



Disease suppressing effects of BSF (black soldier fly, *Hermetia illucens*) larvae frass in bioassays with cress and sugar beet plants

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Disease suppressing effects of BSF (black soldier fly, *Hermetia illucens*) larvae frass in bioassays with cress and sugar beet plants

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Summary: This report describes two bioassays for testing disease suppression by BSF frass in an agricultural soil with two crops: sugar beet (*Beta vulgaris*) and cress (*Lepidium sativum*). In the bioassay with beet, soil was infected with the plant-pathogenic fungus *Rhizoctonia solani* while in the bioassay with cress, soil was non-infected or infected with the plant-pathogenic fungus *Pythium ultimum*. Both concentrations of the BSF frass (7.5 and 15 g/kg soil) proved to be useful as soil application, leading to good plant emergence and growth of sugar beet and cress plants. The highest concentration of BSF frass (15 g/kg soil, both as total product) had a large fertilizer effect. In experiments with cress without addition of *Pythium* (i.e. with only natural soil infections), disease suppression with BSF frass was found. In all tests, keratin was added as a control treatment, which had a higher disease suppression effect than BSF frass but comparable fertilizer effects.

Keywords: black soldier fly, BSF, frass, disease suppression, bioassay, sugar beet, cress

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Preface

In the Netherlands, there are surpluses of manure and digestates/biogas slurries (digested manure and residual flows), which represent a negative value. At the same time, these residual flows contain valuable ingredients for the production of biomass (as raw material for food and feed products), for improving soil quality and for energy production. The number of feasible business cases in which the residual flow is upgraded has so far been limited. This is due both to the efficiency of the technologies used and the legislation and regulations related to the residual flows.

Recent information from research, scientific literature and companies provides new starting points for a biobased valorization of manure/digestate streams and improving the efficiency of anaerobic digestion. The innovative aspect of our research is the cultivation of new types of biomass on the residual flows and the use of the conversion products to improve anaerobic digestion. This involves the use of separated manure and digestate products for the cultivation of mushrooms/fungi, worms, insects, specific bacteria and aquatic biomass. The resulting biomass can be further refined and marketed as food, feed and bio-based feedstock. There are also processed manure and digestate products that are valuable as fertilizer products for soil and plant growth, as substrate for improvement of anaerobic digestion or for export/use besides in agriculture. This gives a new interpretation to obligatory manure processing.

The aim of this project is to further explore and substantiate/test these ideas on lab and practical scale, leading to a proof of principles for new bio-based upgrading methods for manure and digestate that can be used in conjunction to better close cycles and/or sell outside regular agriculture.

Bottlenecks in legislation and regulations are explored and put on the agenda. Key figures are also calculated that are necessary for assessing sustainability (e.g. costs, environmental effects) and for supporting legislation (e.g. minerals, food safety).

The livestock sector gains insight into the possibilities of biobased valorisation and better marketing of their most important residual flows. For the SMEs involved, this research provides proof of principle for their technology and input in their business cases. The combined effects of the technologies provide new knowledge, methods and research directions for science. In a social context, the use and upgrading of manure and digestates in other ways also contributes to the transition to a circular bio-economy with an efficient and sustainable agri-food sector.

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Summary

This report describes two bioassays for testing disease suppression by BSF frass in an agricultural soil with two crops: sugar beet (*Beta vulgaris*) and cress (*Lepidium sativum*). In the bioassay with beet, soil was infected with the plant-pathogenic fungus *Rhizoctonia solani* while in the bioassay with cress, soil was non-infected or infected with the plant-pathogenic fungus *Pythium ultimum*. Both concentrations of the BSF frass (7.5 and 15 g/kg soil) proved to be useful as soil application, leading to good plant emergence and growth of sugar beet and cress plants. The highest concentration of BSF frass (15 g/kg soil, both as total product) had a large fertilizer effect. In experiments with cress without addition of *Pythium* (i.e. with only natural soil infections), disease suppression with BSF frass was found. In all tests, keratin was added as a control treatment, which had a higher disease suppression effect than BSF frass but comparable fertilizer effects.

1 Introduction

During the production of BSF (black soldier fly, *Hermetia illucens*) larval faeces are excreted, so-called BSF frass or compost or digestate or residue. Salomone et al (2017) show an overview of the main inputs and outputs for their LCA (life cycle analysis) of food waste bioconversion by BSF larvae (Table 1).

Table 1 Overview of inputs and outputs of a food waste bioconversion process by BSF larvae (From: Salomone et al, 2017).

Description	Unit	Amount
<i>Input</i>		
Food Waste	t	1
Transport	tkm	24.3
Pruning waste	kg	5.5
Water	kg	61.1
Electric energy	kWh	12.9
<i>Output</i>		
Compost	kg	334.6
Dried larvae	kg	29.6
CO ₂	kg	16
CH ₄	g	51.2

It is clear that insect frass is one of the main outputs of the process. This frass contains nutrients, such as N and P, but also the polysaccharide chitin, which is assumed to have resulted from shed integuments of the BSF larvae (Temple et al, 2013). The composition of the larval frass (e.g. N, P, DM content) will however depend on the type of substrate fed, as can be seen in Oonincx et al (2015) in which BSF larvae were fed with three different types of manure. Salomone et al (2017) mention the following characteristics of the frass: a low humidity value of 25.7 %, pH 6.95, a well-balanced average NPK content of 1.49 %, 0.98 %, and 1.03 % respectively, comparable with other organic manures and easy storage, packing and transport qualities without requirements for further transformation or stabilization processes. Toxic and essential metal concentrations fell within fertilizer limits. They found it an excellent bio-fertiliser without pre/post treatments. Kebli and Sinaj (2017) concluded the same based on organic matter, NPK and microelements content such as Cu and Zn and greenhouse experiments on three types of soil and two crops, ryegrass and lettuce. In addition to these 'regular' fertilizer values of the frass added value of the frass is also expected from its chitin content. Zahn (2017) tested the effect of BSF frass on growth of spring onion and concluded that the frass has potential as a viable biofertiliser and suggests to do longer term growth experiments with higher replication numbers to prove a significant effect on plant growth, due to the potential presence of high microbial biomass, chitin, growth hormones and soil enzymes. Spranghers et al (2016) found a chitin content of BSF prepupae reared on different wastes ranging between 56 and 67 g kg/DM. For comparison: Protein contents of the prepupae are around 40 % of DM. It is, up till now, not clear what is the chitin content of the BSF frass, since commercial products and research papers merely mention that the frass contains chitin, but not its concentrations. Vickerson et al (2015) have filed a patent on behalf of Enterra producing insecticidal BSF frass using it for nutritional and insect pest control activity in soils and/or on foliage. The methods include processes to reduce damage to crops caused by wireworms (i.e., click beetle larvae, in the Elateridae family) and/or other Coleopteran (i.e. beetle) insect pests.

According to several authors, disease suppressive properties of soils can be enhanced by the addition of chitin rich products. Several authors mention suppression of plant-parasitic fungi (e.g. Korthals et al, 2014; Postma and Schilder, 2015) and nematodes (e.g. Oka, 2010). The idea is that because chitin is present in nematode egg shells as well as in most plant-pathogenic fungal cell walls soil application of chitinous materials will increase the number of chitinolytic microorganisms and chitinase activity in the soil, which in turn has an adverse effect on the nematodes and fungi present (Oka, 2010).

The bioassays described in this report were done to test the disease suppression (the fungal species *Rhizoctonia solani* and *Pythium ultimum*) by BSF frass in an agricultural soil with two crops: sugar beet (*Beta vulgaris*) and cress (*Lepidium sativum*).

2 Materials and methods

2.1 Materials

BSF frass was obtained from Bestico BV (Berkel en Rodenrijs, the Netherlands). The frass had been dried overnight at 60 °C prior to transport for hygienisation purposes. The composition of the frass was analysed by Eurofins (Graauw, the Netherlands) (Appendix I) and had a dry matter percentage of 42.1 %. Nitrogen and phosphate concentrations in the dry matter were 37.41 g N and 24.36 g P₂O₅ per kg respectively.

A sandy soil from arable farming which was sensitive to disease development was used (Vredepeel, the Netherlands). Based on the nitrogen concentration, dosages of 7.5 and 15 g frass/ kg of soil were applied, which equals N concentrations of 0.12 and 0.24 g N/kg soil respectively. Besides the control soil without additions, the addition of feather meal (keratin) was used as reference at a concentration of 1.5 g/kg soil, equivalent to 0.2 g N/kg soil.

The mixed soils were used to evaluate their suppressiveness against *Rhizoctonia solani* in sugar beet seedlings and *Pythium ultimum* in cress seedlings. As a control for natural infection in the soil, cress was also grown in the absence of *P. ultimum* inoculum. Table 2 shows the different experimental treatments and bioassays.

Table 2 Experimental treatments in tests with BSF frass.

# of replicates	Control	Keratin	BSF 7.5	BSF 15
Sugar beet + <i>R. solani</i>	5	5	5	5
Cress	5	5	5	5
Cress + <i>P. ultimum</i>	5	5	5	5

2.2 Methods

The soil was mixed with each of the three different products (keratin plus two concentrations of BSF frass), and incubated for two weeks at 20 °C. With these treated soils two bioassays were done.

- 1) Sugar beet with *R. solani*: Per replicate (total 5), 1.3 litres of soil was inoculated in a tank and sown with 22 sugar beet seeds in two rows (every seed at two cm distance). After germination, the soil in front of the tank was inoculated with *R. solani*. The disease progress was scored during 3.5 weeks.
- 2) Cress with or without *P. ultimum*: Per replicate (total 5) 200 grams of soil was inoculated with or without *P. ultimum* and incubated for two days. Then the soil was divided in two small pots (=two sub replicates), and cress seeds were sown on the surface. After 1 week, the disease was scored (%) and the fresh weight of the crop was measured in grams.



Figure 1 Sugar beet bioassay.



Figure 2 *Cress bioassay.*

3 Results and discussion

3.1 Sugar beet with *R. solani*

Germination of the seeds was good in all the treatments (between 95.5 and 98.2 % on average) and not significantly influenced by the different amendments (Table 3). After germination of the seeds, the pathogenic fungus *R. solani* was added to the first plant in every row. At the end of the experiment (24 days after pathogen inoculation) most of the plants got symptoms; percentages of diseased plants were between 94.5 and 97.3 %. There was not a significant reduction in the percentage of infected plants in any of the treatments. Since the plants were not only attacked by the inoculated *R. solani* but also by a natural infection present in the soil (possibly *Aphanomyces*), the progress of the *R. solani* disease from plant to plant could not be scored, which is the normal indicator that we use for soil suppression.

Table 3 Results of the bioassay with sugar beet.

%	Germinated seeds	Natural infection	<i>R. solani</i>	Total infection
Control	95.5	14.8	81.6	96.5
Keratin	97.3	19.1	78.2	97.3
BSF 7.5	95.5	18.2	76.4	94.5
BSF 15	98.2	7.3	88.2	95.5
LSD (P=0.05)	5.0	11.9	14.6	8.1



a



b

Figure 3 a Germination of sugar beet plants with *R. solani* infection with different treatments (from left to right): BSF 7.5, control, BSF 15, keratin. b Plant growth after 14 days with *R. solani* infection (same order of treatments as in a).

3.2 Cress with and without *P. ultimum*

In the bioassay with cress clear differences were found between non-infected and infected soils (Figure 4 left and right respectively). However, the effect between the soils with different amendments was less clear.

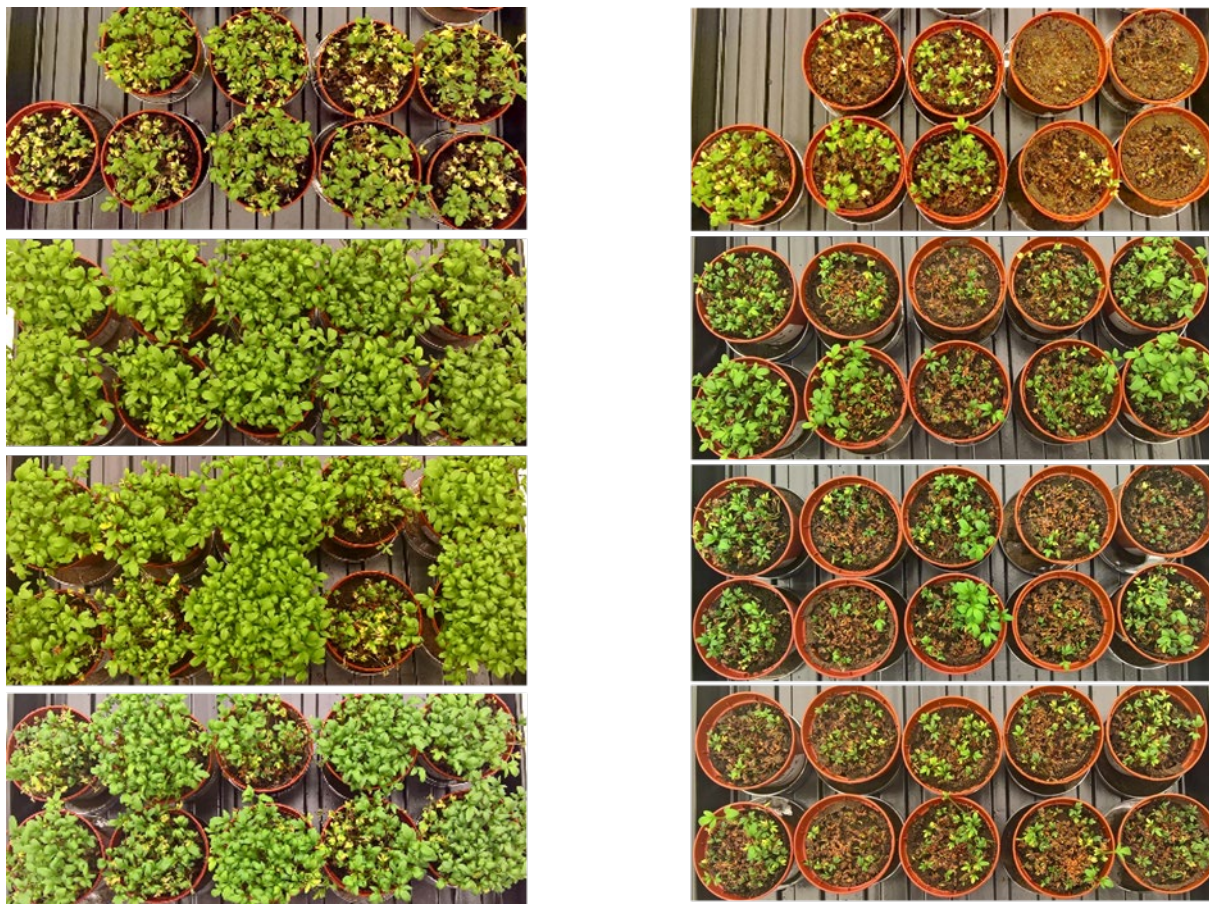


Figure 4 Growth in cress bioassay with different treatments without (left column) and with (right column) *P. ultimum* infection. From top to bottom treatments were: control, keratin, BSF 15 and BSF 7.5 ($n=5$ replicates each with 2 pots, except in control experiment: 1 sub repeat missing in both tests).

3.2.1 Cress without *P. ultimum*

In the cress bioassay without *P. ultimum* inoculation the soil appeared to contain a natural infection; on average 54 % of the plants got symptoms in the control (Figure 5). Less plants became infected by this unknown natural infection when BSF frass (both concentrations) or keratin were added (Figure 5). Both additions also led to more crop growth, with a higher effect for BSF 15 and keratin vs. BSF 7.5, which is the result of the number of healthy plants combined with the increased nitrogen availability. N content of the products was 0.24, 0.2 and 0.12 g N/kg soil respectively for BSF 15, keratin and BSF 7.5, which is proportional to the total plant weight.

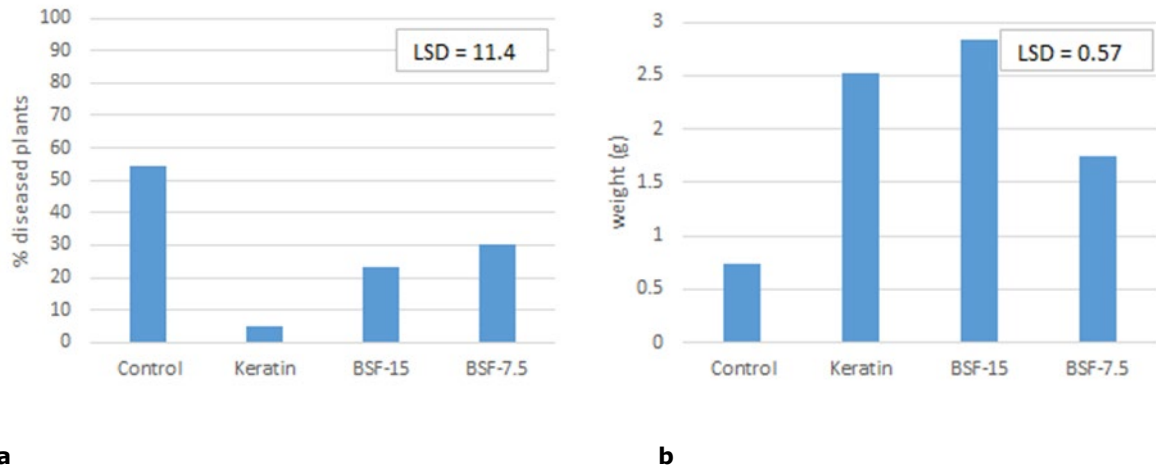


Figure 5 Plant disease percentage (a) and total plant weight (b) for different treatment in bioassays with cress without *P. ultimum* infection ($n=5$).

3.2.2 Cress with *P. ultimum*

In the cress bioassay with *P. ultimum* infection a high disease incidence occurred (Figure 6). There was no disease suppressive effect found for BSF frass, but there was a small effect for keratin. No significant increase or decrease was found in plant growth induced by BSF frass. We assume that there were too few healthy plants to notice any effect on growth.

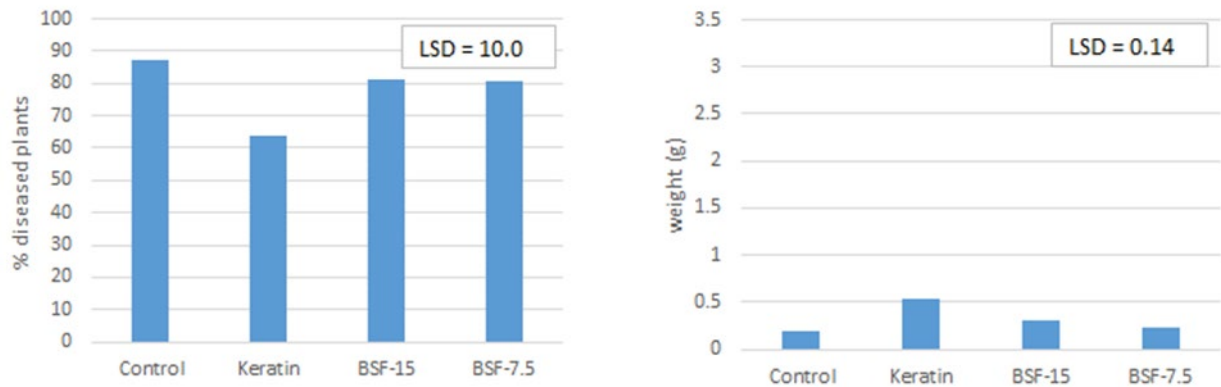


Figure 6 Plant disease percentage (a) and total plant weight (b) for different treatment in bioassays with cress with *P. ultimum* infection ($n=5$).

4 Conclusions

Germination of the sugar beet seeds was not reduced by both concentrations of BSF frass additions to the soil. In the sugar beet assay no disease suppression of *R. solani* was found by any of the amended products. Keratin amendment was used as a reference since this product is known to be able to enhance the disease suppression. We have no explanation for the absence of disease suppression in this experiment, although the presence of natural infections in the soil could have disturbed the bioassay to some extent.

In the bioassay with cress it was found that natural infections (possibly *Pythium* species, as similar symptoms, such as decreased seed germination and rot in the juvenile plants, were observed) in the soil were suppressed by BSF 7.5 and BSF 15 (both g frass/kg soil), which led to less diseased plants. In addition, BSF 15 had a strong fertilizer effect, which led to increased plant growth. After *P. ultimum* infection in the cress bioassay, disease pressure was very high and no suppression was found through both dosages of BSF frass, but reference keratin amendment did result in some disease suppression. It is not clear whether the effects on disease suppression can be ascribed to (the unknown content of) chitin or other components of the Bestico BSF frass.

5 Recommendations

Based on the experiments with cress in this report, there is suppression of natural infections in soil by Bestico BSF frass and also strong fertilizer effects, most likely due to its macromineral content. More research into the mechanisms behind this disease suppression and analysis of chitin/chitosan components in the frass is recommended, as well as testing the hypothesis of disease suppression under field conditions. Care should be taken to pinpoint possible beneficial effects to the right components (e.g. chitin/chitosan, N or P). Also, the frass composition and its 'source' (the substrate on which the larvae were fed) should be accurately described since this is highly variable between BSF larvae production facilities.

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Annex 1 BSF frass analysis



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Report number: 819584-672550

BGM analysis

In order of	Relation
Koppert Biological Systems BV Postbus 155 2650 AD Berkel en Rodenrijs	Koppert Biological Systems BV Postbus 155 2650 AD Berkel en Rodenrijs

Lab code:	L18AD354D	Sampling date:	26-4-2018
Report number:	819584-672550	Sample taker:	Oprichtgever
Date report:	14-5-2018	Date received:	2-5-2018
Starting date analysis:	2-5-2018		
Sample description:	18FE181A BSF compost		

Former labnumber:		Export RVO:	
Productcode:	compost (zwarte grond)	Name producer:	Bestico BV
Address:	Postbus 155	Zipcode and place:	2650 AD Berkel en Rodenrijs
Foreign producer:	N	Relation number producer	
Number of samples:	1	Remark:	BSF Compost
Barcode:		Kind of compost:	
Aantal grepen:	7 grepen van 200 ml	Way of sampling:	

Analysis / Research method / Executive laboratory

Calcium carbonate / Own method, volumetry, WVS-102 / LZV

Calcium carbonate % d.m. 1.0

Dry matter / Own method (execution conform NEN-EN 15934), gravimetry, WVS-003 / LZV

Dry matter kg/ton 421
Dry matter weight % 42.1 Q

Loss on ignition / Own method, loss in ignition method with correction lutum, WVS-035 / LZV

Organic matter weight % d.m. 83.1 Q Samenstellingseis BC >10
Ash weight % d.m. 16.9

Main elements / Own method, spectrofotometry, WVS-136 / LZV

Potassium kg/ton 19.0
Magnesium kg/ton <1.0
Sodium kg/ton 4.7
Calcium kg/ton 1.2

Ing. R. Thomaes
(BU Manager Dioxins)

The results marked with a 'Q' are performed according to the by the Raad voor Accreditatie accredited processes (registration number L201). The results refer to the sampled object, when the sample is taken by Eurofins Lab Zeeuws-Vlaanderen (LZV) B.V. The methods of analyzes, limits of quantitation and features of performance are retrievable. Advice, opinions and interpretations are not covered by accreditation. Without written permission of Eurofins Lab Zeeuws-Vlaanderen (LZV) B.V. it is not allowed to reproduce this report.

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Potassium	g/kg d.m.	45.17
Magnesium	g/kg d.m.	<2.00

Metals / Own method (destruction conform NEN 6961 and measurement conform NEN-EN-ISO 17294-2), ICP-MS, WVS-006 and WVS-071 / LZV

Samenstellingseis BG

Arsenic	mg/kg d.m.	<3.0	Q	<15
Cadmium	mg/kg d.m.	0.11	Q	<1
Chrome	mg/kg d.m.	<10	Q	<50
Copper	mg/kg d.m.	19	Q	<90
Nickel	mg/kg d.m.	<3.0	Q	<20
Lead	mg/kg d.m.	<5.0	Q	<100
Zinc	mg/kg d.m.	70	Q	<290
Mercury	mg/kg d.m.	<0.050	Q	<0.3
Iron	mg/kg d.m.	1200	Q	
Sulfur	mg/kg d.m.	8000		

Nitrogen and Phosphate / Own method (execution based on NEN 7434 and o-NEN 7435), continuous flow spectrofotometry, WVS-022 / LZV

Nitrogen (N)	kg/ton	15.73
Phosphate (P2O5)	kg/ton	10.25
Nitrogen (N)	g/kg d.m.	37.41
Phosphate (P2O5)	g/kg d.m.	24.36

Nutrients / Own method, spectrofotometry, WVS-118 / LZV

Ammonium	kg/ton	2.69
Chlorine (Cl)	mg/kg d.m.	7500

pH / Own method, electrochemistry, WVS-149 / LZV

pH	7.1
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Specific weight / Own method, gravimetry / LZV

EC	uS/cm	1700
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 Ing. R. Thomaes
 (BU Manager Dioxins)

To explore
the potential
of nature to
improve the
quality of life



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