

# ***TECHNICAL NOTE 78.8***

## **REVIEW OF C-IVB BIOMASS COMPOSITION UNDER DIFFERENT OPERATIONAL CONDITIONS**

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### 1. Introduction

The photoautotrophic compartments are key elements of the MELISSA loop providing food and oxygen to the crew. Currently the Higher Plant Compartment (HPC or CIVb) is under design phase in order to provide the equipments to be incorporated into the Pilot Plant (Masot *et al.*, 2006). At present design stage it is very important to have a proper evaluation on the impact of HPC performance on the operation of the Pilot Plant and by extension on the MELISSA loop scaled to one man.

An initial evaluation should rely on the already available empirical data of the higher plant components to be included in the HPC. The most relevant data at this stage refers to the mass balances of the compartment, the nutritive value for the crew and any related dynamic aspect providing information on production rates and possible control variables.

In particular the main aspects proposed to be considered at this stage are:

1. The modification in elemental mass balances of HPC as a result of different operational conditions (only limiting factors corresponding to illumination, carbon source and nitrogen source will be considered at this initial step).
2. The macromolecular composition variations resulting of the modification on the previous experimental conditions, and its potential effects on the crew's nutrition.
3. The determination of the degree of coupling with the rest of Pilot Plant compartments in terms of nutrient supply and consumption and in terms of kinetics as far as possible. Evaluation will be based on the existing data and taking into account the use of these compartments to sustain human life
4. The determination of the range conditions under which HPC will fulfill its role in the whole MELISSA loop, based on the previous analysis. Also, and very importantly, the critical conditions where unbalances could arise shall be foreseen and identified. This is basic information to establish the operational constraints of the compartment.
5. Confirmation of the sizes and the optimal range of conditions to sustain the life of one human being.

As a first step to fulfill those objectives, the available data relevant to the previous issues will be collected, summarized and initially evaluated. The sources of relevant data will be the following:

1. Existing HPC's operational data at University of Guelph relevant to the MELISSA Pilot Plant operation.
2. Bibliographic review of the already available studies concerning higher plant quality under different conditions and higher plants models (C and N limitations, different light levels).

Therefore the aim of this document is to summarize the results of the review on biomass composition and quality under different operational conditions.

During the bibliographic review process it appeared that some interesting and relevant data on plant composition existing at UoG was only available as untreated raw data from several lettuce and beet cultures. Therefore, in order to extract the interesting parameters from UoG composition analyses for the current technical note, this raw data was also processed. The result of UoG raw composition data treatment is included as an annex of this document (Annex A).

## 2. Bibliographic review

Although an important amount of bibliographic references for beet, lettuce and wheat were reviewed, only few of them contained relevant information related to the mineral, proximate and fiber composition of the 3 MELISSA candidate crops.

Elemental and macromolecular composition under different conditions is summarized for each crop in several tables presented in this section and the corresponding references are reported in section 4. Nonetheless, a complete list of all the bibliographic references reviewed is also included in Annex B.

### 2.1. Beet

#### 2.1.1. Elemental Composition

The composition of beet in terms of Na<sup>1</sup>, K, P, Mg, Ca, N and C content is presented below. Data is grouped separately for hypocotyl, leaves and roots in Table 2.1, Table 2.2 and Table 2.3 respectively. In this way, the comparison of mineral composition can be made not only between the morphological sections but also within them. Culture conditions, under which this data was obtained, has been included when available; unfortunately in some cases growth conditions weren't specified in detail.

Table 2.1 Beet Hypocotyl Elemental and Mineral Composition

(1) Culture conditions: sunlight, 12/12 h day/night photoperiod, 15 °C, 350 ppm CO<sub>2</sub>, plant density ranging between 4·10<sup>5</sup> and 12·10<sup>5</sup> plants/ha under different K and N application (0-160 kg K/ha and 120-240 kg N/ha).

(2) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of 450 μmol ·m<sup>-2</sup>·s<sup>-1</sup>, 14/10 h day/night photoperiod, 25/20 °C day/night and 1000 ppm CO<sub>2</sub>

(3) Trace mineral composition also available: Zn 0.03%, Mn 0.003%, Fe 0.01%; Cu 0.001%, Se 0.00001%

Growth Conditions	Irrigation solution	Cultivar	Crop Age	Beet Hypocotyl Elemental Composition							Reference
				Na	K	P	Mg	Ca	N	C	
				d	%	%	%	%	%	%	
soil+ NK fertilizer	Rain water	-	150	0.07	0.93	-	-	-	0.07	-	Mahn <i>et al.</i> 2002 (1)
NFT (Nutrient Film Technique)	Table 5.1	Detroit Medium Top	72 <sup>(a)</sup>	-	3.33	0.59	0.07	0.26	3.31	39.20	UoG Annex A (2) (a) Batch cult. (b) Staged cult.
			68 <sup>(a)</sup>	-	3.52	0.72	0.08	0.25	3.69	39.03	
			66 <sup>(a)</sup>	-	2.92	0.57	0.06	0.19	3.08	39.13	
			40 <sup>(b)</sup>	-	3.17	0.66	0.16	0.50	3.06	39.20	
			50 <sup>(b)</sup>	-	3.13	0.60	0.14	0.36	3.04	41.90	
			60 <sup>(b)</sup>	-	3.05	0.60	0.13	0.29	3.02	41.87	
			70 <sup>(b)</sup>	-	2.81	0.55	0.11	0.24	2.82	40.54	
Soil	-	-	-	0.63	2.62	0.32	0.19	0.13	-	-	USDA 2005 (3)

Beet hypocotyl grown using a hydroponic system presents a higher mineral content than beets sown in soil by Mahn *et al.* (2002) and only slightly higher than compositions presented in USDA (2005).

<sup>1</sup> As a convention along the text ionic species in solution (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>,... ex. in the nutrient solution) are stated including their charge. Alternatively the total content in a tissue of the corresponding element is named without stating its charge (Na, K, Cl, ...).

Table 2.2 Beet Leaves Elemental and Mineral Composition

(1) Culture conditions: sunlight, 12/12 h day/night photoperiod, 15 °C, 350 ppm CO<sub>2</sub>, plant density ranging between 4·10<sup>5</sup> and 12·10<sup>5</sup> plants/ha under different K and N application (0-160 kg K/ha and 120-240 kg N/ha).

(2) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of 450 μmol · m<sup>-2</sup>·s<sup>-1</sup>, 14/10 h day/night photoperiod, 25/20 °C day/night and 1000 ppm CO<sub>2</sub>

Growth Conditions	Irrigation solution	[NaCl] mM	Cultivar	Crop Age d	Beet Leaves Elemental Composition									Reference
					Na	K	P	Mg	Ca	Cl	NO <sub>3</sub> <sup>-</sup>	N	C	
					%	%	%	%	%	%	%	%	%	
Soil + NK fertilizer	Rain water	-	-	150	0.18	1.34	-	-	-	-	0.03	0.16	-	Mahn <i>et al.</i> 2002 (1)
				150	0.08	0.97	-	-	-	0.05	0.10	-		
NFT	Table 5.1	Na+ 0.008 Cl- 0.075	Detroit Medium Top	72 <sup>(a)</sup>	-	5.88	1.30	0.47	1.90	-	-	5.40	35.40	UoG Annex A (2) (a) Batch cult. (b) Staged cult..
				68 <sup>(a)</sup>	-	6.57	1.56	0.28	1.87	-	-	4.56	35.20	
				66 <sup>(a)</sup>	-	6.11	1.50	0.28	1.63	-	-	4.62	36.23	
				40 <sup>(b)</sup>	-	2.50	0.81	0.39	0.72	-	-	3.70	41.30	
				50 <sup>(b)</sup>	-	5.22	1.35	0.94	1.87	-	-	5.26	39.03	
				60 <sup>(b)</sup>	-	5.90	1.32	1.23	2.39	-	-	4.87	37.27	
				70 <sup>(b)</sup>	-	5.41	1.42	0.81	1.73	-	-	4.79	38.40	
Sand + NPK fertilizer	½ strength Hoagland	0	Zwaanpoly	60	0.8	4.5	-	-	-	5.5	4.2	-	-	Ghoulam <i>et al.</i> 2002
			Kawemegapoly		1	5.5	-	-	-	6	4.5	-	-	
			Top		0.8	4.7	-	-	-	6	2.2	-	-	
			Desprez poly		0.8	5	-	-	-	6	3.6	-	-	
			Nejma		1	5.2	-	-	-	5	2.4	-	-	
		50	Zwaanpoly	60	2.6	4	-	-	-	8	4.5	-	-	
			Kawemegapoly		2.7	3.8	-	-	-	8	4.8	-	-	
			Top		2.7	3.3	-	-	-	8.5	2.9	-	-	
			Desprez poly		2.9	4.5	-	-	-	9	4.1	-	-	
			Nejma		3	4.1	-	-	-	8.5	4.9	-	-	
		100	Zwaanpoly	60	2.7	2.8	-	-	-	13	3.4	-	-	
			Kawemegapoly		3	3.7	-	-	-	14	4.7	-	-	
			Top		3	3	-	-	-	10	3.7	-	-	
			Desprez poly		3	3.1	-	-	-	8	2.4	-	-	
			Nejma		3.1	4	-	-	-	10	5	-	-	
		200	Zwaanpoly	60	3.3	3.1	-	-	-	14	5	-	-	
			Kawemegapoly		4.2	4.1	-	-	-	16	5.1	-	-	
			Top		3.5	2.8	-	-	-	12	4.3	-	-	
			Desprez poly		4	3.7	-	-	-	12	3.1	-	-	
			Nejma		4.1	3.8	-	-	-	14	2.6	-	-	

Comparing beet mineral composition for different crop ages, obtained by UoG (Annex A), it can be observed a fairly constant composition in each beet part along the plant growth. However, phosphorous content in hypocotyl seems to diminish along plant age while its content in roots increases. Moreover, calcium percentage in hypocotyl and

potassium composition in roots has a statistically significant decrease along beet maturity.

Table 2.3 Beet Roots Elemental and Mineral Composition

(1) Culture conditions: sunlight, 12/12 h day/night photoperiod, 15 °C and 350 ppm CO<sub>2</sub>

(2) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of 450 μmol · m<sup>-2</sup> · s<sup>-1</sup>, 14/10 h day/night photoperiod, 25/20 °C day/night and 1000 ppm CO<sub>2</sub>

Growth Conditions	Irrigation solution	[NaCl]	Cultivar	Crop Age	Beet Roots Elemental Composition								Reference
					Na	K	P	Mg	Ca	Cl	N	C	
		mM		d	%	%	%	%	%	%	%	%	
soil+ NK fertilizer	Rain water	-	-	150	0.04	0.84	-	-	-	-	0.04		Mahn <i>et al.</i> 2002 (1)
NFT	Table 5.1	Na <sup>+</sup> 0.008 Cl <sup>-</sup> 0.075	Detroit Medium Top	72 <sup>(a)</sup>	-	1.54	0.48	0.48	2.19	-	4.45	37.5	UoG Annex A (2) (a) Batch cult. (b) Staged cult.
				68 <sup>(a)</sup>	-	1.2	0.74	0.16	1.04	-	4	39.4	
				66 <sup>(a)</sup>	-	1.32	0.50	0.38	1.83	-	4.03	38.4	
				40 <sup>(b)</sup>	-	3.39	0.58	1.10	4.74	-	3.72	29.80	
				50 <sup>(b)</sup>	-	4.17	0.73	0.90	2.81	-	4.23	33.23	
				60 <sup>(b)</sup>	-	3.94	0.92	0.98	3.03	-	4.32	29.33	
Sand + NPK fertilizer	½ strength Hoagland	0	Zwaanpoly	60	0.2	2.1	-	-	-	2	-	-	Ghoulam <i>et al.</i> 2002
			Kawemegapoly		0.3	2.9	-	-	-	3	-	-	
			Top		0.3	2.7	-	-	-	2	-	-	
			Desprez poly		0.2	2.9	-	-	-	2	-	-	
			Nejma		0.4	2.9	-	-	-	2	-	-	
		50	Zwaanpoly	60	0.9	2.1	-	-	-	4	-	-	
			Kawemegapoly		0.9	2.3	-	-	-	4	-	-	
			Top		0.8	2.3	-	-	-	4.5	-	-	
			Desprez poly		1	2.5	-	-	-	5	-	-	
			Nejma		0.8	2.1	-	-	-	5	-	-	
		100	Zwaanpoly	60	1.2	2.3	-	-	-	7	-	-	
			Kawemegapoly		1	1.9	-	-	-	7	-	-	
			Top		1.6	2.4	-	-	-	6	-	-	
			Desprez poly		2.3	3.4	-	-	-	9.5	-	-	
			Nejma		1.1	2.1	-	-	-	6	-	-	
		200	Zwaanpoly	60	1.6	2	-	-	-	7	-	-	
			Kawemegapoly		1.6	1.8	-	-	-	7	-	-	
			Top		2	1.9	-	-	-	11	-	-	
Desprez poly	2.2		2.7		-	-	-	10	-	-			
Nejma	2.2		2.7		-	-	-	9	-	-			

The effect of salinity on mineral composition in plants of 5 varieties of beet has been investigated in Ghoulam *et al.* (2002). They concluded that high NaCl concentrations caused a decrease in K content, but Na and Cl contents were highly increased in the leaves. Subbarao *et al.* (2001) already mentioned the enormous capability by red beet to take up Na<sup>+</sup> and utilize it in non-specific functions instead of K<sup>+</sup>. Nearly 95% of the K in red-beet can be replaced by Na, with Na levels reaching close to 2000 μmol/g dw (Subbarao *et al.*, 1999).

The effects in different cultivars shows a not significant effect by  $\text{Na}^+$  but significant for  $\text{K}^+$  and  $\text{Cl}^-$ . In addition to this, the effect of salt concentration on  $\text{NO}_3^-$  content was not significant. Under salt stress, the tested varieties accumulated more inorganic ions in the leaves than in the roots (Ghoulam *et al.*, 2002). Therefore, salinity stress plays an important role in mineral composition of beets.

Comparing mineral content between morphological parts, a non uniform distribution of elements within beet is detected. Data reported by Mahn *et al.* (2002) show a considerable increase of K, Na and N content from the root to the upper stem. In contrast to this, nitrate content doesn't change markedly. Accordingly, Ghoulam *et al.* (2002) reports that beets without salinity stress have a higher K, Na and Cl content in leaves than in roots. Focusing the attention in mineral composition of different plant parts reported in UoG data, K and P content also increases from roots to leaves. However, Mg and Ca content in leaves and roots are similar but higher than hypocotyl.

### 2.1.2. Macromolecular Composition

The main bibliographic source found containing proximate (macromolecular) analysis of red beet was data obtained in UoG (Table 2.4), which reports similar values to the ones presented in USDA (2005).

In beet leaves the content of fat, protein, energy and moisture has no significant variation along plant growth, whereas ash composition increases and carbohydrates diminish along plant maturity. With regards to beet hypocotyl composition only fat, protein and carbohydrates are significantly affected by plant age.

Table 2.4 Beet Macromolecular Composition

(1) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of  $450 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 14/10 h day/night photoperiod, 25/20 °C day/night and 1000 ppm  $\text{CO}_2$

Growth Conditions	Irrigation solution	Cultivar	Crop Age	Beet Macromolecular Composition					Energy	Moisture	Reference
				Protein	Fat	Ash	CH	Fiber			
			d	%	%	%	%	%	cal/g	%	
<b>Leaves</b>											
NFT	Table 5.1	Detroit Medium Top	40	20.8	1.4	11.52	56.3	-	3210	9.97	UoG Annex A (1)
			50	28.35	1.1	18.97	40.8	-	2870	10.69	
			60	29.96	1.1	22.96	35.7	-	2730	10.31	
			70	28.12	1.27	21.66	39.23	-	2810	9.73	
<b>Hypocotyl</b>											
Soil	-	-	-	12.96	1.37	8.70	76.97	22.54	3460	-	USDA, 2005
NFT	Table 5.1	Detroit Medium Top	40	17.79	-	-	-	-	-	-	UoG Annex A (1)
			50	19.3	0.5	8.37	59.7	-	3200	12.22	
			60	18.19	0.3	7.99	61.1	-	3200	12.45	
			70	15.60	0.23	7.51	65.17	-	3250	11.51	
<b>Roots</b>											
NFT	Table 5.1	Detroit Medium Top	70	20.43	0.30	31.59	39.20	-	2410	8.46	UoG Annex A (1)



The nutrient composition of foods found in USDA nutrient database includes extensive and detailed information about mineral and proximate content in plants. The lipid, vitamin and amino acid composition of beet provided by USDA can be of interest for further studies, thus it was decided to be included in Table 2.5.

Table 2.5 Beet lipid, vitamin and amino acid composition (USDA, 2005)

Lipids	Units	Value per 100 g dw	Amino Acids	Units	Value per 100 g dw
Fatty acids, total saturated	g	0.22	Tryptophan	g	0.15
16:00	g	0.21	Threonine	g	0.38
18:00	g	0.01	Isoleucine	g	0.39
Fatty acids, total monounsaturated	g	0.27	Leucine	g	0.55
18:1 undifferentiated	g	0.27	Lysine	g	0.47
Fatty acids, total polyunsaturated	g	0.49	Methionine	g	0.14
18:2 undifferentiated	g	0.45	Cystine	g	0.15
18:3 undifferentiated	g	0.04	Phenylalanine	g	0.37
Phytosterols	mg	0.20	Tyrosine	g	0.31
<b>Vitamins</b>			Valine	g	0.45
Vitamin C, total ascorbic acid	mg	3.95E-02	Arginine	g	0.34
Thiamin	mg	2.50E-04	Histidine	g	0.17
Riboflavin	mg	3.22E-04	Alanine	g	0.48
Niacin	mg	2.69E-03	Aspartic acid	g	0.93
Pantothenic acid	mg	1.25E-03	Glutamic acid	g	3.45
Vitamin B-6	mg	5.39E-04	Glycine	g	0.25
Folate, total	mcg	8.78E-04	Proline	g	0.34
Vitamin A, IU	IU	2.66E-04	Serine	g	0.48
Vitamin E (alpha-tocopherol)	mg	3.22E-04			
Vitamin K (phylloquinone)	mcg	1.61E-06			

## 2.2. Lettuce

### 2.2.1. Elemental Composition

The elemental composition (Na, K, P, Mg, Ca, N, C, Mo, An, B, Mn, Fe, Cu) of lettuce is shown in Table 2.6 for each morphological part and under different growing conditions. The K, P, Mg and Ca content of lettuce leaves grown using hydroponics were increased compared to the field and were decreased for Zn, B, Mn, Fe and Cu (McKeehen, 1994). Analyzing the effect of CO<sub>2</sub>, the composition of K, P, Mg, Ca and N has lower values at the highest CO<sub>2</sub> level (10000ppm). Therefore, CO<sub>2</sub> level is a factor to be considered as an environmental condition influencing elemental condition.

In order to consider the change in composition along plant growth, staggered cultures performed in UoG provide highly valued information. However in one of the cultures most of the elements content has a statistically significant dependency on age, some discrepancies exist with the other replication due to significant differences between replication (Annex A). Hence, at least a third replication should be of high interest to study more in detail the composition changes along plant maturity.

Table 2.6 Lettuce Elemental and Mineral Composition.  
 (1) Culture conditions: Field Purdue University, sunlight, 12/12 h day/night photoperiod.  
 (2) Data obtained by Wheeler. Culture conditions: daylight and fluorescent lamps providing a PPF of 325  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 18/4 h day/night photoperiod, 23 °C, relative humidity 65%, using a modified 1/2 Hoagland nutrient solution.  
 (3) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of 450  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 14/10 h day/night photoperiod, 25/20 °C day/night, plant density 24 plant/m<sup>2</sup>, using nutrient solution shown in Table 5.1.

Growth Conditions	Cultivar	CO <sub>2</sub> levels ppm	Crop Age d	Lettuce Elemental Composition											Reference					
				Na %	K %	P %	Mg %	Ca %	N %	C %	Mo %	Zn %	B %	Mn %		Fe %	Cu %			
Leaves	Field	350	-	0.57	3.94	0.59	0.26	0.73	-	-	-	4.0E-03	-	1.0E-02	2.0E-02	2.0E-02	1.0E-03	USDA,2005		
	Field	350	-	0.0446	1.31	0.12	-	0.34	-	-	-	-	-	-	6.9E-03	-	-	Watt and Merrill, 1975		
	Soil+ NPK	Waldmann's Green	-	51	0.008	3.12	0.31	0.18	0.63	3.088	-	-	5.0E-03	2.0E-03	2.0E-02	1.4E-01	1.3E-03	1.3E-03	McKeehen,1994 (1)	
					0	8.27	0.68	0.23	0.72	4.72	-	-	1.0E-04	1.4E-03	1.3E-02	6.4E-03	4.0E-04	-	-	
					0	8.21	0.62	0.20	0.63	4.464	-	-	1.0E-04	1.5E-03	6.1E-03	5.2E-03	4.0E-04	-	-	McKeehen,1994 (2)
					0	8.48	0.63	0.22	0.70	4.768	-	-	1.0E-04	1.5E-03	9.3E-03	5.3E-03	4.0E-04	-	-	
	NFT	Waldmann's Green	10000	53	-	5.85	0.95	0.16	0.94	4.83	38.1	-	-	-	-	-	-	-	-	
					-	5.29	0.70	0.14	0.61	3.89	39.4	-	-	-	-	-	-	-	-	
					-	6.02	0.84	0.15	0.70	4.67	38.03	-	-	-	-	-	-	-	-	
					-	2.93	0.42	0.18	0.64	3.63	42.03	-	-	-	-	-	-	-	-	
					-	4.74	0.63	0.27	0.82	5.02	40.17	-	-	-	-	-	-	-	-	
	NFT	Grand Rapids	1000	30	-	4.74	0.87	0.29	0.77	5.25	39.43	-	-	-	-	-	-	-	-	
					-	7.84	0.77	0.29	1.30	5.40	41.10	-	-	-	-	-	-	-		
					-	7.71	0.94	0.30	1.13	5.32	40.07	-	-	-	-	-	-	-		
					-	8.04	1.16	0.32	1.09	5.65	37.80	-	-	-	-	-	-	-		
-					1.27	1.21	0.21	1.72	3.82	35	-	-	-	-	-	-	-			
Roots	Field	1000	53	-	1.27	1.21	0.21	1.72	3.82	35	-	-	-	-	-	-	-	-		
				-	1.74	1.58	0.12	1.35	4.00	35.1	-	-	-	-	-	-				
				-	1.99	1.32	0.18	1.31	3.80	37.3	-	-	-	-	-	-				
	Soil+ NPK	Waldmann's Green	1000	30	-	3.16	0.68	0.26	1.31	5.26	37.80	-	-	-	-	-	-	-		
					-	3.12	0.89	0.28	1.59	5.62	37.87	-	-	-	-	-	-			
					-	2.54	1.09	0.40	1.56	4.48	34.18	-	-	-	-	-	-			
					-	5.09	0.93	0.75	2.70	4.25	32.30	-	-	-	-	-	-			
					-	6.23	1.16	0.70	2.48	4.60	30.10	-	-	-	-	-	-			
	NFT	Grand Rapids	1000	40	-	6.23	1.16	0.70	2.48	4.60	30.10	-	-	-	-	-	-	-		
					-	7.48	1.46	0.56	2.53	4.59	32.50	-	-	-	-	-	-			
					-	1.27	1.21	0.21	1.72	3.82	35	-	-	-	-	-	-			
					-	1.74	1.58	0.12	1.35	4.00	35.1	-	-	-	-	-	-			
					-	1.99	1.32	0.18	1.31	3.80	37.3	-	-	-	-	-	-			

A study of Na, K, and Ca composition of lettuce under salinity stress was carried out by Bie *et al.* (2003). The study reports that increasing salinity levels, values of leaf area, dry weight, photosynthetic rate and stomatal conductance diminished. Moreover, using Na<sub>2</sub>SO<sub>4</sub>, the content of K and Ca decreased, whereas Na content increased (Table 2.7). In comparison to this, under NaHCO<sub>3</sub> stress the K content decreased and Na content increased. They mentioned that the rapid uptake of Na and the decrease in K content, leading to a decrease in K/Na ratio, would have influenced the K/Na selectivity in the root system and disrupted the regular osmotic adjustment resulting in osmotic stress (Bie *et al.*, 2003).

Table 2.7 Lettuce Elemental and Mineral Composition under salt stress (Bie *et al.*, 2003). Culture conditions: butterhead cultivars ('P' Sumitomo Chemistry, and 'L-2', Mikado Seed) growth using the hydroponics technique of Deep Water Culture under PPF of 1150  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Crop age at harvest is 39 days for sulphates cultures and 34 days for carbonates cultures.

Na <sub>2</sub> SO <sub>4</sub> mM	Cultivar	CO <sub>2</sub> uptake $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	Mineral Composition		
			Na %	K %	Ca %
<b>Leaves</b>					
0	P	8.6	0.23	14.976	1.12
	L-2	9.3	0.299	13.845	0.88
20	P	8.5	1.449	11.544	0.56
	L-2	8.8	2.714	10.686	0.56
40	P	8.4	2.208	9.048	0.44
	L-2	8.4	3.358	9.36	0.44
60	P	7.7	2.76	8.229	0.4
	L-2	7.9	4.071	8.229	0.4
<b>Roots</b>					
0	P	8.6	0.276	11.778	0.92
	L-2	9.3	0.322	10.998	0.68
20	P	8.5	2.507	10.647	0.44
	L-2	8.8	1.265	9.75	0.48
40	P	8.4	3.519	9.126	0.36
	L-2	8.4	2.139	8.385	0.4
60	P	7.7	3.887	8.463	0.36
	L-2	7.9	2.668	7.761	0.36

NaHCO <sub>3</sub> mM	Cultivar	Mineral Composition		
		Na %	K %	Ca %
<b>Leaves</b>				
0	P	0.276	16.419	1.04
	L-2	0.23	15.912	1.24
2.5	P	0.506	14.742	1.04
	L-2	0.529	15.951	1
5	P	0.828	14.898	1.04
	L-2	0.805	14.391	0.96
7.5	P	1.081	14.898	1
	L-2	1.035	13.455	0.92
<b>Roots</b>				
0	P	0.253	12.09	1.32
	L-2	0.23	11.466	0.96
2.5	P	0.391	11.973	1.88
	L-2	0.391	11.037	1.8
5	P	0.483	12.909	1.12
	L-2	0.529	11.505	1.12
7.5	P	0.598	11.466	1.36
	L-2	0.713	10.998	1.2

### 2.2.2. Macromolecular Composition

The macromolecular composition of lettuce under different culture conditions is present in Table 2.8. Although most of the data correspond to protein, fat, ash, carbohydrate, energy and moisture content, some data related to the total fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin has also been found.

Table 2.8 Lettuce Proximate Composition

- (1) Culture conditions: Field Purdue University, sunlight, 12/12 h day/night photoperiod.  
 (2) Data obtained by Wheeler, R.M. in Growth Chamber located at Kennedy Space Center. Culture conditions: daylight and fluorescent lamps providing a PPF of 325  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 18/4 h day/night photoperiod, 23 °C, relative humidity 65%, using a modified 1/2 Hoagland nutrient solution.  
 (3) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF between 280-336  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 16/8 h day/night photoperiod, 23 °C, relative humidity 75%, plant density 19.2 plant/m<sup>2</sup>, using a modified 1/2 Hoagland nutrient solution, biomass between 5.8-7.7 g dw/plant and Edible yield between 5.4 and 7.1 g dw/(m<sup>2</sup> d). Energy calculated by assigning 4 kcal/g CH, 4 kcal/g protein and 9 kcal/g fat.  
 (4) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF of 450  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 14/10 h day/night photoperiod, 25/20 °C day/night, plant density 24 plant/m<sup>2</sup>, using nutrient solution shown in Table 5.1.

Growth Condition	Cultivar	CO <sub>2</sub> levels ppm	Crop Age d	Proximate composition										Energy cal/g	Moisture %	Reference	
				Protein %	Fat %	Ash %	CH %	Fiber %	ADF %	NDF %	Lignin %						
<b>Leaves</b>																	
Field	-	350	-	27.59	3.04	12.58	56.59	26.37	-	-	-	-	-	-	3043	-	USDA,2005
Field	-	350	-	21.7	5	15	58.3	-	-	-	-	-	-	-	-	-	Watt and Merrill, 1975
Soil+ NPK	Waldmann's Green	-	51	17.7	4.2	15.6	62.5	-	-	-	-	-	-	-	3905	-	McKeehen, 1994 (1)
NFT	Waldmann's Green	400		24	3.6	18.4	54	-	-	-	-	-	-	-	3798	-	McKeehen, 1994 (2)
		1000		21.8	3.2	16.9	58.1	-	-	-	-	-	-	-	3690	-	
		5000		22	3.5	17.6	56.9	-	-	-	-	-	-	-	3698	-	
NFT	Waldmann's Green	10000		19.7	2.6	15.3	62.4	-	-	-	-	-	-	-	3711	-	Wheeler, 1995 (3)
		1000		24.6	8.2	22.4	33.6	11.1	-	-	-	-	-	-	3070	-	
		1000		30.2	4.1	22	32.7	11.1	-	-	-	-	-	-	2880	-	
NFT	Grand Rapids	1000		27.2	4.5	21.8	37	9.4	-	-	-	-	-	-	2970	-	UoG Annex A (4)
				27.8	1.6	21.1	39.8	9.7	-	-	-	-	-	-	2850	-	
				22.58	2.00	6.88	58.00	-	-	-	-	-	-	-	3400	10.59	
				30.35	2.10	10.84	45.50	-	-	-	-	-	-	-	3220	11.22	
				31.58	1.92	11.28	44.12	-	-	-	-	-	-	-	3200	11.13	
NFT	Grand Rapids	1000		32.68	1.70	16.30	41.80	-	-	-	-	-	-	3130	7.51	UoG Annex A (4)	
				32.14	2.33	16.83	40.30	-	-	-	-	-	-	3107	8.39		
				33.94	2.27	18.56	36.87	-	-	-	-	-	-	3033	8.37		
<b>Roots</b>																	
NFT	Grand Rapids	1000	50	26.58	0.97	22.88	42.10	-	-	-	-	-	-	-	2837	7.46	UoG Annex A (4)
			40	29.76	1.20	30.70	32.30	-	-	-	-	-	-	-	2590	6.03	
			50	28.50	1.20	28.10	36.20	-	-	-	-	-	-	-	2690	6.06	

In comparison with field grown plants, lettuce leaves have higher protein and ash levels. In hydroponics cultures, nutrients are available in higher levels than in soil. Wheeler *et al.* (2005) suggest that this fact may have led to a luxuriant uptake of some nutrients (particularly K and N), which might increase ash and protein levels in plant tissue. Accordingly, Davis *et al.* (1988) found enhanced leaf protein content (27-36%) compared to the field caused by all N nutrition treatments applied under controlled environments. Moreover and increase in nitrogen leads to a better yield per unit area, but also an increase in non protein N, such as nitrate (Aldrich, 1980).

Carbohydrates have lower values in lettuce if grown using hydroponics than cultivated in field. Under a CO<sub>2</sub> enriched atmosphere higher lettuce yield is obtained (Knecht and O’Leary, 1983; Knight and Mitchell, 1988). High CO<sub>2</sub> concentrations produce not only a lower protein and fat content, but also a decrease in the nitrate accumulation in lettuce. Due to this fact, CO<sub>2</sub> levels is attempted to be an interesting strategy for controlling nitrate accumulation in tissue other than diminishing nitrate composition in solution (McKeehen, 1994).

In Table 2.9 lipid, vitamin and amino acid composition of lettuce is shown.

Table 2.9 Lettuce lipid, vitamin and amino acid composition (USDA, 2005)

Lipids	Units	Value per 100 g dw	Amino Acids	Units	Value per 100 g dw
Fatty acids, total saturated	g	0.406	Tryptophan	g	0.18
16:00	g	0.365	Threonine	g	1.20
18:00	g	0.041	Isoleucine	g	1.70
Fatty acids, total monounsaturated	g	0.122	Leucine	g	1.60
16:1 undifferentiated	g	0.041	Lysine	g	1.70
18:1 undifferentiated	g	0.101	Methionine	g	0.32
Fatty acids, total polyunsaturated	g	1.663	Cystine	g	0.32
18:2 undifferentiated	g	0.487	Phenylalanine	g	1.12
18:3 undifferentiated	g	1.176	Tyrosine	g	0.65
Phytosterols	mg	0.771	Valine	g	1.42
<b>Vitamins</b>			Arginine	g	1.44
Vitamin C, total ascorbic acid	mg	3.65E-01	Histidine	g	0.45
Thiamin	mg	1.42E-03	Alanine	g	1.14
Riboflavin	mg	1.62E-03	Aspartic acid	g	2.88
Niacin	mg	7.61E-03	Glutamic acid	g	3.69
Pantothenic acid	mg	2.72E-03	Glycine	g	1.16
Vitamin B-6	mg	1.83E-03	Proline	g	0.97
Folate, total	mcg	7.71E-04	Serine	g	0.79
Vitamin A, IU	IU	1.50E-01			
Vitamin A, RAE	mcg	7.51E-03			
Vitamin E (alpha-tocopherol)	mg	5.88E-03			
Tocopherol, gamma	mg	7.51E-03			
Tocopherol, delta	mg	2.03E-04			
Vitamin K (phylloquinone)	mcg	3.52E-03			

### 2.3. Wheat

Wheat growth rate increases in direct proportion to increase in PPF (Salisbury *et al.*, 1987). Wheat yields were increased a 25% by elevating CO<sub>2</sub> from 350 to 700 ppm (Bugbee and Salisbury, 1985).

Smart *et al.* (1998) examined the hypothesis that elevated CO<sub>2</sub> concentrations would increase nitrate absorption. The cultivar “Veery-10” were grown hydroponically (NFT), with HPS lamps providing 1000 μmol/(m<sup>2</sup> · s), under a photoperiod of 18/6 day/night and with a high plant density (1780 plants/m<sup>2</sup>) under two levels of CO<sub>2</sub> (360 and 1000 ppm) and two different nitrate concentration in the nutrient solution (100 and 1000 mmol/m<sup>3</sup>). The productivities obtained were higher when nitrate and/or CO<sub>2</sub> had elevated values, ranging 43 g dw/(m<sup>2</sup> d) for grain and 6-8 g dw/(m<sup>2</sup> d) for roots. Evapotranspiration rates were 4.82 L H<sub>2</sub>O/(m<sup>2</sup>d) at 360 ppm CO<sub>2</sub> and 3.26 at 1000 ppm CO<sub>2</sub>. They concluded that under high CO<sub>2</sub> levels (1000 ppm), wheat presents higher nitrate consumption, but most of this increase did not lead to higher nitrogen content in plant tissue.

André *et al.* (1989) performed a wheat culture in controlled environment chambers under high CO<sub>2</sub> concentration (800 ppm) with an irradiance of 800 μmol/(m<sup>2</sup> · s), photoperiod of 14/10 and plant density of 80 plants/m<sup>2</sup>. The evapotranspiration was found to decrease a 20% under high CO<sub>2</sub> values, from 6 to 4.62 L H<sub>2</sub>O/(m<sup>2</sup>d). Nutrient uptake was slightly higher at elevated CO<sub>2</sub> levels, with the following averages (expressed in mmol/(m<sup>2</sup>d)): 60 NO<sub>3</sub><sup>-</sup>, 12 K, 6 NH<sub>4</sub><sup>+</sup> and HPO<sub>4</sub><sup>2-</sup>.

#### 2.3.1. Elemental Composition

Mineral composition reported in Table 2.10 include Na, K, P, Mg, Ca, N, Mo, Zn, B, Mn, Fe, and Cu content for each wheat part (grain, chaff, straw and roots).

McKeehen (1994) concluded that grain Zn and Fe were decreased in controlled environments compared to the field, whereas other elements in the grain maintained similar values. In addition to this, the controlled environment straw had higher contents of K, P, Ca and Cu compared to field straw.

#### 2.3.2. Macromolecular Composition

Proximate composition for each wheat part is presented in the following tables. First, Table 2.11 shows protein, fat, ash, carbohydrate, fiber and energy content for wheat grown in field and in controlled environments. Then, amino acid composition in wheat grain is listed in Table 2.12 under different growing conditions. Finally, lipid and vitamin composition is included in Table 2.14.

The proximate composition of wheat changes along morphological parts. Protein and fat content in grain is higher than in the other parts, whereas chaff and straw are richer in ashes, carbohydrates and fibers. Protein composition in grain usually present higher values than the range of 11.18% mentioned in Hoff *et al.* (1982) and Gauer *et al.* (1992). Fat composition is about 0.4 - 1.8 % in Yecora Rojo cultivar and 0.6 – 2.3 % in Veery-10 variety for all plants parts.

Table 2.10 Wheat elemental composition

(1) Data obtained by Bugbee, B. in the field at Utah State University. Culture conditions: Sunlight, 12/12 h day/night photoperiod  
 (2) Data obtained by Bugbee, B. in Growth Chamber located at Utah State University. Culture conditions: High Pressure Sodium (HPS) lamps providing a PPF of 1200  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 24 h photoperiod, 23 °C, relative humidity 70%, using a modified 1/2 Hoagland nutrient solution with Deep Root Zone (DRZ) technique.  
 (3) Data obtained by Wheeler, R.M. in Biomass Production Chamber located at Kennedy Space Center. Culture conditions: HPS lamps providing a PPF of 750  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 20/4 h day/night photoperiod, 24-20/16 °C day/night, relative humidity 75%, using a modified 1/2 Hoagland solution with nutrient film technique (NFT).

Growth Conditions	Cultivar	CO <sub>2</sub> levels ppm	Crop Age d	Mineral Composition (%)													Reference
				Na	K	P	Mg	Ca	N	Mo	Zn	B	Mn	Fe	Cu		
				%	%	%	%	%	%	%	%	%	%	%	%	%	
<b>Grain</b>																	
Soil	-	350	-	0.01	1.00	0.95	0.27	0.04	-	-	0.01	-	-	0.01	0.01	0.001	USDA, 2005
Soil	-	350	-	3.4E-03	0.42	0.42	-	0.05	-	-	-	-	-	-	3.8E-03	-	Watt and Merrill, 1975
Soil	Yecora Rojo	350	105	1.9E-03	0.43	0.35	0.13	0.06	3.71	0	5.0E-03	0	3.4E-03	3.7E-03	5.0E-04	6.0E-04	McKeehen, 1994 (1)
	Veery-10			1.9E-03	0.49	0.42	0.15	0.06	3.66	1.0E-04	7.7E-03	0	4.2E-03	6.6E-03	6.0E-04		
DRZ	Yecora Rojo	1000	64	0	0.44	0.40	0.14	0.08	4.13	0	3.0E-03	0	3.8E-03	1.8E-03	8.0E-04	8.0E-04	McKeehen, 1994 (2)
	Veery-10			0	0.59	0.43	0.13	0.05	3.10	0	3.0E-03	0	3.1E-03	1.5E-03	6.0E-04	6.0E-04	
NFT	Yecora Rojo	1200	85	0	0.55	0.48	0.19	0.04	3.57	0	2.5E-03	0	4.0E-03	2.6E-03	5.0E-04	5.0E-04	McKeehen, 1994 (3)
<b>Chaff</b>																	
Soil	Yecora Rojo	350	105	2.0E-04	0.16	0.03	0.03	0.09	1.42	0	5.0E-04	0	7.0E-04	7.9E-03	2.0E-04	2.0E-04	McKeehen, 1994 (1)
	Veery-10			6.3E-03	0.73	0.22	0.17	0.40	2.03	1.0E-04	3.9E-03	2.0E-04	4.3E-03	4.1E-02	1.1E-03	1.1E-03	
DRZ	Yecora Rojo	1000	64	8.6E-03	1.27	0.32	0.12	0.25	1.94	1.0E-04	1.0E-03	3.1E-03	3.6E-03	5.3E-03	6.0E-04	6.0E-04	McKeehen, 1994 (2)
	Veery-10			8.9E-03	2.31	0.21	0.08	0.32	1.76	0	1.6E-03	2.9E-03	2.8E-03	4.9E-03	5.0E-04	5.0E-04	
NFT	Yecora Rojo	1200	85	2.4E-03	1.93	0.43	0.34	0.32	2.11	0	1.3E-03	5.8E-03	1.0E-02	1.1E-02	5.0E-04	5.0E-04	McKeehen, 1994 (3)
<b>Straw</b>																	
Soil	Yecora Rojo	350	105	1.2E-02	2.73	0.07	0.09	0.31	0.88	0	2.1E-03	0	1.7E-03	2.0E-02	4.0E-04	4.0E-04	McKeehen, 1994 (1)
	Veery-10			8.31	2.11	0.11	0.11	0.37	1.39	1.0E-04	2.4E-03	0	2.2E-03	1.8E-02	4.0E-04	4.0E-04	
DRZ	Yecora Rojo	1000	64	9.9E-03	4.52	0.18	0.10	0.53	2.30	3.0E-04	1.4E-03	2.3E-03	1.8E-03	1.0E-02	8.0E-04	8.0E-04	McKeehen, 1994 (2)
	Veery-10			1.3E-02	4.59	0.16	0.19	0.80	2.34	4.0E-04	2.6E-03	3.5E-03	2.8E-03	9.3E-03	8.0E-04	8.0E-04	
NFT	Yecora Rojo	1200	85	9.9E-03	6.69	0.35	0.31	0.52	3.73	0	8.0E-04	9.3E-03	5.8E-03	1.6E-02	5.0E-04	5.0E-04	McKeehen, 1994 (3)
<b>Roots</b>																	
NFT	Yecora Rojo	1200	85	1.2E-02	4.56	0.24	0.12	0.18	5.33	0	1.5E-03	4.8E-03	3.5E-03	8.4E-02	5.6E-03	5.6E-03	McKeehen, 1994 (3)

Table 2.11 Wheat macromolecular Composition.

(1) Data obtained by Bugbee, B. in the field at Utah State University. Culture conditions: Sunlight, 12/12 h day/night photoperiod

(2) Data obtained by Bugbee, B. in Growth Chamber located at Utah State University. Culture conditions: High Pressure Sodium (HPS) lamps providing a PPF of 1200  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 24 h photoperiod, 23 °C, relative humidity 70%, using a modified 1/2 Hoagland nutrient solution with Deep Root Zone (DRZ) technique.

(3) Data obtained by Wheeler, R.M. in Biomass Production Chamber located at Kennedy Space Center. Culture conditions: HPS lamps providing a PPF of 750  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 20/4 h day/night photoperiod, 24-20/16 °C day/night, relative humidity 75%, using a modified 1/2 Hoagland solution with nutrient film technique (NFT).

(4) Culture conditions: Metal Halide and High Pressure Sodium Lamps providing a PPF between 280-336  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 16/8 h day/night photoperiod, 23 °C, relative humidity 75%, plant density 19.2 plant/m<sup>2</sup>, using a modified 1/2 Hoagland nutrient solution, biomass between 5.8-7.7 g dw/plant and Edible yield between 5.4 and 7.1 g dw/(m<sup>2</sup> d). Energy calculated by assigning 4 kcal/g CH, 4 kcal/g protein and 9 kcal/g fat.

Growth Conditions	Cultivar	CO <sub>2</sub> levels ppm	Crop Age d	Proximate composition					Energy cal/g	Reference	
				Protein	Fat	Ash	CH	Fiber			
				%	%	%	%	%			
<b>Grain</b>											
Soil	-	350	-	26.05	10.94	4.74	58.28	14.85	4005	USDA, 2005	
Soil	-	350	-	16.3	2.3	1.9	79.5	-	-	Watt and Merrill, 1975	
Soil	Yecora Rojo	350	105	16.7	1.5	1.9	79.9	-	4173	McKeehen, 1994 (1)	
	Veery-10			18.6	1.4	2.1	77.9	-	4172		
DRZ	Yecora Rojo	1000	64	18.9	1.8	1.9	77.4	-	4122	McKeehen, 1994 (2)	
	Veery-10			16.5	2.3	2.1	79.1	-	4053		
NFT	Yecora Rojo	1200	85	18.9	1.4	2.3	77.3	-	4105	McKeehen, 1994 (3)	
NFT	Yecora Rojo	1000	85	77	18.4	3.2	2	73.3	2.5	3980	Wheeler, 1995 (4)
				86	20.9	3.1	2.1	71.6	3.2	3940	
				85	20.1	3.3	1.9	72.3	2.8	3950	
				85	17	2.9	2	75.7	2.4	4050	
<b>Chaff</b>											
Soil	Yecora Rojo	350	105	5.2	1.1	13.9	79.8	-	-	McKeehen, 1994 (1)	
	Veery-10			6.5	1.9	10.7	80.9	-	-		
DRZ	Yecora Rojo	1000	64	5.4	0.7	4	89.9	-	-	McKeehen, 1994 (2)	
	Veery-10			6.4	0.8	5.6	87.2	-	-		
NFT	Yecora Rojo	1200	85	8	1.1	6	84.9	-	-	McKeehen, 1994 (3)	
<b>Straw</b>											
Soil	Yecora Rojo	350	105	3.4	1	11	84.6	-	-	McKeehen, 1994 (1)	
	Veery-10			4.3	1.3	9.6	84.9	-	-		
DRZ	Yecora Rojo	1000	64	4.5	1	10.7	83.9	-	-	McKeehen, 1994 (2)	
	Veery-10			4.8	1.4	11.6	82.2	-	-		
NFT	Yecora Rojo	1200	85	5.6	1.7	16.1	76.6	-	-	McKeehen, 1994 (3)	
<b>Roots</b>											
NFT	Yecora Rojo	1200	85	14.9	0.4	10.9	73.8	-	-	McKeehen, 1994 (3)	



Table 2.12 Wheat amino acid composition.

(1) Data obtained by Bugbee, B. in the field at Utah State University. Culture conditions: Sunlight, 12/12 h day/night photoperiod

(2) Data obtained by Bugbee, B. in Growth Chamber located at Utah State University. Culture conditions: High Pressure Sodium (HPS) lamps providing a PPF of  $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 24 h photoperiod, 23 °C, relative humidity 70%, using a modified 1/2 Hoagland nutrient solution with Deep Root Zone (DRZ) technique.

(3) Data obtained by Wheeler, R.M. in Biomass Production Chamber located at Kennedy Space Center. Culture conditions: HPS lamps providing a PPF of  $750 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , 20/4 h day/night photoperiod, 24-20/16 °C day/night, relative humidity 75%, using a modified 1/2 Hoagland solution with nutrient film technique (NFT).

Reference	USDA, 2005	McKeehen,1994 (1)		McKeehen,1994 (2)		McKeehen,1994 (3)	Sosulski,1990	
Growth conditions	Soil	Soil		DRZ		NFT	Soil	
Cultivar	-	Yecora Rojo	Veery-10	Yecora Rojo	Veery-10	Yecora Rojo	-	
CO <sub>2</sub> levels (ppm)	350	350		1000		1200	-	
Crop Age (d)	-	105		64		85	-	
Amino Acid (%)	Tryptophan	0.36	0.191	0.192	0.179	0.162	0.175	0.102
	Lysine	1.65	0.474	0.492	0.466	0.413	0.473	0.361
	Histidine	0.72	0.462	0.457	0.441	0.349	0.454	0.301
	Arginine	2.1	0.883	0.905	0.847	0.719	0.878	0.484
	Aspartic Acid	2.33	0.936	0.967	0.899	0.815	0.851	0.649
	Threonine	1.09	0.513	0.542	0.504	0.439	0.5	0.367
	Serine	1.24	0.855	0.917	0.893	0.734	0.853	0.605
	Glutamic Acid	4.49	5.848	6.121	6.444	4.858	5.974	3.68
	Cystine	0.52	0.433	0.439	0.424	0.392	0.42	0.315
	Glycine	1.6	0.764	0.81	0.801	0.67	0.788	0.511
	Alanine	1.66	0.775	0.753	0.724	0.632	0.718	0.468
	Valine	1.35	0.798	0.835	0.79	0.67	0.778	0.54
	Methionine	0.51	0.298	0.285	0.295	0.237	0.286	0.177
	Isoleucine	0.95	0.66	0.707	0.683	0.542	0.652	0.553
	Leucine	1.77	1.258	1.345	1.288	1.05	1.271	0.864
	Tyrosine	0.79	0.564	0.67	0.615	0.537	0.646	0.44
Phenylalanine	1.04	0.906	0.951	1.008	0.708	0.949	0.607	

Table 2.13 Wheat lipid and vitamin composition (USDA, 2005)

Lipids	Units	Value per 100 g dw
Fatty acids, total saturated	g	1.87
14:00:	g	0.01
16:00:	g	1.79
18:00:	g	0.06
Fatty acids, total monounsaturated	g	1.54
16:1 undifferentiated	g	0.04
18:1 undifferentiated	g	1.50
Fatty acids, total polyunsaturated	g	6.76
18:2 undifferentiated	g	5.95
18:3 undifferentiated	g	0.81

Vitamins	Units	Value per 100 g dw
Thiamin	mg	2.1E-03
Riboflavin	mg	5.6E-04
Niacin	mg	7.7E-03
Pantothenic acid	mg	2.5E-03
Vitamin B-6	mg	1.5E-03
Folate, total	mcg	3.2E-04
Folate, food	mcg	3.2E-04

The amino acid composition presents similar values for all the different cultures. Hoff *et al.* (1982) suggest that a complementation with proteins with higher levels of lysine and tryptophan is recommended, since wheat is deficient in them.

### 3. Conclusions

After the literature review, a list of key parameters driving the modification of the biomass quality has been identified and is presented next:

- Growing conditions (soil, hydroponics)
- Salinity of the irrigation solution
- N content in the nutrient solution
- CO<sub>2</sub> levels

The collected data does not record any significant influence of light intensity on biomass composition. Nevertheless it is known to have a significant influence on evapotranspiration rate and growth rate (productivity).

Not sufficient composition data in young plants has been found to evaluate the possible change in mineral and proximate tissue content along plant age.

Most of the references with elemental or macromolecular composition of the plants didn't provide the corresponding yield and kinetic information. Due to this fact, it is concluded that no sufficient data is currently available to develop a dynamic model for the biomass quality under varying conditions. Therefore, further experiments should be planned to provide this kind of information.

Nevertheless the available data allows to make an initial evaluation of composition of mature plants and global nutrient consumption to produce them. With this information preliminary elemental mass balances at steady state (assuming collection of only mature plants) could be done to complement previously done evaluations.

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### 5. Annex A: Higher plant composition data from University of Guelph

Empirical production trials have been carried out in UoG to collect baseline data sets for two of the three MELiSSA candidate crops: beet (*Beta vulgaris* cv. Detroit Medium Red) and lettuce (*Lactuca sativa* L. cv. Grand Rapids).

Since 2004 part of the data collected from the batch (single seeding date) and staged (multiple seeding dates) cultures performed in SEC chambers is lettuce and beet tissue composition analysis for each part of the part. Data available corresponds to three batch replications and two staged replications of each crop at the full canopy scale.

After describing the materials and methods of the UoG cultures, beet and lettuce compositions are presented and discussed separately.

#### 5.1. Materials and methods

##### 5.1.1. Higher Plant Chamber description

All experiments were conducted in the SEC chambers located at the UoG CESRF. These two identical plant growth chambers, measuring 4.5 m x 2.8 m x 2.3 m (LxWxH) internally, have a 5 m<sup>2</sup> growing culture area. Although a detailed description of the chamber can be found in the paper authored by Dixon et al. (1999) a brief one is included next.

###### 5.1.1.1. Lighting system

Overhead irradiation was provided by six 400 Watt metal halide (MH) and nine 600 Watt high pressure sodium (HPS) lamps (PL Light Systems, Grimsby, Ontario), positioned over each chamber and mounted externally to the chambers.

Photosynthetically active radiation (PAR), measured at bench height using a LI-COR LI-915A Quantum Sensor (LI-COR Inc, Lincoln, NE, USA), was between 400-450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Plants were grown under conditions of 14/10 hr light/dark (20:00 - 10:00) photoperiod.

###### 5.1.1.2. Liquid system

Plants were watered using nutrient film technique (NFT). The nutrient solution, stored in a 200L Teflon<sup>TM</sup> tank located outside the chamber, is continuously pumped (Model OM-3435: Setcho, Hauppauge, NY) and distributed into the 10 stainless steel growing trays. The hydroponic solution flows by gravity along the 2.5m long trays to a stainless steel 250L tank located inside the chamber and finally it returns to the outside tank.

Flow regulation between the inside and outside tank is regulated by a valve, depending on an electro optic level sensor (GLL 110020, Levelite/Genelco, Port Huron, MI) located in the exterior tank to maintain to a constant level.

Evapotranspired water is condensed and collected by gravity into an outside tank. A floating level sensor activates a metering pump (Model HD; Barnant Co., MA), which returns condensed water into the external nutrient tank.

### 5.1.1.3. *Gas system*

Both chambers operate isolated from the exterior. All the major variables in the aerial environment, such as temperature, humidity, CO<sub>2</sub> and O<sub>2</sub> composition and pressure are monitored with several sensors and analytical systems.

- **Temperature Control**

Two fans mounted inside the chamber distribute the air through the growing area. Air temperature is controlled by modulated steam and chilled water valves (M100 Motor Activator, Johnson Controls). Temperature control range is 10-40 °C ± 0.2°C and the mean value among the culture is 26/20 °C day/night temperature

- **Pressure Control**

Atmospheric pressure inside the chamber is passively controlled by ten 200L double sealed Teflon TM liners (Now Technologies Inc., Minneapolis, MN) manifolded on a 50 mm diameter stainless steel tube which protrudes through the rear wall of the chamber. This provides a total expansion volume potential of 2 m<sup>3</sup> ± 1m<sup>3</sup>, which represent about 7% ± 3% volume expansion/contraction.

- **Atmospheric Composition Control**

Oxygen concentration is monitored continuously while atmospheric CO<sub>2</sub> concentration is maintained at 1000 µL L<sup>-1</sup> CO<sub>2</sub>, as supplied through an external tank and computer regulated compensatory system using bottled CO<sub>2</sub>.

### 5.1.1.4. *Data Acquisition System*

An Allen-Bradley PLC-5/10 was used to achieve the control. Data acquisition is done using L.W. Anderson Software Consultant.

## 5.1.2. **Crops and culture media**

### 5.1.2.1. *Seeds*

Lettuce seeds (*Lactuca sativa* L., Grand Rapids, 45 days) and beet seeds (*Beta vulgaris* L., Betterave Detroit Medium Top, 63 days) have been provided by Stokes Seeds (Thorold, Ontario, Canada).

### 5.1.2.2. *Culture media*

The nutrient composition used for watering has the composition proposed by Hoagland, 1950 (Table 5.1). The pH of the solution is adjusted to 6 with the addition of 1 M NaHCO<sub>3</sub> solution. This solution had an average EC of 2000 µS cm<sup>-1</sup>.

**Table 5.1 Nutrient solutions composition used for beet and lettuce cultures (Hoagland, 1950). Solution A is separated from the remaining components in solution B to prevent precipitation.**

SOLUTION TYPE	COMPONENTS	CONCENTRACIÓN (mmol/L)
Macronutrients A	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	3.62
Macronutrients B	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.00
	$\text{KNO}_3$	5.00
	$\text{NH}_4\text{H}_2\text{PO}_4$	1.50
	$(\text{NH}_4)_2\text{SO}_4$	1.00
Micronutrients	$\text{H}_3\text{BO}_4$	0.020
	$\text{FeCl}_3$	0.025
	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.005
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.0035
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.0008
	$\text{H}_2\text{MoO}_4(85\%\text{MoO}_3)$	0.0005

### 5.1.3. Experimental Procedure

#### 5.1.3.1. Daily operation

Gas bottles pressure used for atmospheric control and analytical system ( $\text{CO}_2$ , Air,  $\text{N}_2$ ,  $\text{H}_2$ ,  $\text{CO}_2/\text{O}_2$  standard) are checked daily. The gas bottle is changed when pressure is lower than 400 psi..

Also the correct nutrient pump and condensed water pump is verified every day.

Gas flows form the gas analysis loop and oxygen composition is recorded daily and the proper operation of the analytical devices is also supervised.

Regarding the control system, alarm comments are checked to detect any control system disfunction.

#### 5.1.3.2. Nutrient Solution Change

The nutrient solution is recirculated for five days and the completely replaced with fresh solution. At the start of each five day cycle solution EC was adjusted to  $2000 \mu\text{S cm}^{-1}$  and pH adjusted to 6.0, but during each period no amendments are made to the solution composition.

Every five days after, the solution is pumped out and its end volume is measured to allow for the correction of elemental analysis results due to evapotranspiration.

Three 25 mL samples are taken of the nutrient solution for off-line HPLC analysis.

#### 5.1.3.3. Harvest and Planting

Beet and lettuce seeds were initially germinated in a research greenhouse using Rockwool<sup>®</sup> cubes. During the germination and true leaf emergence period, seedlings were watered regularly with distilled water and once weekly with a dilute fertilizer solution (20-8-20 ppm N-P-K commercial. After 30 days for beet and 20 days for lettuce, the Rockwool<sup>®</sup> cubes containing seedlings were positioned in larger cubes (4" x 4" x 2.5", 625 cm<sup>3</sup>) to improve water distribution in the hydroponics channels. Trays were

covered once the blocks were in position so as to minimize the growth of algae on the surface of the Rockwool<sup>®</sup>.

In batch cultures all the seeds were planted at once and harvested the same day after their growing period.

In staggered cultures a 10 days cycle between planting, based on an optimized menu system developed by Waters *et al.* (2002), was used.

For beet staged experiments 24 seedlings were transferred inside the chamber after 30 days of growing. Following the initial planting, the chamber was planted with 24 additional plants of beet at 10 day intervals. Thus, after 40 days in chamber the first plants reached the mature age of 70 days old and they were harvested while transferring 24 new seedlings. Since then a mature harvest was obtained during the full chamber stocking at a 10 day interval until the final harvest, where a 40, 50, 60 and 70 days old beets were harvested.

For lettuce staged experiments 36 seedlings were transferred inside the chamber after 20 days of seeding. Following the initial planting, the chamber was planted with 36 additional plants of beet at 10 day intervals. Thus, after 30 days in chamber the first plants reached the mature age of 50 days old and they were harvested while transferring 36 new seedlings. Since then a mature harvest was obtained during the full chamber stocking at a 10 day interval until the final harvest, where a 30, 40 and 50 days old lettuce were harvested.

### 5.1.4. Analytical methods

#### 5.1.4.1. Biomass Analysis

After harvesting, plant parts were sampled at the individual plant scale with the exception of roots. Harvested root material was pooled by each trough in the chamber.

- **Fresh weight**

All plant parts are weight just after harvesting.

- **Dry Weight**

Dry weight of each plant part is obtained after at least four days in a drying oven at 60°C. Water content was measured as the difference between fresh and dry weights.

- **Leaf Area**

Leaf area was measured on the plants harvested using a Li-Cor 3100 Leaf Area Meter (Li-Cor, Lincoln, NE, USA). Initial leaf area was determined on the remaining (unplanted) seedlings using a Li-Cor 3100 Leaf Area Meter (Li-Cor, Lincoln, NE, USA).

- **Mineral Analysis**

Harvested tissue was pooled for mineral (Ca, K, Mg, P, N and C) analysis. Edible and inedible fractions of each crop were analyzed using UoG Laboratory Services Protocol SNL-1020 and SNL-104.



The concentration of Ca, K, Mg, and P in plant materials was determined using a high temperature dry oxidation of the organic matter and the dissolution of the ash with hydrochloric acid. Mineral concentrations are determined using Varian atomic absorption and Technicon auto analyzer.

The method used to quantify the total N in plant samples is based on the Dumas Method, which consists of converting all the forms of N into gaseous nitrogen oxides (NO<sub>x</sub>) by combustion in an oxygen-rich atmosphere at about 1000°C, reducing the NO<sub>x</sub> gases catalytically (metallic copper, tungsten) to N<sub>2</sub> and quantifying the amount of nitrogen gas by thermal conductivity (Nollet, 2004).

The total carbon content in plant samples were measured using the combustion method E1019, approved by the American Society for Testing and Material, in a LECO SC-444. Plant samples are combusted at 135°C and the CO<sub>2</sub> produced is measured by an infrared detector.

- **Proximate Analysis**

The proximate analysis includes the determination of fat, protein, ash, carbohydrates, calorie and moisture content. Proximate tissue analysis was performed by UoG Laboratory Services.

Fat content was measured using the Soxhlet extraction procedure (AOAC-920.39, 1990). This method typically uses anhydrous diethyl ether as the non-polar solvent.

Protein content was determined as described in 'Crude protein in Food & Feed products by Combustion' (FC-PR-109).

Ash determination was done in accordance with AOAC Method 930.05 (1990). After determining the ash, fat and protein contents, carbohydrates content was calculated by difference.

Calorie content is obtained using a bomb calorimeter.

Plant tissue was analyzed for moisture content according to the AOAC Method 930.04 (1990)

- **Fiber Analysis**

The analyses of Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and Lignin from plant samples were carried out by Agri-Food Laboratories (AgTest) in Guelph.

Lignin and ADF content were determined simultaneously using a titration method as described in AOAC-973.18 (1990). ADF consists of cellulose, lignin, bound protein, and acid insoluble ash portions of a feed. Since these constituents are quite indigestible, ADF is a negative indicator of energy level in grains, i.e., as ADF increases, digestible energy is decreased.

NDF was measured using a gravimetric determination of amylase treated ADF in feeds (Merters, 2002). In addition to the components which make up ADF, NDF contains hemicelluloses, which are a more digestible fibre fraction. NDF values are good predictors of dry matter intake.

### 5.1.4.2. *Nutrient Solution Composition Analysis*

Nutrient solution samples are analyzed using the Dionex HPLC Model DX500 (Sunnyvale, CA, USA) for the ions of interest which included  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ .

### 5.1.4.3. *Atmospheric Composition Analysis*

SEC chambers are equipped a  $\text{CO}_2$  gas analyser IRGA LiCor LI6262 (Li-Cor Inc. Lincoln, NE, USA) which measures online the  $\text{CO}_2$  composition every 3 minutes. Oxygen is measured with an  $\text{O}_2$  analyser Model 100P (California Analytical Instruments).

### 5.2. Beet Experimental Data Available

Data collected in UoG corresponding to beet includes 3 replicates of batch cultures and 2 independent staged cultures. In Table 5.2 a summary of the main operational parameters for each of the replicates can be found.

**Table 5.2 .Experiment summary sheet for beet (*Beta vulgaris* cv. Detroit Medium Red) batch and staged cultures.**

Parameter	Batch Cultures			Staged Cultures	
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2
Identification	BB1	BB2	BB3	BS1	BS2
Photoperiod (h day/night)	14/10	14/10	14/10	14/10	14/10
Temperature (°C day/night)	25/20	25/20	25/20	25/20	25/20
Demand CO <sub>2</sub> (ppm)	1000	1000	1000	1000	1000
Hydroponics system	NFT	NFT	NFT	NFT	NFT
Number of plants in chamber	120	120	120	96	96
Production area (m <sup>2</sup> )	5	5	5	4	4
Planting density (plants m <sup>-2</sup> )	24	24	24	24	24
Crop Age at harvest (day)	72	68	66	40,50,60,70	40,50,60,70
Composition data collected	Mineral			Mineral, Proximate	Pending

Harvest and yield data collected for beet batch cultures are reported in TN 75.3 Table 2.2.1.

#### 5.2.1. Batch Cultures: Tissue Mineral Composition

A summary of data collected on tissue mineral composition for beet batch cultures is presented next.

Due to that plants were dissected at harvest and the different parts grouped into similar types of tissues, mineral composition (Ca, K, Mg, N, P and C) is quantified in a dry weight basis for each beet part (roots, hypocotyl and leaves). Therefore, if a mineral composition on a total plant biomass basis is desired, yield data at harvest should be used. First, composition results are shown separately for the 3 replicates in Table 5.3, Table 5.4, Table 5.5. Finally in Table 5.6, mean values over the experiments are calculated.

All the tables includes the following parameters: mean value, number of samples (n), variance, standard deviation (Std Dev.), standard error of the mean (SE Mean), lower confidence limit (LCL) and upper confidence limit (UCL) of the mean with a 0.95 probability.

Table 5.3 Beet mineral composition for experiment BB1 on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

Beet	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	1.90	5.88	0.47	5.40	1.30	35.40
n	3	3	3	3	3	3
Variance	0.0330	0.0489	0.0094	0.0004	0.0026	0.2100
Std Dev.	0.18	0.22	0.10	0.02	0.05	0.46
SE Mean	0.10	0.13	0.06	0.01	0.03	0.26
LCL Mean	1.45	5.33	0.23	5.35	1.17	34.26
UCL Mean	2.35	6.43	0.71	5.46	1.42	36.54
<b>Hypocotyl</b>						
Mean	0.26	3.33	0.07	3.31	0.59	39.20
n	3	3	3	3	3	3
Variance	0.0016	0.0044	-	0.0037	0.0001	0.4300
Std Dev.	0.04	0.07	-	0.06	0.01	0.66
SE Mean	0.02	0.04	-	0.04	0.00	0.38
LCL Mean	0.16	3.17	0.07	3.16	0.57	37.57
UCL Mean	0.36	3.50	0.07	3.46	0.60	40.83
<b>Roots</b>						
Mean	2.19	1.54	0.48	4.45	0.48	37.50
n	1	1	1	1	1	1

Table 5.4 Beet mineral composition for experiment BB2 on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

Beet	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	1.87	6.57	0.28	4.56	1.56	35.20
n	3	3	3	3	3	3
Variance	0.0497	0.0142	0.0004	0.0091	0.0044	0.0900
Std Dev.	0.22	0.12	0.02	0.10	0.07	0.30
SE Mean	0.13	0.07	0.01	0.06	0.04	0.17
LCL Mean	1.31	6.28	0.23	4.32	1.39	34.45
UCL Mean	2.42	6.87	0.33	4.80	1.72	35.95
<b>Hypocotyl</b>						
Mean	0.25	3.52	0.08	3.69	0.72	39.03
n	3	3	3	3	3	3
Variance	0.0001	0.0097	0.0001	0.0052	0.0001	0.4433
Std Dev.	0.01	0.10	0.01	0.07	0.01	0.67
SE Mean	0.01	0.06	0.01	0.04	0.0049	0.38
LCL Mean	0.22	3.28	0.05	3.51	0.70	37.38
UCL Mean	0.28	3.76	0.11	3.87	0.74	40.69
<b>Roots</b>						
Mean	1.04	1.20	0.16	4.00	0.74	39.40
n	1	1	1	1	1	1

Table 5.5 Beet mineral composition for experiment BB3 on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

Beet	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	1.63	6.11	0.28	4.62	1.50	36.23
n	3	3	3	3	3	3
Variance	0.0072	0.0212	0.0007	0.0090	0.0023	0.0633
Std Dev.	0.09	0.15	0.03	0.10	0.05	0.25
SE Mean	0.05	0.08	0.02	0.05	0.03	0.15
LCL Mean	1.42	5.75	0.21	4.38	1.38	35.61
UCL Mean	1.84	6.48	0.35	4.85	1.62	36.86
<b>Hypocotyl</b>						
Mean	0.19	2.92	0.06	3.08	0.57	39.13
n	3	3	3	3	3	3
Variance	0.0007	0.0309	-	0.0156	0.0001	0.4233
Std Dev.	0.03	0.18	-	0.13	0.01	0.65
SE Mean	0.02	0.10	-	0.07	0.01	0.38
LCL Mean	0.12	2.48	0.06	2.77	0.54	37.52
UCL Mean	0.26	3.36	0.06	3.39	0.59	40.75
<b>Roots</b>						
Mean	1.83	1.32	0.38	4.03	0.50	38.40
n	1	1	1	1	1	1

Table 5.6 Beet mineral composition mean over experiments (BB1, BB2 and BB3) on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

(1).- A statistically significant effect among the 3 replicates has found in the ANOVA analysis.

(2).- Not enough data to perform an ANOVA analysis.

Beet	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>		(1)	(1)	(1)	(1)	(1)
Mean	1.80	6.19	0.34	4.86	1.45	35.61
n	9	9	9	9	9	9
Variance	0.0389	0.1152	0.0113	0.1713	0.0163	0.3161
Std Dev.	0.20	0.34	0.11	0.41	0.13	0.56
SE Mean	0.07	0.11	0.04	0.14	0.04	0.19
LCL Mean	1.65	5.93	0.26	4.54	1.35	35.18
UCL Mean	1.95	6.45	0.42	5.18	1.55	36.04
<b>Hypocotyl</b>		(1)		(1)	(1)	
Mean	0.23	3.26	0.07	3.36	0.62	39.12
n	9	9	9	9	9	9
Variance	0.0016	0.0820	0.0001	0.0766	0.0051	0.3294
Std Dev.	0.04	0.29	0.01	0.28	0.07	0.57
SE Mean	0.01	0.10	0.003	0.09	0.02	0.19
LCL Mean	0.20	3.04	0.06	3.15	0.57	38.68
UCL Mean	0.26	3.48	0.08	3.57	0.68	39.56
<b>Roots</b>		(2)	(2)	(2)	(2)	(2)
Mean	1.69	1.35	0.34	4.16	0.58	38.43
n	3	3	3	3	3	3
Variance	0.3460	0.0297	0.0268	0.0633	0.0201	0.9033
Std Dev.	0.59	0.17	0.16	0.25	0.14	0.95
SE Mean	0.34	0.10	0.09	0.15	0.08	0.55
LCL Mean	0.23	0.92	-0.07	3.54	0.22	36.07
UCL Mean	3.15	1.78	0.75	4.78	0.93	40.79

### 5.2.2. Staged Cultures: Tissue Mineral and Proximate Composition

Although two replicates for beet staged cultures have already been performed, only the tissue composition data for one of them was available when writing this document.

For staggered cultures, tissue composition was not only analysed by plant part but also by plant age, so that any composition change along growth could be detected. As mentioned in the methodology section, every 10 days during the full chamber stocking 24 beets were harvested until the final harvest, where 24 plants of each age (40, 50, 60 and 70 days old) were collected.

Thus, in order to visualize the tissue composition among the plant age, mineral and proximate composition for each beet part is depicted in Figure 5.1 and in Figure 5.2.

A linear regression analysis was done to evaluate whether the mineral and proximate composition significantly change among the plant growth. Table 5.7 and Table 5.8 summarize the statistics parameters values of this analysis, which include the linear regression slope, its standard error, t- statistics (t), p-value (p) and the degrees of freedom (df).

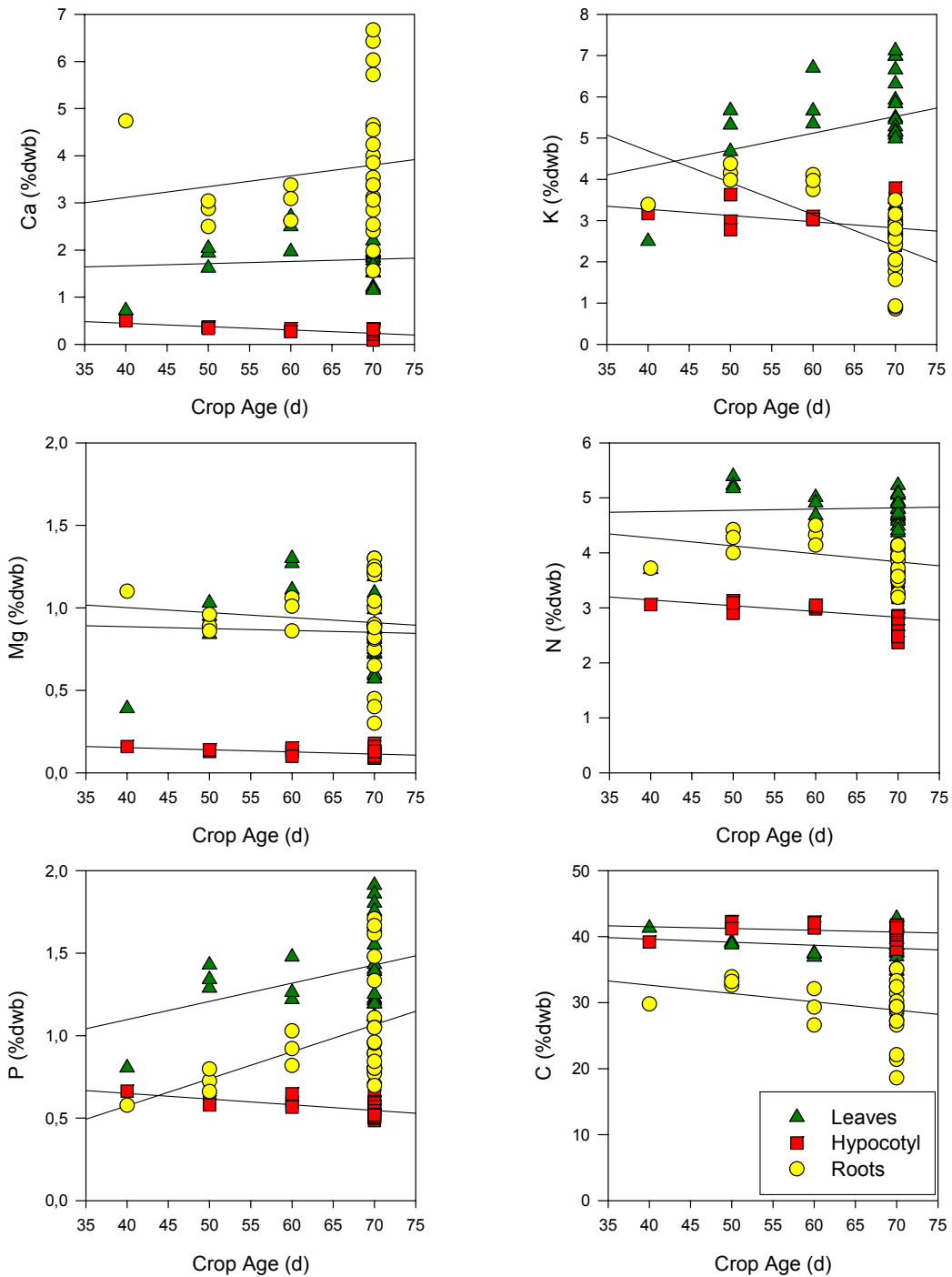


Figure 5.1 Beet mineral composition for experiment BS1 on a percent dry weight (dwb) basis for each part (▲ leaves; ■ hypocotyl, ● roots).

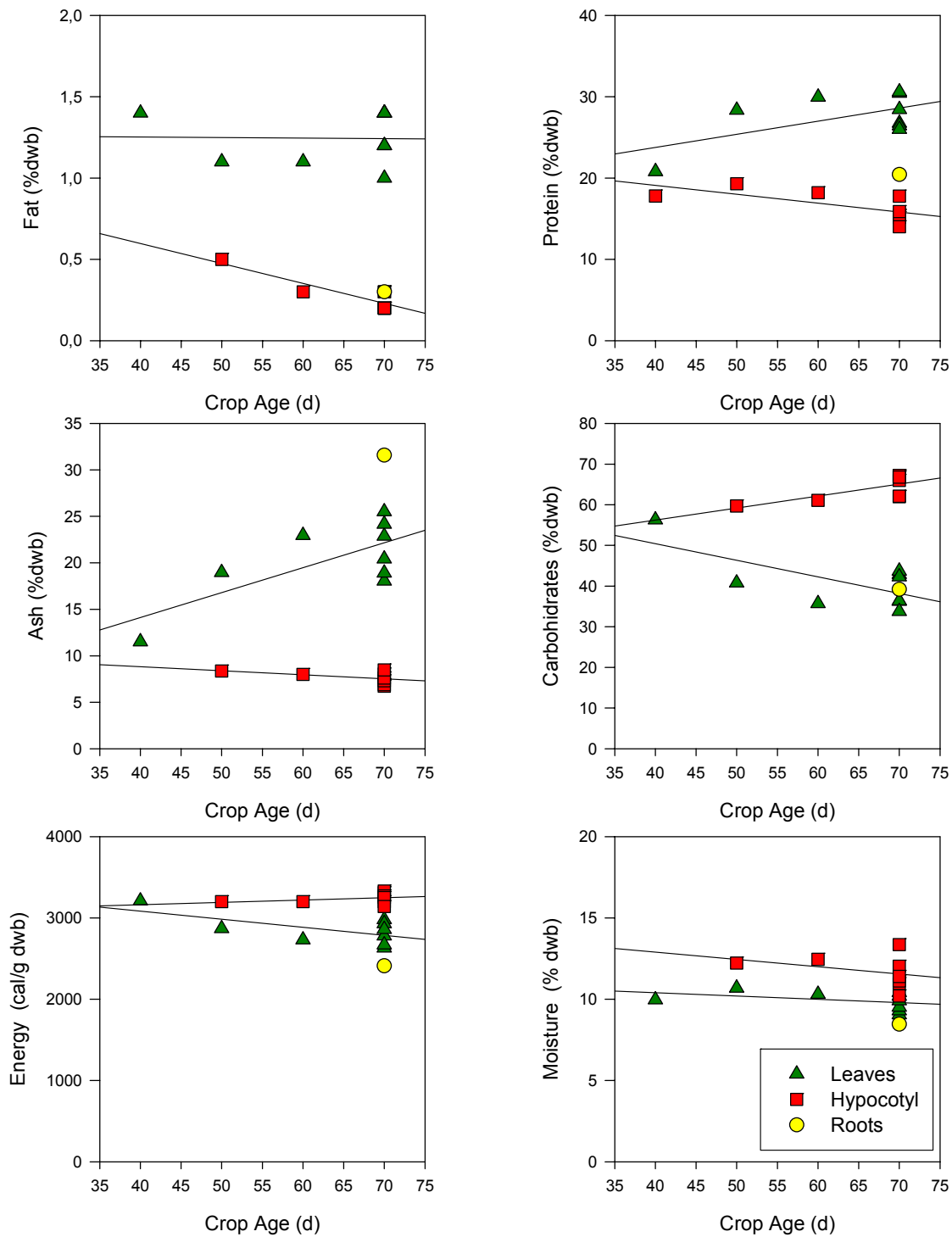


Figure 5.2 Beet proximate composition for experiment BS1 on a percent dry weight (dwb) basis for each part (▲ leaves; ■ hypocotyl, ● roots).



Table 5.7 Linear regression parameters for beet mineral composition at different crop ages of staggered culture BS1. Statistics parameters include t-statistics, p-values and degrees of freedom (df).

(\*) Significant differences exist among the crop age

Mineral	Slope	Std. Error	t	p	df
<b>Leaves</b>					
Ca	0.0046	0.0099	0.4620	0.6482	24
K	0.0405	0.0268	1.5133	0.1433	24
Mg	-0.0011	0.0054	-0.2136	0.8326	24
N	0.0023	0.0082	0.2761	0.7849	24
P	0.0110	0.0066	1.6855	0.1049	24
C	-0.0458	0.0529	-0.8660	0.3951	24
<b>Hypocotyl</b>					
Ca	-0.0071	0.0018	-3.9426	0.0006*	24
K	-0.0151	0.0083	-1.8314	0.0795	24
Mg	-0.0013	0.0005	-2.6059	0.0155	24
N	-0.0105	0.0061	-1.7277	0.0969	24
P	-0.0034	0.0009	-3.8184	0.0008*	24
C	-0.0271	0.0264	-1.0251	0.3155	24
<b>Roots</b>					
Ca	0.0230	0.0317	0.7266	0.4745	24
K	-0.0771	0.0185	-4.1608	0.0004*	24
Mg	-0.0031	0.0063	-0.4876	0.6303	24
N	-0.0145	0.0074	-1.9501	0.0629	24
P	0.0164	0.0067	2.4423	0.0223*	24
C	-0.1265	0.0933	-1.3562	0.1877	24

Upon the results obtained, beet mineral composition remains almost constant in each beet part along the plant growth. However, phosphorous content in hypocotyl diminishes along plant age while its content in roots increases. Moreover, calcium percentage in hypocotyl and potassium composition in roots has a statistically significant decrease along beet maturity.

In beet leaves the content of fat, protein, energy and moisture has no significant variation along plant growth, whereas ash composition increases and carbohydrates diminish along plant maturity. In regard to beet hypocotyl composition, only fat, protein and carbohydrates are significantly affected by plant age.

No linear regression analysis in roots proximate content was performed, since its value was only available for the oldest plants.

Table 5.8 Linear regression parameters for beet proximate composition at different crop ages of staggered culture BS1. Statistics parameters include t-statistics, p-values and degrees of freedom (df).

(\*) Significant differences exist among the crop age

Proximate	Slope	Std. Error	t	p	df
<b>Leaves</b>					
Fat	-0.0003	0.0054	-0.0620	0.9523	7
Protein	0.1614	0.0834	1.9347	0.0943	7
Ash	0.2681	0.0999	2.6848	0.0313*	7
Carbohydrates	-0.4073	0.1707	-2.3866	0.0484*	7
Energy	-0.9933	0.4703	-2.1122	0.0726	7
Moisture	-0.0202	0.0167	-1.2061	0.2670	7
<b>Hypocotyl</b>					
Fat	-0.0123	0.0027	-4.5637	0.0038*	6
Protein	-0.1090	0.0430	-2.5342	0.0390*	7
Ash	-0.0438	0.0313	-1.4004	0.2109	6
Carbohydrates	0.2948	0.1160	2.5407	0.0440*	6
Energy	0.2903	0.3311	0.8769	0.4143	6
Moisture	-0.0448	0.0514	-0.8725	0.4165	6

### 5.3. Lettuce Experimental Data Available

Experimental conditions used for lettuce cultures (3 batch and 2 staggered replications) are summarized in Table 5.9.

Table 5.9 .Experiment summary sheet for lettuce (*Lactuca sativa* L. cv. Grand Rapids) batch and staged cultures.

Parameter	Batch Cultures			Staged Cultures	
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2
Identification	LB1	LB2	LB3	LS1	LS2
Photoperiod (h day/night)	14	14	14	14	14
Temperature (°C day/night)	25/20	25/20	25/20	25/20	25/20
Demand CO <sub>2</sub> (ppm)	1000	1000	1000	1000	1000
Hydroponics system	NFT	NFT	NFT	NFT	NFT
Number of plants in chamber	120	120	120	108	108
Production area (m <sup>2</sup> )	5	5	5	4.5	4.5
Planting density (plants m <sup>-2</sup> )	24	24	24	24	24
Crop Age at harvest (day)	53	57	64	30, 40, 50	30, 40, 50
Composition data collected	Mineral			Mineral, Proximate	Mineral, Proximate, Fiber

Harvest and yield data collected for batch beet cultures are reported in TN 75.3 Table 2.2.2.

### 5.3.1. Batch Cultures: Tissue Mineral Composition

In the following tables tissue mineral composition for lettuce batch cultures, expressed as a percentage of dry weight for each lettuce part, is shown. Results are presented using the same structure as in the previous section for beet batch cultures. Thus, mineral composition is reported first individually for each experiment in Table 5.10, Table 5.11 and Table 5.12 and later a mean value among the 3 replications is included in Table 5.13. Statistical parameters included refer to the number of samples sent for analysis (n), variance, standard deviation (Std Dev.), standard error of the mean (SE Mean), lower and confidence limits (LCL and UCL respectively) at 95%.

Table 5.10 Lettuce mineral composition for experiment LB1 on a percent dry weight (dwb) basis for each part (leaves and roots).

Lettuce	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	0.94	5.85	0.16	4.83	0.95	38.10
n	3	3	3	3	3	3
Variance	0.0037	0.0433	0.00003	0.1570	0.0004	0.1600
Std Dev.	0.06	0.21	0.01	0.40	0.02	0.40
SE Mean	0.04	0.12	0.003	0.23	0.01	0.23
LCL Mean	0.79	5.33	0.15	3.85	0.90	37.11
UCL Mean	1.09	6.37	0.18	5.82	1.00	39.09
<b>Roots</b>						
Mean	1.72	1.27	0.21	3.82	1.21	35.00
n	1	1	1	1	1	1

Table 5.11 Lettuce mineral composition for experiment LB2 on a percent dry weight (dwb) basis for each part (leaves and roots).

Lettuce	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	0.61	5.29	0.14	3.89	0.70	39.40
n	3	3	3	3	3	3
Variance	0.0049	0.0039	0.00003	0.0032	0.0054	0.2500
Std Dev.	0.07	0.06	0.01	0.06	0.07	0.50
SE Mean	0.04	0.04	0.003	0.03	0.04	0.29
LCL Mean	0.44	5.13	0.12	3.75	0.52	38.16
UCL Mean	0.78	5.45	0.15	4.03	0.88	40.64
<b>Roots</b>						
Mean	1.35	1.74	0.12	4.00	1.58	35.10
n	1	1	1	1	1	1

Table 5.12 Lettuce mineral composition for experiment LB3 on a percent dry weight (dwb) basis for each part (leaves and roots).

Lettuce	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	0.70	6.02	0.15	4.67	0.84	38.03
n	3	3	3	3	3	3
Variance	0.0069	0.1486	0.00003	0.0254	0.0099	0.1233
Std Dev.	0.08	0.39	0.01	0.16	0.10	0.35
SE Mean	0.05	0.22	0.003	0.09	0.06	0.20
LCL Mean	0.49	5.07	0.13	4.27	0.59	37.16
UCL Mean	0.90	6.98	0.16	5.06	1.09	38.91
<b>Roots</b>						
Mean	1.31	1.99	0.18	3.80	1.32	37.30
n	1	1	1	1	1	1

Table 5.13 Lettuce average mineral composition over experiments (LB1, LB2 and LB3) on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

- (1).- A statistically significant effect among the 3 experiments has been found in the ANOVA analysis.  
 (2).- Not enough data to perform an ANOVA analysis.

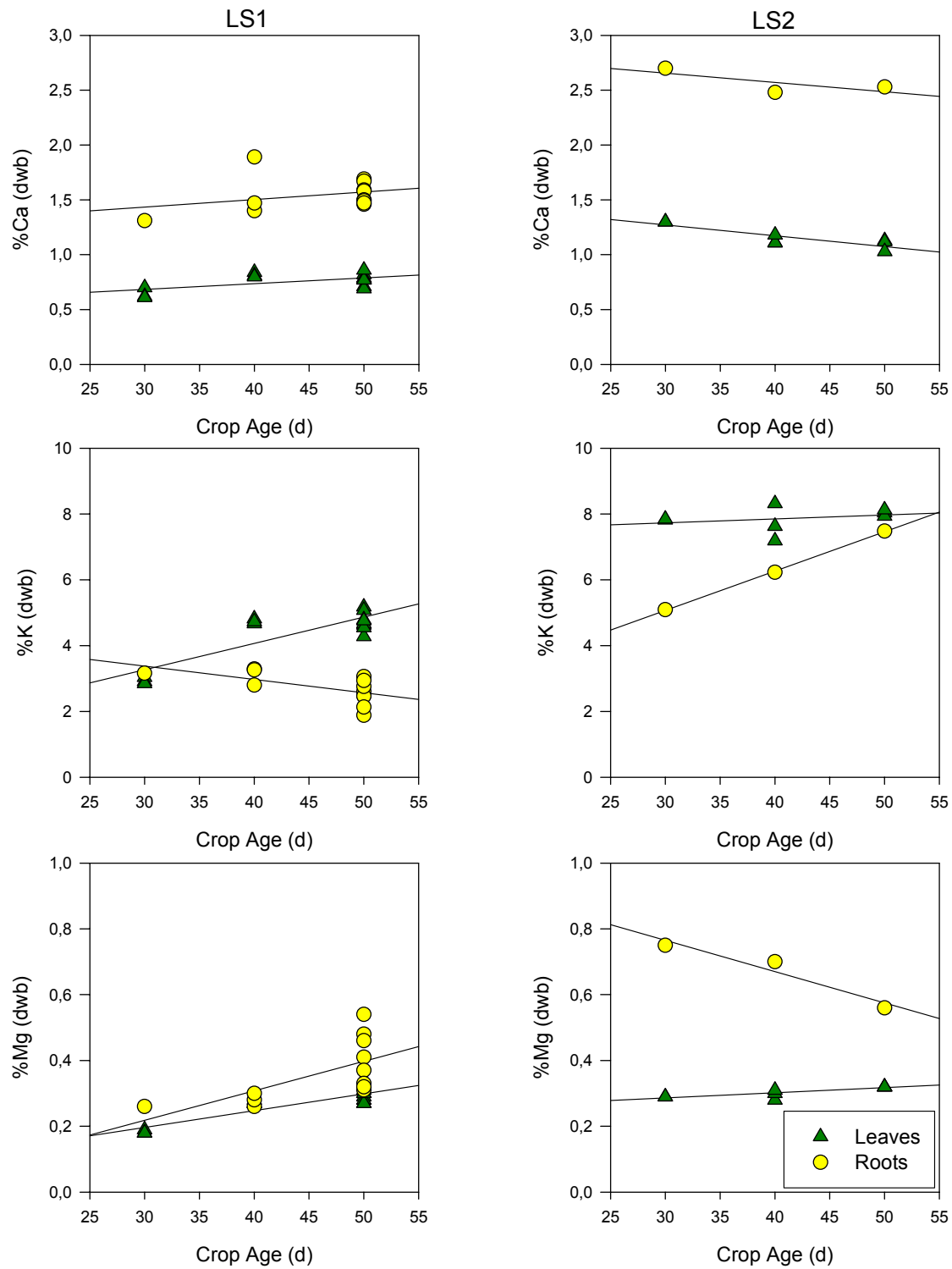
Lettuce	Mineral concentration (% dwb)					
	Ca	K	Mg	N	P	C
<b>Leaves</b>						
Mean	(1)	(1)	(1)	(1)	(1)	(1)
n	9	9	9	9	9	9
Variance	0.0258	0.1591	0.0002	0.2380	0.0159	0.5786
Std Dev.	0.16	0.40	0.01	0.49	0.13	0.76
SE Mean	0.05	0.13	0.004	0.16	0.04	0.25
LCL Mean	0.63	5.41	0.14	4.09	0.73	37.93
UCL Mean	0.87	6.03	0.16	4.84	0.93	39.10
<b>Roots</b>						
Mean	(2)	(2)	(2)	(2)	(2)	(2)
n	3	3	3	3	3	3
Variance	0.0511	0.1336	0.0021	0.0121	0.0364	1.6900
Std Dev.	0.23	0.37	0.05	0.11	0.19	1.30
SE Mean	0.13	0.21	0.03	0.06	0.11	0.75
LCL Mean	0.90	0.76	0.06	3.60	0.90	32.57
UCL Mean	2.02	2.57	0.28	4.15	1.85	39.03

### **5.3.2. Staged Cultures: Tissue Mineral, Proximate and Fibre Composition**

In lettuce staggered cultures, during the full chamber stocking 36 plants were harvested every 10 days until the final harvest, where 36 plants of each plant age inside chamber (30, 40 and 50 days old) were gathered.

Mineral and proximate composition was analysed for the 2 staggered replications, whereas fiber composition is only available for one of the cultures.

Similarly to staggered beet section, first lettuce composition is plotted versus crop age (figures 5.3, 5.4, 5.5, 5.6). Afterwards an evaluation of the existing quality differences between plant ages is done taking into account the linear regression statistical values (Table 5.14, Table 5.15 and Table 5.16).



**Figure 5.3** Lettuce mineral composition (Ca, K, Mg) for experiment LS1 and LS2 on a percent dry weight (dwb) basis for each part (▲ leaves; ● roots).

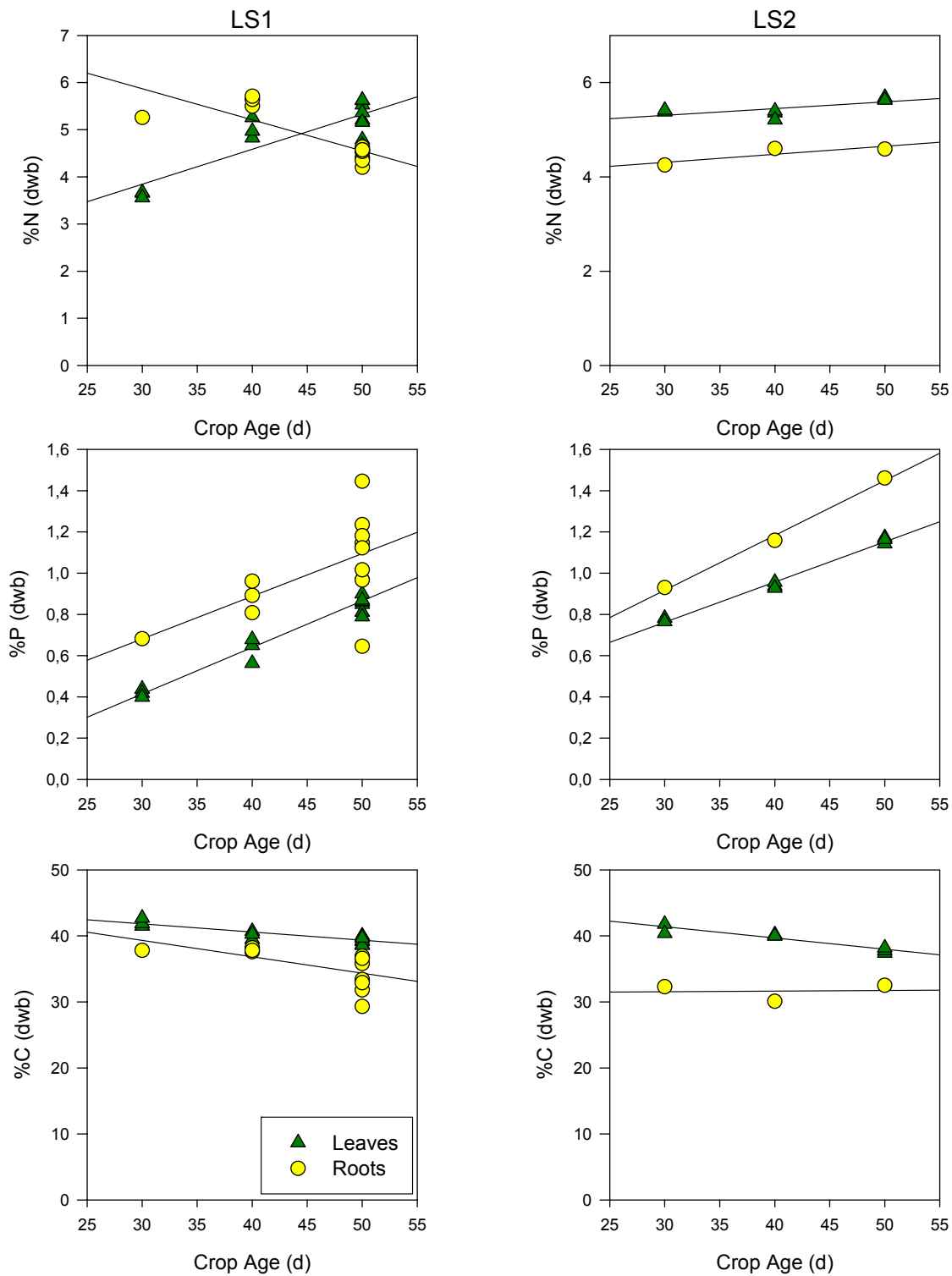


Figure 5.4 Lettuce mineral composition (N, P, C) for experiment LS1 and LS2 on a percent dry weight (dwb) basis for each part (▲ leaves; ● roots).

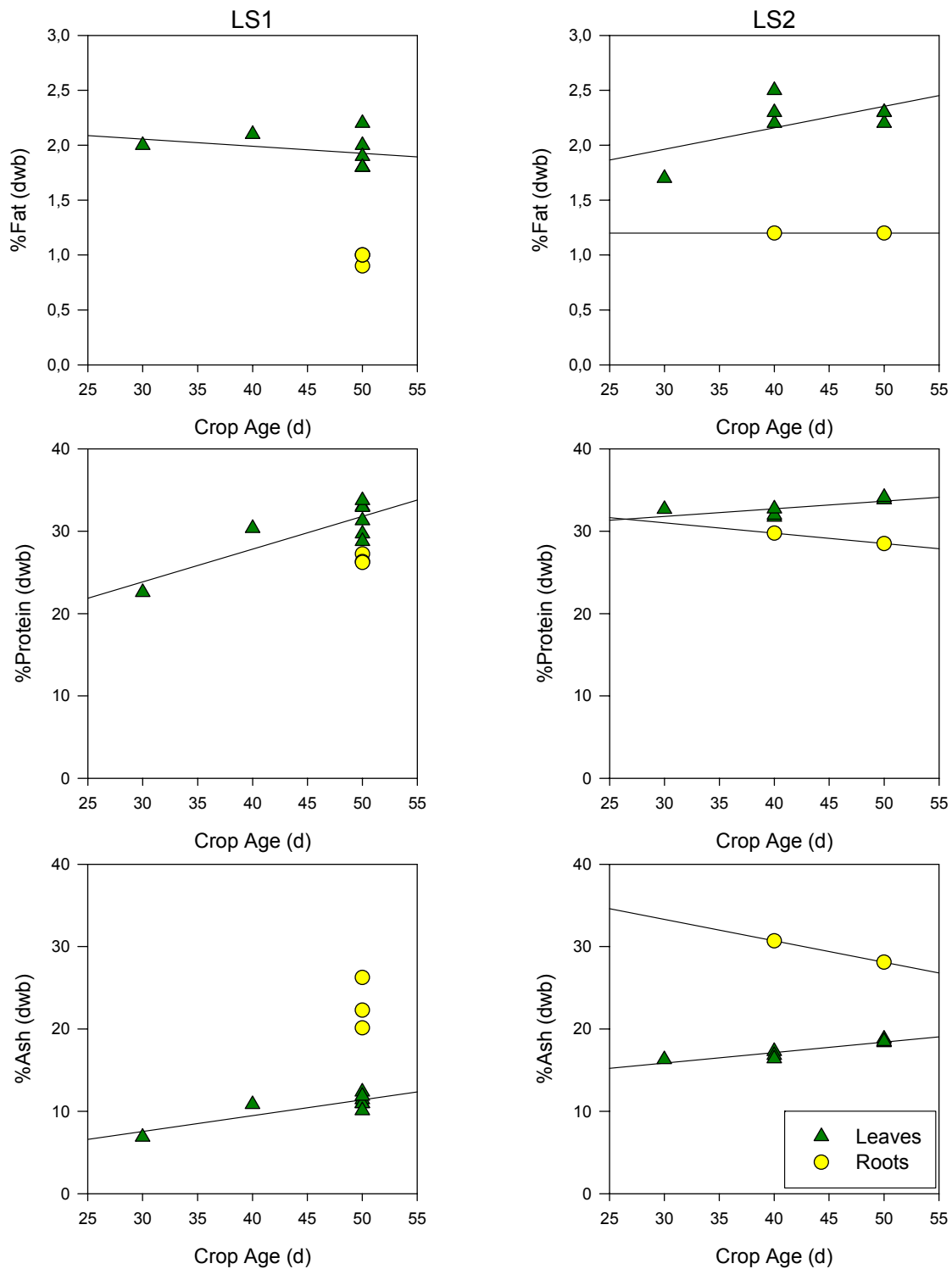
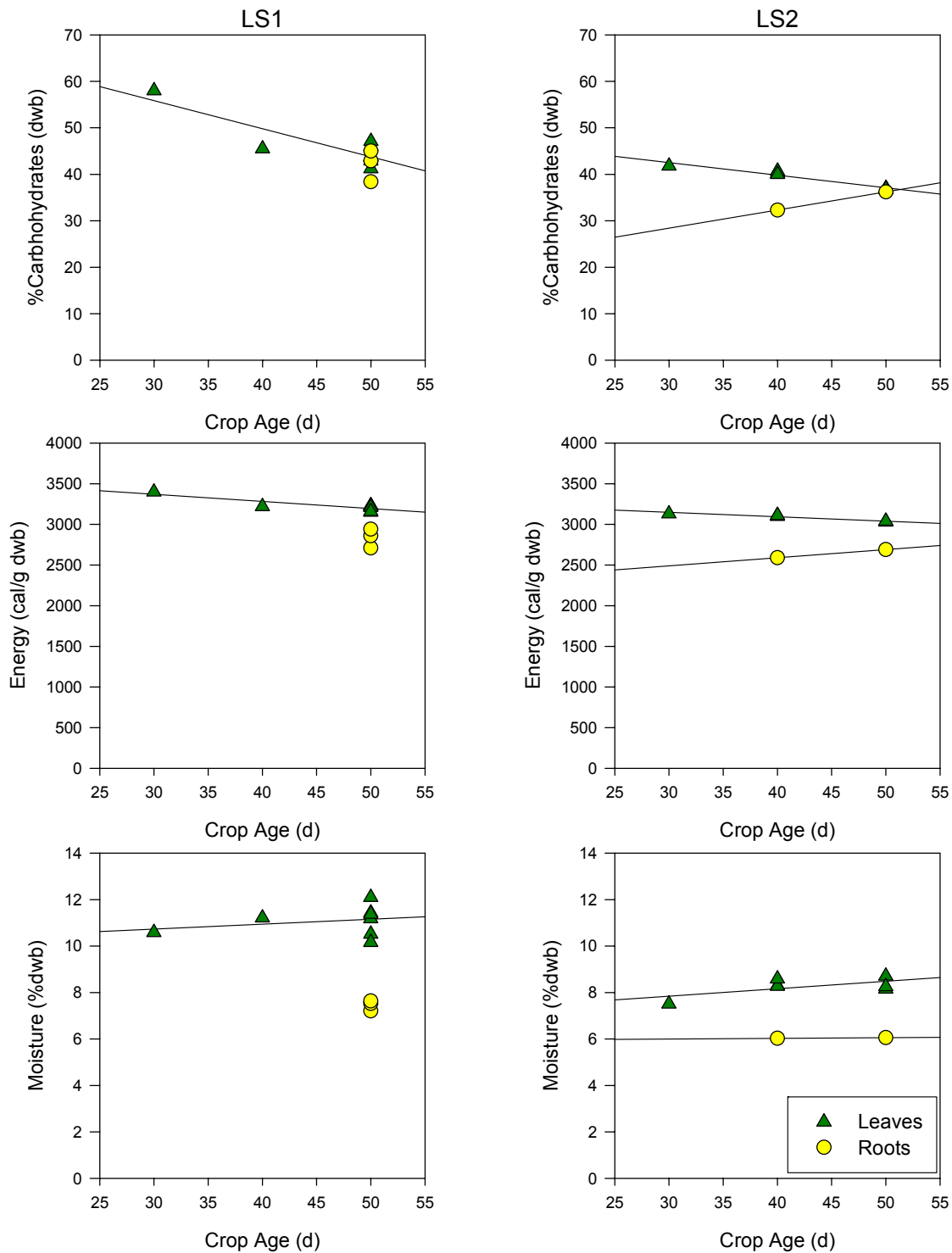
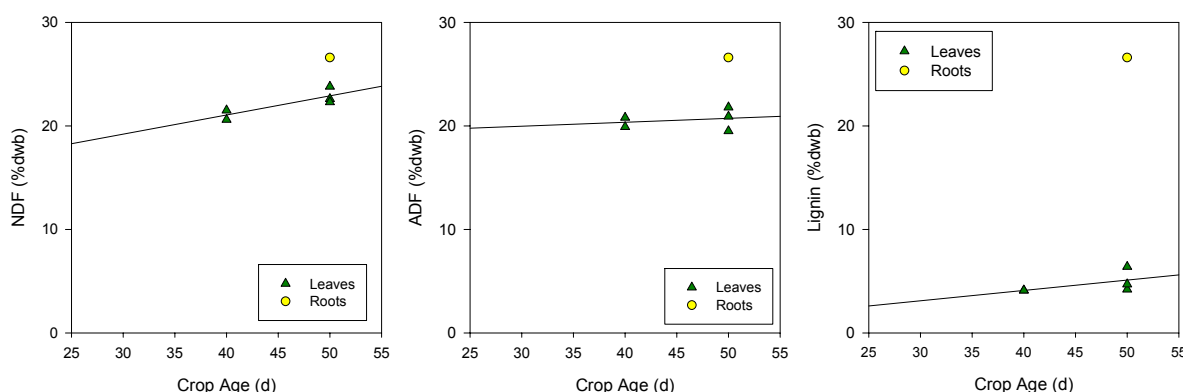


Figure 5.5 Lettuce proximate composition (Fat, Protein, Ash) for experiment LS1 and LS2 on a percent dry weight (dwb) basis for each part (▲ leaves; ● roots).





**Figure 5.6 Lettuce proximate composition (Carbohydrates, Energy, Moisture) for experiment LS1 and LS2 on a percent dry weight (dwb) basis for each part (▲ leaves; ● roots).**



**Figure 5.7 Lettuce fiber composition (NDF, ADF, Lignin) for experiment LS2 on a percent dry weight (dwb) basis for each part (▲ leaves; ● roots).**

Table 5.14 Linear regression parameters for lettuce mineral composition at different crop ages in a staggered culture. Statistics parameters include t-statistics, p-values and degrees of freedom (df).

(\*) Significant differences exist among the crop age

Mineral	LS1					LS2				
	Slope	Std. Error	t	p	df	Slope	Std. Error	t	p	df
<b>Leaves</b>										
Ca	0.0053	0.0022	2.4082	0.0330*	12	-0.0098	0.0024	-4.1160	0.0062*	6
K	0.0800	0.0148	5.4013	0.0002*	12	0.0118	0.0161	0.7323	0.4916	6
Mg	0.0051	0.0007	7.0501	1.3·10 <sup>-5</sup> *	12	0.0016	0.0005	3.4683	0.0133*	6
N	0.0741	0.0127	5.8258	0.0001*	12	0.0142	0.0058	2.4429	0.0503	6
P	0.0226	0.0018	12.6655	2.6·10 <sup>-8</sup> *	12	0.0195	0.0009	21.8848	5.9·10 <sup>-7</sup> *	6
C	-0.1239	0.0200	-6.1855	4.6·10 <sup>-5</sup> *	12	-0.1697	0.0263	-6.4453	0.0007*	6
<b>Roots</b>										
Ca	0.0068	0.0069	0.9892	0.3459	10	-0.0085	0.0078	-1.0906	0.4724	1
K	-0.0406	0.0167	-2.4368	0.0350*	10	0.1195	0.0032	37.6327	0.0169*	1
Mg	0.0089	0.0033	2.6751	0.0233*	10	-0.0095	0.0026	-3.6566	0.1699	1
N	-0.0659	0.0148	-4.4456	0.0012*	10	0.017	0.0104	1.6358	0.3493	1
P	0.0207	0.0089	2.3209	0.0427*	10	0.0265	0.0021	12.5989	0.0504	1
C	-0.2481	0.1104	-2.2483	0.0483*	10	0.0100	0.1328	0.0753	0.9521	1

The mineral content dependency on age is commented next for each element:

- Ca: Significant differences exist between replications regarding calcium content in lettuce. Moreover statistics values show that calcium content in leaves depends on lettuce age, but the tendency along plant age differs in each experiment.
- K: Significant differences exist between replications regarding potassium content in lettuce. Furthermore, statistics values show that K content in roots depends on lettuce age, but the tendency along plant age differs in each experiment, while its content in leaves increases significantly only in one of the experiments.

- Mg: Any significant difference exists between replications regarding Mg content in a 50 days old lettuce. Upon its regression values, Mg percentage in leaves increases in both replications along the crop age.
- N: No significant difference was found between replications in the N composition of a 50 days old lettuce. A significant increase of N content in lettuce leaves exist along plant growth. However, roots have any change along age in N percentage.
- P: Significant differences in P leaves composition are detected between replications. Nevertheless, there is a significant increase in P leaves content in both experiments.
- C: Carbon content in lettuce leaves decrease significantly along plant growth.

Upon the statistics parameters for lettuce proximate composition (Table 5.16), the following statements can be done.

- Fat: Only fat leaves content of the 50 days old lettuce present a significant difference between replications. Fat content doesn't vary significantly along age.
- Protein: No significant difference was found between experiments. However, only in one of the replications present a protein significant increase along plant growth.
- Ash: Significant differences in P leaves composition are detected between cultures, but both show a significant increase of P content along lettuce age.
- Carbohydrates (CH): Significant differences exist in CH leaves composition between replications. Nonetheless, in both cultures the CH content diminishes significantly along crop age.
- Energy: Although significant differences in P leaves composition are found between experiments, there is a significantly energy decrease along growth in both cases.
- Moisture: Significant differences were found between replications, but no dependency on age is detected.

Table 5.15 Linear regression parameters for lettuce proximate composition at different crop ages in a staggered culture. Statistics parameters include t-statistics, p-values and degrees of freedom (df).

(\*) Significant differences exist among the crop age

Proximate	LS1					LS2				
	Slope	Std. Error	t	p	df	Slope	Std. Error	t	p	df
Leaves										
Fat	-0.0065	0.0079	-0.8201	0.4435	6	0.0196	0.0118	1.6652	0.1567	5
Protein	0.3971	0.1104	3.5953	0.0114*	6	0.0925	0.0394	2.3493	0.0656	5
Ash	0.1915	0.0489	3.9195	0.0078*	6	0.1277	0.0246	5.1975	0.0035*	5
CH	-0.6045	0.1416	-4.2687	0.0053*	6	-0.2708	0.0314	-8.6301	0.0003*	5
Energy	-0.8710	0.2210	-3.9412	0.0076*	6	-0.5458	0.0790	-6.9062	0.0010*	5
Moisture	0.0211	0.0329	0.6421	0.5445	6	0.0320	0.0177	1.8044	0.1310	5

Any significant difference in fiber content between 40 and 50 days old lettuce was found (Table 5.16).

Table 5.16 Linear regression parameters for lettuce fiber composition at different crop ages of staggered culture LS2. Statistics parameters include t-statistics, p-values and degrees of freedom (df).

Fiber	Slope	Std. Error	t	p	df
<b>Leaves</b>					
ADF	0.0383	0.0927	0.4136	0.7069	3
NDF	0.1850	0.680	2.7203	0.0725	3
Lignin	0.1000	0.0860	1.1634	0.3288	3

In Table 5.17, Table 5.18 and Table 5.19 the mineral and proximate content mean among replications are summarized for several crop ages.

As some significant differences exist among experiments, more replications for staggered cultures are recommended.

Table 5.17 Lettuce-30-days-old composition mean over experiments (LS1, LS2) on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

(1).- A statistically significant effect among the 3 experiments has found in the ANOVA analysis.

(2).- Not enough data to perform an ANOVA analysis.

Lettuce	Crop Age: 30 d											
	Mineral concentration (% dwb)						Proximate concentration (% dwb)					
	Ca	K	Mg	N	P	C	Fat	Protein	Ash	CH	Energy	Moisture
<b>Leaves</b>	(1)	(1)	(1)	(1)	(1)		(2)	(2)	(2)	(2)	(2)	(2)
Mean	0.91	4.89	0.23	4.34	0.56	41.66	1.85	27.63	11.59	49.90	326.50	9.05
n	5	5	5	5	5	5	2	2	2	2	2	2
Variance	0.13	7.21	0.003	0.94	0.04	0.69	NA	NA	NA	NA	NA	NA
Std Dev.	0.36	2.69	0.06	0.97	0.20	0.83	NA	NA	NA	NA	NA	NA
SE Mean	0.16	1.20	0.03	0.43	0.09	0.37	NA	NA	NA	NA	NA	NA
LCL Mean	0.46	1.56	0.15	3.13	0.32	40.63	NA	NA	NA	NA	NA	NA
UCL Mean	1.35	8.23	0.30	5.54	0.80	42.69	NA	NA	NA	NA	NA	NA
<b>Roots</b>	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
Mean	2.01	4.13	0.51	4.76	0.81	35.05	-	-	-	-	-	-
n	2	2	2	2	2	2	-	-	-	-	-	-

Table 5.18 Lettuce-40-days-old composition mean over experiments (LS1, LS2) on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

(1).- A statistically significant effect among the 3 experiments has found in the ANOVA analysis.

(2).- Not enough data to perform an ANOVA analysis.

Lettuce	Crop Age: 40 d														
	Mineral concentration (% dwb)						Proximate concentration (% dwb)						Fibre (% dwb)		
	Ca	K	Mg	N	P	C	Fat	Protein	Ash	CH	Energy	Moisture	NDF	ADF	Lignin
Leaves	(1)	(1)			(1)				(1)	(1)	(1)	(1)	(2)	(2)	(2)
Mean	0.98	6.23	0.29	5.17	0.79	40.12	2.28	31.69	15.34	41.60	313.50	9.10	20.35	21.05	4.10
n	6	6	6	6	6	6	4	4	4	4	4	4	2	2	2
Variance	0.03	2.78	0.0003	0.05	0.03	0.16	0.03	0.98	9.116	6.85	32.33	2.02	NA	NA	NA
Std Dev.	0.18	1.67	0.02	0.23	0.17	0.40	0.17	0.99	3.02	2.62	5.69	1.42	NA	NA	NA
SE Mean	0.07	0.68	0.01	0.09	0.07	0.16	0.09	0.49	1.51	1.31	2.84	0.71	NA	NA	NA
LCL Mean	0.79	4.48	0.27	4.94	0.60	39.70	2.00	30.12	10.53	37.44	304.45	6.83	NA	NA	NA
UCL Mean	1.16	7.98	0.30	5.41	0.97	40.53	2.55	33.26	20.14	45.76	322.55	11.36	NA	NA	NA
Roots		(1)	(1)	(1)		(1)	(2)	(2)	(2)	(2)	(2)	(2)			
Mean	1.81	3.90	0.39	5.36	0.96	35.93	1.20	29.76	30.70	32.30	259.00	6.03	-	-	-
n	4	4	4	4	4	4	1	1	1	1	1	1	-	-	-
Variance	0.25	2.47	0.044	0.27	0.02	15.14	NA	NA	NA	NA	NA	NA	-	-	-
Std Dev.	0.50	1.57	0.21	0.52	0.15	3.89	NA	NA	NA	NA	NA	NA	-	-	-
SE Mean	0.25	0.79	0.11	0.26	0.07	1.95	NA	NA	NA	NA	NA	NA	-	-	-
LCL Mean	1.02	1.39	0.05	4.54	0.72	29.73	NA	NA	NA	NA	NA	NA	-	-	-
UCL Mean	2.60	6.40	0.72	6.18	1.19	42.12	NA	NA	NA	NA	NA	NA	-	-	-

Table 5.19 Lettuce-50-days-old composition mean over experiments (LS1, LS2) on a percent dry weight (dwb) basis for each part (leaves, hypocotyl and roots).

(1).- A statistically significant effect among the 3 experiments has found in the ANOVA analysis.

(2).- Not enough data to perform an ANOVA analysis.

Lettuce	Crop Age: 50 d														
	Mineral concentration (% dwb)						Proximate concentration (% dwb)						Fibre (% dwb)		
	Ca	K	Mg	N	P	C	Fat	Protein	Ash	CH	Energy	Moisture	NDF	ADF	Lignin
Leaves	(1)	(1)			(1)	(1)	(1)		(1)	(1)	(1)	(1)	(2)	(2)	(2)
Mean	0.86	5.64	0.30	5.36	0.95	38.98	2.03	32.37	13.70	41.70	314.44	10.21	20.73	22.90	5.10
n	11	11	11	11	11	11	9	9	9	9	9	9	3	3	3
Variance	0.02	2.43	0.0004	0.13	0.02	0.83	0.05	3.92	13.650	15.98	77.53	2.22	1.34	0.63	1.33
Std Dev.	0.16	1.56	0.02	0.36	0.15	0.91	0.22	1.98	3.69	4.00	8.80	1.49	1.16	0.79	1.15
SE Mean	0.05	0.47	0.01	0.11	0.04	0.27	0.07	0.66	1.23	1.33	2.93	0.50	0.67	0.46	0.67
LCL Mean	0.75	4.59	0.29	5.12	0.85	38.37	1.87	30.84	10.86	38.63	307.68	9.06	17.85	20.93	2.24
UCL Mean	0.97	6.69	0.32	5.60	1.05	39.59	2.20	33.89	16.54	44.77	321.21	11.36	23.61	24.87	7.96
Roots		(1)										(1)	(2)	(2)	(2)
Mean	1.66	3.09	0.42	4.49	1.14	33.99	1.03	27.06	24.19	40.63	280.00	7.11	26.60	43.70	10.50
n	9	9	9	9	9	9	4	4	4	4	4	4	1	1	1
Variance	0.11	2.85	0.009	0.02	0.06	7.06	0.02	1.15	13.207	16.28	144.67	0.52	NA	NA	NA
Std Dev.	0.34	1.69	0.09	0.14	0.25	2.66	0.13	1.07	3.63	4.04	12.03	0.72	NA	NA	NA
SE Mean	0.11	0.56	0.03	0.05	0.08	0.89	0.06	0.54	1.82	2.02	6.01	0.36	NA	NA	NA
LCL Mean	1.41	1.79	0.35	4.38	0.94	31.95	0.82	25.35	18.40	34.20	260.86	5.96	NA	NA	NA
UCL Mean	1.92	4.39	0.49	4.60	1.33	36.03	1.23	28.76	29.97	47.05	299.14	8.26	NA	NA	NA

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