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PRELIMINARY STUDY REGARDING THE USE OF FOLIAR BIOMASS AS FERTILIZER

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SUMMARY

Biomass as chemical solar energy accumulated in the form of vegetable or animal matter is one of the most precious and diverse resource of the earth. A resource that is still unexploited is the leaf litter known to be a natural fertilizer after decomposition. Throughout the degradation and leaching of leaf litter, "new born" substances are continuously generated and may aggregate to form humic substances. These findings suggest that leaf litter degradation extracts may be an important environmental factor influencing community structure within ecosystems. In this research we tried to decompose leaf litter with chemical reagents in order to obtain a liquid fertilizer as well as trying to use foliar biomass in combination with soil. When trying to decompose foliar biomass with chemical reagents, the development of the studied plants (petunia - *Petunia hybrida*, cress - *Lepidium sativum*) was inhibited. This process occurred in case of using agar gel and in case of the cultivated plants in pots as well. The direct use of foliar biomass in combination with soil, after being preceded by an inhibitory process in the development of the plant, seems to have the role of a fertilizer.

Keywords: foliar biomass; fertilizer; leaf litter; biodiesel.

INTRODUCTION

Biomass as chemical solar energy accumulated in the form of vegetable or animal matter is one of the most precious and diverse resource of the earth. The term is applied to the mass of substance generated by the development of living organisms, be they microorganisms, plants or animals, or plant biomass including organic organisms, animal biomass, microbial biomass, aquatic biomass, less studied until now but an important source for the future because submerged aquatic plants in temperate zone produce, on average, 5-7 tons dry biomass per hectare a year. Terrestrial plant species are very different in terms of conversion efficiency of solar energy and biomass production. The potential of these species is evaluated in the temperate zone at 20- 30 tons of dry biomass/ ha/ year, but these values are achievable only by the most productive species and in very favorable conditions [1, 2].

Under EU legislation, biomass has a broader sense as the biodegradable fraction of waste products and residues from agriculture (including organic and animal substances), forestry and related industries, as well as the biodegradable fraction of municipal and industrial wastes.

The conversion of solar radiation into chemical energy via photosynthesis results in the growth of woody, herbaceous, and aquatic biomass and the formation of many organic compounds in situ, each of which has an intrinsic energy content. Cellulose are the more commonly known (40-45%), being usually the chief structural element and principal constituent of many biomass species, but is not always the dominant carbohydrate, especially in aquatic species. The lignin (15-30%) and hemicelluloses (20-35%) comprise most of the remaining organic components. In addition, other polymers and a large variety of non polymeric organic solids are formed naturally, although not equally, in biomass. Many natural glycerides can be finding in many biomass species [3].

Energy production from biomass currently has a high political priority, as for example shown by the European Union target of a 20% share of renewable energy by 2020. It can be expected that the cultivation of short rotation coppice and perennial energy grasses for heat and power generation will become more important after 2010 when new technologies enter the market and bio-heat options are further developed [4].

A resource that is still unexploited is the leaf litter known to be a natural fertilizer after decomposition. Leaf litter is one of the major input sources of organic carbon and nutrients in ecosystems. Throughout the degradation and leaching of leaf litter, ‘‘new born’’ substances are continuously generated and may aggregate to form humic substances. These findings suggest that leaf litter degradation extracts may be an important environmental factor influencing community structure within ecosystems [5].

Leaves from grassland plant species had higher decomposition rate compared to the stems due to high N and low C content. The amount of nutrients released during crop residue decomposition is highly important in both organic and conventional farming systems; in the former, it has a decisive influence on crop yield and in the latter, it could lead to a reduction in mineral fertilizer application. Litter decomposition is an important process in the global carbon cycle. It accounts for most of the heterotrophic soil respiration and results in formation of more stable soil organic carbon which is the largest terrestrial carbon stock [6].

The physicochemical nature of soil habitat, the quality and quantity of organic matter and the community composition of decomposers control the process of litter decomposition in terrestrial ecosystems. The influence of soil fauna on litter decomposition includes both direct and indirect effects. Small organisms, such as bacteria, fungi and protozoa, are the key drivers of energy and nutrient transformations, whereas larger organisms, earthworms, millipedes and isopods, are the dominant habitat transformers [7].

In the litter of six deciduous tree species (*Fagus sylvatica, Tilia spp., Fraxinus excelsior, Carpinus betulus, Acer pseudoplatanus and Acer platanoides)* and in stand-specific litter mixtures, mass loss was compared and nutrient release across diversity levels along a gradient of decreasing proportion of *Fagus* in stands with similar environmental and physical soil conditions. The litterbag studies ran over 22 months [8].

Decomposition of fresh leaf litters and fine roots was studied in laboratory microcosms, consisting of airtight glass jars. There was generally a substantial decrease in K concentration in the decomposed litters. The C/N ratios in the decomposed layer were lower than those of fine roots and leaf litter [9]. Lignin is a decay-resistant biopolymer usually regarded as a rate-regulating factor in leaf litter decomposition. Hence, lignin content may be used to predict the decomposition rate and weight loss of leaf litter [10].

A group of researchers in Bangladesh obtained a natural and effective biofertilizer produced from fruits and vegetables waste. The Process uses biotechnology to convert organic material into a valuable, pathogen-free organic fertilizer in less than 72 hours using naturally occurring, heat-generating thermophilic bacteria.

There are a few works in literature that investigated the foliar biomass, either by describing the decomposition process to mold, or by qualitative and quantitative analysis of soil on which it was laid. All natural decomposition taking place under the action of bacteria, fungi and worms are slow processes, lasting for months.

We tried to find a version of decomposition of leaf litter under the influence of chemicals and to provide nitrogen and phosphorus, necessary elements for cultivated soil. The idea that was started was to find a quick and efficient exploitation variant of foliar biomass available in very large quantities at fall, for obtaining a fertilizer.

On the other hand, because the aquatic biomass is recently in the researcher's

attention, as a possible source of biodiesel, we investigated an aquatic plant (mud sedge - *Carex limosa*) as a potential source of fat.

MATERIALS AND METHODS

Was used:

a) Aquatic plants (mud sedge - *Carex limosa*) harvested from the lake Secu in Reşiţa in fall 2009. For storage until processing the plants have been frozen.

b) Mixed foliar mass (hornbeam leaves - *Carpinus betulus*, and acacia leaves - *Robinia pseudacacia*) collected from parks in Timisoara and Reşiţa town. Some of the leaves were frozen; others were dried and kept in a dry and airy place.

For analysis of the fat leaf material we used a Varian 2000 FT- IR spectrometer and to assess the fertilizer potential we made experiments using plants of petunia- *Petunia hybrida* grown in pots of 10x10 cm and seeds of cress (*Lepidium sativum*) grown on agar gel. Cress was chosen because it is a fast growing plant; its complete cycle is 14 days.

To prepare 500 mL of gel, 2.5 g of agar was used and added to the liquid (water or extract) warmed to 65-70 ºC while stirring. After the agar was dissolved, the solution slowly became a gel while cooling.

RESULTS

A. Fats in the aquatic plant mud sedge - *Carex limosa*

70 g of crushed aquatic plant were placed to soak with 100 mL ethyl ether for 24 hours, and then filtered to remove plant material. The ethereal extract was concentrated by distillation up to two thirds its original volume, then dried on anhydrous salt (anhydrous Na_2SO_4) and evaporated to dryness on a water bath. An oily residue not homogeneous was obtained, with areas of brown. The FT-IR analysis indicates the presence of triglycerides by vibration from 1726 cm^{-1} specific to this ester group.

B. The use of foliar biomass as fertilizer

Foliar biomass was crushed to a fine uniform powder, using a laboratory mill; 6 g of this powder was mixed with 60 g of soil and put in pots of 10x10 cm. In these pots decorative flowers (petunia, *Petunia hybrida*) were planted. These represents sample B1. As control samples we used different pots of the same sizes with plant soil in which identical flowers were planted (samples B2). Plants were watered every two days with equal volumes of distilled water. For a few days the soil in samples B1 has been much less penetrable for water than the soil in the control samples and the plants suffered. After two week there were

some changes, both in the soil structure, and in the development of the plants. After one month we noticed that the plants B1 developed better than the control samples (B2).

C. Using the foliar biomass after decomposition/ chemical extraction

Initially we intended to find some reagents to decompose foliar mass and/ or to extract chemicals from it. We used a number of tubes in which we put one spatula tip of leaf powder and different reagents. We watched the appearance of the solution and the decomposing of the foliar mass. The observations are placed in Table I.

Table I. Tests with different reagents for decomposing/ extraction of leaf powder

Reagent	Appearance after 30 minutes of contact	Appropriate (A)/ Inappropriate (I)
H_3PO_4 (85%)	Reddish - brown extract, decomposed foliar biomass	A
H_2SO_4 (96%)	Decomposed, dark colored biomass	I
HNO_3	Yellow solution, partially dissolved biomass.	A
NH_4OH (19.5%)	Orange extract, brown biomass.	A
KOH (50%)	Reddish - brown extract, brown biomass	A
$NaHPO_4$ H_2O solution	Yellow extract, brown biomass	I
Na_2HPO_4 12 H_2O solution	Reddish brown extract, brown color	A
$(NH_4)_2HPO_4$ solution	Blurry yellow extract, dark brown biomass	I
$NH_4H_2PO_4$ solution	Yellow extract, brown biomass	I
$NaNH_4HPO_4$ $4H_2O$ solution	Bright yellow extract, brown biomass.	A
H_2O	Slightly yellow extract, brown biomass	I

Obvious results were obtained with solutions of H_3PO_4, NH_4OH, H_2SO_4, HNO_3 and Na_2HPO_4. Strong acids and NH_4OH decompose a good part of the foliar mass. Concentrated HNO_3 (69% and 50%) produces noticeable decomposition by release of gas. This process is not observed when we used more dilute HNO_3 (solution 25% in water).

From all the reagents that we used we selected only those which contain elements with a role for plant macronutrients (N, P): 34.5% HNO_3 solution (obtained by diluting the solution 1:1 HNO_3 69%), 19.5% NH_4OH solution and 10% disodium phosphate solution in water.

The working plan is shown below:

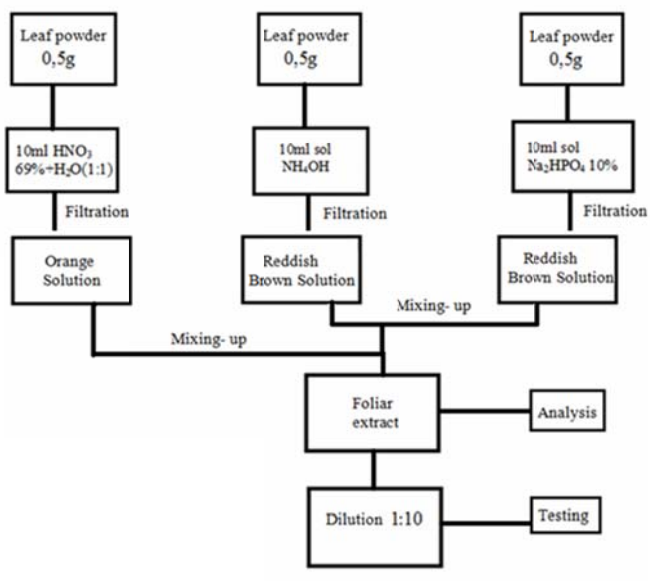

Figure 1. The working plan in obtaining the foliar extract

The leaf extract is diluted 10 times with distilled water and a homogenous yellow-brown liquid is obtained noted (F). The liquid was used to assess the fertilizer potential in the two versions listed below:

C1: We used control samples with plants exactly the same size that were watered every other day with equal volumes of distilled water (samples MA) and of the solution (F), sample noted MR. After almost two weeks the MR plant dried completely and the MA plants developed normally. After one month the results show that the plants MR died. The samples F survived but underdeveloped comparing with samples MA.

C2. Tests on plants cultivated on agar gel

On a plate with wells 10 mL of agar gel and seeds of cress (*Lepidium sativum*) were placed in each well. For the preparation of agar gel 1.25 g of agar were dissolved in 250 mL of distilled water (control samples MAA), 250 mL of MR (MRA samples) and 250 diluted foliar extract (sample FA). We observed the plants germination, development and after a week they were weighed and measured individually. We calculated averages for each group of plants (in each well) and global averages. The results are shown in Table II.

Two series of experiments were made to verify the reproducibility. The results are placed in Table II.

Table II. The results from testing the ability of fertilization of the foliar extract on agar gel

Experimental series	Samples	Seeds that were not germinated (%)	Medium length/ plant (cm) *	Changes in plant length (%)	Medium weight/ plant (mg)*	Changes in plant weight (%)
I	FA	33.3	2.58	- 39.0	16.5	-31.2
	MRA	100.0	0	0	0	0
	MAA	10.0	4.22	reference	24.0	reference
II	FA	12.0	2.79	- 23.0	20.0	- 13.0
	MRA	100.0	0	0	0	0
	MAA	16.0	3.62	reference	23.0	reference

DISCUSSIONS

A. The aquatic plant (mud sedge - *Carex limosa*) that we investigated contains a small amount of raw fat triglycerides which represents only 0.15% of the mass of plant taken into work. The exploitation of these lipids to obtain biodiesel requires a prior purification and losing a part of the product. For one tone of aquatic biomass only 1.5 kg of lipids would be obtained. This amount does not justify the processing costs. In conclusion the analyzed aquatic plant does not represent an efficient source for isolating the triglycerides for obtaining biodiesel.

B. The use of chopped leaf litter mixed with soil in a report of 1:10 produced changes in water permeability which becomes normal after about two weeks. In this period the cultivated plants (petunia) suffered. Therefore, we recommend planting the plants after about two weeks from the soil preparation which contains sprayed foliar biomass.

C1. The use of (MR) solutions with a pH of 5.9 for watering the pots destroys the plant, probably because the solution is too concentrated. The plants watered with distilled water (MA) were taken as a control sample. The plants from the pot noted (F), after one month survived but were underdeveloped compared to samples MA.

C2. The experiments on agar gel with cress seeds (*Lepidium sativum*) shows that the germination and the development of the plants is inhibited when diluted reagents solution 1:10 (MRA) is used (100% inhibition). When (F) is used on agar gel (samples FA) we saw a reduction in the length (with 23-39%) and in the weight (with 13-31%) of the plants. We noticed that the results from the two series of experiments were different. This can be explained by the infestation of the samples FA in series I with mold. The foliar extract obtained by decomposition/ chemical extraction of the foliar biomass does not meet the fertilizing expectations. This could be due to the reduced bioavailability of the nutrients, the high concentration of the reagents or the high concentration of the watering solution.

CONCLUSIONS

1. We investigated the content of triglyceride lipids from the aquatic biomass of *Carex limosa* for obtaining biodiesel. The small content of lipids (0.15%) does not justify economically the use of this natural resource.
2. A method of decomposition/chemical extraction of foliar biomass using inorganic reagents (HNO_3 solution 34.5%, NH_4OH solution 19.5 % and Na_2HPO_4 solution 10%) was discovered.
3. The diluted extract 1:10 obtained from the foliar biomass was tested as a fertilizer in two different experiments: on decorative plants (petunia, *Petunia hybrida*) planted in pots and on cress (*Lepidium sativum*) cultivated on agar gel. The results show an inhibition in the development - length and weight, of the cress. In the case of petunia the development of the plant was also inhibited.
4. The results show that the foliar biomass as it is, but finely crushed, mixed with soil serves as fertilizer, but only after two weeks since inclusion in the soil, in which time the permeability of the soil is diminished.

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