bind the absorbed boron in the cell walls in form of easily hydrolyzable boromonoesters and of very stable borate diesters (DUVAL et al. 1980, THELLIER et al. 1979).

#### **1.1.5. Variation of mineral content due to different measuring techniques and different environmental conditions**

Washing of the Lemnaceae before analyzing yields different results compared with unwashed samples (MUZTAR et al. 1978). If washed under acid conditions, an additional amount of  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$  is washed away (up to more than 50%). Evidently, these elements are incrustated on the surface of the frond (ALLENBY 1981). For this reason the upper limits of mineral content in table 1.1 may well be too high.

The concentration of the minerals in the nutrient solution has a primary influence on the mineral content within the frond. The N and P content rose from 2.8 to 5.6% N and 0.4 to 1.4% P, respectively between low and

high N and P content in the culture medium. The maximum content in the frond was reached at a concentration of about 4 mg of N and P, respectively (REJMANKOVA 1981, see fig. 1.1). AEBLI (1986) got similar results with L. minor. The P content varied between 0.05% of the dry weight (at a concentration of 0.025 mg P per liter culture solution), 0.1% (0.1 mg/l), 0.37% (0.4 mg/l), and 1% (1.55 mg/l). At higher P concentrations in the nutrient solution, the P content of the fronds did not enhance considerably.

The content of heavy metals is correlated with the concentration of the metal in the nutrient solution (fig. 1.2).

The concentration of a mineral is also dependent on the concentration of other minerals. For instance, the potassium content of L. gibba and L. minor can rise from 2.5% of the dry weight under normal conditions up to 5% under low nitrogen supply (ERICSSON et al. 1982). The copper content is raised to about 130% if Cd is added to the culture solution together

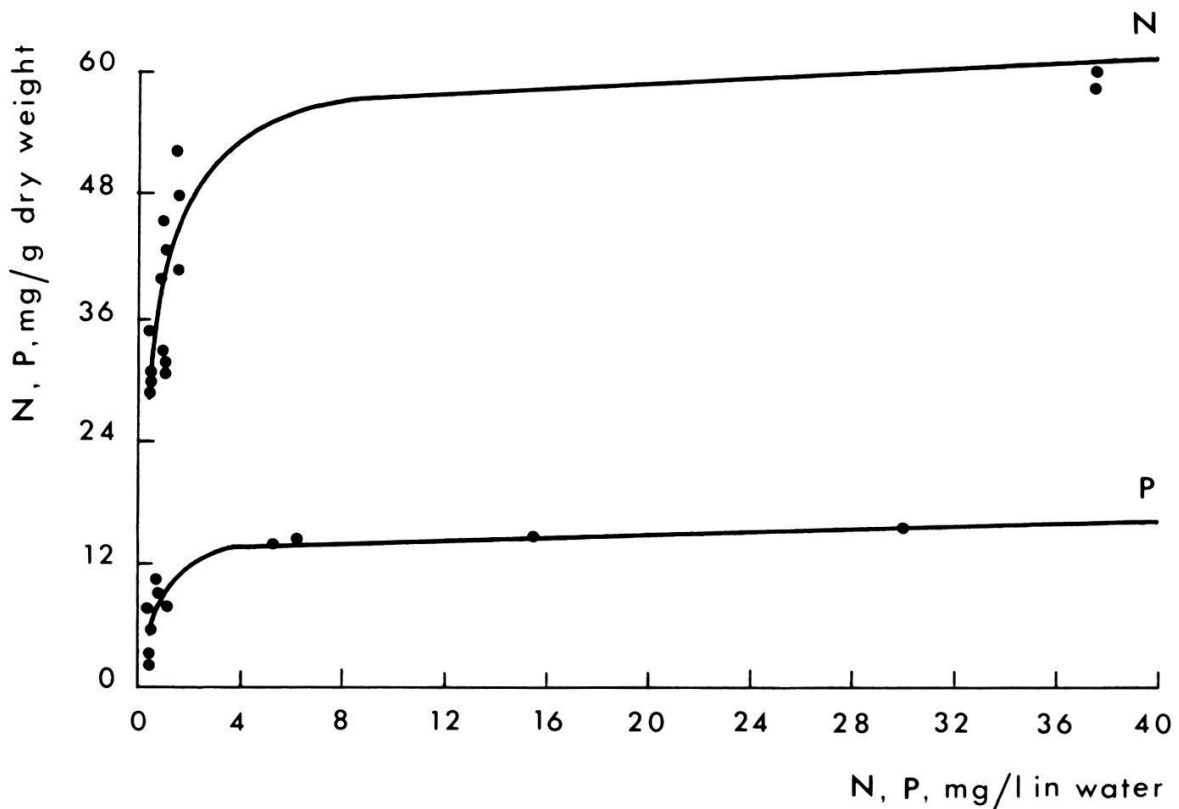


Fig. 1.1. The N and P content of Lemna gibba in relation to the N and P content of the nutrient solution (from REJMANKOVA 1981)

with copper. On the other hand, the Cd content is lowered by the simultaneous addition of Cu, As, or Zn; the Mn content is lowered by Cu, Cd, and Zn in the solution, and the Zn content by Cu and As (NASU et al. 1985). The C/N ratio is significantly higher in N-deficient cultures and in turions. It can reach up to 20. The calcium content of L. gibba is enhanced by the addition of ABA to the medium. It is lowered by BA (DEKOCK and HALL 1981). On the other hand, ABA at a concentration of 10 ppm diminishes the potassium content of L. gibba from 60 mg to 15 mg per g dry weight (LIEBERT 1977). The lowest P content under which growth of L. minor is still possible lies between 0.036 and 0.053% of the dry weight (FEKETE et al. 1976).

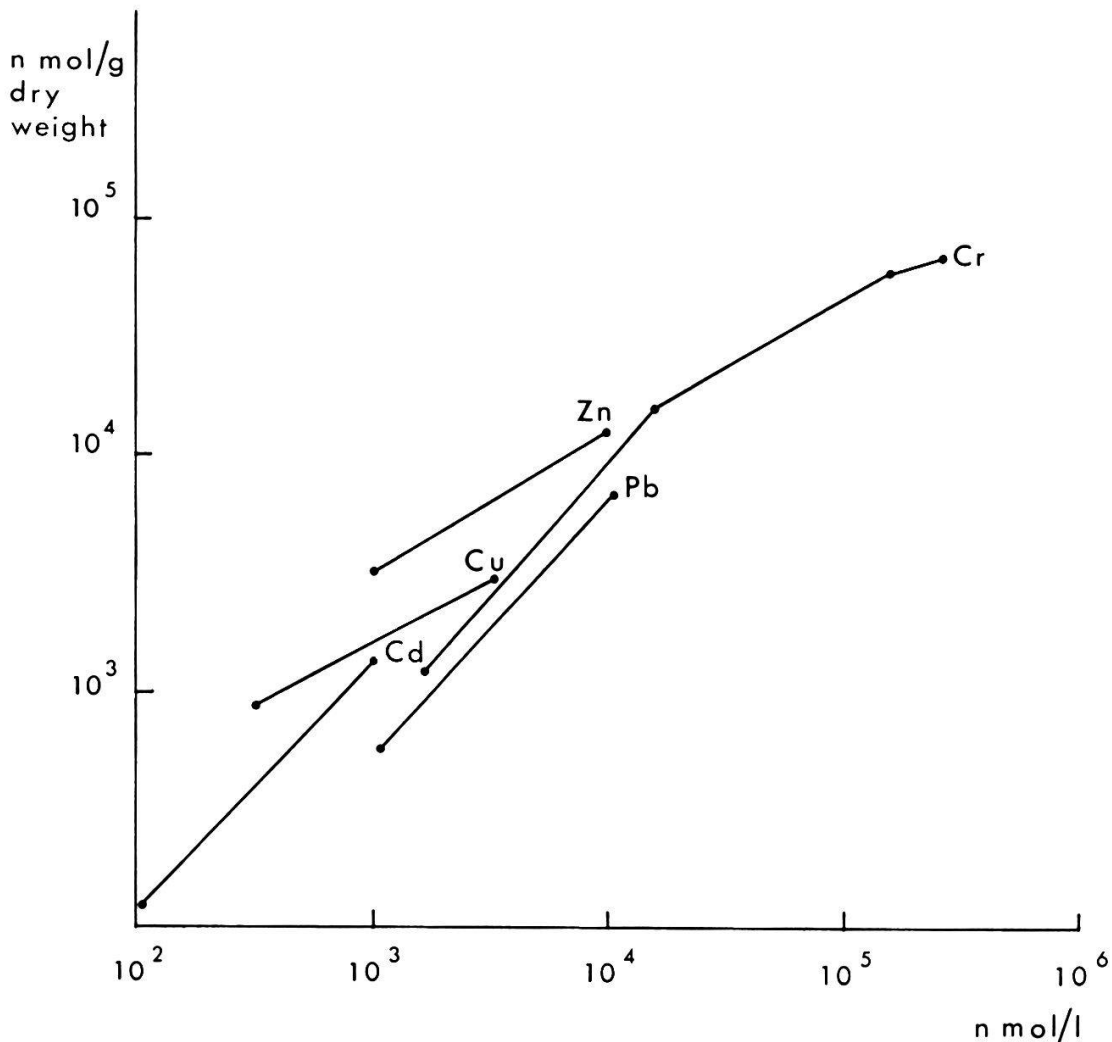


Fig. 1.2. The Cd, Cr, Cu, Pb, and Zn content of Spirodela polyrrhiza in relation to the concentration of the metals in the nutrient solution (data for Cd, Cu, Pb, and Zn from VAN DER WERFF 1981, for Cr from STAVES and KNAUS 1985).



The upper limit of mineral content which still allows an optimal growth rate in L. minor proved to be 0.08% of the dry weight for boron (FRICK 1985a), 0.05% for iron, 0.18% for manganese, 0.03% for copper, 0.11% for zinc, and 0.56% for molybdenum (FRICK 1985b).

#### **1.1.6. Variation of the mineral content during different seasons and during lifetime**

RAKHIMOV et al. (1981) measured the nitrogen content of L. minor during different seasons. In winter the nitrogen content as well as the content of sucrose and mannose are distinctly higher than during the summer; for the cellulose content it is vice versa. In table 1.3, another example is given. Main changes in this example are within the organic compounds. The content of certain elements during lifetime is not stable. FISCHER (1949) showed that the Ca content of the fronds of L. minor rises and the K content diminishes with age. LIEBERT (1980b) measured 80 mg Ca per g dry weight in old fronds and old cultures whereas the Ca content of young fronds and of young cultures was only 28 mg per g dry weight.

#### **1.1.7. Variation of mineral content in different species and clones**

In table 1.4, differences in mineral content between S. polyrrhiza, L. minor, and L. trisulca are shown (KARPATI and POMOGYI 1979). S. polyrrhiza and L. minor were collected in the same pond, L. trisulca originated from a neighbouring one. Table 1.5a gives an example of three Lemna species cultivated under the same conditions (ERICSSON et al. 1982). KOLES (1986) put some results from literature together of minimum and maximum nitrogen and phosphorus content in different species (table 1.5b). Since the study conditions of the different authors were not identical, the results are only partly comparable. DOCAUER (1983) measured the mineral content which is necessary to achieve half of the maximum growth rate in five different Lemnaceae species (table 1.6). From tables 1.4 to 1.6 it can be drawn that there are distinct differences between different species. Since only one clone of each species was investigated, it is not known if the differences are species specific or only characteristics of different clones. The relatively big differences

Table 1.3. Variation of chemical composition of Wolffia globosa from Japan (named as W. arrhiza) between summer and winter (after SEKINE 1979) in % of the dry weight

	protein	fat	carbo- hydrate	cellu- lose	minerals	H <sub>2</sub> O	chloro- phyll
summer	40-45	10-14	25-30	1-2	6-8	3	1-2
winter	8-10	18-20	60-65	1-2	6-8	3	1-2

Table 1.4. Differences in mineral content between Spirodela polyrrhiza, Lemna minor, and L. trisulca (according to KARPATI and POMOGYI 1979). Content in % of the dry weight (converted from the values of the fresh weight by the factor 20)

Species	K	Na	Ca	Mg	Mn	Zn	Fe	P	N
S. polyrrhiza	0.27	0.12	0.31	2.17	0.54	0.05	0.74	0.97	3.99
L. minor	1.53	0.02	0.18	1.92	0.03	0.05	0.06	0.83	8.74
L. trisulca	0.26	0.05	0.34	2.90	0.55	0.04	0.44	1.81	4.79

Table 1.5a. Mineral content of three Lemna species cultivated under identical conditions (according to ERICSSON et al. 1982) in % of the dry weight

Species	Elements				
	N	K	P	Ca	PO <sub>4</sub>
L. gibba	4.6	5.3	1.1	0.57	0.35
L. minor	4.7	5.7	0.86	0.47	0.40
L. aequinoctialis	4.1	4.9	0.74	0.33	0.30

in table 1.3 may be attributable to local environmental differences rather than purely to differences in species. From the results of ERICSSON et al. (1982) it seems that L. aequinoctialis generally has a lower level of minerals than the other two species investigated. The investigations of DOCAUER (1983) indicate that the Wolffias need to accumulate

Table 1.5b. Differences of minimum and maximum content of N and P between different species (modified from KOLES 1986)

Species	content in % of the dry weight			
	N min	N max	P min	P max
<i>Spirodela polyrrhiza</i>	1.7 (a)	7.9 (b)	0.3 (c)	2.1 (d)
<i>S. punctata</i>	1.7 (d)	7.2 (e)	0.6 (f)	2.4 (g)
<i>Lemna gibba</i>	1.5 (d)	7.7 (b)	0.7 (d)	2.6 (d)
<i>L. obscura</i>	0.8 (h)	4.6 (i)		
<i>L. minor</i>			0.4 (k)	2.4 (g)

**References**

- |                           |                       |
|---------------------------|-----------------------|
| a SUTTON and ORNES 1977   | f CULLEY et al. 1981  |
| b ORON et al. 1985        | g ALLENBY 1981        |
| c SUTTON and ORNES 1975   | h TRIDECH et al. 1981 |
| d CULLEY et al. 1981      | i HARVEY and FOX 1973 |
| e HILLMAN and CULLEY 1978 | k MUZTAR et al. 1976  |

Table 1.6. Mineral content of Lemnaceae species grown at concentrations of minerals to accomplish half of the maximum growth rate (in moles per mg wet weight) (from DOCAUER 1983)

Species	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>
<i>S. polyrrhiza</i>	0.14	0.011
<i>L. minor</i>	0.12	0.008
<i>L. turionifera</i>	0.14	0.006
<i>W. borealis</i>	0.17	0.018
<i>W. columbiana</i>	0.08	0.016

more phosphates to reach a good growth rate than S. polyrrhiza and the two Lemnas. W. borealis has an extremely high and W. columbiana an extremely low level of nitrate when half the growth rate is reached.

Of the three investigated species S. polyrrhiza, S. punctata and L. gibba, S. punctata accumulates most Cr and L. gibba least at low Cr concentrations. At higher concentrations (1 ppm and more) there is no longer any difference between the species (STAVES and KNAUS 1985). In a comparison between S. polyrrhiza and L. minor, VAN DER WERFF (1981) observed more Cd, Cu, and Pb and less Zn in L. minor than in S. polyrrhiza at concentrations between 0.1 to 10 mM in the solution.

## 1.2. ORGANIC COMPONENTS

### 1.2.1. General remarks

The variation of the different main groups of organic components in Lemnaceae is summarized in table 1.7. The data were taken from the same authors as in table 1.1. CHANG et al. (1978) measured much higher content of lipids (up to 26.2%) than other authors, probably due to different analyzing methods. JOHRI and SHARMA (1980) report 47% ash in L. minor. This great amount can scarcely be explained. The percentage of ash remains relatively constant under different temperature conditions of growth (MESTAYER et al. 1984). The carbohydrates are not always defined and measured in the same way. In starving cultures and in turions the content of carbohydrates (especially starch) can reach much higher values. REID and BIELESKI (1970a) observed 75% starch in S. punctata grown in P-deficient cultures.

The density of the culture did not exert any influence on the content of ash, carbohydrate, and protein, nor on the amount of the cell wall fraction in L. minor (TUCKER 1981). Differences between species may occur. However, no investigations have been made with more than one clone (except the amino acid studies of AMADO et al. 1980, see chapter 1.2.2). CHANG et al. (1978) measured the crude fibre content of S. polyrrhiza, S. punctata, L. aequinoctialis, and W. globosa (named as W. arrhiza) with 7.4%, 6.1%, 7.0%, and 7.4%, respectively.

1.1 to 2.6% of the total carbon fixed by L. minor is released to the water (BAKER and FARR 1987).

Table 1.7. Variations of organic components in Lemnaceae (data from many authors) in % of the dry weight

proteins	6.8 - 45.0
lipids	1.8 - 9.2
crude fibres	5.7 - 16.2
carbohydrates	14.1 - 43.6
ash	12.0 - 27.6

## 1.2.2. Proteins and amino acids

### 1.2.2.1. Proteins

The protein content of L. minor is composed of 49% albumine, 1.7% globuline, 32.1% gluteline, and 11.2% prolamine, according to MACIEJEWSKA-POTAPCZYK et al. (1970). The corresponding values for S. polyrrhiza amount to 25-55% albumine, 0.7-5% globuline, 15-30% gluteline, and 18-49% prolamine (included other remaining proteins), the variation depending on the preceding growth conditions (BYTNIIEWSKA and POTAPCZYK 1981).

The total protein content of the Lemnaceae varies between 6.8 and 45% of the dry weight (e.g. WHITE 1939, KESER 1955, MACIEJEWSKA-POTAPCZYK et al. 1970, BHANTUMNAVIN and MCGARRY 1971, TULGANOW 1972, CHANG et al. 1977, 1978, PORATH et al. 1979, AMADO et al. 1980, ROBINETTE et al. 1980, RUSOFF et al. 1980, BYTNIIEWSKA and POTAPCZYK 1981, APPENROTH et al. 1982).

The total content of protein in Lemnaceae is, under favourable conditions, one of the highest within the plant kingdom. ANTANINENE and TRAI-NAUSKAITE (1985) found L. trisulca, in Lithuania, one of the four species with the highest protein content out of 74 macrophyte species tested.

The protein content is much dependent on the growth conditions. It rises with higher light intensity (WHITE 1939); also it is distinctly higher at 24°C than at 18°C (LEHMANN et al. 1981). Young cultures contain more protein than old ones (CHANG et al. 1978) and fronds more than roots (LEHMANN et al. 1981). Fronds of L. aequinoctialis with a size of 5-6% of the final one only reach about 10% of that of the full-grown fronds (DATKO et al. 1980b). The mineral content of the nutrient solution (especially the nitrogen content) greatly influences the protein content of three nutrient solutions tested (Hoagland, Hutner 1/5, and Pirson-Seidel). Hoagland gave rise to the highest protein content (AMADO et al. 1980). This is probably due to the fact that the nitrogen content is highest in the Hoagland solution (see also KRZECZOWSKA et al. 1975). Indeed, the nitrogen content of the nutrient solution (at least for lower concentrations, see fig. 1.1) is in the first place positively correlated with the protein content of the fronds (CULLEY and EPPS 1973, SUTTON and ORNES 1975, SAID et al. 1979, REJMANKOVA 1979). ORON et al. (1987) measured the protein content of L. gibba at different concentrations of

ammonium. It amounted to c. 17% at an ammonium concentration of 5 mg/l and to c. 28% at 10 mg/l. BORNKAMM (1970a) measured the protein content of L. minor and L. trisulca in solutions with 0.5 and 1.0 mM N. In the first-named concentration the protein content was 7.1 and 5.0%, respectively, in the second concentration 18.2 and 9.9%, respectively. According to MALEK and COSSINS (1983b), the protein content of nitrogen- and sulfur-deficient cultures of S. polyrrhiza was only 8 to 31% of that of the controls. Higher levels of Ca in the medium consequently lowered the protein content of S. polyrrhiza (LECHEVALLIER 1977a). Exposure to ozone resulted in a 25% reduction of the protein content of L. minor within two hours (CRAKER 1972). The density of the culture, however, has no influence on the protein content of L. minor.

The differences in protein content between different species are generally smaller than between different clones of the same species. In 5 clones of L. trisulca cultivated under identical conditions, AMADO et al. (1980) measured the following protein content in % of the dry weight: 6.8, 15.4, 17.7, 18.0, 23.0. The total variation of the protein content of all 94 clones of 28 species was between 6.8 and 37.0%.

All stress situations cause a loss of soluble protein per frond due to a decrease in the rate constant of protein synthesis and an increase in the rate constant of protein degradation (DAVIES 1978, COOKE et al. 1979b) (see also chapter 2.4.1.4). Extensin, a component part of stress-produced proteins was analyzed in L. minor (BIGGS and KOSSUTH 1985). Stresses which increased levels of extensin include temperature extremes, intense photosynthetically active radiation, UV-B-radiation, and strong osmoticum.

GTB-binding proteins were detected in the extract of L. aequinoctialis containing membrane components (HASUNUMA and FUNADERA 1987). NECHUSHTAI et al. (1987a,b) isolated two chlorophyll-protein complexes of the photosystem I complex of L. gibba.

#### 1.2.2.2. Amino acids

KESER (1955) detected 28 amino acids in S. polyrrhiza. L. minor also contained 28 amino acids which were however partly different from those of S. polyrrhiza. Table 1.8 gives a survey of the content of principal amino acids in Lemnaceae, according to different authors. MACIEJEWSKA-POTAPCZYK et al. (1970) and TULGANOW (1972) investigated L. minor; CHANG

et al. (1977, 1978) S. polyrrhiza, L. aequinoctialis, and W. globosa (named as W. arrhiza); BYTNIIEWSKA and MACIEJEWSKA-POTAPCZYK (1980) S. polyrrhiza; PORATH et al. (1979) S. polyrrhiza, L. gibba, L. minor, and W. arrhiza; RUSOFF et al. (1980) S. polyrrhiza, S. punctata, L. gibba, and W. columbiana; APPENROTH et al. (1982) W. arrhiza, and AMADO et al. (1980) 94 clones of 28 species of all 4 genera of Lemnaceae. In addition to the amino acids mentioned in table 1.8, KESER (1955) noted within the free amino acids of S. polyrrhiza and L. minor glutathione, taurine,  $\beta$ -alanine,  $\gamma$ - and  $\alpha$ -aminobutyric acid but no oxyproline. Oxyproline was only observed in the protein.

The percentage of the different amino acids is very different depending on growth conditions, age of cultures, and methods of analysis. Lack of nitrogen alone lowers the content of free amino acids (especially asparagine) in S. polyrrhiza and L. minor and raises the content of glutamine. K- and P-deficiency in the solution had no influence, however (KESER 1955). The percentage of the content of different amino acids of S. polyrrhiza in N- and S-deficient cultures varies between 7% for methionine (controls 100%) and 1793% for asparagine (MALEK and COSSINS 1983b). Urea as nitrogen source leads to distinctly lower portions of asparagine, aspartic acid, arginine, glutamine, glutamic acid, and alanine (COOK 1968). Raising CO<sub>2</sub> supply enhances the content of alanine, arginine, glutamic acid, aspartic acid, glutamine, and asparagine and lowers that of glycine, serine, and histidine (MUELLER et al. 1977). HUBALD et al. (1979) investigated the influence of asparagine, glutamic acid and glycine on amino acid and protein content of L. gibba. The light quality (white, red, blue) has no influence on the composition of amino acids of the soluble proteins in W. arrhiza (APPENROTH et al. 1982). CHANG et al. (1978) demonstrated the effect of growth substances and age of culture on the composition of amino acids in Lemnaceae. PERRY (1963) observed different protein constituents in turions and normal fronds of S. polyrrhiza. Differences in the amino acid content between different species do not show up, however, differences between clones are frequent (AMADO et al. 1980).

In general, Lemnaceae, if compared with other plant families, contain a relatively great amount of leucine, threonine, valine, isoleucine, and phenylalanine whereas the content of cysteine, methionine, tyrosine, and taurine is relatively low.



Table 1.8. Amino acid composition of the proteins of Lemnaceae in % of the whole protein content

\* threonine included in proline, \*\* isoleucine included in glycine, nd. not determined

amino acids	Authors									
	1	2	3	4	5	6	7	8	9	10
asparagine + aspartic acid	11.0-13.9	9.9	5.5- 9.1	10.0-27.3	8.9-11.7	10.7	5.6-7.5	9.4	11.3	6.6
threonine	nd.	5.1	2.5-14.3	3.6- 5.2	4.3- 6.8	4.1	2.5-3.4	6.3	4.5	*
serine	5.9- 7.7	4.0	2.1- 4.8	4.4- 5.3	4.4- 6.0	4.3	2.3-2.8	5.4	4.0	5.0
glutamine + glutamic acid	11.4-14.4	13.5	5.7-10.5	10.7-14.7	10.7-19.2	8.8	5.8-8.0	10.6	10.8	9.9
proline	nd.	4.9	3.1- 3.2	1.5- 5.3	3.2- 7.1	5.9	2.4-3.3	4.4	7.3	10.9*
glycine	5.3- 6.4	7.4	2.8- 4.9	4.7- 5.9	5.2- 8.2	5.1	3.0-3.9	7.0	5.7	14.0**
alanine	6.4- 9.2	7.9	2.2- 5.0	5.9- 6.8	4.9- 7.6	9.2	3.7-4.8	7.7	7.2	0.8
cysteine	1.6- 2.9	tr.	4.7- 8.7	0.1- 0.4	0.1- 1.7	nd.	0.05	nd.	0.9	3.2
valine	4.6- 6.4	7.7	0.1- 5.7	5.4- 6.8	4.5- 8.0	5.7	3.5-5.0	6.3	6.6	8.2
methionine	nd.	0.4	1.1- 4.7	0.6- 2.6	0.1- 3.0	tr.	0.8-1.1	1.2	1.7	5.0
isoleucine	3.3- 3.9	5.9	3.8- 7.3	3.4- 5.0	2.8- 5.7	6.0	3.1-3.9	4.9	5.4	**
leucine	5.8- 8.1	10.3	1.0-10.4	6.7- 9.4	6.3-11.4	9.0	5.8-7.1	10.3	8.9	1.6
tyrosine	3.5- 4.2	2.8	3.1- 9.9	2.2- 5.7	1.0- 4.1	4.6	2.2-3.1	3.3	3.5	12,2
phenylalanine	6.8- 7.9	6.3	4.7-44.4	5.0- 7.2	4.4- 7.2	4.9	3.6-4.4	7.0	5.5	2.8
histidine	0.9- 2.9	2.3	0.9- 6.9	1.5- 2.8	1.0- 2.8	2.8	1.2-2.1	nd.	1.5	5.2
lysine	6.1- 6.4	6.2	0.7- 8.2	5.0- 7.5	5.6- 8.5	4.9	3.4-4.3	nd.	5.9	1.6
arginine	5.6- 9.4	4.7	2.0- 9.8	6.3- 9.3	5.8-15.4	5.7	3.8-5.2	nd.	6.7	4.3
tryptophane	nd.	0.9	nd.	nd.	nd.	1.3	nd.	nd.	0.5	3.0
hydroxyproline	nd.	nd.	nd.	0.4- 0.9	nd.	nd.	nd.	nd.	nd.	nd.
hydroxylysine	nd.	nd.	nd.	nd.	nd.	1.2	nd.	nd.	nd.	nd.

Table 1.8 (p. 24, continued)

**References**

- 1 TULGANOW 1972
- 2 MACIEJEWSKA-POTAPCZYK et al. 1970, 1975
- 3 CHANG et al. 1978, 1979
- 4 PORATH et al. 1979
- 5 AMADO et al. 1980
- 6 BYTNIIEWSKA and MACIEJEWSKA-POTAPCZYK 1980
- 7 RUSOFF et al. 1980
- 8 APPENROTH et al. 1982
- 9 BONOMI et al. 1981
- 10 YAMANI et al. 1978

RUSOFF et al. (1980) distinguish between crude protein and true protein. In their results, the sum of all amino acids only amount to 52.8-86.3% instead of 100% as for the other authors. To compare their values with those of other authors, a multiplication by the factor 1.5 must be made.

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**1.2.3. Carbohydrates**

The total content of carbohydrates of normal fronds varies between 14% and 43% of the dry weight (TAN 1970, BHANTUMNAVIN and MCGARRY 1971, CHANG et al. 1978, RAKHIMOV et al. 1981). The content (especially the starch content) can rise in old cultures up to the double of that in young cultures (CHANG et al. 1978).

MUZAFFAROV et al. (1968) and TULGANOW (1972) measured 20-35% starch in L. minor. A still higher starch content is reached in turions or in starving cultures. The normal starch content of L. minor was 3%, when grown in boron-deficient cultures it reached 6.5% (SCHOLZ 1962). REID and BIELESKI (1970a) measured a starch content of up to 75% of the dry weight in P-deficient cultures of S. punctata. Turions of S. polyrrhiza contained 62% starch compared with 16% in normal fronds (HENSSEN 1954). PANKEY et al. (1965) studied starch from S. polyrrhiza. The starch grains varied between 1 and 8  $\mu\text{m}$  (average 3.85). The amylose content in the starch was 21%; gelatinization occurred between 94°C and 98°C.

Some results of carbohydrate analysis are summarized in table 1.9.

AMADO et al. (1980) determined the percentage of certain sugars compared with the total amount of neutral sugars in L. valdiviana (1 clone), L. minuscula (3 clones) and W. neotropica (2 clones). The total amount of all sugars was not measured for methodical reasons. No differences be-

Table 1.9. Carbohydrate content in Lemnaceae, in % of the dry weight

Elements	Species						
	L. minor		L.tri- sulca	L.aequi- noctialis	L.minus- cula	"duck- weed"	
	Authors						
	1	2	3	4	5	6	7
soluble sugars		4.8	1.8-13.6	1.3		1	
starch		4.8	5.8- 8.7	0.25	4.6-13.8	3.0	
hemicelluloses			0.9- 9.5				21.7
celluloses			4.1-16.6			14.0	10.0
apiose	4.8						
xylose	2.5						
glucose	14.0						
mannose			0.2- 4.0				
sucrose			0.1- 1.5				

**References**

- |                        |                               |
|------------------------|-------------------------------|
| 1 DUFF and KNIGHT 1963 | 5 CHANG et al. 1978           |
| 2 MUZTAR et al. 1979   | 6 STEUBING et al. 1980        |
| 3 RAKHIMOV et al. 1981 | 7 WOLVERTON and McDONALD 1981 |
| 4 JANAUER 1982         |                               |

Table 1.10. Percentage of sugars in Lemnaceae (total amount of neutral sugars is 100%) (after AMADO et al. 1980)

glucose	55.3-57.3	arabinose	8.2-11.8
galactose	13.3-16.4	fucose	2.4- 5.6
mannose	1.9- 3.9	rhamnose	1.9- 2.6
xylose	7.1-14.2		

tween the different species could be observed (table 1.10). In addition to the neutral sugars mentioned, small amounts of glucosamine and galactosamine were detected as well as a sugar which probably was identical with apiose. Apiose was observed in Lemnaceae at a content of 4-8% of the dry weight (BELL et al. 1954, DUFF and KNIGHT 1963, DUFF 1965, VAN BEUSEKOM 1967, HART and KINDEL 1970a,b). The apiose was isolated mostly from the pectin fraction. According to HART (1969), 83% of the apiose is incorporated in the cell wall where it is present up to 20%, as a component of the polysaccharide apiogalacturonan (BECK 1964, BECK and KANDLER 1965, HART 1969, MASCARO 1975). Among others, PICKEN and MENDICINO (1967), GUSTINE (1969), and GRISEBACH et al. (1972a,b) report on the biosynthesis of apiose and xylose in L. minor (see chapter 2.5.5.4). Apiose occurs, within the monocotyledons, in some water and shore plants, especially in species of Hydrocharitaceae, Potamogetonaceae, and Zannichelliaceae which live submerged in brackish water (VAN BEUSEKOM 1967). No apiose was found in the families Araceae and Najadaceae. MUZTAR et al. (1978) analyzed the cell wall of L. minor. They observed 30.3% of the dry weight hemicelluloses and 19.2% celluloses (beside 2.4% lignin). According to these authors, L. minor has by far the highest hemicellulose content of 11 water plants studied.

The sugar content of the three carbohydrate components pectins, hemicelluloses and celluloses is given in table 1.11 (data from DEKOCK et al. 1979).

Table 1.11. Content of different sugars within the 3 carbohydrate groups of pectins, hemicelluloses and celluloses in L. gibba, in % of the total amount (after DEKOCK et al. 1979). The pectin fraction and to a lesser degree the hemicellulose fraction contain components of starch

carbohydrate groups sugars	pectins	hemicelluloses	celluloses
rhamnose and fucose	11	0	0
galactose	22	11	0
arabinose	18	8	0
glucose	7	41	75
xylose and apiose	40	38	25
mannose	2	2	0

AMBROSE (1978) investigated the carbohydrates released into water by different Lemnaceae (L. gibba, L. minor, L. trisulca). She always found glycerol and arabinose. L. gibba and L. trisulca emitted in addition fructose, glucose, and some unidentified sugars. Chemical analysis of L. aequinoctialis (named as L. minor) revealed the presence of the reducing sugars cardenolide and digitoxose (YONG and THO 1976).

#### 1.2.4. Lipids and fatty acids

The lipid content of Lemnaceae varies between 2 and 9% (LECHEVALLIER 1966, 197a7, BHANTUMNAVIN and McGARRY 1971, SU et al. 1973a, HILLMAN and CULLEY 1978b, STEUBING et al. 1980). CHANG et al. (1978) analysed between 6.3 and 26.2% lipids. The highest lipid content was measured in W. globosa cultures supplied with gibberellins. Perhaps the striking deviation of the result of CHANG et al. from other results can be explained by different methods of analysis. Like other organic compounds of Lemnaceae, the lipid content is conditioned by the age of the cultures (CHANG et al. 1978) and by the composition of the nutrient solution (LECHEVALLIER 1977a, GRENIER et al. 1979). 63 percent of the lipids of Lemnaceae belong to the phospholipids (with palmitic acid, linoleic acid and linolenic acid), 31% to the galactolipids (mostly linolenic acid), and 6% are neutral lipids (GRENIER et al. 1979). The indications of CHECHENKIN (1955) that no high saturated acids occur in L. minor, are not confirmed. The acid lipids of S. polyrrhiza contain 65% linolenic acid, 15% palmitic acid and 8% linoleic acid; quantitative determinations of fatty acids have also been made for single phospholipids, lipids, and sulfolipids (LECHEVALLIER 1966, 1967). The addition of polyethylene glycol to the nutrient solution results - among other things - in a reduced lipid content of S. polyrrhiza. The relative content of linoleic acid was enhanced and that of linolenic acid lowered (LECHEVALLIER 1977b). A similar shifting of the ratio of linoleic acid/linolenic acid showed up in S. punctata after the addition of glucose to the nutrient solution (GROB and EICHENBERGER 1969). In both cases, the changes of lipid composition occur in connection with enhanced frond senescence (yellowing of chloroplasts). There is another effect of sucrose in younger cultures of S. polyrrhiza, the lipid as well as the chlorophyll and protein content per chloroplast is highest with 2% sucrose supply

(LECHEVALLIER et al. 1971). Also the addition of benzyladenine to the culture medium changes the lipid composition of Lemnaceae. The percentages of total palmitic and  $\alpha$ -linolenic acids increase and the percentage of linoleic acid decreases in L. minor after supply of benzyladenine. At 2 and 5 ppm BA the proportion of phospholipids increases greatly as compared with galactolipids (especially the diacylgalactosylglycerol) and total neutral lipids. Also the  $\alpha$ -linolenic acid content of total neutral lipids is considerably enhanced (BERUBE et al. 1982). Benzyladenine ( $10^{-3}$  mM) increases the total phospholipid content by 20% in S. polyrrhiza. The degree of unsaturation of polar lipid fatty acids rises slightly due to greater amounts of linoleic and linolenic acids (LE PABIC 1980). Atrazine in sublethal doses stimulates the lipid metabolism of chloroplasts in L. minor (higher desaturation of fatty acids) (GRENIER and BEAUMONT 1983). Lipids of chloroplasts are described by BAHL et al. (1971) in S. polyrrhiza. EICHENBERGER (1975) studied the lipids of microsomes in L. minor. The hydrocarbons of S. polyrrhiza and L. trisulca were investigated comparatively by LECHEVALLIER (1969). He observed distinct differences between the two species: the hydrocarbons of S. polyrrhiza mostly contain long saturated and unsaturated chains ( $C_{17}$  to  $C_{29}$ ) which could be identified partly as squalenes and partly as alkanes and alkenes; the hydrocarbons of L. trisulca show shorter chains ( $C_{12}$  to  $C_{23}$ ) and only slight amounts of squalenes and few unsaturated chains (alkenes). Sterols of both species are frequent in form of campesterol, stigmasterol, and  $\beta$ -sitosterol. The proportion of stigmasterol is high in S. polyrrhiza (c. 70%) and low in L. trisulca (c. 25%), the percentage of  $\beta$ -sitosterol is high in L. trisulca (c. 55%) and low in S. polyrrhiza (c. 10%) (LECHEVALLIER 1970). D'HARLINGUE et al. (1976) measured the ratio of campesterol, stigmasterol and  $\beta$ -sitosterol from S. polyrrhiza held in solutions without sugar or with 2% sucrose. The ratios amounted to 26:43:30 and 20:48:30, respectively. The fatty acid composition of L. minor was studied by PREVITERA and MONACO (1983). The triacylglycerol fraction contained mainly 16:1 and 16:3 fatty acids and a hydroxy  $C_{16}$  acid. The same authors (1984) analyzed sitosterol, phytadiene, lycopersene, phytol, and (4R)-4-hydroxy-isophytol in L. minor. Different oxygenated fatty acids have been isolated from L. trisulca by MONACO and PREVITERA (1987), e.g. (12S)-hydroxyhexadeca-8Z,10E,14Z-trienoic acid and a prostaglandin-like  $C_{16}$  fatty acid.

### 1.2.5. Organic phosphorus compounds and nucleic acids

After 9 days supply of radioactive phosphate to S. punctata, BIELESKI (1968a) was able to extract phosphorus compounds and to identify them by means of chromatographic and autoradiographic methods. Most of the organic-bound phosphate (5.1  $\mu\text{mol P}$  per g fresh weight) occurs in the RNA. The dominant phospholipid is phosphatidyl choline (1.6  $\mu\text{mol P}$  per g fresh weight). In addition, there are phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, and phosphatidic acid. Glucose-6-phosphate (0.5  $\mu\text{mol P}$  per g fresh weight) and phosphoglyceric acid (0.25  $\mu\text{mol P}$  per g fresh weight) are the quantitatively prominent phosphate-esters. Furthermore, the author was able to demonstrate and determine quantitatively most of the nucleotides, sugar phosphates, and some more products of the intermediary metabolic processes as well as several inositol phosphates. The inositol phosphates and especially inositol hexaphosphate (phytic acid) have also been noted in several other Lemnaceae species: S. polyrrhiza, L. gibba, L. minor, W. gladiata (INHUELSEN and NIEMEYER 1978, SCHEINER et al. 1978, ROBERTS and LOEWUS 1968). WEIMER and ARMSTRONG (1979) measured 0.37% myo-inositol hexaphosphate in Lemna spp. Phytic acid is produced mainly in turions and resting fronds as a phosphate deposit. Factors which induce the formation of turions and resting fronds (nitrogen deficiency, sugar supply, short-day conditions at temperatures of 8-10°C) consequently enhance the accumulation of phytic acid (SCHEINER et al. 1978). Phosphatidylcholine and phosphoethanolamine bases as intermediates in the synthesis of the first substance have been demonstrated in L. aequinoctialis by MUDD and DATKO (1986b).

D'HARLINGUE et al. (1976) measured the content of NADP and NAD (about 3 to 4  $\mu\text{mol}$  per mg dry weight) in S. polyrrhiza. About 75% of the pyridine nucleotides were oxydized. Pyridine nucleotides of the same species have been investigated by LECHEVALLIER et al. (1977).

HASANUMA (1986) reports on cyclic nucleotides in L. aequinoctialis.

VAN EE and PLANTA (1982) isolated and characterized polyribosomes and non-ribosomal ribonucleoprotein particles in S. punctata. TOBIN and KLEIN (1975) report on the isolation and translation of mRNA in L. gibba. A similar RNA in Spirodela was detected by ROSNER et al. (1977a). Further studies on RNA were made by DYER and BOWMAN (1976), BOWMAN and DYER (1979), GRESSEL (1978), EICHHORN (1984, 1986), EICHHORN and AUGSTEN

(1984), KEUS et al. (1984a,b), VANDENBERGHE et al. (1984), DE HEIJ et al. (1985), KARLIN-NEUMANN et al. (1985). Investigations on RNA metabolism and on DNA structure and function are summarized in chapters 2.5.7.4 and 2.5.7.5.

The banding pattern of chloroplast DNAs have been used to distinguish different clones of L. aequinoctialis (BEPPU et al. 1985) and to characterize different species of Lemna (BEPPU in lit. 1986).

GEBER (in lit. 1986) measured the DNA content of the chromosomes of some Lemnaceae which varied according to the species (see table 3.2 in LANDOLT 1986).

#### **1.2.6. Chlorophylls, carotenoids, phytochrome, and cytochrome**

Results on chlorophyll and carotenoid content from the literature are put together in table 1.12. The content of both substances is dependent on culture conditions and species. Some of the variations in table 1.12 are probably also caused by different analyzing techniques. Very low chlorophyll contents must be attributed to a partial destruction during analysis. Low chlorophyll content was observed at low nitrogen and phosphorus concentration of the nutrient solution (REJMANKOVA 1979). The content was 1.2% of the dry weight in solutions with 375 mg N per liter and 154 P per liter and 0.4% if the N and P content was only 1/25 (for L. minor). Low copper concentration (FILBIN and HOUGH 1979) and low boron concentration (EICHHORN and AUGSTEN 1974) resulted also in a low chlorophyll content. According to ROMBACH (1976), the chlorophyll and carotenoid content in L. minor rises with increasing light intensity, whereas REJMANKOVA (1979) observed lower chlorophyll content in S. polyrrhiza and L. minor at high photosynthetically active radiation. The chlorophyll content is lower in solutions with sugar than without (in S. polyrrhiza) (D'HARLINGUE et al. 1976). In S. polyrrhiza, it rises with age up to the 15th day of frond growth (GAPONENKO and STAZHETSKII 1969). Also it is higher in normal fronds of W. arrhiza (0.9%) than in resting fronds (0.6%) or turions (0.16%) (GODZIEMBA-CZYZ 1970).

According to ANTONIELLI and CAGIOTTI (1976), the chlorophyll content is higher in L. trisulca than in S. polyrrhiza and L. gibba. REJMANKOVA (1979) reports a higher chlorophyll content in S. polyrrhiza (0.5-1.2%) than in L. minor (0.2-0.9%). SINHA and SAHAI (1975) compared the rela-



Table 1.12. Chlorophyll and carotinoid content in Lemnaceae, in % of the dry weight.

\* the original data were measured in relation to fresh weight  
 + consisting of 0.034  $\beta$ -carotene and 0.096 lycopene and xanthophyll  
 " about 1/4 of it  $\beta$ -carotene and 3/4 mixed xanthophylls

L.: Lemnaceae, L.m.: L. minor, S.p.: S. polyrhiza, W.a.: W. arrhiza

authors	1	2	3	4	5	6*	7	8	9	10	11
species	L.	W.a.	L.m.	L.m.	S.p.	L.m.	S.p.	L.m.	L.m.	L.	W.a.
chlorophyll total		4-7	0.5	0.3	1.6	1.7	0.6	0.02		0.2-1.2	0.35-0.63
chlorophyll a						1.3	0.4				0.11-0.24
chlorophyll b						0.4	0.2				
carotinoid	0.006-0.01				0.25	0.27		0.13+	0.08		0.15-0.21"

**References**

- |                             |                            |                      |
|-----------------------------|----------------------------|----------------------|
| 1 CULLEY and EPPS 1973      | 5 LECHEVALLIER 1977b       | 9 MUZTAR et al. 1979 |
| 2 EICHHORN and AUGSTEN 1974 | 6 McLAREN and SMITH 1976   | 10 REJMANKOVA 1979   |
| 3 FILBIN and HOUGH 1979     | 7 MONEGER 1968e            | 11 TOTH 1962         |
| 4 KAVANAGH 1941             | 8 MUELLER and LAUTNER 1954 |                      |

tive chlorophyll content of 4 Lemnaceae species under given culture conditions. It amounted to 1.3, 1.28, 1.06, and 1, respectively, for S. polyrrhiza, L. aequinoctialis (named as L. minor), W. globosa (named as W. arrhiza), and W. microscopica.

The ratio of chlorophyll a to b varies between 1 and 3.7. It is higher for L. minor (1.7-3.7) than for S. polyrrhiza (1.0-2.2) (REJMANKOVA 1979). Other values are 2.2 for S. polyrrhiza (LECHEVALLIER et al. 1976), 3 for L. minor (ARO 1982), and 4 for W. arrhiza (TOTH 1962). The ratio is low in solutions with a low nitrogen level and at high temperatures (37° compared with 22°C) (REJMANKOVA 1979). It increases with the age of the fronds (up to the 15th day) (GAPONENKO and STAZHETSKII 1969). However it does not change very much with changing light intensities (ARO 1982).

The ratio of chlorophylls to carotinoids was determined in L. minor by McLAREN and SMITH (1976) as 6.4. MUZTAR et al. (1979) analysed the carotinoid content of different water plants. For L. minor they noted the following components (in % of the dry weight): Carotene (0.015), xanthophylls (0.056 : 0.003 as monohydroxy pigment, 0.039 as dihydroxy pigment, and 0.014 as polyhydroxy pigment). In comparison to terrestrial plants (Medicago sativa, Zea mays) the total content of carotinoids is 10 times higher in Lemnaceae. BONOMI et al. (1981) noted, in L. minor,  $\beta$ -carotene (0.03), and xanthophyll (0.05). MONEGER (1968a,d) observed, in S. polyrrhiza, the following carotinoids (in % of the dry weight):  $\beta$ -carotene (0.04), lutein (0.04), lutein-epoxyde (0.01), violaxanthin (0.01) and neoxanthin (0.003). In L. gibba, the following carotinoids were demonstrated by SIEFERMANN (1971, 1972):  $\beta$ -carotene, lutein, neoxanthin, zeaxanthin, antheraxanthin, and violaxanthin. The carotinoid content is dependent on the light. It increases with light duration after dark growth. With short illumination, the maximum content of carotinoids is formed under green, red, and blue light (MONEGER 1968a,c, MONEGER and JACQUES 1968). SIEFERMANN (1971, 1972) observed more zeaxanthin and, to a lesser extent, more antheraxanthin and less violaxanthin with increasing light intensities. Lutein isolated from L. aequinoctialis revealed an inhibiting effect on BEA induced flowering of the same species (FUJIOKA et al. 1986c).

To investigate possible differences in carotinoid composition of Lemnaceae, Prof. Dr. C.H. Eugster and Dr. R. Buchecker of the Institute of Organic Chemistry, University of Zürich, in 1970 kindly analysed the ca-

rotinoids of 5 Lemnaceae species (S. polyrrhiza, L. gibba, L. valdiviana, W. hyalina, W. brasiliensis) (unpublished results). By thin-layer chromatography it could be demonstrated, that all species contain the same carotinoids of which the following have been identified:  $\beta$ -carotene, lutein, zeaxanthin (only few), and neochrome. Of the green and grey pigments (chlorophylls and possibly phaeophytins) 9 different substances could be recognized. In the composition of chlorophylls, L. valdiviana was distinctly different from L. gibba, W. hyalina, and W. brasiliensis, which all had 4 identical chlorophylls; in contrast, L. valdiviana showed 5 different chlorophylls of higher polarity (EUGSTER and BUCHECKER, unpublished). L. valdiviana is the only investigated species which is able to live submerged. A comparison with other submerged species would be desirable.

LAM and MALKIN (1985) studied a photosynthetic mutant of L. aequinoctialis lacking the cytochrome  $b_6$ -f complex but with a similar amount of the light harvesting chlorophyll-protein complex of photosystem II as in the wild-type Lemna. Further studies on the light harvesting chlorophyll a-b protein were made by KOHORN et al. (1986) and MAEENPAEAE and ARO (1986). THORNBUR et al. (1986) detected six different chlorophyll-proteins in L. gibba by electrophoretic separation.

Investigations of BIGGS and KOSSUTH (1980) with UV irradiation show that the carotinoids as well as the flavonoid pigments play an important role in photoprotection mechanisms of S. punctata and L. minor (and other higher plants).

In several heterotrophically cultivated Spirodela and Lemna species (with etiolated fronds), phytochrome has been demonstrated spectrophotometrically (ROMBACH 1965, ROMBACH and SPRUIT 1968, PORATH and BEN-SHAUL 1973, ROMBACH 1978). After illumination of plants, dark reversion of Pfr (half-life of 2-6 hours) as well as decay of phytochrome have been observed. The phytochrome content of plant parts with and without meristem tissues was determined on a dry weight basis, but did not differ significantly in L. minor (ROMBACH and SPRUIT 1968). The phytochromes extracted from etiolated tissues of duckweed do not show any cross-reactions with 6 investigated monoclonal anti-rye phytochrome antibodies and 6 monoclonal anti-pea phytochrome antibodies (SAJI et al. 1984).

### 1.2.7. Flavonoids

Whereas anthocyanins are often easily visible (Spirodela, some species of Lemna), other flavonoids can only be recognized by chemical tests or analysis. The anthocyanins which are dissolved in the cell sap are not identical with the brownish red pigment which can be observed as small dots in special pigment cells of dead fronds of Spirodela, Wolffiella, and Wolffia. According to HEGELMAIER (1868) this pigment can be dissolved in boiling potassium carbonate in Wolffiella and Wolffia but not in Spirodela. There must be a chemical difference between the pigments of the Wolffioideae and Spirodela, which has never been verified chemically. These pigments also belong to the flavonoid group and develop in S. punctata and W. borealis after UV irradiation or drying out from phlobaphene-like compounds by oxidation and polymerisation (WITZTUM 1974a). They are probably identical with the flavonols observed by McCLURE and ALSTON (1966).

THIMANN and collaborators investigated the anthocyanin of S. punctata and the conditions which lead to its production (see chapter 2.5.8.5). They determined the anthocyanin to be petunidin 3-glucoside (NG and THIMANN 1962), but later on they corrected this statement into the 3,5-diglucoside of petunidin (NG et al. 1964). In S. polyrrhiza KRAUSE and STRACK (1979a) identified cyanidin 3-monoglucoside (as was already found by McCLURE 1964 as the major anthocyanin of S. polyrrhiza), and in addition malonylcyanidin 3-monoglucoside. The anthocyanin cyanidin-bioside was found by REZNIK and NEUHAEUSEL (1959) in L. minor and L. trisulca. In L. trisulca, cyanidin-glycoside could be detected in addition. These chemical compounds exist as colourless pseudobases in the living plants and become red if treated with mineral acids. The anthocyanin in S. polyrrhiza is zwitterionic by acylation with aliphatic dicarboxylic acids (HARBORNE 1986). In S. punctata, JURD et al. (1957) identified the glycoflavones saponarin, isosaponarin, vitexin, and the flavone apigenin. McCLURE studied the flavonoids of Lemnaceae extensively and published his results in a series of papers (McCLURE 1964, 1967a,b, 1968, 1970, 1975; McCLURE and ALSTON 1963, 1964a,b, 1966). As an example of a relatively well marked biochemical differentiation within a whole family which is morphologically not very distinctly structured and therefore difficult to handle, the results of McCLURE received broad attention and recognition. That is why they will be discussed here in more detail. A

survey of the results is given in table 1.13. In this table, only groups of flavonoids but not single identified flavonoids are stated. However, McCLURE and ALSTON (1966) looked upon these single compounds as especially typical for a Lemnacean species. They distinguished 40 identified flavonoids, 7 unidentified flavonoids, and 27 possible flavonoids. The 5 groups of flavonoids distinguished by McCLURE and coworkers are: 1) glycoflavones: orientin, vitexin, isosaponarin, homo-orientin, luto-narin, lucenin, vicenin, saponaretin, and 4 acylated glycoflavones; 2) anthocyanins: petunidin-3,5-diglucoside, cyanidin-3-glucoside, and two additional not identified compounds (another anthocyanin, malonylcyanidin-3-glucoside, was identified by KRAUSE and STRACK 1979a); 3) flavonols: 11 quercetin compounds and 4 kaempferol compounds; 4) flavones: 5 apigenin compounds and 4 luteolin compounds.

According to McCLURE and ALSTON (1966), the only variation of the flavonoid composition within a species occurred in L. aequinoctialis (named as L. perpusilla) where a single flavonoid was not found in all samples. Later (1967a) McCLURE showed that clones from Africa and Asia differed in at least two flavonoids from American clones. Even mutants of the clone no. 6746 show some variability in the presence of a certain flavonoid. A morphological examination of the living clones of McCLURE (see table 1.13) showed that the delimitation of species by McCLURE was not the same as that of the present author. Especially surprising are his results with the two species L. valdiviana and L. minuscula which are extremely difficult to identify morphologically. However, they look very different in their flavonoid composition. McCLURE investigated, according to the present author, 5 clones of L. valdiviana under the name of L. minima and 11 clones of L. minuscula and in addition 2 clones of L. valdiviana under the name of L. valdiviana. Eleven flavonoids were detected in "L. minima" and 4 in "L. valdiviana" (in addition 3 possible flavonoids); the 2 groups had only 1 flavonoid in common. This big difference in flavonoid composition without any intermediate pattern can only be explained if it is assumed that this difference is caused by a

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Table 1.13 (p. 37). Flavonoids of Lemnaceae (according to McCLURE and ALSTON 1966, McCLURE 1970).

The species name of the key clone of McCLURE is underlined.

species name according to McCLURE	identification by LANDOLT (unpublished)	number of clones	number of identified flavonoids				number of unidentified compounds	
			glyco-flavones	antho-cyanins	flavonols	flavones	flavonoids	possible flavonoids
<i>S. intermedia</i>	<i>S. intermedia</i>	1	3	2	5		1	1
<i>S. biperforata</i>	<i>S. polyrrhiza</i>	1	4	2		3	1	1
<i>S. polyrrhiza</i>	<i>S. polyrrhiza</i>	21	4	2		3	1	1
<i>S. oligorrhiza</i>	<i>S. punctata</i>	9	8	2	2		5	5
<i>L. gibba</i>	<i>L. gibba</i>	12	4	2			3	3
<i>L. turionifera</i>	<i>L. turionifera</i>	10	4	2			3	3
<i>L. minor</i>	<i>L. minor</i>	34	8				7	7
<i>L. obscura</i>	<i>L. obscura</i>	6	5	2			4	4
	<i>L. turionifera</i>	3	5	2			4	4
<i>L. trisulca</i>	<i>L. trisulca</i>	5	7	2			3	3
<i>L. perpusilla</i>	<i>L. aequinoctialis</i>	8	3		1		3	3
<i>L. trinervis</i>	<i>L. aequinoctialis</i>	1	3		2		1	1
<i>L. valdiviana</i>	<i>L. valdiviana</i>	2	4				3	3
	<i>L. minuscula</i>	11	4				3	3
<i>L. minima</i>	<i>L. valdiviana</i>	5	3		4		4	
<i>W. lingulata</i>	<i>W. oblonga</i>	3			3			
<i>W. oblonga</i>	<i>W. oblonga</i>	2			4			
<i>W. gladiata</i>	<i>W. oblonga</i>	1			4			
<i>W. floridana</i>	<i>W. gladiata</i>	1			1			
<i>W. microscopica</i>	<i>W. microscopica</i>	1			8		1	4
<i>W. papulifera</i>	<i>W. brasiliensis</i>	6			10		4	4
<i>W. punctata</i>	<i>W. borealis</i>	1			8		4	4
<i>W. arrhiza</i>	<i>W. arrhiza</i>	2	4			2	1	3
	<i>W. globosa</i>	1	4			2	1	3
<i>W. columbiana</i>	<i>W. columbiana</i>	5	5			2	3	3
	<i>W. globosa</i>	1	5			2	3	3
<i>Spirodela</i>	<i>Spirodela</i>	32	9	4	6	4	4	4
<i>Lemna</i>	<i>Lemna</i>	97	11	2	0	7	7	7
<i>Wolffiella</i>	<i>Wolffiella</i>	7	0	0	5	0	0	0
<i>Wolffia</i>	<i>Wolffia</i>	17	5	0	12	2	2	2

single gene. McCLURE had identical conditions for the growth of his clones which is of course a precondition for reliable results. To test the influence of different conditions, he studied the formation of flavonoids in 7 clones of S. punctata under 26 different conditions. Out of 28 compounds (including 13 flavonoids) recognized in the chromatograms, only 9 (8 of which flavonoids) could be observed under all conditions (McCLURE and ALSTON 1964a). However all compounds except one could be detected if there was sufficient material for investigation (McCLURE 1975). BALL et al. (1967) investigated the chromatographical spot patterns of S. polyrrhiza and S. punctata in relation to growth conditions and to different clones. They reported differences between two clones of the same species under identical conditions, as well as within the same clone under different environmental conditions. Especially N and P deficiency and day-length caused deviations of the pattern. The investigations were repeated by H. CLARK (in PARKS et al. 1972). He found many more constituents in his extracts than the former authors reported, thus showing that certain substances might be overlooked depending on the methods used and the amount of plant material investigated. One clone of S. polyrrhiza was studied by McCLURE (1968) to identify the formation of flavonoids under different culture conditions. Cyanidin glucoside is only formed under white or blue light but not under red or in the dark. Contrary to anthocyanins, glycoflavones are also produced in the dark, though not in the same quality as in the light. Under optimal light conditions, S. intermedia contains 58  $\mu\text{mol}$  vitexin per 100 g fresh weight, 12  $\mu\text{mol}$  orientin, and 36  $\mu\text{mol}$  cyanidin glucoside; in the dark, the values are 17, 4, and 0, respectively. The cyanidin glucoside production seems to be phytochrome-regulated. In S. polyrrhiza, light-dependent anthocyanin synthesis is most effective under blue and red light. Far red has only 10% or less of the effectiveness of blue or red light (MANCINELLI 1977). The author also sees some evidence for an involvement of photosynthesis in anthocyanin synthesis of S. polyrrhiza (compare chapter 2.5.8.5).

In S. polyrrhiza, SAUNDERS and McCLURE (1976) observed a quantitatively different flavonoid pattern in chloroplasts compared with whole fronds (much more luteolin-7-glucoside) whereas in S. intermedia no differences showed up. Differences in the flavonoid pattern also occur between normal fronds and turions of S. polyrrhiza (REZNIK and MENSCHICK 1969, MENSCHICK 1970). The flavonoid content was influenced by the nitrogen

source. Turions showed 9 different flavonoids (including one anthocyanin) in addition to the ones mentioned by McCLURE and ALSTON (1966). The additional substances are characterized by a higher degree of glycosylation. HOESEL et al. (1972) demonstrated, in L. minor, the existence of a flavonol transforming enzyme. They think it possible that the lacking accumulation of flavonols in L. minor is due to the action of this enzyme. McCLURE (1975) mentions some biosynthetic steps leading to the construction of flavonoids in S. intermedia under the influence of external factors. The synthesis of the C<sub>15</sub> ring, the closure of the heterocyclic ring, and the 3-hydroxylation are stimulated by a low intensity of red light but proceed also in darkness. Introduction of the 3'-hydroxyl group needs low-energy blue or white light whereas the oxydation of the heterocyclic ring is only performed in high-energy blue or white light. The anthocyanin content of S. intermedia is highest in full-grown fronds whereas the orientin content is highest in still growing fronds. The vitexin content does not change between the two growth stages. Certain factors in the nutrient medium also influence the content of 3',4'-hydroxylated flavonoids (cyanidin-3-glucoside and orientin) but not of the 4'-hydroxylated flavonoid (vitexin) (McCLURE 1973, 1975). Kinetin (at 10<sup>-3</sup> mM) promotes and gibberellin (at 10<sup>-2</sup> mM) inhibits flavonoid accumulation in the light (McCLURE 1973).

In contrast to McCLURE and ALSTON (1966), VEEN (1975) found variations of the flavonoid pattern in L. gibba and L. minor not only between the species but also between different clones of the same species. The flavonoid pattern was similar but not identical to that of McCLURE and ALSTON (1966) for the same species. Also contrary to McCLURE, SU et al. (1973a) detected in L. minor flavones as well as flavonols. According to McCLURE L. minor contains neither flavones nor flavonols. However, there is a slight possibility that SU et al. confused L. minor with S. punctata which looks similar and has flavones and flavonols according to McCLURE. Since SU et al. had their material from Minnesota where S. punctata does not occur and since the material has been checked by taxonomists, the identification can scarcely be doubted. The following conclusions do not consider the results of SU et al. and might have to be revised. The conclusions of the extensive studies on flavonoids of Lemnaceae in respect to evolution and taxonomy of the family are as follows:



- 1) The genus Spirodela shows the highest differentiation in flavonoid formation. The investigated species contain not only anthocyanins but also 3 additional flavonoid groups.
- 2) Glycoflavones are evidently typical for the Lemnoideae. In the Wolffioideae they occur very rarely (only in W. arrhiza, W. columbiana, and W. globosa).
- 3) Flavonols are characteristic for species with pigment cells (which are only visible in dead fronds) (cf. WITZTUM 1974a). The only species which has flavonols but no pigment cells in the vegetative fronds is W. microscopica. Wolffia and Wolffiella species have pigment cells in the anthers and on the stigma. Since W. microscopica flowers under the culture conditions applied the presence of flavonols is understandable. It is to be expected that all Wolffia and Wolffiella species contain flavonols when flowering.
- 4) It does not seem very likely that Wolffia has a biphyletic origin (one group deriving from Lemna and the other from Wolffiella) as was proposed by TURNER (1967) established only by the presence or absence of flavonols.
- 5) Anthocyanins are restricted to the genus Spirodela and to the most differentiated sections of Lemna (sections Lemna and Hydrophylla). Contrary to the indications of McCLURE, anthocyanins do exist in L. minor (REZNIK and NEUHAEUSL 1959, VEEN 1975). S. punctata differs in the anthocyanins from all other species.
- 6) The genus Wolffiella as far as investigated has no flavonoids other than flavonols, however only a few species have been investigated.
- 7) It is not yet clear how far the flavonoid pattern of a clone can help in identifying the species within morphologically poorly differentiated groups. More clones of each known species must be investigated.

In general, most flavonoids are spread throughout the whole plant kingdom. They can therefore not be used in the taxonomy of higher categories.

Possible physiological significance of flavonoids for Lemnaceae is, according to McCLURE (1975): "light absorbance, chelating, and antioxidant properties, ability to hydrogen-bind with proteins, possible participation in electron transport systems, steric mimicry of certain hormones, and frequent antimicrobial effectiveness".

### 1.2.8. Lignins

According to WOLVERTON and McDONALD (1981), a mixture of Lemna and Spirodela contained 2.7% lignin related to the dry weight. DYKE and SUTTON (1977) measured 1.7% lignin from L. obscura (named as mixture of L. minor and L. gibba). From cell walls of L. minor MUZTAR et al. (1978) analysed 2.4% acid-detergent lignin per wall dry weight. STEUBING et al. (1980) report a much higher content of lignin (17% of the dry weight) in L. minuscula (named as L. valdiviana) from Chile. Since L. minuscula has practically no sclerenchymatic tissue and only very few vascular cells (less than L. minor), it must be concluded that some precursory compound was included in the lignin by the analysis method used. STAFFORD (1964) observed lignin reactions in 15 plant species. He did not get any reaction with L. minor (as well as Elodea) in phloroglucinol or with other histochemical tests. Only trace amounts of a blue colour in the phenol test showed up, but no p-hydroxycinnamic acid or ferulic acid were de-

Table 1.14. Content of lignin components in Lemnaceae, in  $\mu\text{mol}$  per 300 g dry weight sample (from BLAZEY and McCLURE 1968)

species	name according to BLAZEY and McCLURE 1968	p-hydroxybenzaldehyde	vanillin	syringaldehyde
S. intermedia	S. intermedia	9.1	4.1	4.9
S. polyrrhiza	S. polyrrhiza	6.6	1.7	1.1
	S. biperforata	5.3	0.1	0.0
S. punctata	S. oligorrhiza	2.8	1.0	0.6
L. gibba	L. gibba	3.0	0.0	0.0
L. minor	L. minor	3.0	0.2	0.0
L. obscura	L. obscura	4.5	0.0	0.0
L. trisulca	L. trisulca	4.3	0.0	0.0
L. aequinoctialis	L. perpusilla	5.5	1.0	0.0
	L. trinervis	3.3	0.4	0.0
L. valdiviana	L. valdiviana	6.8	0.0	0.0
	L. minuscula	4.9	0.1	0.0
W. oblonga	W. oblonga	4.2	0.0	0.0
W. microscopica	W. microscopica	6.1	2.1	0.0
Hordeum vulgare		10.5	11.7	9.7
Elodea densa		4.3	0.0	0.0

tectable in the acidified ether extracts. The lignification of 11 Lemna-ceae species (1 clone of each species) was investigated by BLAZEY and McCLURE (1968). Beside p-hydroxybenzaldehyde they found vanillin and syringaldehyde (see table 1.14). Vanillin and syringaldehyde are supposed to originate from lignin, however p-hydroxybenzaldehyde is known from fractions which do not originate from lignin. As is recognizable from table 1.14, there are 3 species (S. polyrrhiza, L. aequinoctialis, and L. valdiviana) which are represented with 2 clones. A certain variability shows up between the 2 different clones, but, in general, the differences between different species are more significant. The quantitative and qualitative formation of lignins probably also varies with the growth conditions. The results of BLAZEY and McCLURE (1968) show that the lignification is highest in Spirodela which is the only genus containing syringaldehyde. This is not surprising because Spirodela develops more vascular cells than Lemna and the Wolffioideae. Vanillin is also present in some species of Lemna. The relatively high content of vanillin in W. microscopica must be attributed to the flowering status of the investigated material. It is known that W. microscopica flowers regularly under culture conditions and that the flower organs are the only parts of the Wolffioideae which contain vascular cells. Furthermore, BLAZEY and McCLURE (1968) found the anther walls of W. microscopica rich in sclereids which can be stained by the phloroglucinol-HCl technique. It is surprising that L. gibba, L. obscura, and L. trisulca with noticeable vascular cell rows in the nerves do not contain vanillin. This suggests that p-hydroxybenzaldehyde originates at least partly from lignin. Anyway, it has to be considered that probably p-hydroxybenzaldehyde is, especially in monocotyledons, beside vanillin and syringaldehyde a decomposition product of lignin since in this plant group p-coumaryl alcohol (beside coniferyl alcohol and sinapoyl alcohol) is incorporated into lignin to a relatively high degree.

#### 1.2.9. Oxalates and other organic anions

L. minor contains up to 4% (of the dry weight) oxalic acid which is precipitated to 80% as raphide crystals and stored in special cells (idioblasts) (BORNKAMM 1965). In Spirodela, oxalates are present in form of raphides and druses besides the free oxalate content. Oxalates remains

dissolved in the cell sap within the Wolffioideae, where it never forms crystals. Evidently, Lemnaceae do not produce oxalate oxidases; Spirode-la and Lemna are dependent on calcium to neutralize accumulations of oxalic acid (LOETSCH and KINZEL 1971). The amount of oxalate is correlated with the protein content.

Low temperatures favour oxalate formation. In L. minor cultivated at 20°C BORNKAMM (1970c) measured 2.3% oxalate, at 25°C 2.4%, and at 30°C 0.8%. Dark-grown L. minor forms 4 times as many crystal cells as do light grown plants (FRANCESCHI 1985). The oxalate is formed by glycolate oxidase (BORNKAMM 1965, 1969a, FRANCESCHI 1985).

WROBLEWSKI (1976) investigated the chemical composition of raphides of L. minor by means of analytical electron microscopy. He distinguishes between two kinds of crystals (table 1.15): smaller ones with hexagonal or rectangular sectional view, and bigger ones with an hourglass-like sectional view. This is the only report on two different forms of crystals in L. minor. May-be the small crystals are young stages of a raphide. It is interesting to note that there is not only a quantitative difference between the two different crystal forms but also a difference in chemical composition (table 1.15). AL-RAIS et al. (1971) measured up to 2% Mg in the raphides of Lemnaceae. The oxalate of raphides is present in the monohydrate form, the oxalate of druses (not measured in Lemnaceae) contains calcium oxalate x 2.25 H<sub>2</sub>O. According to WATTENDORF (1985) the crystalline Ca oxalate occurs in Lemna as the stable monohydrate (whewellite) as well as the dihydrate. As a rule, the dihydrate does not show up in crystal idioblasts, but is found dissolved in all living cells. L. minor is able to incorporate Sr (but not Ba, Cd, Co, and Mn) into the raphides (FRANCESCHI and SCHUEREN 1986).

Table 1.15. Relative chemical composition of raphide idioblasts in L. minor (from WROBLEWSKI 1976)

Crystals	Chemical elements			
	Mg	S	Cl	Ca
big form	0.41	1.34	-	41000.0
small form	-	1.94	0.84	40.1

The presence of malate in Lemnaceae was demonstrated by KOPP et al. (1974a), KANDELER and HELDWEIN (1979), HELDWEIN and KANDELER (1981) and many other authors. The accumulation of malate only proceeds during photosynthesis and seems to be connected with nitrate metabolism. The malate content of L. aequinoctialis is about 10 times higher in the light (0.003 mmol per g fresh weight) than in darkness (KANDELER and HELDWEIN 1979). JANAUER (1982) identified, from L. trisulca, 0.1 mval organic anions per g dry weight. The following anions could be demonstrated in the sequence of decreasing content: fumarate, malate, glycolate, and citrate.

#### 1.2.10. Vitamins and plant growth substances

CLARK and THOMAS (1934) and CLARK et al. (1938) successfully fed rats nourished with a vitamin-free food with S. polyrrhiza. In this way they demonstrated marked amounts of vitamins A, B<sub>1</sub>, and C within S. polyrrhiza. Sixty-six to 110 mg per kg fresh weight carotene, an indicative of vitamin A, was determined by CULLEY and EPPS (1973) in Lemnaceae. FRAHM (1938) proved that S. polyrrhiza contains vitamin B<sub>1</sub>. The presence of vitamin C in L. minor was corroborated by RAKHIMOV and RAKHIMOVA (1975). NAKAMURA (1960) noted the vitamins A, B<sub>2</sub>, B<sub>6</sub>, and C in W. globosa (named as W. arrhiza). MUZAFFAROV et al. (1971) isolated the vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, E, and PP from L. minor. Especially the content of vitamin E (0.02-0.04 mg per g fresh weight) and of vitamin PP (0.04-0.06 mg per g fresh weight) is remarkable. Nicotinic acid was isolated by FUJIOKA et al. (1986a) from flowering plants of L. gibba and L. aequinoctialis. It is supposed that nicotinic acid influences the flowering process by interacting with other plant hormones (FUJIOKA et al. 1986b).

THIMANN and SKOOG (1940) achieved auxin-like effects on Avena coleoptiles by applying ether extracts of S. punctata (named as L. minor). In a further publication, THIMANN et al. (1942) demonstrated that the yield of auxins from S. punctata is greatly increased by a short incubation with chymotrypsin or trypsin; ficin has a similar though smaller effect. Therefore, a major part of the auxin seems to be bound to protein or a protein-like substance in S. punctata. SARGENT (1957) isolated four growth-stimulating substances and one growth-inhibiting substance from L. minor. The mainly active substance was identified as IAA. According

to SLOVIN and COHEN (1985b), the IAA level in Lemnaceae is unusually high. They measured, in L. gibba, per kg fresh weight 6.4 µg free IAA, 51.5 µg esterified IAA and 9.2 µg amide IAA. WITZTUM et al. (1978) measured up to 2.2 mg IAA per kg fresh weight in S. punctata; the addition of sucrose enhances the IAA content in S. punctata, UV irradiation lowers it (WITZTUM et al. 1978). The endogenous IAA level of L. gibba was also measured by COHEN et al. (1986). It is difficult to explain the great difference in IAA content between the results of SLOVIN and COHEN (1985b) and WITZTUM et al. (1978).

PIETERSE et al. (1971a) and PIETERSE (1972) observed two gibberellin-like substances in several species of Lemnaceae. Preliminary determinations of the gibberellin content in L. aequinoctialis and L. gibba cultivated under identical conditions showed higher values in the short-day plant L. aequinoctialis than in the long-day plant L. gibba (LADENBURGER, BAUER and KANDELER, unpubl. results). In L. aequinoctialis, substances were found with auxin-like and gibberellin-like activities as well as neutral and acidic inhibitors. The endogenous level of gibberellins increases during the night if the plants are treated with a short far-red irradiation at the end of the day. Supposedly, the content of gibberellins is dependent on the active form of phytochrome (PEKIC and NESKOVIC 1982). BEZEMER-SUBRANDY and VELDSTRA (1971a) analyzed cytokinins in alkaline hydrolysates of t-RNA of L. minor. In n-butanol extracts of W. arrhiza some cytokinin-like compounds can be separated by thin-layer chromatography and quantitated by the Amaranthus bioassay (EICHHORN and AUGSTEN 1980, EICHHORN 1986). The fraction which has the same  $R_f$  value as zeatin dominated in all investigated extracts. The authors found 98-244 µg zeatin equivalent per kg fresh weight. Cultivation of plants under blue light increased the cytokinin level (in comparison to white light) if the population was growing fast (good supply of phosphate). Red light on the other hand raised the content of the zeatin-like compound if the plants were growing slowly (low supply of phosphate). Another factor which modifies the cytokinin content in Lemnaceae is nitrate (LOEPPERT, GRUNTZEL and KANDELER, unpubl. results). Omission of nitrate from the medium lowers the level of cytokinins in L. aequinoctialis within 24-48 hours (GRUNTZEL 1982).

The levels of ABA which have been determined in Lemnaceae differ by several orders of magnitude. WITZTUM and KEREN (1978a,b) found  $2-14 \times 10^{-4}$  g ABA per g fresh weight in S. polyrrhiza, HUBER and SANKHLA (1979) 5x

$10^{-6}$  g ABA per g fresh weight in L. minor, and MERTEN (1979)  $2-9 \times 10^{-7}$  g ABA per g fresh weight in winter material of L. gibba and L. aequinoctialis, and  $4-42 \times 10^{-9}$  g ABA per g fresh weight in summer material of the same species. Accumulation of ABA within the fronds or release to the surrounding medium may be one of the causes for the differing results. Release of ABA to the nutrient medium was observed by MERTEN (1979) and SAKS et al. (1975, 1980). HUBER (1985) detected an increasing amount of xanthoxin and a decrease of ABA in pentachlorophenol-treated L. minor. The formation of xanthoxin, which is supposed to be a stress indicator, must be coupled with a membrane damage of the bleached fronds permitting the release of unsaturated fatty acids and herewith the peroxidation of xanthophylls (e.g. violaxanthin).

FUJIOKA et al. (1983a, 1985) established the presence of benzoic acid (BEA) in L. aequinoctialis and L. gibba (30-44  $\mu$ g per kg fresh weight). No positive correlation was found between the endogenous level of BEA and flowering of Lemna. BEA has also been demonstrated by TAKIMOTO and TAKAHASHI (cited from CLELAND and BEN-TAL 1983).

MATTOO et al. (1983, 1984d, 1986a) measured less than 0.05 ppm ethylene in S. punctata when cultivated in Hutners solution. If 0.02 mM Cu is added to the culture solution, the production of ethylene is 15- to 30-fold. The authors think that the stimulation of ethylene production in  $\text{Cu}_{(II)}$ -supplemented solutions is caused by  $\text{O}_2$  mediated damage of cellular membranes. FAERBER (1984), SCHARFETTER et al. (1984) report on the ethylene production of L. gibba. FAERBER et al. (1986) and KANDELER and FAERBER (1987) observed ethylene production under uncrowded and crowded conditions. Under uncrowded conditions, the production of ethylene amounted to 0.25-0.5 nl per g fresh weight and hour in L. gibba and L. minor, and 1.5-2.0 nl per g fresh weight and hour in S. polyrrhiza. Under crowded conditions it increased slowly in L. gibba and immediately in S. polyrrhiza and L. aequinoctialis. The addition of ABA did not result in a higher ethylene production. The production and release of ethylene in L. minor from aminocyclopropane-1-carboxylic acid (ACC) was studied by FUHRER (1985).

GIOVANELLI et al. (1981) demonstrated spermidine in L. aequinoctialis, and FLORES and GALSTON (1982) found a great amount of agmatine and spermidine in vegetative and flowering fronds of L. gibba.

LEROITH et al. (1985) and COLLIER et al. (1986) isolated 3.0 ng per g wet weight of a somatostatin-like substance from L. gibba. Somatostatin

is a hormone known from hypothalamus of vertebrates. The role of the hormone in Lemna as well as in bacteria and in spinach where it has also been found is not known. The authors conclude that vertebrate-type peptide hormones which are spread through many organisms have early evolutionary origins. Similarly, an insulin-related substance has been found in Lemna gibba G3 (and also in spinach) by COLLIER et al. (1987). The substance stimulates glucose oxidation and lipogenesis in isolated adipocytes from young rats. The role of this insulin-like compound in plants is unknown.

#### 1.2.11. Enzymes

Occurrence, properties, and regulation of more than hundred enzymes have been established within members of the Lemnaceae (table 1.16). The known enzymes are listed in alphabetical order with indications of the investigated species and references (as numbers). The enzyme content is dependent on the culture conditions. APPENROTH et al. (1986) showed that the light quality effects the activity of alanine aminotransferase and aspartate aminotransferase in W. arrhiza. Blue light stimulates the activity of both enzymes and red light decreases it. The significance of the enzymes for metabolism and developmental processes will be discussed in chapter 2.5. PERRY (1963) showed that in S. polyrrhiza many enzymes in normal fronds are essentially absent from turions.

GILES (1977-1979, unpublished results) examined 11 Lemnaceae species for 7 enzyme systems and studied the banding pattern on different gels: general protein (GP), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), alcohol dehydrogenase (ADH), peroxidase (PER), and glutamate oxaloacetic transaminase (GOT). In the staining reactions (see table 1.17) and in the banding pattern (fig. 1.3), genera and species as well as different clones of the same species showed differences. There are probably biochemical differences in the structure of the enzymes in different groups resulting in different response to the treatments. The lack of reaction of Wolffiella species might be due partly to relatively unfavourable conditions during culture and partly to the submerged life form. Unfortunately, GILES did not follow up the investigations. The following preliminary conclusions might be drawn from her results (see p. 55):



Table 1.16. Enzymes determined in members of Lemnaceae  
(for references see at the end of the table)

name of enzyme	test species	references
o-acetylserine sulfhydrylase	<i>L. aequinoctialis</i>	137
adenosine 5'-phosphosulfate sulfotransferase (APSSTase)	<i>L. minor</i>	10a,b,11,12a, 114,133b,150, 151
adenosine 5'-triphosphate-ase (ATPase)	<i>S. polyrrhiza</i> <i>S. punctata</i> <i>L. minor</i>	12b 50 133b
ATP-phosphohydrolase	<i>W. arrhiza</i>	66
ATP-sulfurylase	<i>L. minor</i> <i>L. gibba</i>	12a,117,133c, 30b,155
alanine aminotransferase	<i>L. minor</i> <i>W. arrhiza</i>	59 66
alanine dehydrogenase	<i>L. minor</i>	63
alanine glyoxylate aminotransferase	<i>L. minor</i>	108
alcohol dehydrogenase	<i>L. gibba</i> 3 <i>Wolffia</i> spp.	39 1b,39
aldolase	<i>L. minor</i>	20,60,111,112
(di)aminopimelate decarboxylase	<i>S. punctata</i> <i>L. aequinoctialis</i>	149 122
$\beta$ -amylase	<i>S. polyrrhiza</i> <i>L. gibba</i> <i>L. minor</i>	51 142 46a
D-apiosyltransferase	<i>L. minor</i>	89a,100,101,102
ascorbic acid oxidase	<i>L. minor</i>	52,53
aspartate aminotransferase	<i>W. arrhiza</i>	1b,66
aspartokinase	<i>L. minor</i>	157
carbonic anhydrase	<i>L. minor</i> <i>L. trisulca</i> <i>W. columbiana</i>	153 153 153
catalase	<i>L. minor</i>	38,52,53
cellulase	<i>L. minor</i>	120
$\beta$ -cyanoalanine synthase	<i>L. trisulca</i>	140
cystathionine $\gamma$ -synthase	<i>L. aequinoctialis</i>	40,41,136-139
cystein synthase	<i>L. minor</i>	10b
cytochrome oxidase	<i>Lemna</i> sp.	16
flavonol-converting enzyme	<i>L. minor</i>	54
$\beta$ -D-fructofuranosidase (invertase)	<i>W. arrhiza</i>	66
D-fructose-6-phosphate 1-phosphotransferase	"duckweed"	109
D-galacturonosyl transferase	<i>L. minor</i>	83
glucose-6-phosphate dehydrogenase	<i>L. gibba</i> <i>L. minor</i> <i>L. aequinoctialis</i> <i>W. arrhiza</i>	91 60,61 103 28,29,30a
glutamate dehydrogenase (see under NAD)		

Table 1.16. (continued)

name of enzyme	test species	references
glutamate glyoxylate aminotransferase	L. minor	108
glutamate oxaloacetic transaminase	S. intermedia	39
	S. punctata	39
	L. gibba	39
	L. minor	39
	L. valdiviana	39
	W. hyalina	39
glutamate pyruvate aminotransferase	L. minor	108
glutamate synthase	L. minor	56,105,107
glutamine synthetase	L. minor	56,104-107
glutathion peroxidase	L. gibba	30b
glyceraldehyde 3-phosphate dehydrogenase (see under NADP)		
glycolate oxidase	L. minor	9,32,33,33a,38 52,53
	L. trisulca	9
$\alpha$ -glycolydase, $\beta$ -glycolydase	W. arrhiza	66
4-hydroxyphenylpyruvate dioxygenase	L. gibba	86,92
hydroxypyruvate reductase	L. minor	1a,33a
invertase	L. gibba	144
isocitric dehydrogenase (see under NADP)		
malate dehydrogenase (see under NADP)		
malic enzyme	L. minor	57,60
mercaptopyruvate sulfotransferase	L. minor	115
myo-inositol kinase	L. gibba	7
myo-inositol-1-phosphate kinase	L. gibba	7
myo-inositol-1-phosphate synthase	L. gibba	96,119
	L. aequinoctialis	87
myo-inositol-triphosphate kinase	L. gibba	7
NAD (and NADP) glutamate dehydrogenase	L. gibba	39,121
	L. minor	26,27a,39,48, 59,60-65,105, 113,130,135
	L. aequinoctialis	23,24,25
	L. valdiviana	39
	S. intermedia	39
	S. punctata	39
	W. hyalina	39
	3 Wolffia spp.	39
NADH cytochrome-c-reductase	S. punctata	27b
NADP glyceraldehyde 3-phosphate dehydrogenase	L. gibba	44,45,46,91,93, 116,158
	L. minor	57

Table 1.16. (continued)

name of enzyme	test species	references
NADP isocitric dehydrogenase	L. gibba	91
	L. minor	60,61
NADP malate dehydrogenase	S. polyrrhiza	18,19
	L. minor	58-61
	11 spp.	39
NAD and NADP nitrate reductase	S. polyrrhiza	131
	S. punctata	34, 35, 36a, 148
	L. gibba	2
	L. minor	56, 60, 62, 97, 98, 99, 123, 127, 128, 129, 132
	L. trisulca	89b
NADP 6-phosphogluconate dehydrogenase	L. aequinoctialis	21, 132, 135
	L. gibba	91
nitrite reductase	S. punctata	34
	L. trisulca	89b
S <sub>1</sub> nuclease	L. gibba	67
	L. minor	120
pectinase	L. minor	125, 126
	11 spp.	39
peroxidase	S. polyrrhiza	42, 43
	S. punctata	81b
phenylalanine ammonia-lyase	L. gibba	42, 43
	L. aequinoctialis	40, 43
	S. punctata	5, 72-81a
phosphatases (acid)	L. gibba	91
	L. minor	133a
	S. punctata	5, 75-80
phosphatases (alkaline)	L. minor	85
	W. arrhiza	65
	S. punctata	78
phosphodiesterase	L. minor	57, 60, 88
phosphoenolpyruvate carboxylase	L. minor	60
phosphofructokinase	L. aequinoctialis	137
o-phosphohomoserine sulfhydrylase	W. arrhiza	66
o-phosphoric acid-monoester phosphohydrolase (acid and alkaline)	L. gibba	142
phosphorylase	L. minor	52, 53
polyphenolase	L. minor	71, 143
protein kinases	L. aequinoctialis	13, 68-70, 152
	L. minor	133a
pyrophosphatase (alkaline)	W. arrhiza	65
pyrophosphate phosphohydrolase	L. gibba	94
pyruvic kinase	S. punctata	73-81
ribonucleases	L. gibba	94
RNA polymerase		

Table 1.16. (continued)

name of enzyme	test species	references
ribulose-1,5-biphosphate carboxylase	<i>S. punctata</i>	50,133c
	<i>L. gibba</i>	37,124,133c, 141,156
	<i>L. minor</i>	36b,57
	<i>L. valdiviana</i>	133c
	11 spp.	14
ribulose-5-phosphate kinase	<i>L. minor</i>	57
serine glyoxylate aminotransferase	<i>L. minor</i>	108
sulfhydrylase	<i>L. aequinoctialis</i>	17
superoxide dismutase	<i>L. gibba</i>	145
tetrazolium oxidase	9 spp.	39
threonine synthase	<i>L. minor</i>	118
	<i>L. aequinoctialis</i>	41
thylakoid superoxide dismutase	<i>S. punctata</i>	84
trehalase	<i>L. aequinoctialis</i>	147a
tyrosine ammonia-lyase	<i>S. polyrrhiza</i>	43
	<i>L. aequinoctialis</i>	43
UDP apiose / UDP-xylose synthase	<i>L. minor</i>	47,90,110,154
UDP D-glucuronic acid cyclase	<i>L. minor</i>	47
UDP D-glucuronic acid carboxy-lyase	<i>L. minor</i>	47
urease	<i>s. punctata</i>	6,15
uridine kinase	<i>L. gibba</i>	95
D-xylosyltransferase	<i>L. minor</i>	102,110,154
enzyme system producing C <sub>6</sub> -aldehydes (from unsatu- rated fatty acids)	<i>S. polyrrhiza</i>	49

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Table 1.17. Enzyme reactions to 7 different stainings of 11 Lemnaceae species (mostly 2 clones per species) (according to GILES, unpubl. results)

ADH alcohol dehydrogenase	++ strong reaction
GDH glutamate dehydrogenase	+ medium reaction
GOT glutamate oxaloacetic transaminase	(+) weak reaction
GP general protein	- no reaction
MDH malate dehydrogenase	
PER peroxidase	
TO tetrazolium oxidase	

Species	Enzymes						
	GP	TO	MDH	GDH	ADH	PER	GOT
<i>S. intermedia</i>	++	++	(+)	+	-	+	+
<i>S. punctata</i>	++	++	+	+	-	+	+
<i>L. gibba</i>	++	++	+	+	(+)	+	+
<i>L. minor</i>	++	++	+	+	(+)	+	+
<i>L. valdiviana</i>	++	++	+	+	-	+	+
<i>W. hyalina</i>	++	++	+	+	-	-	+
<i>W. neotropica</i>	++	-	(+)	-	-	-	-
<i>W. gladiata</i>	++	-	(+)	-	-	-	-
<i>W. australiana</i>	++	++	+	+	+	+	-
<i>W. arrhiza</i>	++	++	+	+	+	+	-
<i>W. globosa</i>	++	++	+	+	+	+	-







the enzyme macromolecule and 8 individual small subunit polypeptids of different isoelectric point (table 1.18). Each species can be characterized by a specific combination. Taxonomically related species do not always have a similar pattern. Since only one clone of a species has been investigated, it is not known if a given combination is typical for a species or only for a clone.

### 1.2.12. Other organic substances

SU et al. (1973a) analyzed, in a Lemnaceae species (called L. minor), tannins and saponins as well as traces of alkaloids and steroids. In contrast, McCLURE (1970) found neither alkaloids nor terpenes in Lemnaceae.

Cardiac glycoside-like substances (belonging to the steroids) were stated to occur in L. aequinoctialis (named as L. minor) (YONG and THO 1976). Acetylcholine was identified from L. gibba extracts by paper chromatography, pharmacological activity on frog muscle, and sensitivity to acetylcholinesterase (HOSHINO and OOTA 1978).

In root cells of a Lemnaceae species (called L. minor) WROBLEWSKI (1976) observed amorphous inclusions which he interprets as tannin idioblasts. Tannins are known from the brownish red pigment cells of Spirodela and certain species of Wolffiella and Wolffia. In the living fronds, the colourless content of these cells consists of a phlobaphene-like compound (WITZTUM 1974a; see also chapter 1.2.7). It is not quite clear if the tannin idioblasts are identical with the pigment cells or if they also occur outside these cells and even in species (e.g. genus Lemna)

Table 1.19. Relative chemical composition of tannin idioblasts of L. minor (from WROBLEWSKI 1976)

	Mg	S	Cl	Ca
Big granules within membrane limited vacuoles	0.7	3.6	1.1	10.4
Medium coarse material within vacuoles	0.0	0.8	1.2	3.4
Finely granular material within vacuoles	0.8	1.0	1.0	4.3

which do not contain pigment cells. A confusion between L. minor and S. punctata cannot be excluded. From the vacuoles of turions of S. polyrrhiza VINTEJOUX (1978) isolated tannin-like polyphenolic substances associated with proteic and polysaccharidic compounds. Other polyphenolic substances are located in intercellular spaces, cell walls, zones of the cytoplasm, and along the plasmalemma and the tonoplast. WROBLEWSKI (1976) analyzed the chemical composition of tannin idioblasts. He detected the following elements (table 1.19).

According to REZNIK and NEUHAEUSEL (1959), L. minor and L. trisulca contain caffeic acid, L. trisulca also ferulic acid. In S. polyrrhiza KRAUSE (1978) analyzed the following derivatives of cinnamic acids: 1-feruloylglucose, 1-sinapoylglucose, 5-p-coumaroylquinic acid and 5-caffeoylquinic acid. 4 known isoprenoids (lycopersene, sitosterol, phytol, and trans-1,3-phytadiene) and a novel diterpene ([4R]-4-hydroxyisophytol) have been detected in L. minor by PREVITERA and MONACO (1984) (see chapter 2.5.8.2).

HCN tests (Picrin test and Feigel's test) were made at the Geobotanical Institute in Zürich with clones of S. intermedia (1 clone), S. punctata (2 clones), L. ecuadoriensis (1 clone), L. turionifera (1 clone) and proved to be negative.

No methane emission nor methane in the fronds could be detected in Lemnaceae by SEBACHER et al. (1985).