



Essential and Toxic Trace Elements in Achenae of the Cup Plant (*Silphium perfoliatum* L.), a Perennial, Alternative Energy and Honey Plant

W. Froelich* and M. Noznic

*Corresponding author email id: Walter.Froelich@gmx.net

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Abstract – The introduction shows the broad forms of use and advantages of the perennial cup plant *Silphium perfoliatum* L., as a fodder, energy and honey crop, in soil protection and accumulation of organic materials. Furthermore, it provides animal shelter and contributes, especially, to the maintenance of insect biodiversity, primarily of hymenopterae.

Main objectives of this study were determination of mineral and micronutrient contents as well as toxic elements in achenae (fruits) and seeds of various accessions of the cup plant. Mean values (MV), coefficients of variation (CV %) and of correlation (r) were calculated.

Clear differences in element contents between achenae and seeds of the cup plant were mainly found with phosphorus, sulfur, calcium, molybdenum, nickel, and cobalt. More investigations are urgently needed regarding contents of calcium in achenae compared to seeds, aiming at its role in seed germination processes.

Field plots destined for harvesting seeds (achenae) should dispose of a high soil pH level.

Mean values for element contents in achenae are presented as follows:

Macronutrients (%): Nitrogen N (4,74), phosphorus P (0,68), potassium K (1,18);

Secondary Nutrients (%): Sulfur S (0,29), calcium Ca (0,49), magnesium Mg (0,36)

Essential micronutrients (mg/kg) - Cations: Iron Fe (67), manganese Mn (16), zinc Zn (36), copper Cu (15); nickel Ni (2,1), cobalt Co (0,1), vanadium V (0,05), tungsten W (0,04)

Anions: Boron B (16), molybdenum Mo (0,9), selenium Se (0,1)

Toxic elements (mg/kg): Chromium Cr (0,8), lead Pb (0,1).

Statistically highly significant / significant, and numerically extremely high coefficients of correlation were determined for the relationships between the following elements: Mg x Pb (r=0,99); Ni x V (r=0,99) / N x Mn (+0,96); N x Se (0,98); K x Cr (0,97); and Fe x W (0,99).

A negative relevant correlation found for phosphorus x cobalt (r=-0,95) appears remarkable once about 96% of the problems observed with trace elements in biogas plants are caused by cobalt deficits.

Keywords – Achenae, Cup Plant, Energy Plant, *Silphium perfoliatum* L., Trace Elements.

I. INTRODUCTION

The perennial frost-resistant cup plant *Silphium perfoliatum* L. having its origin in the North American prairie is an insectogamous cross-pollinating species which has become native to European and non-European botanical gardens long time ago. Abundantly flowering from June to September in a dichasium shaped inflorescence, it is an attractive, commercially interesting honey plant (BIERTUEMPFEL & CONRAD 2012, HORN 2012). It is also

sought for by wild and bumble bees, hovering flies and other hymenopterae, thus contributing to protection and conservation of biodiversity. Even small game seek shelter in cup plant stands, especially when they are established as biotops and stalks left till spring.

Formation of a compact rootstocks enables the cup plant to protect and stabilize erosion-prone soils. Non-tilling practices and increased production of organic matter, in addition stimulated by earthworm activities, lead to a continuous humus accumulation (EMMERLING 2014, 2016)

It is emphasized that the cup plant as a perennial crop - it may be harvested for 10, 15 or more years - after the year of establishment will not need an application of synthetic fertilizers or agrochemicals anymore. The nutrients extracted by ensiling are recycled through applying the fermentation residuals from the biogas plant.

Attempts to establish it as a fodder plant in central Europe have failed, it is only accepted by rabbits (SCHELLHARDT 2008, pers. comm.). About ten years ago, its suitability as a biogas substrate complementary to maize silage has been proven (CONRAD *et al.*, 2007). However, in the beginning establishing a plant stand was based upon an expensive planting of pre-germinated seedlings (BIERTUEMPFEL 2015). It should be realized that the actually available plant material consists of clones selected and adapted in central Germany, from accessions which had originally been received from European botanical gardens (NEUMERKEL 1980). By subsequent hybridization, they have developed into obviously heterogeneous mixtures.

An overview on experimental results obtained with the cup plant (*Silphium perfoliatum* L.) in Baden-Wuerttemberg is given by STOLZENBURG *et al.* (2016). Crucial and very successful steps towards a fast expansion of the cup plant growing area, based upon a practice-oriented direct-sowing method using maize as a cover crop, developed near the upper Danube valley, are reported by FROELICH *et al.* (2016 a).

When used as a co-substrate in the biogas plant, the cup plant is significantly different in its contents of mineral nutrients, as well as of trace elements being essential in methanogenesis, and of toxic elements, too, from other substrates, especially the hybrid maize (SAUER 2011). BOEHM *et al.* (2012) and HOSCHKE (2015) report on contents of macro- and secondary nutrients being substantially higher in above-ground parts of the cup plant compared to silage maize. Values for nutrient extraction by the cup plant, as determined by Bavarian analyses, are presented by HARTMANN & LUNENBERG (2016) and LUNENBERG & HARTMANN (2016).



In addition to parameters obtained from a group of elements as published by FROELICH & STOLZENBURG (2012) for mineral nutrients in achenae of the cup plant, and in continuation of a study initiated by FROELICH *et al.* (2015), this investigation is focused on trace elements.

In order to facilitate comparisons with results of preceding analyses, achenae - which contribute about 1-2% to the whole plant matter - were again used as analytical objects. Achenae, from the botanical view, are fruits which are covered by a coat which contains the real seeds in its interior. There is an important difference: the fruit coat is to be seen as a vegetative, the seed as a generative plant component. For some elements, permeability exists between these two components - i.e. translocation (FIEDLER & ROESLER, 1993) from fruit coat into seed - for other ones a more or less strict barrier was found (FROELICH *et al.*, 2016 b).

Moreover, a mixed seed sample could be analyzed after the achenae had lost their fruit coat due to threshing and cleaning. The share of the seed in the whole achena, in various accessions, was found to be about 70 % (FROELICH *et al.*, 2016 b).

The crude ash contents (following the Weender Analysis) in the cup plant vary between 11 and 12 % (STOLZENBURG 2010; BRODMANN 2016; pers. comm. each), thus, being approximately three times as high as in the silage maize plant. We may assume that high contents of mineral nutrients in substrates open the chance for a satisfactory supply with essential trace elements, too.

In addition, it should be remarked that crude fat contents in the dry matter of cup plant fruits vary around 25 % (JUERGENS 2011; SCHWADORF 2015, 2016; pers. comm. each). In comparison to that, fruits of the spice/aromatic plants coriander, anise and fennel harvested from biannual trials showed crude fat contents between 17 and 21 % (EL-GAMAL & HAMDINO 2017). Surprisingly, a very close relationship between the development of the fruit maturity and the germinability and, in a very conspicuous manner, the crude ash content was found.

In general, metal ions are toxic, however, a good number disposes of functions as an essential micronutrient. By all means, the toxicity also depends upon their concentration.

As described in the following, possible options are presented to make future use of the relatively high mineral nutrient contents of the cup plant - as far as they can be identified in achenae, for improving substrates in biogas plants, with the aim to increase the energy yield.

These elements are, in a sequence given by BISCHOFF (2009): Nickel (Ni), cobalt (Co), molybdenum (Mo), selenium (Se), tungsten (W), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn). Vanadium (V) can be substituted by W (SCHERER (2015, pers. comm.).

Three so-called ferromagnetic elements from this group, namely Fe, Ni, and Co, in their metallic form, can be magnetically oriented. This points to a special path in the metabolism of the pro-caryotic archaea-bacteria, to be seen as an early expression form of life.

The elements nickel and cobalt show important functions as enzymatic co-factors in eu-caryonta, too, Ni in the urea dissolving enzyme urease, and Co in symbiotic N-fixation.

Recently, Ni has been identified as another stabilizing factor for seed vitality (CAKMAK 2016).

FIEDLER & ROESLER (1993) state that the mechanism of translocation, the ion transfer from roots, stalks, and leaves into storage organs, plays an especially important role for essential nutrients boron (B), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), zinc (Zn), as well as for the toxic elements cadmium (Cd) and thallium (Tl). They classify chromium (Cr) as an essential trace element which, however, can act toxic even in very low concentrations. Besides Cd and Tl, toxic heavy metals occurring frequently but without any known function in the plant metabolism are lead (Pb) and arsenic (As).

Both REKULTA (2013) and SAUER & RUPPERT (2011) discuss the decontamination or phytoremediation of soils in heavy-metal contaminated (former) mining areas with cadmium as a major contaminant. In a highly noteworthy manner, as for the Cd concentration, the cup plants ranks last among the analyzed species. ZHANG *et al.* (2010) see it as a candidate for a kind of phytostabilization. ADLE *et al.* (2009) describe intracellular Cd detoxification and excretion - processes which, possibly, could very well be studied in the cup plant.

The major objectives of these investigations were to determine in achenae of the cup plant contents of those trace elements which are, on one side, essential in the methanogenesis by archaea-bacteria, e.g. nickel and cobalt, or on the other hand, are important indicators in the phytoremediation of soils, e.g. cadmium.

II. MATERIALS AND METHODS

Samples of achenae (fruits) being widely homogeneous in form and size, of five accessions of the cup plant harvested during 2011 to 2014, as well as a hand-selected single sample of coatless seeds, collected after threshing and cleaning, were analyzed. The State Institute for Agricultural Chemistry, Stuttgart-Hohenheim, determined a total of 22 mineral nutrients, applying ICP-OES analytics, whilst for nitrogen (N) the Dumas elemental analysis was used.

Tab. 1 presents and discusses mean values (MV) and coefficients of variation (CV %). Biological materials are characterized by a natural variability expressed by the coefficient of variation whereby a range of 10 to 15 % is seen to be normal.

A complete diagram of correlation coefficients (Pearson's r) cannot be presented here. Tab. 2 shows so-called *relevant* ($+0,8 < r < -0,8$) coefficients of correlation. Due to the small sample size and few degrees of freedom, only a few, numerically extremely high coefficients proved to be statistically (highly) significant. Starting from a value of the coefficient of correlation of approximately $\pm 0,8$, an important or "relevant" interdependence of two characteristics, normally underlain with a physiological base, too, may be plausible. This assumption can be supported by another statistical parameter, the coefficient of determination $B = r^2$ (as %). The value $r^2 = 0,64$ or $B = 64\%$ indicates that almost two thirds of the variation of one characteristic can be explained by the variation of the other



characteristic, an association which can easily be seen as relevant. Both positive and negative coefficients of correlation which exceed the accepted critical value of 0,8 have been entered into Tab. 2. Statistically (highly) significant coefficients are additionally marked (P < *0,05 or **0,01).

III. RESULTS AND DISCUSSION

Tab. 1 shows mean values (MV) of achenae as compared to the seed single value (SV). Clear differences between achenae and seeds were found for nitrogen N (4,74 / 7,17 %), phosphorus P (0,68 / 0,92%), sulfur S (0,29 / 0,35%), calcium Ca (0,49 / 0,11%), molybdenum Mo (0,86 / 0,49 mg/kg). Nickel Ni (2,1 / 2,9 mg/kg) showed a remarkable tendency to be stored in seeds.

The surprisingly great difference of the Ca contents between achenae and seeds leads to the conclusion that here for this element an almost permeable layer translocation barrier exists. HANNSS (2016, pers. comm.) states that calcium is the most important element in the initiation of germination processes. Then, calcium deficiency in the seed - independent from the achena - appears to be a fundamental reason for seed germination problems of the cup plant. TROELENBERG *et al.* (2012) conclude that its low germination ability does not have its origin in dormancy; however, mineral nutrient contents are not presented in this study.

With regard to the different groups of mineral nutrients, for contents of elements in achenae the following values were obtained:

Macro-Nutrients (%): Nitrogen N (4,74), phosphorus P (0,68), potassium K (1,18) - with coefficients of variation (CV) between 6 and 20%.

Secondary Nutrients (%): Sulfur S (0,29), calcium Ca (0,49), magnesium Mg (0,36); CV between 12 and 16%.

Micro-Nutrients (mg/kg):

Cations:

Iron Fe (67), manganese Mn (16), zinc Zn (36), copper Cu (15); CV between 19 and 56%.

Anions:

Boron B (16), molybdenum Mo (0,86), selenium Se (0,1); CV between 15 and 91%.

Trace elements essential in methanogenesis (mg/kg):

Nickel Ni (2,1), cobalt Co (0,1), vanadium V (0,05), tungsten W (0,04); CV from 22 to 81%.

Toxic elements (mg/kg): Chromium Cr (0,8), lead Pb (0,1); CV 27 or 71%

Below detection level: Cadmium Cd, as well as arsenic As and thallium Tl

With regard to the respective coefficients of variation in maize grains, FROELICH *et al.* (2014) had already found values in a normal range, between 4 and 21%, for macro- and secondary nutrients used in mineral fertilization. However, in this study, micronutrients and trace elements being essential in methanogenesis showed high to extremely high coefficients of variation of up to 91%. This offers, on the level of trace elements, very promising aspects for a breeders' selection.

Table 1. Mean Values (MV), Single Value (SV), Coefficients of Variation (CV) measured for elements in five accessions of cup plant *Silphium perfoliatum* L.

Element	Symbol	Mean Value Achenae	Single Value Seeds Dry Matter	Contents in Achenae	CV (%)
Nitrogen	N	4,74	7,17	%	6,1
Phosphorus	P	0,68	0,92	%	5,9
Potassium	K	1,18	0,97	%	19,7
Sulfur	S	0,29	0,35	%	11,7
Magnesium	Mg	0,36	0,46	%	11,7
Calcium	Ca	0,49	0,11	%	14,6
Iron	Fe	67	78	mg/kg	19,7
Manganese	Mn	16	15	mg/kg	27,5
Copper	Cu	15	9	mg/kg	18,6
Zinc	Zn	36	41	mg/kg	30,5
Boron	B	17	11	mg/kg	14,9
Molybdenum	Mo	0,86	0,49	mg/kg	91,6
Selenium	Se	0,10	0,07	mg/kg	56,7
Nickel	Ni	2,10	2,90	mg/kg	55,9
Cobalt	Co	0,12	0,06	mg/kg	38,3
Tungsten	W	0,04	0,04	mg/kg	22,4
Vanadium	V	0,05	0,05	mg/kg	81,3
Chromium	Cr	0,78	0,40	mg/kg	27,2
Lead	Pb	0,10	0,06	mg/kg	71,4

Cadmium	Cd	Not detectable
Arsenic	As	" "
Thallium	Tl	" "

Table 2 shows relevant coefficients of correlation ($r > +0,8$ or $r < -0,8$) in an approach which has first been published by FROELICH *et al.* (2015). Remarkable observations are commented, the negative relationship between phosphorus and cobalt and potential consequences discussed.

With $r = 0,99$ highly significantly positive correlations were found between the elements magnesium Mg and lead Pb, as well as for nickel Ni and vanadium V.

Significantly positive coefficients were obtained for the correlations between nitrogen N and manganese Mn ($r=0,96$), N and selenium Se ($r=0,98$), potassium K and chromium Cr ($r=0,97$) as well as iron Fe and tungsten W ($r=0,99$).

The relation between phosphorus P and cobalt Co has proven to be negative ($r=-0,95$). This is of great significance, from the following reasons: phosphorus is immobile in the soil solution, and deficit supply of crops has to be avoided, above all in the juvenile stage (LUETKE ENTRUP *et al.*, 2013). However, in maize crops where the basic mineral underground fertilization is amended by liquid (slurry, fermenter residues) farmyard manure (JACOBS & REMMERSMANN, 2013), an excess P concentration might reduce the Co availability to such an

extent that it is taken up by the substrate plant only insufficiently. BISCHOFF (2016, pers. comm.) observed that 96% of all problems with trace elements in biogas plants have to do with cobalt deficits - presumably explainable by the **phosphorus-cobalt antagonism** as presented for discussion here first. A phosphate application not carefully enough controlled could have a double negative effect on the economic level even: unnecessary costs for mineral fertilizer and serious problems with Co deficits in the biogas plant. Inevitably, the latter ones lead to suboptimal energy yields unless they are compensated by costly chemical additives.

The relevant negative relationship between N and Mo ($r=-0,82$) appears to be especially dis-advantageous once Mo is essential not only in seed germination but also in methanogenesis. Manganese Mn affects the uptake of Se negatively ($r=-0,89$). The relevant positive correlation ($r=0,81$) found between Mn and vanadium V could be helpful in case uptake of tungsten W is blocked by Mn ($r=-0,87$). On the other hand, the relevant negative relationship found between calcium Ca and lead Pb has to be seen positive. The relevant positive relationship ($r=0,85$) found for P and Mo is a strong advantage for the interaction of these two elements.

Table 2. Relevant Coefficients of Correlation (Pearson's r)

Relevant Coefficients of Correlation ($r > +0,8$ oder $r < -0,8$)		r =	Probability P *0,05 **0,01
Element 1	Element 2		
Nitrogen	Iron	0,803	
	Manganese	0,960	*0,05
	Molybdenum	-0,818	
	Selenium	0,983	*0,05
Phosphorus	Molybdenum	0,855	
	Cobalt	-0,956	*0,05
Potassium	Cobalt	0,802	
	Chromium	0,975	*0,05
Sulfur	Iron	0,856	
	Zinc	0,883	
	Cobalt	0,822	
	Tungsten	-0,846	
	Lead	-0,889	
Magnesium	Calcium	0,943	
	Copper	0,849	
	Lead	0,990	**0,01
Calcium	Copper	0,933	
	Lead	-0,889	
Iron	Manganese	0,917	
	Tungsten	0,989	*0,05
Manganese	Nickel	0,837	
	Selen	-0,895	
	Tungsten	-0,866	

Relevant Coefficients of Correlation ($r > +0,8$ oder $r < -0,8$)		r =	Probability P *0,05 **0,01
	Vanadium	0,808	
Copper	Boron	0,851	
	Molybdenum	0,834	
Zinc	Chromium	0,817	
Boron	Molybdenum	0,831	
	Nickel	-0,829	
Molybdenum	Selenium	0,890	
Nickel	Vanadium	0,993	**0,01
Tungsten	Lead	0,834	
	0		

IV. CONCLUSIONS

Remarkably high contents of some essential trace elements offer a great chance in practical agriculture, primarily in biogas production, to compensate existing mineral deficits in maize silage, especially in trace elements essential in methanogenesis, by adding cup plant substrate.

The negative relevant relation phosphorus x cobalt ($r = -0,95$) requires special attention once about 96% of all the problems observed with trace elements in biogas plants are caused by a deficit in cobalt, and presumably induced by an antagonism between P and Co which urgently needs to be studied.

Moreover, it is referred to the content of cadmium Cd - ranking first as a toxic element in former mining areas - which was below detection level, as well as arsenic As and thallium Tl. Consequently, it should be investigated whether cup plant stands could be grown on soils contaminated with heavy metals, and the harvest crop be utilized without any risk through biogas plants.

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AUTHOR'S PROFILES

Authors are Integrated Expert and Head of Department of Industrial crops, respectively, Agricultural Institute, Banja Luka, Bosnia & Hercegovina. Cup plant (*Silphium perfoliatum* L.) is an accession in the efforts of the Institute to intensify research activities on sustainable and renewable energy sources.

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Contact Address

Dr. Walter Froelich - Agricultural Institute, Knjaza Milosa 17, 78000 Banja Luka, Republika Srpska, Bosnia & Hercegovina E-Mail: Walter.Froelich@gmx.net



Fig. 1. Fruit stands of cup plant *Silphium perfoliatum* L., wreath-like arrangement of achenae