



Co-funded by the Eco-innovation
Initiative of the European Union



eco-innovation
WHEN BUSINESS MEETS THE ENVIRONMENT

CIP Eco-innovation
Pilot and market replication projects
Call 2012

Call Identifier: CIP-EIP-Eco-Innovation-2012

D3.4 REPORT ON THE ANALYSIS, REGULATIONS AND FIELD PERFORMANCE OF THE MINERAL FERTILISERS PRODUCED

DIGESMART

CONTRACT ECO/12/332882



European
Commission | Executive Agency for
Small and Medium-sized Enterprises



Project website: www.digesmart.eu

DIGESMART PROJECT: **DIGES**tate from **MA**nure Recycling Technologies

www.DIGESMART.eu

Disclaimer

The responsibility for the content of this report lies with the authors. It does not necessarily represent the opinion of the European Community. The EACI is not responsible for any use that may be made of the information contained herein. The information contained is given for information purposes only and does not legally bind any of the parties involved.

TABLE OF CONTENT

Introduction	1
Product analysis	2
Regulation	3
Field Performance.....	4
Italy	4
Belgium.....	20
Overall remarks and conclusion	28



INTRODUCTION

This report is the deliverable 4 in work package 3 of the DIGESMART-project. It consists of three chapters related to the products produced with the DIGESMART solution. The major product is the ammonium nitrate (hereby DIGESMART-fertiliser) produced by stripping and subsequently acid scrubbing. Furthermore, some analysis results are included on the products after the solar drying step, which are ammonium water and less moist thin fraction.



PRODUCT ANALYSIS

This part briefly discussed the product analysis made during the project for various goals such as use in field trials and transport.

The main parameter that is necessary to assess is the total nitrogen content, this is a means to know how much nitrogen is present in the product sample. Based on this, proper advices concerning the fertiliser use could be given. Also this is a means of assessing process quality of the stripping/scrubbing set-up.

To attain a measure of acidity the pH value can be determined. Ammonium nitrate acts as a weak acid in water, so the pH should not be too low, otherwise this could mean an anomaly in the stripping/scrubbing process.

Organic carbon content in the form of COD should also be minimal to nothing in the product sample. Traces of metals such as Zn, Cu, Cd, Hg, Pb, Cr and Ni should also be low.

Microbiological parameters such as analysing the presence of *E. coli* could also be tested if the product is used as a fertiliser in crops used for fresh consumption, but it seems not to be a prerequisite. As the liquid fraction is stripped of ammonium by means of caustic substance addition or temperature, the gaseous phase is scrubbed with a highly concentrated acid which no micro-organisms can endure for a long time. On the other side, to comply to the animal by-products legislation, microbiological assays could be requested.

Following items are to be concluded from the product analysis done during the project duration:

Ammonium nitrate

- Increasing quality and stabilisation in product composition achieved during the project: Total N from 13.2% (Inagro-trial) to above goal content 19,2% (UGent-trial)
- Technical pure product
- Below threshold of heavy metals

Condensate after evaporation

- Clear solution
- Containing around 0,8 % of NH₃ per liter

Product after solar drying

- Increase in dry matter content in the range of 1 to 3%
- Need of additional drying



REGULATION

According to the Nitrate directive ([91/676/EEC nitrate](#)), the maximum application of manure corresponds to 170 kg N/ha/year (as general limit value, also for digestate). The main objective of the Directive is to reduce water pollution caused or induced by nitrates from agricultural sources and prevent further pollution. It has been implemented into the member states regulations through Action Programs for vulnerable areas to nitrates pollution that include good agricultural practices specially related to fertilisers application. This directive regulates the application of all kind of fertilisers onto the soil and applies to digestate. It considers a chemical fertiliser as a fertiliser manufactured by an industrial process and livestock manure as waste products excreted by livestock, even in processed form (digestate). In particular, the DIGESMART products application in vulnerable areas have to take into account maximum nutrients application as well as good agricultural practices developed by each member state.

The animal by-products Regulation ([EC/1069/2009](#); [EC/142/2011](#)) lays down special health related rules for non-food animal “waste”. Organic fertilisers and soil conditioners which contain biomass from animal sources are included in this legislation. Commercialization, storage and transport of those products are regulated by them. The organic fertilisers must fulfil end-product requirements in case that they are commercialized (thermal treatment of at least 70 °C 1 hour, maximum microorganisms content, such as *Salmonella* and *E. coli*, or other parameters authorized by the competent national authority). Digestate from animal by-products digestion can be used as organic material to elaborate organic fertilisers and it must also fulfil the requirements mentioned before (see national regulations for further detail on permit procedures). In case of DIGESMART solution, it will be applied a thermal treatment of the liquid part of digestate, which will be refined into different marketable products.

In case substances cease to be waste and become products, they may become subject to EU chemical legislation (Registration, Evaluation, Authorization and Restriction of Chemicals, REACH). This issue should be also considered in the future End-of-Waste criteria development and future revision of Fertiliser Regulation. After receiving the product status, digestate products may fall under the REACH, but could be exempted from registration because of their recycled status and similarity to existing chemical products.

As the DIGESMART-products are obtained from animal manure in essence, the legislation sees the products as still animal manure in most cases. Future outlooks give the possibilities that the ammonium nitrate will be accepted as chemical fertiliser as is the case for scrubber waters for instance.



FIELD PERFORMANCE

Field trials are the most important way to assess the agronomical potential of any (mineral) fertilisers. In order to validate the use of the mineral fertiliser produced by the DIGESMART technology, the consortium set up two clusters of trials in two different crop systems in Italy and Belgium. The goal was to identify the most suitable crops and utilisation ways to the characteristics of the DIGESMART fertiliser.

An overview of the trials performed in each country is presented in the following sections. For each trial, the trial, the set-up, the results and the data analysis are described in detail. Finally, a general conclusion is made for the use of DIGESMART fertiliser in the European agricultural system.

ITALY

In Italian agriculture, liquid fertilisers are mostly used in fertigation systems and for foliar application. They are seldom directly applied to the soil, mainly because of the great number of commitments to protect the environment and machinery problems. Therefore, the decision was to test the DIGESMART fertiliser on a leafy vegetable grown in greenhouse, maize grain with fertigation system in open-field and superior bread wheat with foliar fertilization. In total the trials add up to 4 assays performed in 2 years. Table 1 summarizes the trials carried out in Italy, including timing and location of each assay:

Table 1. Overview of trials in Italy

Trial Number	Green-house	Timing	Type	Fertiliser application
1	Lettuce - spring cycle	Feb 2015 - May 2015	Greenhouse	Fertigation
2	Lettuce - fall cycle	September 2015 - Oct 2015	Greenhouse	Fertigation
3	Superior bread making Soft wheat	Oct 2015 - June 2016	Open field	Foliar
4	Maize grain	May 2015 - Oct 2015	Open field	Fertigation

All trials were set up in a randomized complete block design (RCB), comprising at least 3 treatments and 3 blocks, for at least 9 replicates each. The treatments followed the scheme shown in Table 2.



Table 2. General treatment scheme

Treatments	Details
T-0	No fertiliser application to the crop (blank)
T-1	Conventional practice for fertilization of the crop: based on integrated agriculture guidelines (which can comprise animal manure + chemical fertiliser)
T-2	Use of the DIGESMART mineral fertiliser instead of the conventional LIQUID fertiliser.

In the following paragraphs, a commentary is given for every trial describing the material and methods, the results and the comments; some pictures are included to illustrate the trials discussion.

LETTUCE TRIAL - SPRING

In the Italian region, lettuce is commonly grown in open field or in a greenhouse. Fertilisation can be done with granular fertiliser before transplanting and during the crop cycle or can be performed through fertigation if a drip system is set-up. While the former is easier and may be used by small scale farm, the latter is more technical and compatible with the trial aim. Due to the complex set-up of the trial, the researchers preferred to host the trial in SATA’s experimental fields, located in Quargento (AL), in the north of Italy.

MATERIALS AND METHODS

For this trial, seedlings were used of lettuce (*Lactuca sativa* L.) var. Sintia produced by VIVAIO Ricca Sebastiano from Carmagnola (TO) who kindly provided the plants for the project. The trial had a RCB design with 3 treatments as described above and 4 blocks. The scheme of the trial is represented in the following outline (Figure 1):

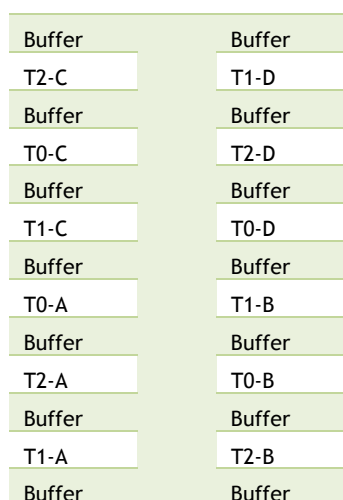


Figure 1. Spring lettuce trial design



For the whole trial, 1000 plants were transplanted on the 5th of March 2015, at 0,30 m distance between the rows and 0,22 m between the plants, counting to around 12 plant per square metre. The trial comprised 75 square meter, including the 12 experimental plots (4 m² each) and the 14 buffers (2 m² each) between the plots.

A maximal dose was considered of 100 kg/N ha in order to calculate the amount of fertiliser for T1 (conventional liquid nitrogen fertiliser - 20% N) and T2 (DIGESMART fertiliser - 16% N). Buffer plants did not receive any fertilization and were grown as T0. Fertilisers were applied in 3 times: on 16/03/2016 (30% of the total dose), on 27/03/2016 (40% of the total) and on 07/04/2016 (30% of the total). Irrigation followed the plant needs. Some illustrative pictures of the trials are given below (Figure 2).



Figure 2. Left: Fertiliser application through mechanical pump during irrigation - Right: The trial with the developed crop

During the crop development an assessment was done to evaluate potential phytotoxicity and plant mortality related to the use of the fertiliser. The harvest took place on 15/04/2015, measuring the following parameters afterwards:

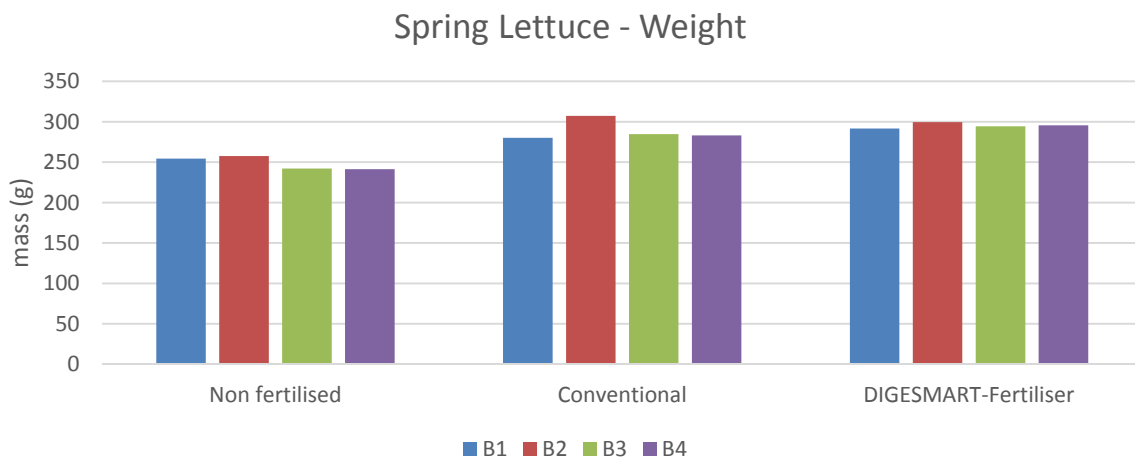
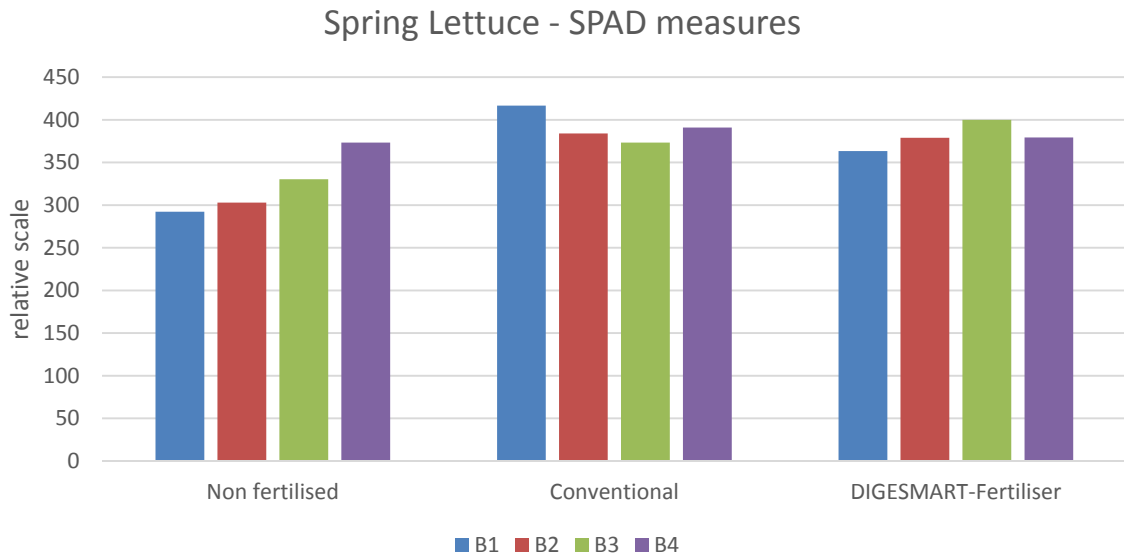
- Weight of plants in the plot (12 plants per plot)
- SPAD (soil plant analysis development) measurements (3 plants per plot)
- Number of rotten plants in the plot.
- Nitrate concentration in the soil before and after harvest of the trial for every treatment (mixing the block samples) and in the plant leaves in every treatment (mixing the block samples).

Weight data were statistically analysed with one-way ANOVA and graphs were elaborated for other parameters.



RESULTS AND DISCUSSION

No phytotoxicity was seen during the development of the crops. Weights and SPAD-data were measured and are represented in the following graphs.



Treatment	T0	T0	T0	T0	T1	T1	T1	T1	T2	T2	T2	T2
Block	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
Mass (g)	254,5	257,5	242,1	241,4	280,2	307,3	284,7	283,2	291,6	299,6	294,3	295,6
SPAD	292,3	303	330,3	373,3	416,7	384	373,3	391	363,3	379	400	379,3

Figure 3. Data results for the lettuce crop experiment

For the weight, data is not normally distributed and as a consequence no ANOVA can be performed. By the graph, it is visible that data from conventional and green fertiliser are similar.



It can be concluded by observing the graph that the data are normally distributed and ANOVA showed significant differences. This from no fertilised treatment (T0) to fertilised treatments (T1 and T2). There are no statistical differences between the use of the DIGESMART fertiliser and a conventional one.

Regarding plant bottom rot it was seen that only with fertilization (T1 and T2) some plants were slightly rotting or partially rotting. This can be due to a higher vigour of the plants and more contact surface with the soil (Table 3).

Table 3. Bottom rot indication of the different trial blocks

Treatment	Block	Rotting bottom	Comments
T0	B1	0	
T0	B2	0	
T0	B3	1	
T0	B4	0	
T1	B1	3	
T1	B2	1	slightly
T1	B3	2	slightly
T1	B4	2	
T2	B1	4	
T2	B2	2	slightly
T2	B3	2	
T2	B4	2	

Regarding nitrate content in soil and leaves reported in Table 4, it is noticeable that the amount is really variable and it is influenced by the residual fertilization of the soil as well as by the use of the DIGESMART fertiliser. No matter what treatment used, all values on leaves comply with the European law.

Table 4. Results of the analysis of nitrates on soil and on leaves of the lettuce trial - spring cycle

Matter	Analysis ref. Number	Date of sampling	Trial ID	Treatment	Nitrate (mg/kg)	Error range (+/-)
Soil	3174	24/04/2015	Lettuce Spring cycle	T0	886	102
Soil	3175	24/04/2015	Lettuce Spring cycle	T1	430	55
Soil	3176	24/04/2015	Lettuce Spring cycle	T2	660	79
Lettuce	3177	24/04/2015	Lettuce Spring cycle	T0	1550	164
Lettuce	3178	24/04/2015	Lettuce Spring cycle	T1	1665	175
Lettuce	3179	24/04/2015	Lettuce Spring cycle	T2	1971	201

In conclusion, it is very clear that in this trial and in this type of crop, the DIGESMART fertiliser worked as a conventional fertiliser. As expected, no observable differences were detected.

LETTUCE TRIAL - AUTUMN

This trial was performed in order to investigate if the season could affect the sensitivity of the crop to some parameter of the fertiliser. The trial did not follow the same set-up of the previous cycle as the researchers preferred to increase the size to have a more representative situation and make a stronger statistical analysis.

MATERIALS AND METHODS

As before, the seedlings used were those of lettuce (*Lactuca sativa* L.) var. Sintia produced by VIVAIO Ricca Sebastiano from Carmagnola (T0) who again kindly offered the plant for the project. The trial had a RCB design with 3 treatments as described above and 4 blocks. The scheme of the trial is represented in Figure 4:

T2-B1	T0-B2	T1-B3	T2-B4
T1-B1	T2-B2	T0-B3	T1-B4
T0-B1	T1-B2	T2-B3	T0-B4

Figure 4. Scheme of the Italian lettuce trial - fall cycle

Each plot comprised 4 rows of 15 meters long (18 square meter). Every block was 0,5 m distance from the others. Plant density was about 11 plants/square meters (0,30 m of distance between the rows and 0,3 m between the plants). The trial comprised a total of 216 square meters for a total of 2300 plants. Due to the larger number of plants, a buffer was not set between the plots.

Calculation of the nitrogen amount followed the previous trial as well as the treatments. The experimental scheme stated to apply the fertilisers in 3 times, but this was undertaken slightly later



than expected because a correct batch of DIGESMART fertiliser did not arrive in time. The schedule was as it follows: on 08/10/2015 (30% of the total dose), on 19/10/2015 (40% of the total) and on 09/11/2015 (30% of the total). Irrigation followed the plant needs. Figure 5 illustrates the trials.



Figure 5. The trial lettuce-fall cycle. Left: 1 week after transplanting - Right: before the harvest

As in the spring trial previously described, the crop development, phytotoxicity and plant mortality related to the use of the fertiliser were monitored.

The harvest took place on 23/11/2015, with measurements on the following parameters:

- Weight of plants in the plot (20 plants per plot) and evaluation of plant development from 1 to 5 (1=small plant and 5=high developed plant)
- SPAD measures (3 plants per plot)
- Number of rotten plants in the plot.
- Nitrate concentration in the soil before and after harvest of the trial for every treatment (mixing the block samples) and in the plant leaves in every treatment (mixing the block samples).

Weight and SPAD-data were statistically analysed with one-way ANOVA and graphs were elaborated for the other parameters.

RESULTS AND DISCUSSION

Results of the trial are reported in Table 5.

Table 5. Data averages of the Italian trial lettuce in fall cycle

Treatment	Block	Plants with diseases	SPAD (average)	Weight (kg)	Development (1= late to 5= advanced)
T0	B1	4	331	0,304	3,65
T0	B2	0	328	0,375	3,85
T0	B3	2	308	0,345	3,85
T0	B4	1	301	0,326	3,6
T1	B1	0	380	0,375	3,95



T1	B2	0	322	0,427	4,15
T1	B3	2	330	0,375	3,95
T1	B4	2	323	0,378947	3,95
T2	B1	0	332	0,397	4,1
T2	B2	1	339	0,371	3,95
T2	B3	3	311	0,436842	4,25
T2	B4	1	333	0,375	4,05

Figure 6 reports the averages in weight for the lettuce crops.

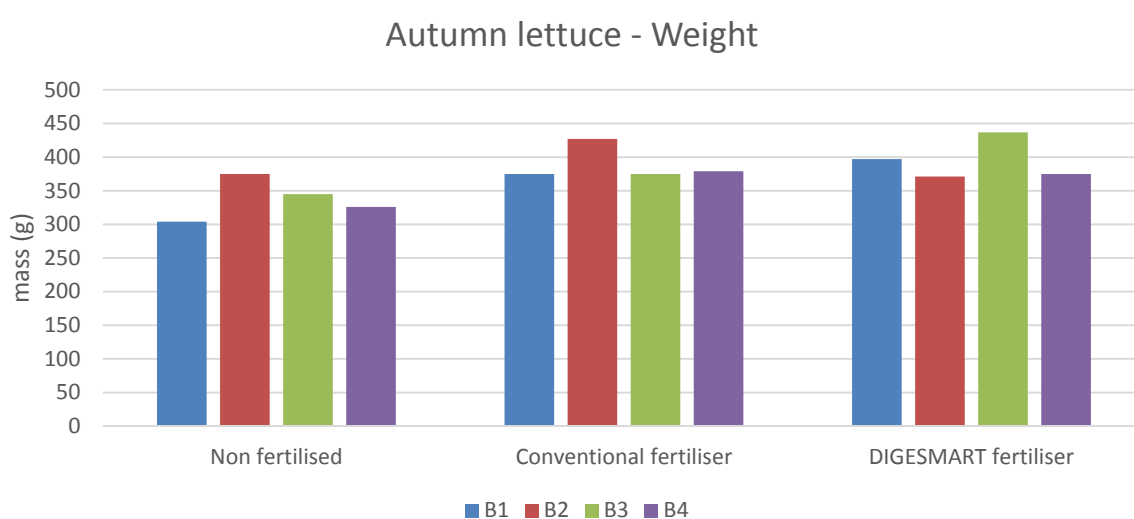


Figure 6. Graph of the weight average of the Lettuce-autumn cycle.

Observations are similar to those found for the previous cycle as no differences were measured between the treatment 1 and 2. Only between the non-fertilized and the fertilized treatments a difference can be noted. For the statistical analysis, data proved to be normally distributed ($W = 0.793$, $p\text{-value} < 2.2e-16$). The ANOVA showed significant differences between the data ($P\text{-value}=0.0152^*$). For SPAD-values, the averages are reported in Figure 7.

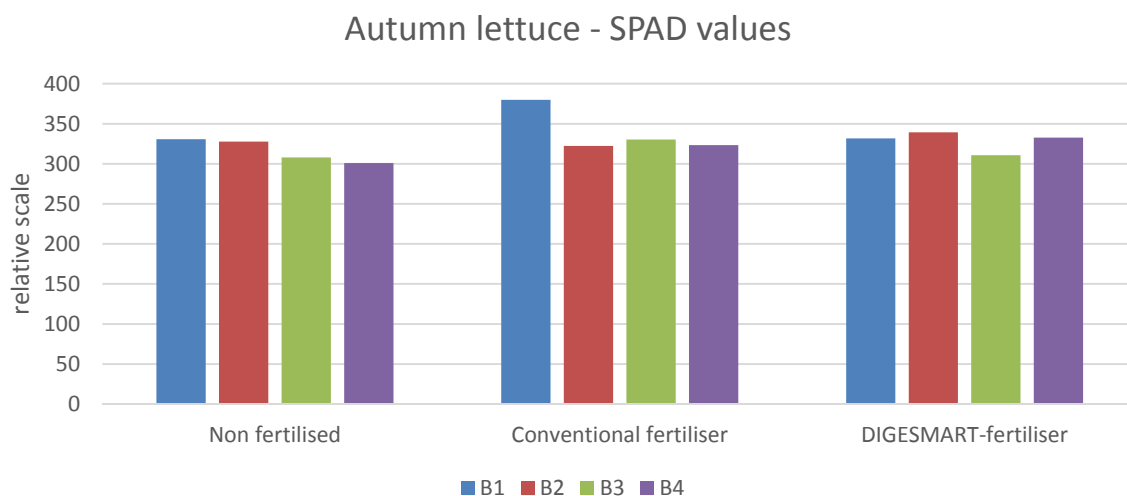


Figure 7. SPAD-values of the lettuce trial - fall cycle

No significant differences were observed, being this confirmed by the statistical analysis with one way ANOVA which showed no statistical difference (P-value = 0,22).

Regarding the number of plant with diseases, no differences were observed among the treatments.

Table 6. Results of the nitrates analysis performed in this trial.

Matter	Analysis ref. Number	Date of sampling	Trial ID	Treatment	Nitrate (mg/kg)	Error range (+/-)
Soil	13846	30/11/2015	Lettuce Fall cycle	T0	640	77
Soil	13847	30/11/2015	Lettuce Fall cycle	T1	86	14
Soil	13848	30/11/2015	Lettuce Fall cycle	T2	1221	134
Lettuce	13849	30/11/2015	Lettuce Fall cycle	T0	1796	184
Lettuce	13850	30/11/2015	Lettuce Fall cycle	T1	1796	186
Lettuce	13851	30/11/2015	Lettuce Fall cycle	T2	1906	196

As visible in the table, the nitrate concentration values showed no interesting trend, while the differences found are related to spatial variability. Values for lettuce are compliant with the EU requirements.

In conclusion, as in the previous trial, the DIGESMART fertiliser worked as a conventional fertiliser; as expected, no visible differences are observed.

MAIZE TRIAL

Maize is one the most important crops in the north of Italy, which requires large amount of water and fertilisers. In the last decade, new techniques have been introduced in order to increase the sustainability and the efficiency of the agricultural system. In fact, in areas where water is scarce, maize is commonly grown with drip irrigation system and often fertigated, where both liquid and solid fertiliser can be used. This is the reason why this type of crop system was chosen for the field trial. In addition, no liquid fertilisers are commonly spread directly on soil.

MATERIALS AND METHODS

The trial was sown on the 8th of May 2015 in SATA's experimental field, in Quargnento (AL). A maize grain medium cycle hybrid from Dekalb[®] (Monsanto) - FAO 500 was used. The trial had a RCB design with 3 treatments as described above (T0, T1 and T2) and 3 blocks. The scheme of the trial is represented in Figure 8.

Border crop																	
Plot	T0-A	b	T2-A	b	T1-A	b	T1-B	b	T0-B	b	T2-B	b	T2-C	b	T1-C	b	T0-C
Drip tape	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Road																	

Figure 8. Maize trial scheme. T0 = no nitrogen; T1 = conventional nitrogen fertiliser; T2= DIGESMART fertiliser; b= buffer

Each plot consisted of 4 maize rows (3 m) of 20 meters long (60 square meter). Since drip tapes are located each two maize rows, the plot included 2 drip-tapes. All plots were divided by 2 maize rows (one drip tape). Plant density was about 11 plants/square meters (0,75 m between the rows and 0,15 m between the plants). The trial comprised a total of 780 square meters for a total of 8500 plants.



Figure 9. Right: the maize trial during ripening of the crop - Left: the mechanical sheller used to get the kernels from the maize cobs



Fertilisation comprised 180 kg/ha nitrogen and followed the schedule reported in Table 7.

Table 7. Fertilisation schedule of the maize trial.

Date	Amount	Fertiliser
Before sowing:	100 K kg/ha + 100 P kg/ha	0-20-20
2 July 2015	50 N kg/ha	Urea (T1) or DIGESMART fertiliser (T2)
13 July 2015	50 N kg/ha	Urea (T1) or DIGESMART fertiliser (T2)
22 July 2015	30 N kg/ha	Urea (T1) or DIGESMART fertiliser (T2)
28 July 2015	30 N kg/ha	Urea (T1) or DIGESMART fertiliser (T2)

During the crop cycle the development of the plant and the presence of phytotoxicity symptoms were analysed. In order to assess the effects of the fertilisation, each plot was harvested manually only in the two central rows of each plot for a length of 10 meters (15 square meters per plot). On harvest the ears were shelled mechanically and the following parameters were measured:

- Total weight (yield)
- Specific weight
- Protein content through NIR technology.

Data were collected and represented in graphs in order to evaluate the different parameters. An inferential statistical analysis (one-way ANOVA) has been performed in order to find significant difference among the treatments.

RESULTS AND DISCUSSION

No difference in plant development between the common fertiliser and the DIGESMART-fertiliser was observed as well as for plant toxicity to the solutions. Regarding the yield and quality parameters of the kernels the average values are reported in Table 8.

Table 8. Average results of the maize trial in Italy.

Treatment	Block	Yield (kg)	Specific Weight	Protein content %
T0 - No fertiliser	A	4,48	67,7	9,70
	B	6,78	69,5	9,85
	C	7,53	68,3	9,75
T1 - Urea	A	10,97	72,2	9,85
	B	11,5	72,7	10,00
	C	11,51	71,4	9,80



T2 –DIGESMART-fertiliser	A	9,58	72,3	9,70
	B	11,07	72,4	9,90
	C	10,14	73,5	10,00

Regarding the yield, the results are depicted in Figure 10.

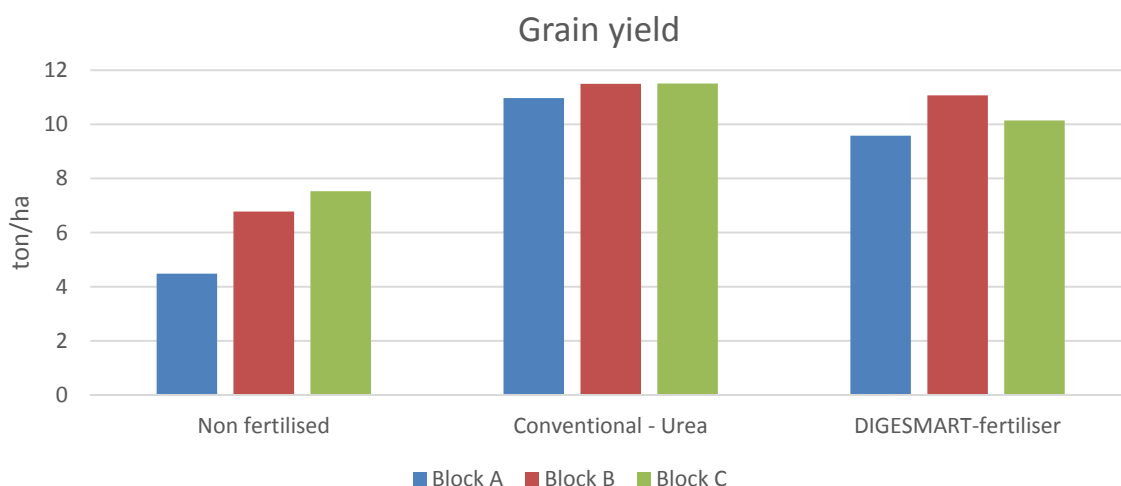


Figure 10. Graph of the yield of the Italian maize trial (grain hybrid)

As visible, the non-fertilized plots have a very low yield while both urea and DIGESMART-fertiliser gave acceptable results. The ANOVA model confirmed the significant difference among the treatments (p-value of 0,002).

Figure 10 gives a visual representation of the data for the specific weight.

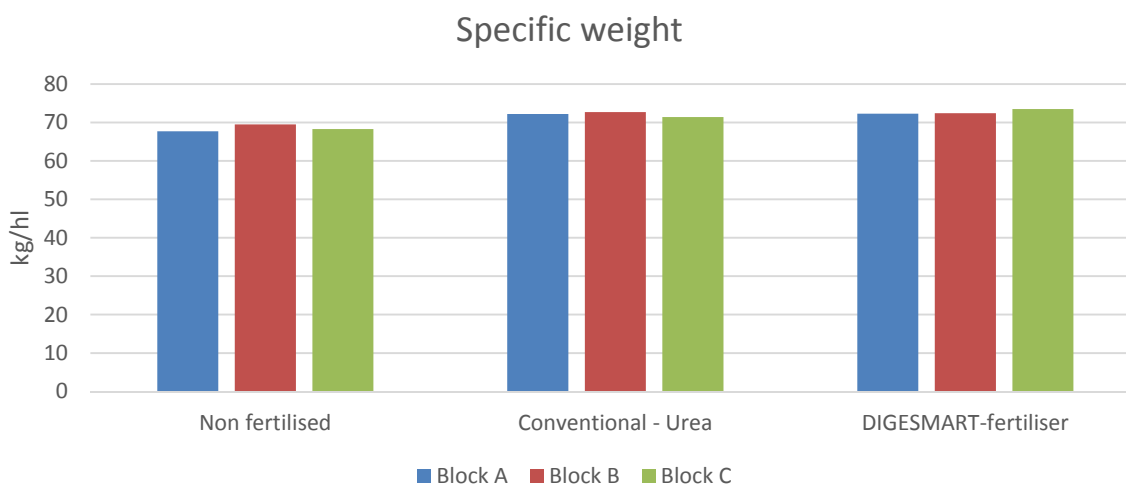


Figure 11. Graph of the specific weight of the maize trial in Italy

As clearly visible, the same differences are observed as with the yield. This is confirmed by the statistical analysis with the ANOVA (p-value of 0,013).

Figure 12 shows the measurements regarding the protein content of the kernel.

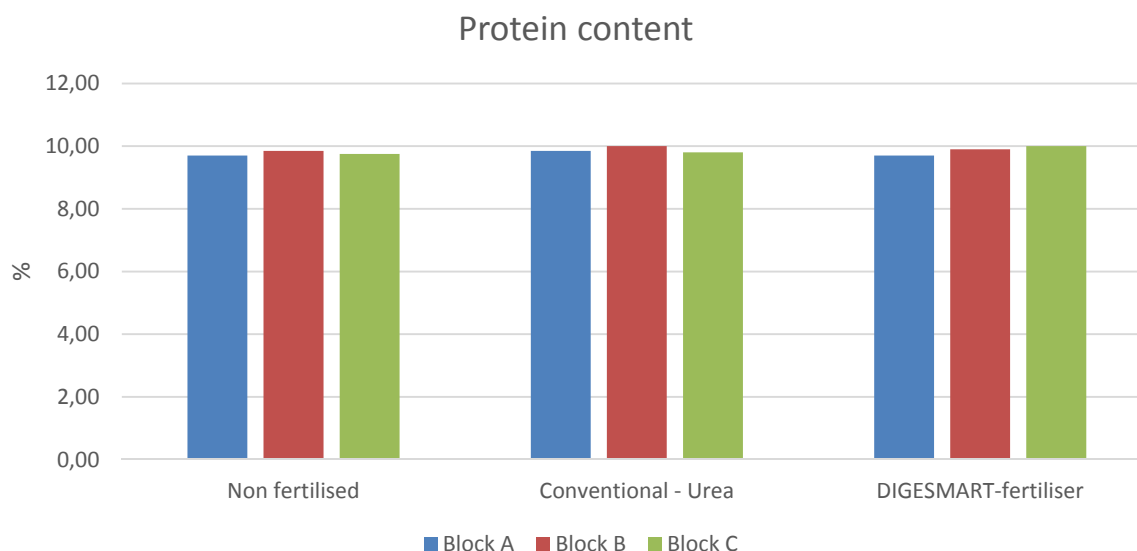


Figure 12. Graph of the protein content measured by the NIR device for the maize trial in Italy

By comparing the results showed in the graphs, it appears that no differences exist among the treatments, which was confirmed by the statistical analysis through ANOVA.

WHEAT TRIAL - FOLIAR APPLICATION

Wheat is another important crop of European agriculture. Even if the most common way of fertilization consists of granular/solid fertiliser, liquid formulas of nitrogen are employed as foliar fertiliser for high quality varieties, so called superior bread wheat. Currently, a wide range of products are available on the market and are commonly used by the farmers. For this reason, in this trial the researchers tested the DIGESMART fertiliser only in foliar application instead of as a substitute of the granular fertiliser.

MATERIALS AND METHODS

The trial comprised a Superior Bread Variety called BOLOGNA from SIS (Società Italiana Sementi), commonly grown in fertile soil to obtain high strength flour ($W > 350$). The field trial was located in SALE (AL).

The trial was set-up with a RCB with the known three treatments (T0 = no foliar, T1 = conventional foliar fertiliser, T2= DIGESMART fertiliser applied on leaves) and 4 blocks, for a total of 12 plots. The scheme of the trial is given in Figure 13.



2C (T1-C)	3C (T2-C)	1C (T0-C)	3D (T2-D)	1D (T0-D)	2D (T1-D)
3A (T2-A)	1A (T0-A)	2A (T1-A)	1B (T0-B)	2B (T1-B)	3B (T2-B)

Figure 13. Trials scheme for the foliar application

The crop was sown on 21/10/2015 at a seed density of 400 seeds per square meter with a mechanical seeder. Plots were 1,5m large and 7 m long for a total of 10 square meter per plot and 90 square meter for the whole trial. Crop protection was applied according to weather conditions and field situation. The farmer applied about 150 kg N/ha of nitrogen through granular fertiliser in all the plots. The foliar fertilisation was applied during earing time (13/05/2016) with a common back sprayer.

For this trial the following parameters were assessed:

- SPAD values
- yield
- Specific weight and protein content

Data were analysed with inferential statistics (ANOVA) and some graphs were elaborated in order to observe the possible differences.



Figure 14. Pictures of the wheat trial in Italy



RESULTS AND DISCUSSION

The results are given for the SPAD-measurements (Figure 15) performed during the development of the crop and about the yield (Figure 16), specific weight (Figure 17) and protein content (Figure 18).

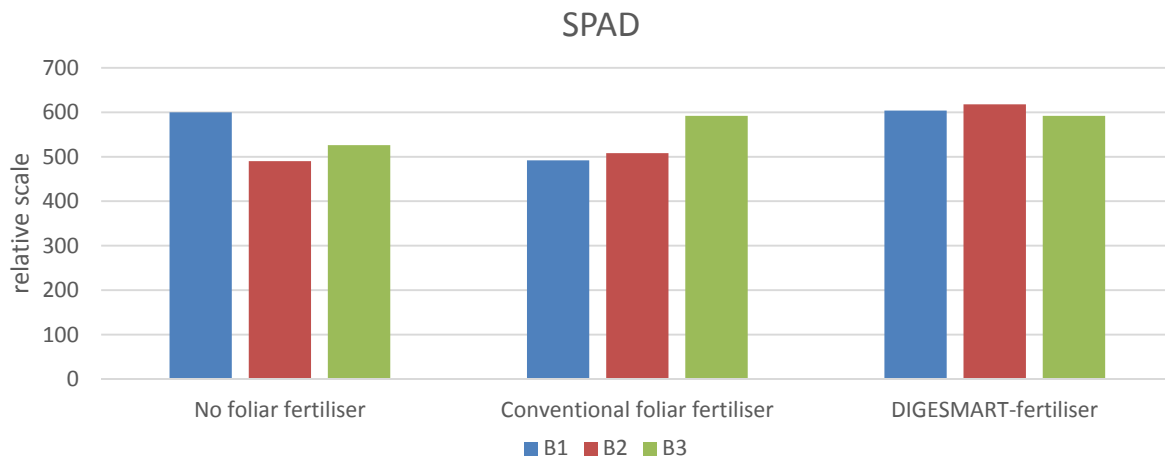


Figure 15. Average SPAD-values of the wheat trial in Italy

Considering the SPAD-measurement (N-tester) of the flag leaves, it seems that the DIGESMART-fertiliser had a slight influence on the chlorophyll content. Besides that, there are no statistical differences (p -value= 0,172).

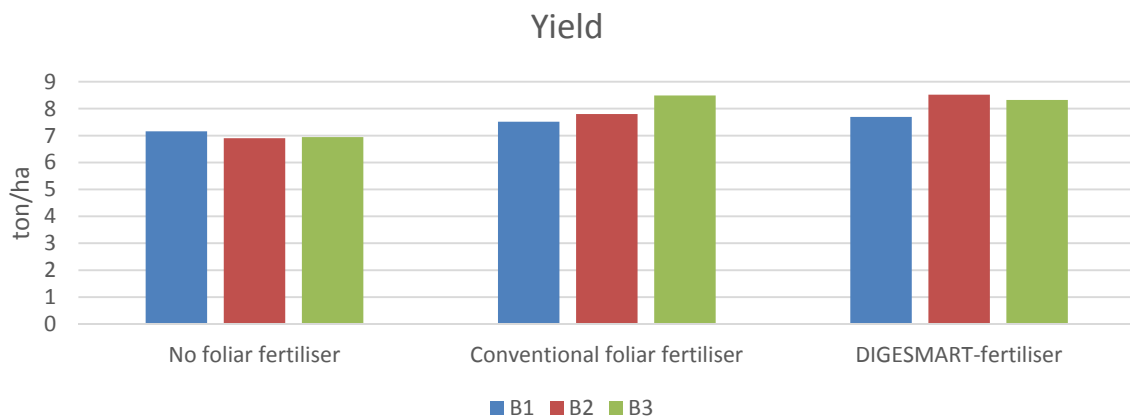


Figure 16. Graph of the average values of the yield of the wheat trial in Italy

Considering yield, the foliar fertilisation provided a slight increase in the yield/ha, considered significant by the statistical analysis through the one-way ANOVA (p -value= 0,02). The DIGESMART-fertiliser had the same effect as the conventional nitrogen fertiliser.

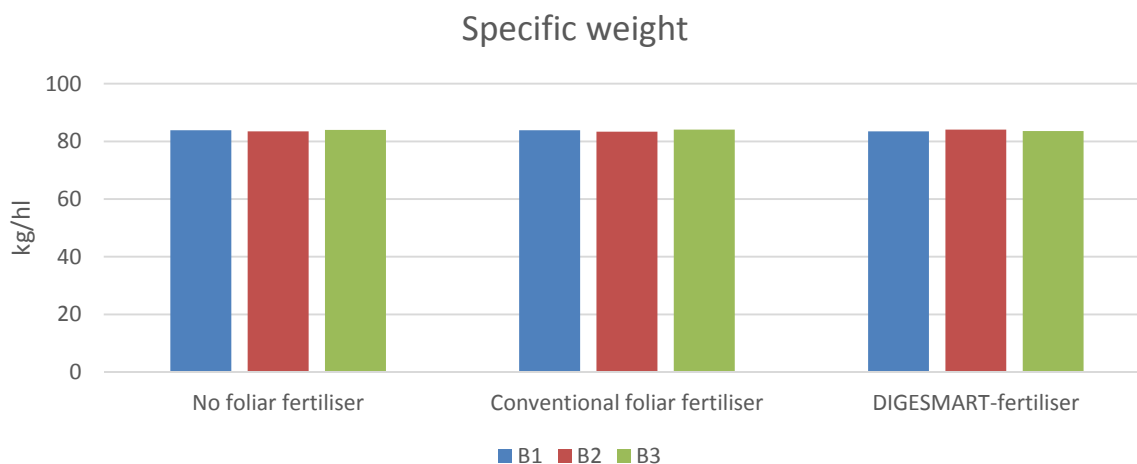


Figure 17. Graph of the average values of the specific weight of the wheat trial

With regard to the specific weight, the foliar fertilisation does not seem to have influenced this parameter. In fact, the statistical analysis did not give any significant difference (p-value=0,97).

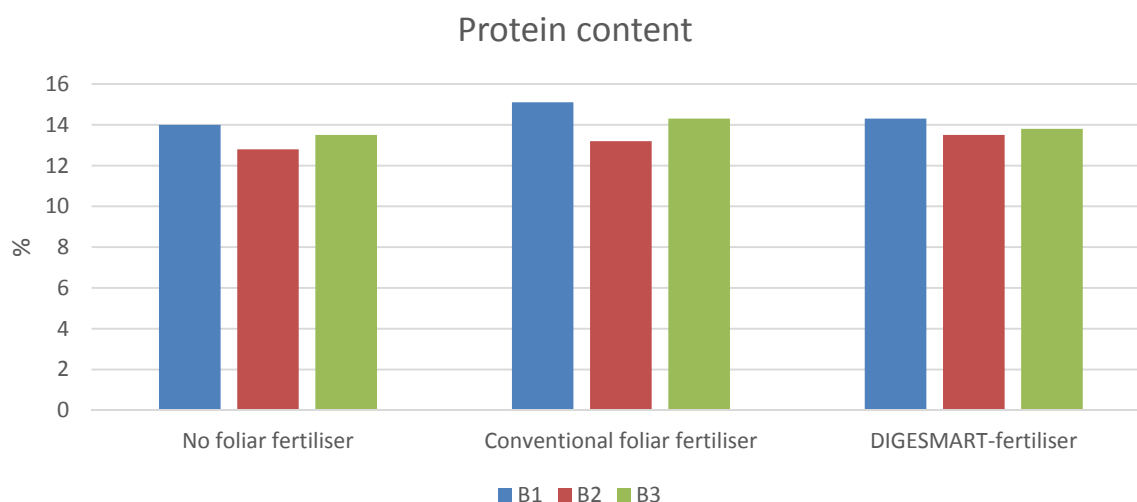


Figure 18. Graph of the average values of the protein content in the wheat trial

As for the protein content of the kernel, the observation shows a slight difference between the untreated and the treated plots. Nevertheless, this is not considered significantly different by the statistical analysis (p-value= 0,446).

In conclusion, the DIGESMART fertiliser can be used on wheat as the conventional fertiliser available on the market.



BELGIUM

Liquid fertilisers are less commonly used by farmers in Belgium, in contrast with commonly used granular solid chemical fertilisers. Nevertheless, given the price competition, the agricultural sector is always interested in innovation that could increase the profit margin. In this sense, the idea was to test the DIGESMART stripping/scrubbing product as an alternative for artificial fertiliser, further denominated as ammonium nitrate. Ammonium sulphate produced by a chemical air scrubber from a nearby pig stable was also included for its test as a competing agent for the DIGESMART-product.

The consortium conducted two trials in Belgium as can be seen in Table 9.

Table 9. Overview of trials in Belgium

Trial Number	Crop	Timing	Type	Fertiliser application
1	Maize	May 2015 - October	Open field	Spreading
2	Lettuce	January 2016 - March 2016	Growth chamber	Mixed with soil

MAIZE TRIAL

The experiment was conducted by Inagro on a field (0,8 ha) near the community of Beernem in Flanders. Silage maize was cultivated during the previous growing season, with a fallow period during winter. Harvesting and sampling was done on the 8th of October 2015.

MATERIALS AND METHODS

Soil samples were taken on 9th of April and 7th of May 2015 prior to sowing on 15th of May 2015. A fertilisation scheme was composed based on the soil analysis, manure analysis and crop needs, resulting in the scheme given in Table 10.

Table 10. Fertilisation scheme for the maize trial in Belgium

Applied fertiliser treatment	Pig slurry (PS) (ton/ha)	Calcium ammonium nitrate (CAN) (ton/ha)	Ammonium nitrate (AN) (DIGESMART) (ton/ha)	Ammonium sulphate (AS) (ton/ha)
Blank	0	0	0	0
PS + CAN	18	0,21	0	0
PS + AN	18	0	0,43	0
PS + AS	18	0	0	1,9



A random block design was constructed with blocks of 6 m to 10 m, and all the treatments were done in quadruple. Figure 19 gives a representation of the applied design.

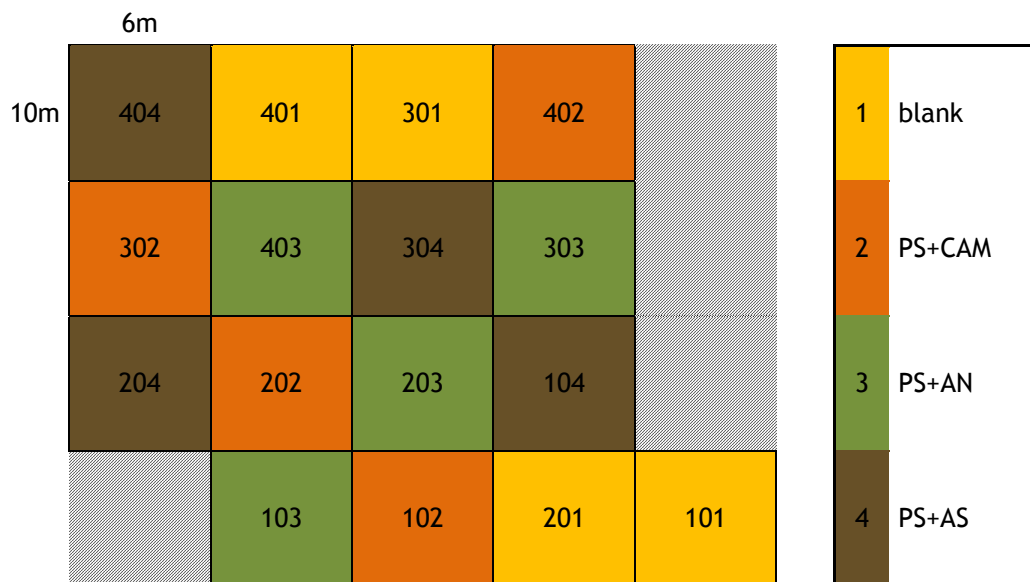


Figure 19. Experiment block outline with different fertiliser treatments.

The experiment was focused on nitrogen, the pig slurry contained the necessary phosphate and a part of the needed potassium for which Patentkali was added to attain the desirable potassium level. Crop yield, protein and N-content, chlorophyll (N-tester) and nitrate residue were determined.

Furthermore, the apparent N recovery (ANR), apparent N efficiency (ANE) and nutrient working coefficients (NWC) were calculated based on the physicochemical data and by using the following equations:

$$\text{ANR} = (\text{N uptake treatment} - \text{N uptake blank}) / \text{amount N applied} \quad (1)$$

$$\text{ANE} = (\text{Dry matter yield treatment} - \text{dry matter yield blank}) / \text{dosed N total} \quad (2)$$

$$\text{NWC}_{\text{ANR}} = (\text{ANR treatment} / \text{ANR reference (PS+CAN)}) \times 100 \quad (3)$$

$$\text{NWC}_{\text{ANE}} = (\text{ANE treatment} / \text{ANE reference (PS+CAN)}) \times 100 \quad (4)$$

RESULTS AND DISCUSSION

The results for crop yield, protein and N-content, chlorophyll and nitrate residue gave no significant differences between the treated plots after statistical analysis. Figure 20 shows the visual representation of these parameters for each treatment.

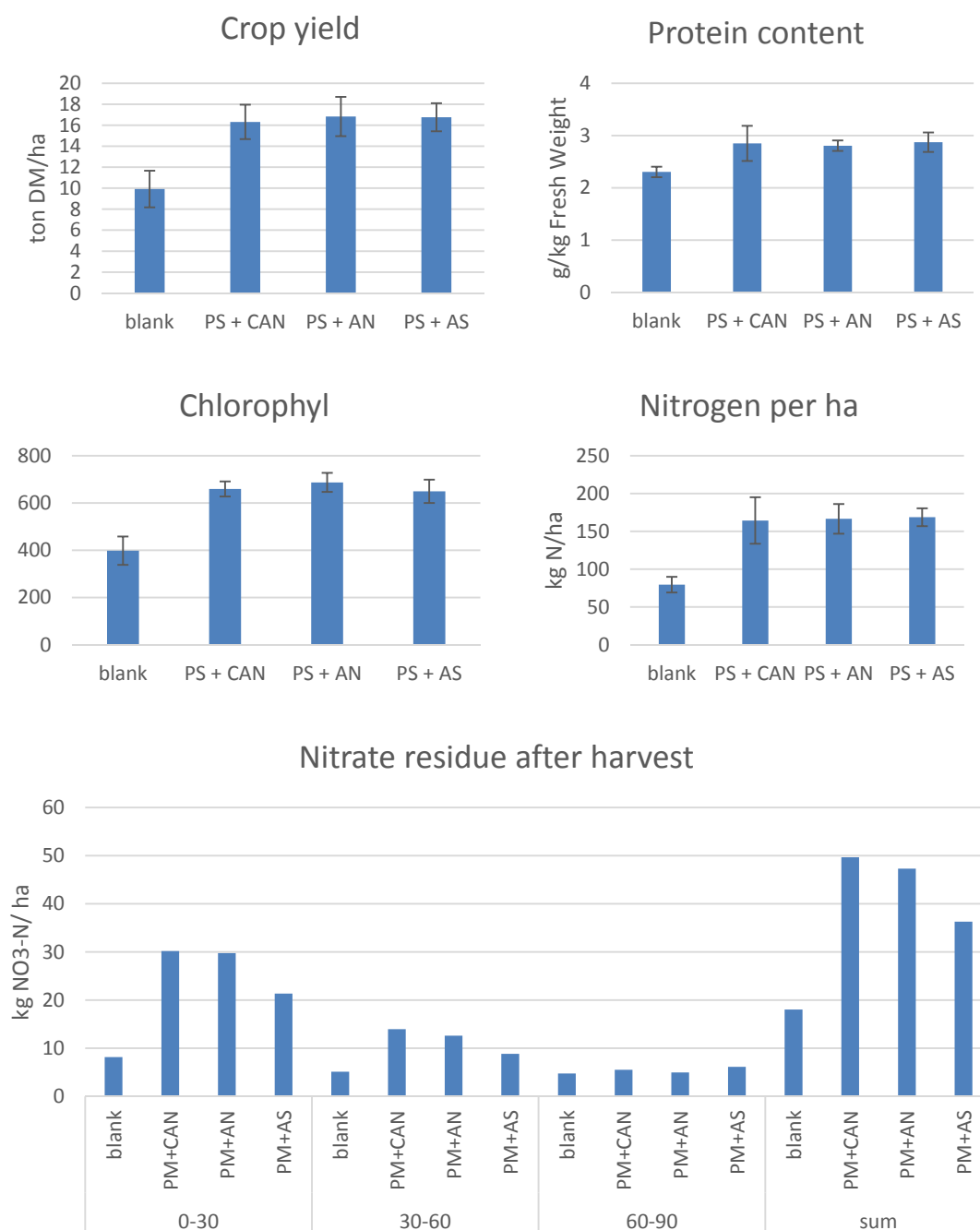


Figure 20. Crop yield, protein and N-content, chlorophyll and nitrate residue for the maize in the DIGESMART field trial in Belgium



It is particularly noteworthy to mention the results concerning the nitrate residue in the topsoil (0-90 cm): while that parameter is supposed to be below 90 kg NO₃-N per hectare according to the manure action plan in Flanders, none of the samples surpassed the limit. The used scrubber water had a significantly lower nitrate residue than the other fertilised plots.

Table 11. Fertiliser performance calculations

Applied fertiliser treatment	ANR	ANE	NWC _{ANR}	NWC _{ANE}
PS + CAN	0,52	39,2		
PS + AN	0,53	42,4	103%	108%
PS + AS	0,55	42,0	106%	107%

Also the fertiliser parameters calculations gave no underperforming for the biobased fertilisers as can be seen in Table 10. For the maize trial in the given circumstances it can be concluded that the alternative fertiliser product performed at least as good as the synthetic calcium ammonium phosphate.

LETTUCE POT EXPERIMENT

This pot experiment assesses the potential of using ammonium sulphate (AS) and ammonium nitrate (AN), obtained by ammonia stripping/scrubbing from animal manure, as potential synthetic N fertiliser replacements. The performance of these two bio-based products was compared to the one of calcium ammonium nitrate (CAN; 27 % mineral N) in a pot experiment, where lettuce (*Lactuca sativa* L.) was used as a test crop.

MATERIALS AND METHODS

At harvest time, crop yield and nutrient uptake were assessed along with soil properties. Crop quality control was conducted and Nitrogen Fertiliser Replacement Value (NFRV), which is the same as the nutrient working coefficient (NWC_{ANR}) was determined.

The pot experiment was conducted in 1,7 l pots at Laboratory of Analytical Chemistry and Applied Ecochemistry (Ghent University, Belgium). Two types of soil (USDA soil texture triangle), loamy sand (LS) and sandy loam (SL), were sampled from an arable field in Beernem (Belgium) and Roeselare (Belgium), respectively. Soil was taken from 0-30 cm soil layer at the beginning of December 2015. It should be noted that loamy sand soil was collected from the arable field which was previously used for maize trial that ended in October 2015.

The AN was collected at stripping/scrubbing unit of Detricon in Ieper (Belgium), while AS was collected at pig farm in Merkem (Belgium). Both products were collected in polyethylene bottles (2 l),

stored ($< 4^{\circ}\text{C}$) and characterized to determine the required total nitrogen application rate for the different cultivation treatments based on the total nitrogen demand of lettuce.

As a test crop, lettuce (*Lactuca sativa* L. cv. *Cosmopolia*) was used. It is a moderately salt sensitive crop whose potentially negative response on product application should be seen quite fast. The required total fertilization dosage was calculated (Table 12) according to the nutrient requirements for lettuce (210 N, 125 P_2O_5 and 240 K_2O kg ha^{-1} ; Sigurnjak et al., 2016) by taking into consideration the nutrient value of fertilisers. Since AN and AS do not contain P and K, triple superphosphate (TSP; 46 % P_2O_5) and patentkali (PAT; 30 % K_2O , 10 % MgO and 42.5 % SO_3) were used as sources of P and K, respectively. The fertilization advice was given on volume basis, since the height of pots was too small in order to apply products on surface basis. Meaning, with application on surface basis pots of 5 l are required, otherwise nutrients would be applied in excess. Also, different soil types have different bulk densities, resulting in small differences in product application amount (Table 12). Along with AN, AS and CAN treatment, a blank treatment (i.e. no fertilization) was introduced.

Table 12. Product amount applied on volume basis (g pot^{-1}) for treatments ($n=4$) on loamy sand (A) and sandy loam (B) soil

Loamy sand	Synthetic fertiliser			Bio-based products	
Treatment	CAN (g)	TSP (g)	PAT (g)	AN (g)	AS (g)
Blank	0	0	0	0	0
CAN	0,284	0,099	0,292	0	0
AS	0	0,099	0,292	0	2,068
AN	0	0,099	0,292	0,386	0
Sandy loam	Synthetic fertiliser			Bio-based products	
Treatment	CAN (g)	TSP (g)	PAT (g)	AN (g)	AS (g)
Blank	0	0	0	0	0
CAN	0,289	0,101	0,298	0	0
AS	0	0,101	0,298	0	2,111
AN	0	0,101	0,298	0,394	0

AN: Ammonium nitrate; AS: Ammonium sulphate; CAN: Calcium ammonium nitrate; TSP: Triple superphosphate; PAT: Patentkali.

The collected soil was first air-dried, passed through a 2 mm sieve mesh and stored. Prior to the pot experiment (January 20, 2016), 100 ml of distilled water was added per 1 kg of air-dried soil in order to assure homogenous mixing with products. On January 21, in each pot 1,7 kg of soil was mixed with the corresponding dosage (Table 12) per treatment ($n=4$). Only the blank treatment did not receive any fertilization. In each pot one lettuce plant was transplanted and additional water was added to reach 60 % of soil field capacity (FC). Pots were placed on a metal shelf where they were exposed to artificial light (Brite-grow bio-growth light) of 2000 LUX for 12 h day^{-1} . The pots were kept at 20°C

for 54 days. On daily basis water was added to maintain FC at 60 %. Once a week, the location of the pots was randomized. On March 15, the lettuce was harvested. Prior to harvest, chlorophyll was measured and crop quality control was conducted.

RESULTS AND DISCUSSION

A picture with the final visual overview of the lettuce plants is given in

Figure 21. The crop fresh weight (FW) yield and nutrient uptake is presented in Table 13. In commercial greenhouse cultivation of lettuce, the plant is harvested once it reaches 500 g of FW yield. In this study, lettuce was cultivated in 1.7 l pots which led to lower FW yields, ranging from 37-89 g pot⁻¹.



Figure 21. Overview of the plants at the time before harvest. Left: loamy sand - Right: Sandy Loam

At harvest time, blank treatments on both types of soil exhibited the lowest lettuce FW yield (Table 13). This is a result of no fertilization, leading to crop nitrogen deficiency. For amended treatments, the highest lettuce FW yield was observed in treatment with ammonium nitrate (AN), on both types of soil. As compared to CAN and AS treatment, the difference was small but still statistically significant ($p < 0.05$). It seems that lettuce in AN treatment has taken up more easily the applied N which has led to higher FW yield. On the other hand, there were no significant differences ($p > 0.05$) with respect to P and K crop uptake between CAN, AN and AS treatments.



Table 13. Crop fresh weight (FW) and nutrient uptake on dry weight (DW) basis at harvest time (March 15, 2016) per treatment (n=4) on loamy sand (LS) and sandy loam (SL) soil

Treatment	FW (g pot ⁻¹ FW)	DW (%)	N (g kg ⁻¹ DW)	P (g kg ⁻¹ DW)	K (g kg ⁻¹ DW)	S (g kg ⁻¹ DW)
Blank_LS	37 ± 1a	7,9 ± 0,9	19,4 ± 1,2a	3,9 ± 0,1	38,5 ± 3,0	1,8 ± 0,1a
CAN_LS	69 ± 2b	7,7 ± 0,6	20,7 ± 0,4a	4,0 ± 0,1	33,8 ± 1,7	2,7 ± 0,1b
AN_LS	76 ± 4c	7,1 ± 0,8	23,3 ± 1,2b	3,7 ± 0,2	35,4 ± 1,9	2,6 ± 0,0b
AS_LS	67 ± 3b	7,2 ± 0,2	23,5 ± 1,5b	3,8 ± 0,2	38,0 ± 2,7	3,1 ± 0,1c
Blank_SL	39 ± 3a	7,5 ± 0,8	16,1 ± 1,3a	4,2 ± 0,1a	57 ± 3	1,7 ± 0,1a
CAN_SL	77 ± 2b	6,6 ± 0,3	20,8 ± 1,7bc	5,4 ± 0,4b	63 ± 3	2,5 ± 0,1b
AN_SL	89 ± 8c	6,5 ± 1,0	22,6 ± 2,3c	5,5 ± 0,6b	61 ± 8	2,9 ± 0,3b
AS_SL	70 ± 8b	7,0 ± 0,5	18,9 ± 0,9b	5,1 ± 0,4b	59 ± 4	2,8 ± 0,3b

Mean values denoted by the same letter in a row are not statistically different according to Tukey's test at probability level. AN: Ammonium nitrate; AS: Ammonium sulphate; CAN: Calcium ammonium nitrate.

As compared to AN and CAN, AS is not only a potential substitute for synthetic N, but it is also rich in S. In this pot trial, significant differences were detected with respect to S uptake on loamy sand soil. However, due to higher standard deviations, no significant differences were observed in crop S uptake on sandy loam soil. On both types of soil, the obtained values of plant S uptake were within the appropriate range of 2-3 g kg⁻¹ DW in lettuce grown within the greenhouse.

The indicators of AN and AS potential to be used as a synthetic N substitute can be found in Table 14. For apparent nitrogen recovery (ANR) along with mean values also standard deviations are reported in order to indicate that there was a higher variability in nitrogen uptake within the treatments on sandy loam, rather than on loamy sand soil. Nevertheless, it can be seen that the performance of AN was higher (CAN_LS) or similar (CAN_SL) to the one of CAN on both types of soil. On the other hand, AS treatment exhibited higher standard deviations, which led to statistically non-significant ANR as compared to CAN and AN treatment. However, on the basis of mean values its NFRV was higher on loamy sand soil, rather than on sandy loam soil.



Table 14. The apparent nitrogen recovery (ANR) and nitrogen fertiliser replacement value (NFRV) for tested treatments (n=4) on loamy sand (LS) and sandy loam (SL) soil

Treatment	ANR	NFRV
CAN_LS	0.71 ± 0.07a	-
AN_LS	0.89 ± 0.08b	125%
AS_LS	0.74 ± 0.10ab	104%
CAN_SL	0.77 ± 0.13ab	-
AN_SL	1.06 ± 0.16b	138%
AS_SL	0.59 ± 0.19a	77%

Mean values denoted by the same letter in a row are not statistically different according to Tukey's test at probability level. AN: Ammonium nitrate; AS: Ammonium sulphate; CAN: Calcium ammonium nitrate; ANR: Apparent nitrogen recovery; NFRV: Nitrogen fertiliser replacement value.

This study confirms that utilization of AN and AS can result in higher or similar lettuce FW yield as compared to CAN fertilization. When comparing two tested bio-based products, AN led, although small in difference, to a significantly higher lettuce FW yield as compared to CAN and AS. At the same time, it did not cause any changes in soil properties at harvest time. On the other hand AS, as a NS-source, has considerably influenced the soil EC and pH values. Finally, on both types of soil, AN resulted in the highest NFRV values, indicating that this product is perfectly suitable to replace the conventional synthetic N fertiliser (CAN; 27 % mineral N).



OVERALL REMARKS AND CONCLUSION

The results observed in the four field trials give a solid base for making the argument that in the European agricultural system, the DIGESMART fertiliser has the same performance of the conventional liquid fertilisers. The trials in lettuce, maize and wheat conducted in two European regions make the validation of the product more solid. Nevertheless, it was noticed during the trials that attaining a stable product concentration is a work in progress. Also, the liquid formulation sets a limit on the adaptation in agriculture of the ammonium nitrate. Most practices in farming use granulated chemical fertiliser products and farmers are used to handling such products. From the agronomists point of view, this could represent a limit for the DIGESMART-product in terms of storage space, packaging and handling.