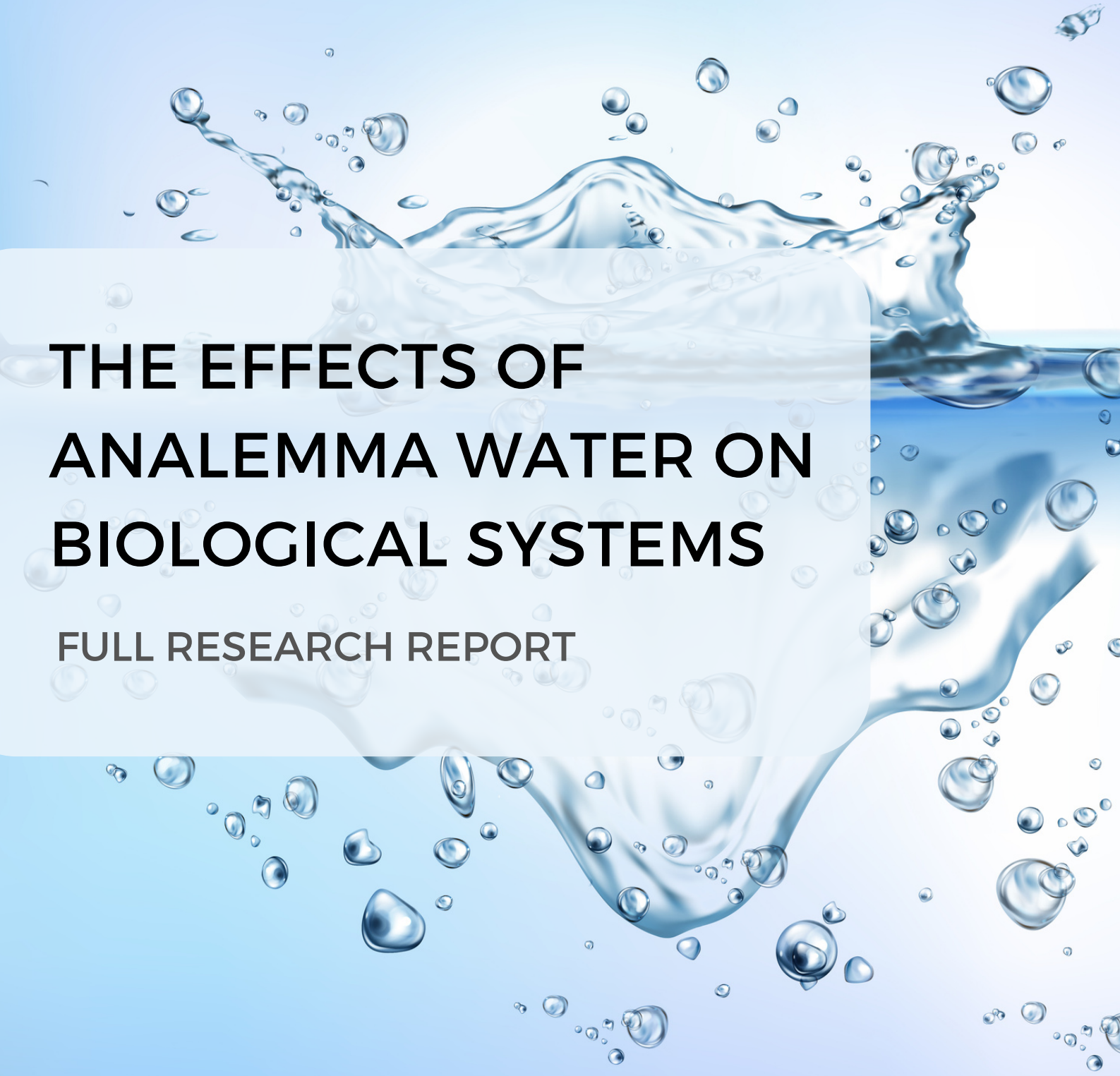
JANUARY 2023



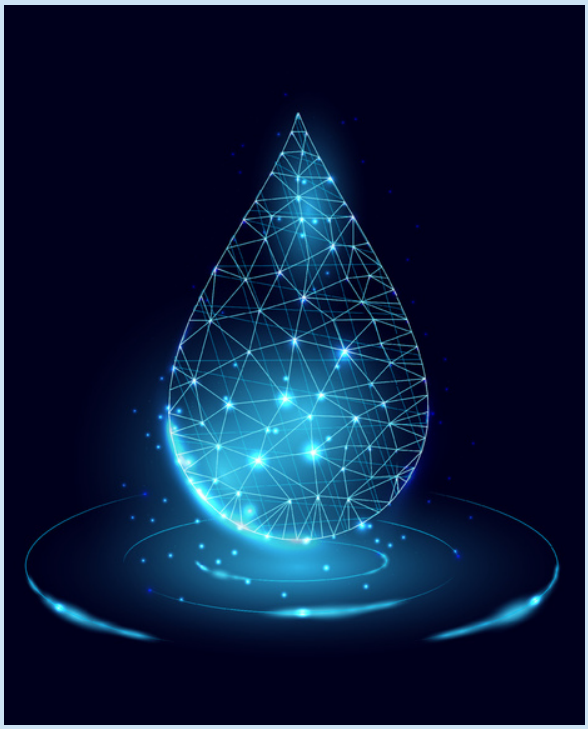
THE EFFECTS OF ANALEMMA WATER ON BIOLOGICAL SYSTEMS

FULL RESEARCH REPORT

www.analemma-water.com

WHAT IS ANALEMMA WATER?

Analemma was conceived in a laboratory in The Netherlands, where discussions about the relationships between life and light led to water as the missing link and a key player.
Mimicking the cycles of water in nature, our water undergoes a year-long physical process which ultimately brings it into a powerful coherent state. We fill this water into a wand made of quartz glass. When the Analemma wand comes into contact with regular water, it transforms its state and renders it coherent.



"Analemma was built on nearly two decades of research."

Beginning in 2014 with our first studies on plant biophoton emission, all the way to 2020s when our focus shifted toward humans, we had always had a uniform goal of exploring the effects of Analemma water on biological systems. As a result, Analemma was built on nearly two decades of research.
This has remained our mission ever since - to use a scientific approach in discovering the true power of coherent water.

IN THIS REPORT

This document summarizes our most important research studies to date. The majority of experiments were performed by independent institutions, whose scientists analyzed the data and presented them in the form of scientific reports. Here, we highlight the most interesting findings and discuss our plans for further research.
The document is divided into two parts, our latest studies on human participants and earlier studies on plants and soil. Additional data is presented in the Appendices.

CONTENTS & TIMELINE

PART I. EFFECTS ON HUMAN BIOLOGY

PART II. EFFECTS ON PLANTS AND SOIL

List of Appendices

RESEARCH REPORT
JANUARY 2023

PART I

EFFECTS ON HUMAN BIOLOGY

The study of ATP levels in humans (2022)

The aim of the study was to measure the effect of prolonged Analemma water consumption on blood ATP levels of human participants.

Adenosine triphosphate (ATP) is commonly referred to as the "energy currency" of the cell. Its structure is simple - it consists of a base, a sugar and three serially linked phosphate groups. The energy of ATP is stored in the bond between its second and third phosphate group. Whenever this bond is broken (hydrolyzed), energy is released.

The majority of cellular ATP is synthesized in the mitochondria and then exchanged as fuel for other biochemical reactions. ATP synthesis and hydrolysis are in a constant cycle and both need to be maintained for proper cellular functioning. Inadequate production of ATP adversely affects numerous cellular processes and can have deleterious effects on human health.

WHAT IS ATP USED FOR?

ATP is utilized in a number of biological processes, such as **DNA and RNA synthesis, muscle contraction, neuronal impulse propagation** and many others. The role of ATP in muscle contraction is multifold - ATP is necessary for generating force and maintaining ion transport across membranes, making it essential for everyday muscle functioning.

Even more demanding is the utilization of ATP in the brain. A single neuron hydrolyzes nearly one billion ATP molecules for a single repolarization event, which happens every time it needs to send an impulse to a neighboring cell. Not surprisingly, the brain is the highest consumer of ATP, spending around 25% of total available energy in the body.

RESEARCH REPORT
JANUARY 2023

The study of ATP levels in humans (2022)

EXPERIMENTAL DESIGN

OBJECTIVE: To assess the effect of prolonged Analemma water consumption on ATP levels in the blood of healthy adult humans.

PARTICIPANTS: 50 healthy adult human subjects, aged 18-60, BMI 18.50-29.99 kg/m^2.

STUDY DESIGN: The study was designed as a **double blind, placebo-controlled, randomized, parallel group clinical study**. Participants were randomly divided into three groups and given adequate amounts of Analemma Water (n=13), Test Water 2 (n=12) or non-treated water (Placebo Control, n=25). The setup was double blind, with neither the participants nor the clinical investigators aware of how the subjects were distributed into groups. All participants were instructed to consume at least 1.5 L of the given water for 60 days, with regular compliance assessment check-ups. ATP levels of all participants were measured in whole blood samples obtained prior to treatment (Day -1) and on the last day of treatment (Day 60). Relative ATP levels were detected using a standardized kit based on firefly luciferase bioluminescence (Wako Pure Chemical Industries, Osaka, Japan).

DATA ANALYSIS: The change in ATP levels between Day -1 and Day 60 was calculated for all groups. Statistical difference between the mean values obtained in the Analemma Water group and the Placebo Control group was analyzed by one-way analysis of variance (ANOVA).

RESULTS

The change in ATP levels in the Analemma group was 26.84 ± 24.90, while the change in ATP levels in the Placebo Control group was 7.32 ± 26.65. The difference between the means was significant. Therefore, this study indicates that **consuming Analemma water for 60 days significantly increases blood ATP levels in humans**.

INSTITUTION

Raptim Research Pvt. Ltd. (Navi Mumbai, India)

RAPTIM RESEARCH LTD.
A CONTRACT RESEARCH ORGANIZATION

CLINICAL INVESTIGATOR: Dr. Yashvant Khaire
Clinical Co-Investigator: Dr. Nandkishor Gameti, M.B.B.S., Dr. Raviraj Jagdhani, M.B.B.S. M.D. Pharmacology
Bioanalytical Investigator: Dr. Milind Bagul, M. Pharm, Ph.D.
Biostatistician: Veerababu Yegi, M.Sc. Statistics
Head of Quality Assurance: Usha Ramakrishnan, B. Pharm

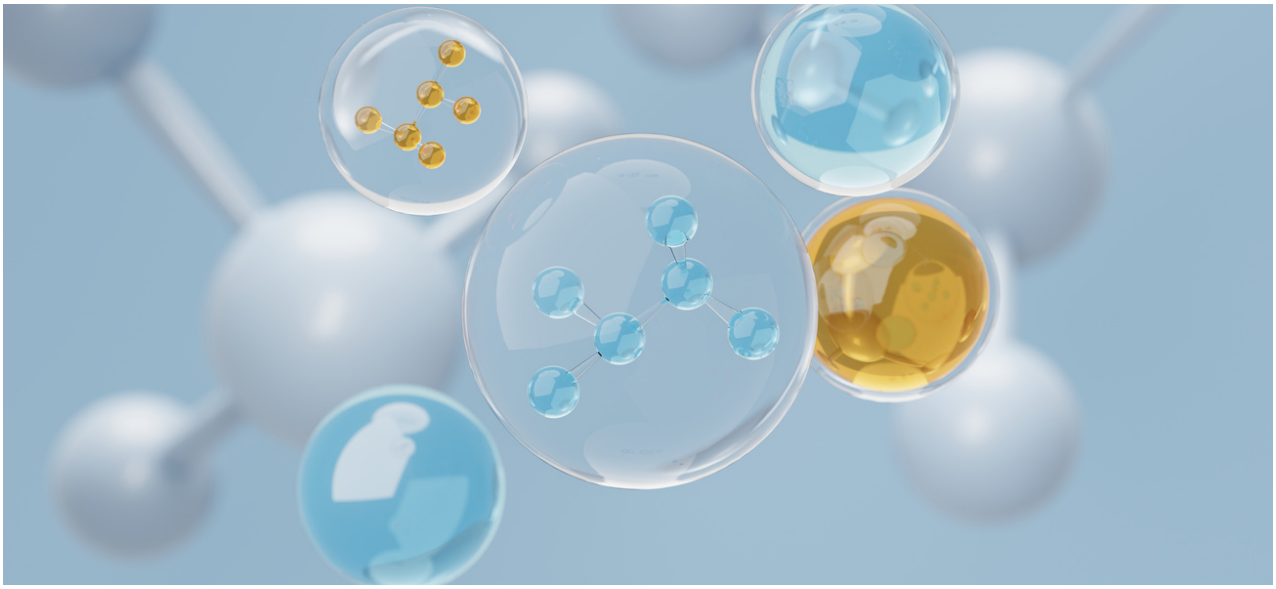
The human microbiome study (2022)

In 2022, a pilot study was performed on the effects of Analemma Water consumption on the microbiome of human adults. At the time of writing this report, only a short summary of the results was available.

The main output of the study was the **dysbiosis index, which describes the degree of deviation in the microbiome**. The dysbiosis index takes into account all the different bacterial phyla and species as well as their weighting factor.

In the Analemma Water group, in which participants consumed Analemma water for a prolonged period of time, the dysbiosis index improved in 7 out of 9 participants (78%). In the Control group, in which participants consumed untreated water, the dysbiosis index improved in 4 out of 10 participants (40%). **Overall, the study shows a 38% improvement of the dysbiosis index in the Analemma Water group compared to Control**.

The full research report of the human microbiome pilot study will be available by **February 15th 2023**.



GlycanAge study (2021)

In 2021, a GlycanAge study was performed on adult humans consuming Analemma Water for three months. The GlycanAge test measures glycan profiles of Immunoglubulin G, which reveals the biological age of the human immune system.

WHAT ARE GLYCANS?

Glycans are sugar molecules attached to proteins. The main antibody proteins of the human immune system, immunoglobulins, can be marked with different glycans, **which determine whether the protein's role will be anti-inflammatory or pro-inflammatory**.

Both functions are necessary, but so is the balance between them. Various environmental factors can shift this balance toward pro-inflammatory glycans, leading to low-grade systemic inflammation which underlies faster aging and increased risk of disease.

The **GlycanAge** test analyzes the presence of G0, G2 and S glycans linked to the most common antibody in human blood, Immunoglobulin G (IgG).

IgGs marked with **G0 glycans are pro-inflammatory** and their levels generally increase with age and in inflammation-associated disease. On the other hand, **G2 and S glycans promote protective, anti-inflammatory roles** of IgG, and are therefore considered to be biomarkers of health and youthfulness. All three markers are used for calculation of overall biological age of an individual.

GlycanAge study (2021)

EXPERIMENTAL DESIGN

OBJECTIVE: To assess the effect of prolonged Analemma Water consumption on the IgG glycan profile (biological age) of healthy adult humans.

PARTICIPANTS: 19 healthy adult human subjects, aged 29-76.

STUDY DESIGN: All participants were given adequate amounts of Coherent Water (Analemma Water, n=19) and instructed to consume at least 1 L (35 oz) of water for 90 days. No other changes in diet, exercise or lifestyle regimes were made. Prior to treatment (Day -1) and on the last day of treatment (Day 90), blood samples were obtained by finger prick from all participants. Both GlycanAge tests were performed in the Genos Glyco Research Laboratory.

DATA ANALYSIS: For each participant, the results of the first and second GlycanAge test were compared to the population average (based on age and gender), and the difference between the first and second GlycanAge test was calculated. This value is referred to as 'the biological age reversal' value. The results were combined to draw conclusions about the changes in biological age which ocurred in the three months of consuming Analemma Water.

RESULTS

An overwhelming majority (17 out of 19) participants experienced biological age reversal (Table 1). **On average, the biological age decreased by 3.79 years after 3 months of consuming Analemma Water.** Naturally, the reversal differed between individual participants, with some participants experiencing 1 year and others up to 12 years of biological age reversal. **These results indicate that Analemma Water enhances anti-inflammatory activity of the human immune system, as indicated by changes in the glycan profile of IgG.**

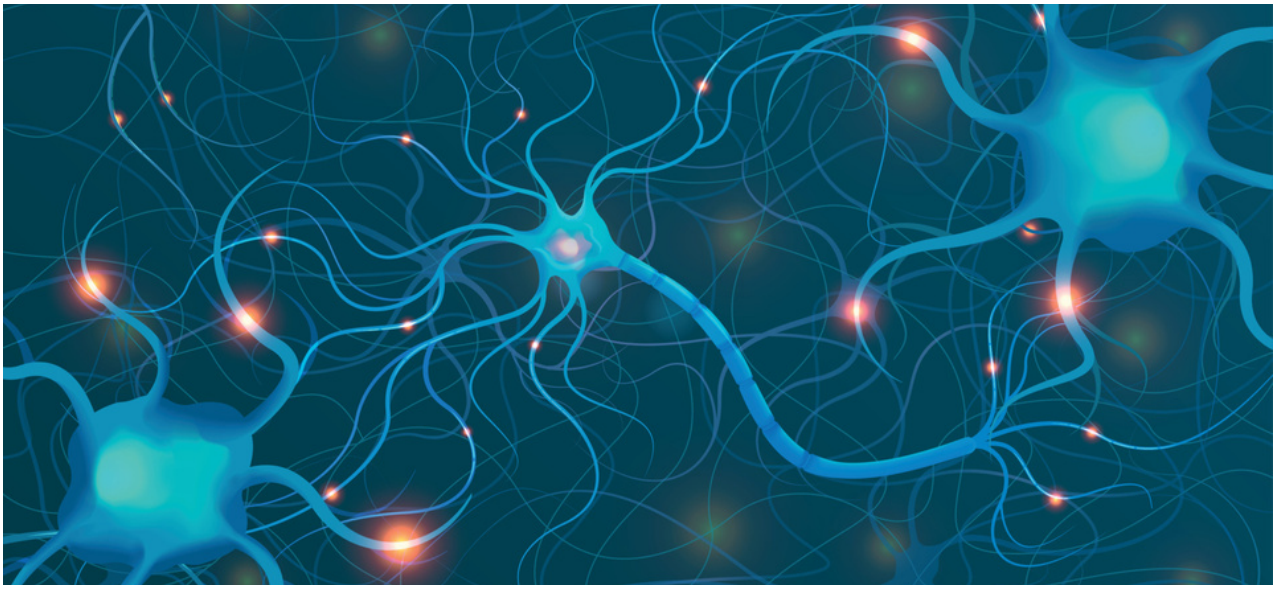
INSTITUTIONS

Genos d.o.o. (Zagreb, Croatia)

GlycanAge study (2021)

Table 1. Results of the GlycanAge study, with chronological and biological age listed for each participant. Changes in chronological (Result 1) and biological age (Result 2) were deducted to obtain the biological age reversal value (End result) measured after 3 months of Analemma Water consumption.

Subject	Chronological age 1. sample	Chronological age 2. sample	Result 1	GlycanAge 1. sample	GlycanAge 2. sample	Result 2	End Result -(1-2)
1	52	53	+1	70	58	-12	-13
2	32	32	0	42	40	-2	-2
3	76	76	0	78	71	-7	-7
4	29	29	0	45	43	-2	-2
5	48	48	0	27	20	-7	-7
6	46	47	+1	20	20	0	-1
7	52	53	+1	56	56	0	-1
8	39	40	+1	20	20	0	-1
9	36	37	+1	44	33	-11	-12
10	46	47	+1	25	21	-4	-5
11	68	68	0	65	64	-1	-1
12	41	42	+1	54	51	-3	-4
13	42	42	0	57	56	-1	-1
14	63	64	+1	59	58	-1	-2
15	51	51	0	59	55	-4	-4
16	38	39	+1	28	20	-8	-9
17	51	51	0	20	20	0	0
18	46	46	0	54	56	+2	+2
19	38	39	+1	30	29	-1	-2



Preliminary research on brainwaves (2019)

In 2019, we performed our first preliminary experiments on the effects of Analemma Water on human brain activity. A short-term experiment was performed on a group of adult humans and a long-term experiment was performed on a patient diagnosed with Alzheimer's disease.

Brainwaves are the synchronized electrical pulses emitted by masses of neurons communicating with each other. Brainwave activity can be measured by quantitative electroencephalogram (qEEG), which is most commonly divided into four frequency bands: delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–30 Hz). The lower frequency bands, delta and theta, are active in more restful states, while high frequency bands, alpha and beta, show increased activity in cognitively engaging states.

An important information about brain function is the efficiency of communication between different brain areas. One way to quantify this is by measuring the coherence (spectral correlation of two qEEG signals) between two different brain locations. In the following experiments, both spectral qEEG signals and the coherence between them were assessed.

Recent studies indicate that exposure to electromagnetic fields (EMFs) generated by mobile phone radiation affect all common frequency bands (delta, theta, alpha, beta and others).

In the following experiments, short-term exposure to mobile phone radiation was used to negatively affect brainwave activity and was immediately followed by consumption of Analemma Water with a repeated measurement of the same aspects. The very small time difference between the two measurements allowed for a reliable assessment of the immediate effects of Analemma Water on human brain activity.

General effects on spectra and coherence

EXPERIMENTAL DESIGN

OBJECTIVE: To determine whether Analemma Water consumption affects **power distribution and communication between brain areas** in adult humans.

PARTICIPANTS: Six adult human subjects, aged 27-73.

STUDY DESIGN: The experiment was performed as a **double blind placebo-controlled study**. Participants were divided into two groups (Analemma Water group, n=3, and Placebo Control group, n=3), with neither the participants nor the investigators aware of the distribution into groups. All participants were exposed to cellphone radiation (via 2 minute cellphone call) and instructed to drink a glass of water after the call. In the Analemma Water group, participants consumed Analemma Water, and in the Placebo Control group, participants consumed untreated water. qEEG measurements were performed at baseline (before call), after the cellphone call and 10 minutes after water consumption, Measurements were taken in the following four conditions; eyes closed (EC), eyes open (EO), while reading (EOR) and while watching a movie (EOM). Spectral information was obtained for 19 different brain locations. In total, 32 aspects were measured: brainwave spectra in all 19 locations (in EC, EO, EOR and EOM) and coherences between different brain locations at seven different frequency bands (from delta to beta 3), measured in EC, EO, EOR and EOM. All data were compared with reference values in the Thatcher qEEG database.

*After evaluation, qEEG data provides two types of information. The **spectra** reflect power distribution over different frequency bands: i.e. how many circuits are firing with a certain frequency at a certain location. The **coherences** between brain locations for all of the different frequency bands give information on the efficiency of communication between different locations.*

This experiment produced a high amount of data. For simplicity, the change in each aspect (detected by visual inspection) was expressed as a numbered value ranging from 0 to 5. Cumulative values were obtained from all 32 aspects and presented as total change values for each of the two states (after the call and after water intake).

INSTITUTIONS

Neurotherapie Centrum Hilversum (Hilversum, The Netherlands)

General effects on spectra and coherence

RESULTS

The results of the experiment are shown in **Figure 1** below. The graph shows total change in spectral activity and coherence between different brain locations, as measured for two states: after exposure to cellphone radiation (blue bars) and after water intake (orange bars). **Overall, more changes in spectral activity and coherence were measured in the Analemma Water group** (orange bars in the left panel), **than the Placebo Group** (orange bars in the right panel). Although the small sample size did not allow for a statistical analysis, **the stark difference between the two groups already indicates that consumption of Analemma Water immediately affects human brainwave activity.** The number of aspects analyzed in this experiment was very large and only allows a general idea of Analemma Water effects on human brain activity. However, the observed trends offer an excellent starting point for further research.

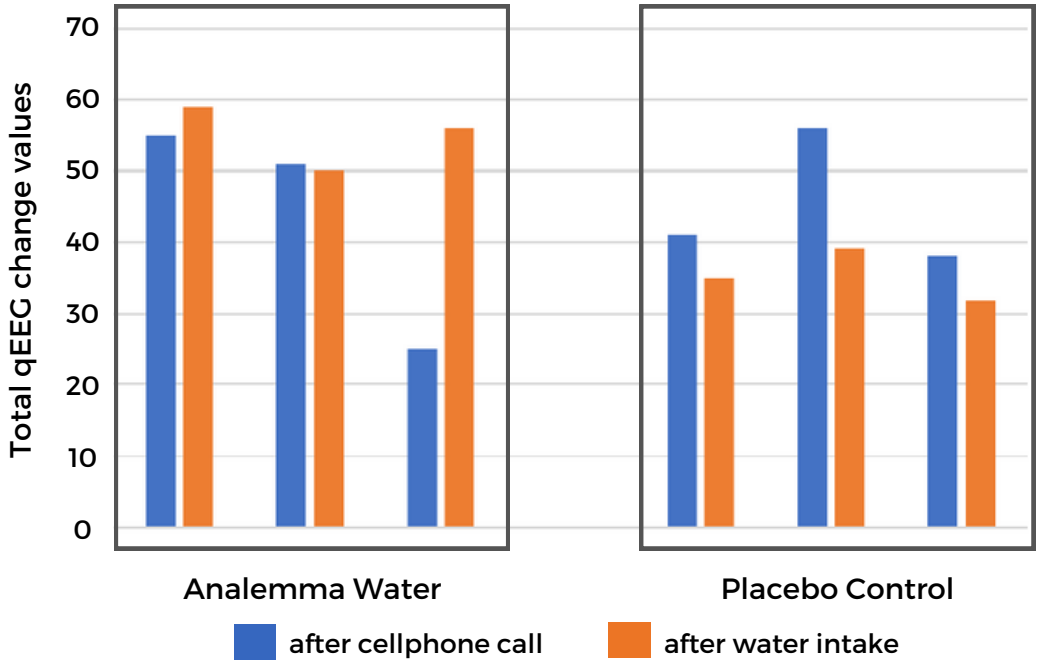
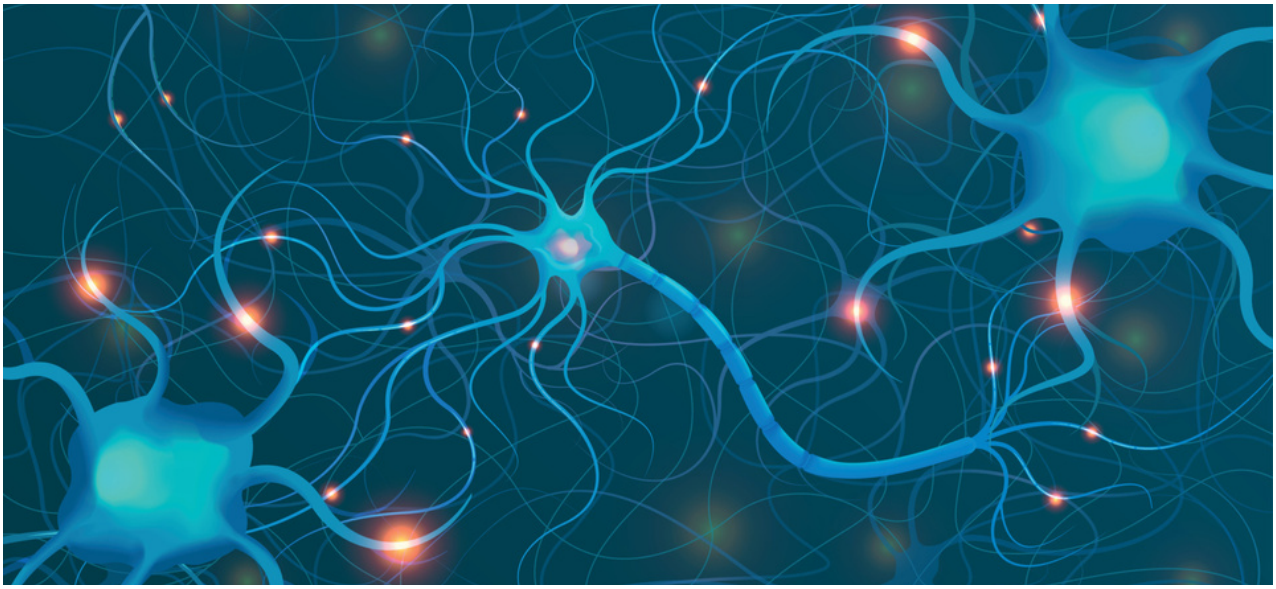


Figure 1. Total change in qEEG aspects (spectra and coherence) measured after 2 min exposure to cellphone radiation (**blue**) and after water intake (**orange**). Values are shown for each individual participant in the Analemma Group where participants consumed Analemma Water (left panel, n=3) and the Placebo Control group where participants consumed untreated water (right panel, n=3). These values were obtained by measuring 32 qEEG aspects describing the power distribution and coherence in and between 19 different brain locations. Each aspect was then given a value (0-5) based on the amount of visual change observed for each of the two states (after call and after water intake) and the cumulative values were depicted here as total change.



Preliminary research on brainwaves (2019)

EFFECTS ON DELTA BRAINWAVES

Alzheimer's disease is a progressive neurodegenerative condition generally characterized by a decline in memory function. Neuronal damage which underlies Alzheimer's disease is caused by accumulation of beta-amyloid plaque outside neuronal cells and neurofibrillary tangles inside the cells.

Biomarkers are measurable biological characteristics often used for diagnosis or tracking disease progression. One type of biomarker which can be used to assess the progression of Alzheimer's disease is the change in brainwave activity measured by the EEG, since abnormalities of electric potentials recorded in the cortex are directly related to the pathological changes of the structure and function of cortical layers.

A number of studies have reported EEG slowing in patients diagnosed with Alzheimer's disease. **EEG slowing is defined as an increase in lower EEG frequencies (delta and theta brainwaves) and a decrease in higher frequencies (mainly alpha brainwaves).**

Here, the activity in delta and theta frequency bands was measured in a female patient diagnosed with Alzheimer's disease. Since EEG slowing is a well known phenomenon linked to Alzheimer's disease, this parameter was selected as a measurable biomarker for assessment of the **effects of prolonged Analemma Water consumption on brain activity**.

Effects on delta brainwaves

EXPERIMENTAL DESIGN

OBJECTIVE: This study aimed to determine the effects of prolonged Analemma Water consumption on delta brainwave activity of an Alzheimer's patient.

PARTICIPANTS: One adult human subject, aged 68, previously diagnosed with Alzheimer's disease.

STUDY DESIGN: The patient was instructed to continually consume Analemma Water over a period of 51 days (6 weeks). The qEEG measurements were taken at baseline (prior to experiment) and at Day 1, 2, 9, 37 and 51 of the experiment. Frequency maps were obtained for delta (1 - 3.5 Hz) and theta (4 - 7.5 Hz) bands. Additionally, the immediate effects (within 10 minutes) of Analemma Water consumption on delta and theta brainwave activity were measured in normal conditions and after exposure to cellphone radiation (via 2 minute call). All measurements were obtained in the Eyes Closed condition.

RESULTS

Over a period of 51 days, **the patient's delta levels exhibited a gradual decrease, as evident by the increasing levels of green color in the delta band (Figure 2).** This was uncommon for an Alzheimer's patient, indicating a strong effect of Analemma Water on brain activity. Since delta brainwaves are most active in deep sleep states, a decrease in delta brainwave activity can be correlated with less drowsiness. Correspondingly, the participant herself reported increased vitality and less tiredness.
Furthermore, frequency maps obtained **immediately after consumption of Analemma Water clearly demonstrate a decrease in delta levels** (**Figures 3 and 4**). This effect was observed both in regular conditions (**Figure 3**) and after exposure to cellphone radiation (**Figure 4**).

INSTITUTIONS

Neurotherapie Centrum Hilversum (Hilversum, The Netherlands)

Guusje Roozemond, MSc., Neurotherapist

Effects on delta brainwaves

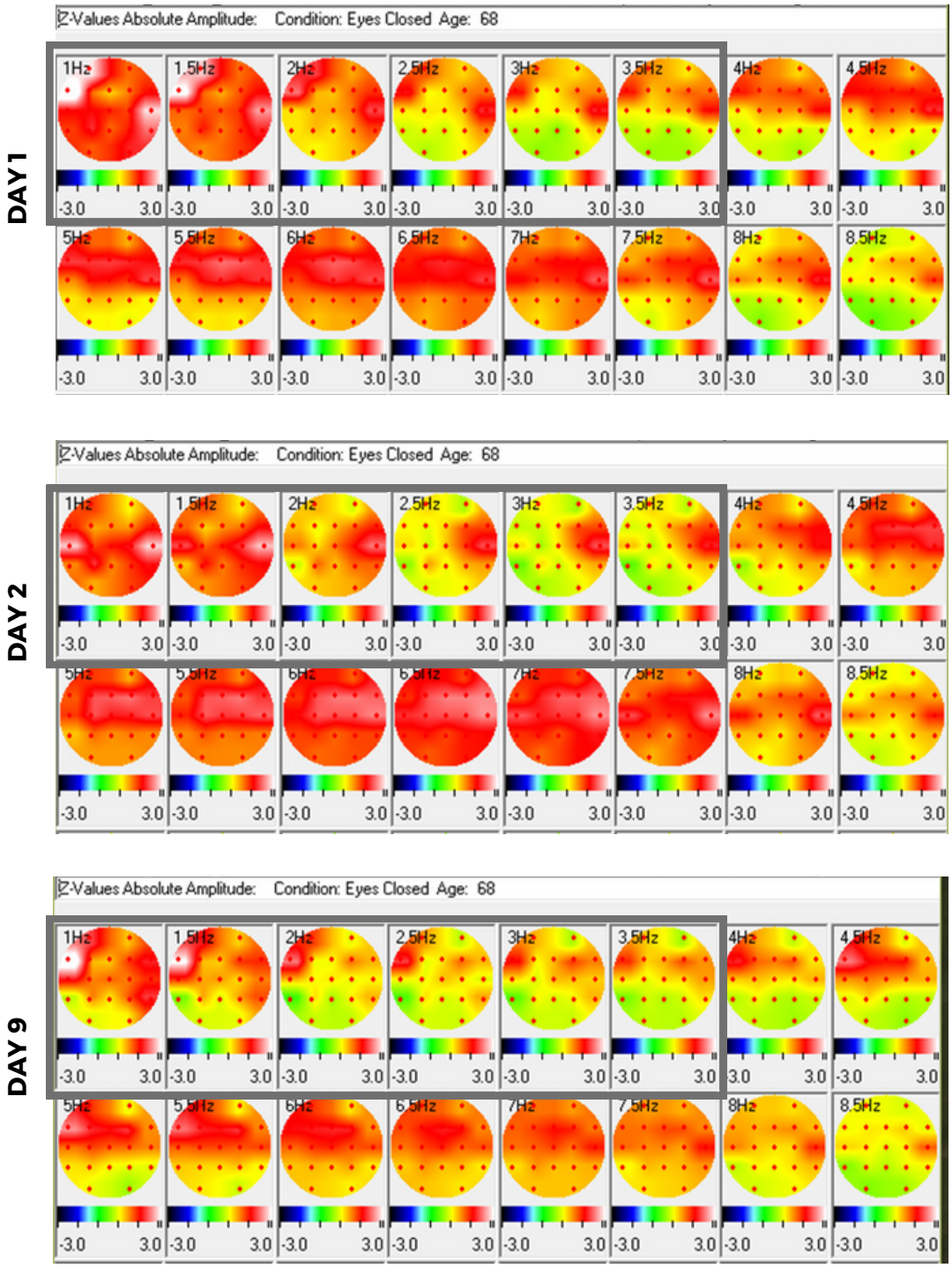


Figure 2 - continued on next page.

Effects on delta brainwaves

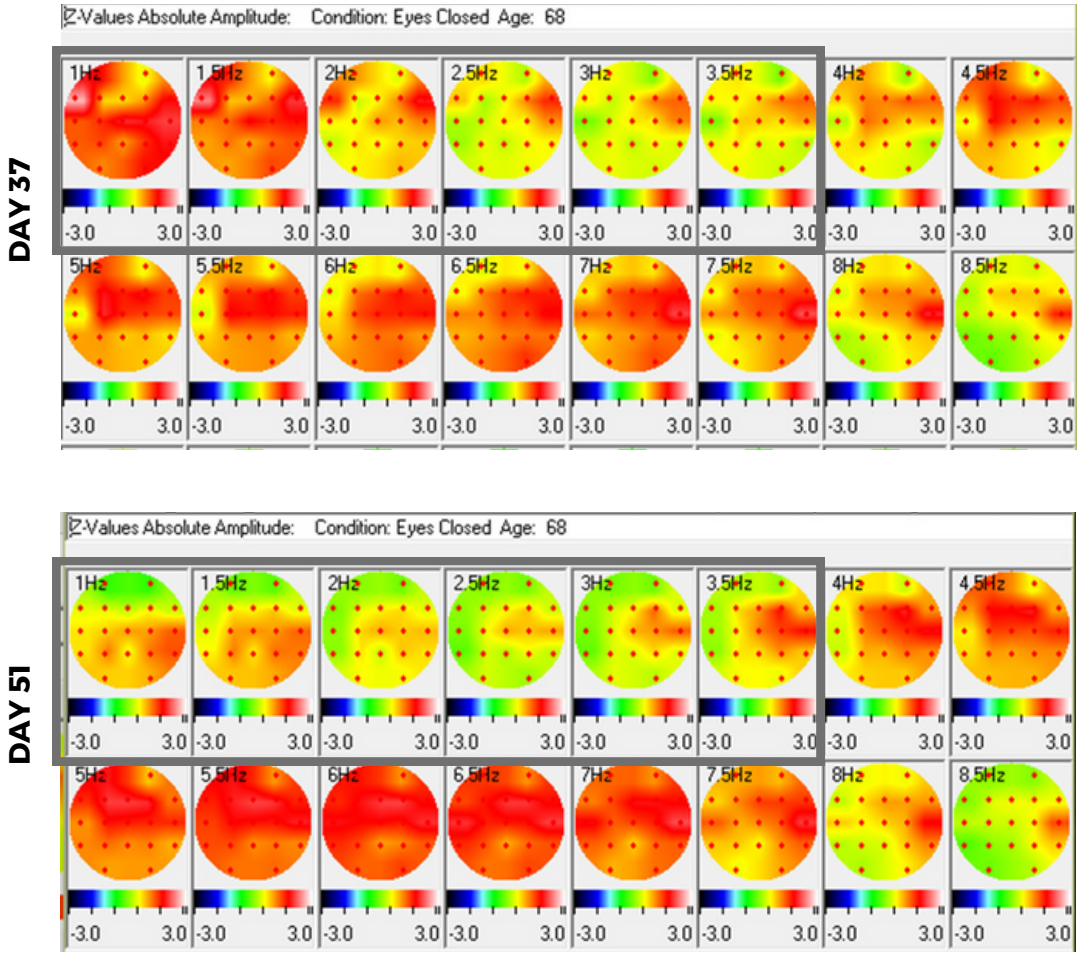
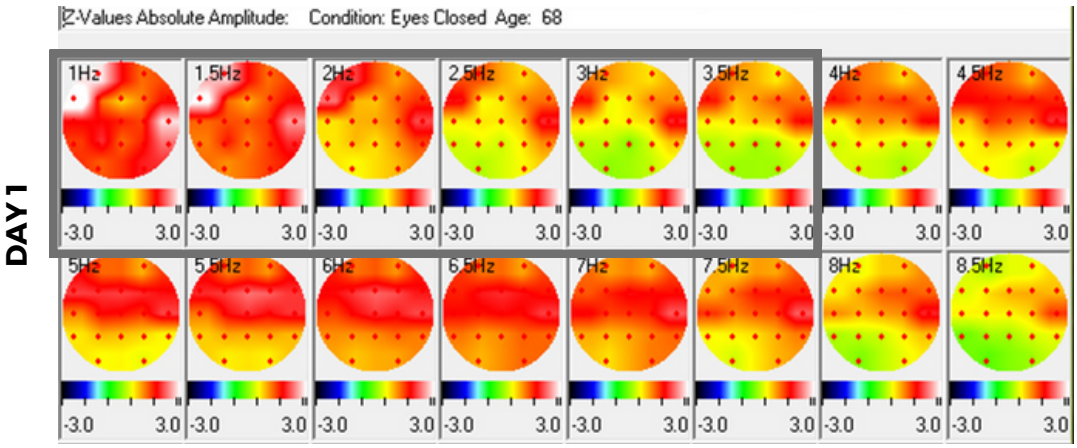


Figure 2. Frequency maps for delta (1 - 3.5 Hz) and theta (4 - 7.5 Hz) bands measured in a patient diagnosed with Alzheimer's disease. Measurements were obtained over the course of 6 weeks, during which the patient consumed Analemma Water. The day of each measurement is indicated on the left. The delta band range is marked with a grey box. **Red** color indicates increasing brainwave activity, while **green** color indicates decreasing brainwave activity. Alzheimer's patients have been reported to exhibit increased delta activity. Here, activity in the delta band gradually decreased in an Alzheimer's patient who continually consumed Analemma Water.

Effects on delta brainwaves

Before Analemma Water consumption



Immediately after Analemma Water consumption

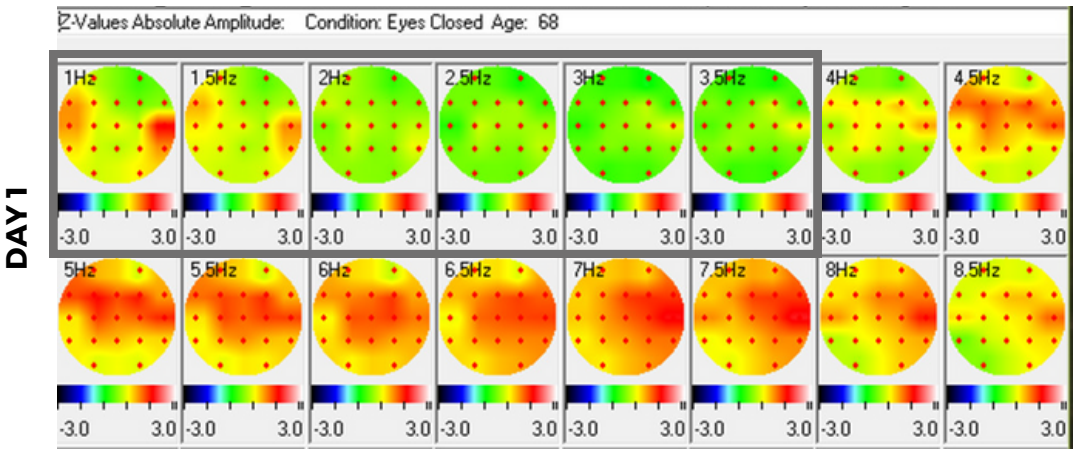
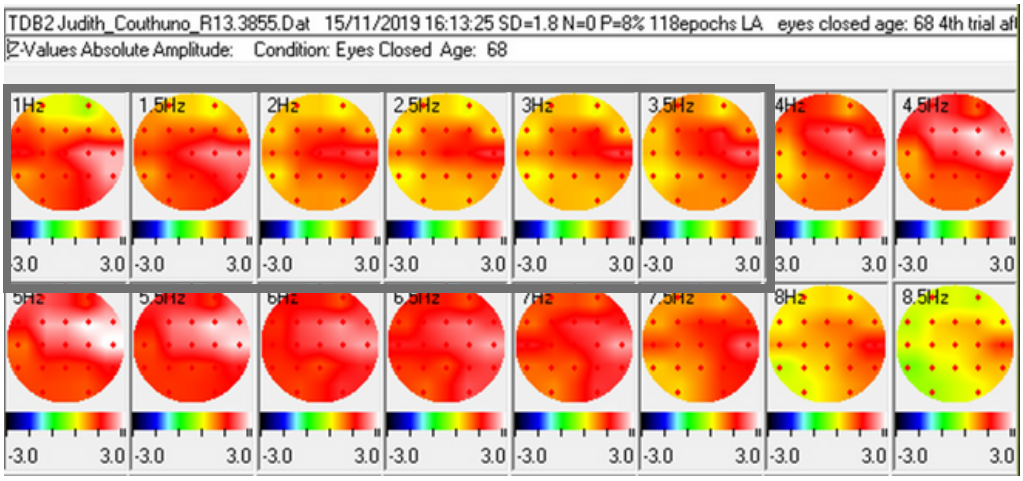


Figure 3. Frequency maps for delta (1 - 3.5 Hz) and theta (4 - 7.5 Hz) bands measured in a patient diagnosed with Alzheimer's disease. Measurements were obtained on Day 1 of a continuous experiment (see the text for details). Here, the measurements were obtained in regular conditions (top panel) and then immediately after consumption of Analemma Water (bottom panel). The delta band range is marked with a grey box. **Red** color indicates increasing brainwave activity, while **green** color indicates decreasing brainwave activity. As evident by the change from predominantly orange-red tones in the top panel toward green tones in the bottom panel, Analemma Water consumption caused an immediate decrease in delta brainwave activity.

Effects on delta brainwaves

After 2 minute exposure to cellphone radiation

DAY 2



Immediately after Analemma Water consumption

DAY 2

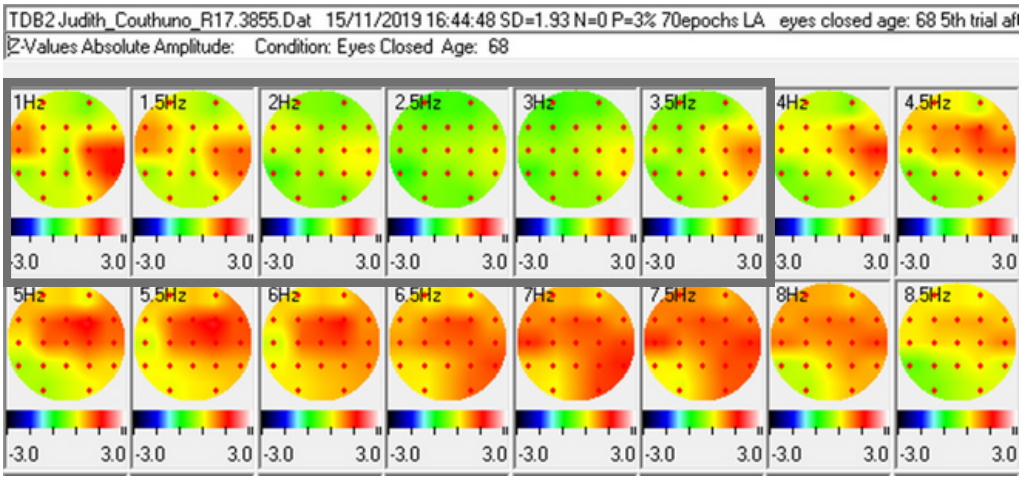


Figure 4. Frequency maps for delta (1 - 3.5 Hz) and theta (4 - 7.5 Hz) bands measured in a patient diagnosed with Alzheimer's disease. Measurements were obtained on Day 2 of a continuous experiment (see the text for details). The patient was exposed to cellphone radiation (2 min phone call) and consumed Analemma Water immediately after. Measurements were taken after the phone call (top panel) and after consumption of Analemma Water (bottom panel). The delta band range is marked with a grey box. **Red** color indicates increasing brainwave levels, while **green** color indicates decreasing brainwave levels. As evident by the change from predominantly red tones in the top panel toward green tones in the bottom panel, Analemma Water consumption caused an immediate decrease in delta levels.

RESEARCH REPORT
JANUARY 2023

PART II

EFFECTS ON PLANTS AND SOIL

The cherry tomato study (2021)

In 2021, a series of experiments was performed on cherry tomato plants grown under different watering regimes with the purpose of assessing the **effects of Analemma Water on plant physiology and soil microbiome**. The most interesting results of this study are presented in the following sections.

Terrestrial pants are tightly linked to soil microorganisms (bacteria and fungi). The importance of soil microbiome is highlighted by the fact that plants invest somewhere between 11 and 40% of their photosynthetically fixed carbon as well as 10–16% of their total nitrogen into compounds used by the microorganisms surrounding their roots. In turn, bacteria and fungi actively participate in organic matter decomposition, thus releasing nutrients and furthering plant growth.
On the other hand, some pathogenic microorganisms (fungi) pose serious threat to plant health. Infestation of crops with pathogenic fungi can have disastrous effects on food production and, consequently, on global economy.

In this study, cherry tomato plants were treated with different watering treatments, including Analemma Water. **Significant effects of Analemma Water were found in several chemical parameters related to the nitrogen cycle in the soil**, linking Analemma Water with factors connected to soil fertility and productivity.

Additionally, **Analemma Water caused a reduction in a genus of pathogenic fungi, and an increase in bacterial diversity.** These preliminary results offer us insight into possible effects of Analemma Water on soil fertility and biodiversity and light our path toward future research.

The cherry tomato study (2021)

EXPERIMENTAL DESIGN

Plant growth conditions: The study took place in a climate-controlled greenhouse located in Schiedam, The Netherlands. On Day 1, the soil was distributed over a concrete surface and larger chunks where crushed to achieve a more even grain size. The soil was randomly distributed into 24 plastic plant pots with a diameter of 39 cm and a height of 36 cm. The plates contained a central hole for drainage. The 6 week-old cherry tomato plants (grown by Kwekerij Poot, Schiedam, The Netherlands) were repotted from their original 10 cm pots into the 24 larger pots at random. The potted plants were kept in an isolated part of the greenhouse, not easily reachable by third parties. The study setup was randomized, with the 24 plants randomly divided into 6 groups (4 plants per treatment), designated to receive 6 different watering treatments. During the first 14 days, all plants received only Rainwater, which was dosed using an automated system. Each plant received 100 mL of rainwater at 7 a.m. CEST.

Water treatments: Two types of water were tested in this treatment, named Water 1 and Water 2. **In the following text, Analemma Water will refer specifically to Water 1**. Coherent water was prepared about a month prior to beginning of the study and stored in standard water jerrycans. On Day 15, the plants belonging to the same treatment group where placed next to each other to allow easier treatment and measurements. The treatments were as follows: (1) Rainwater (2) Rainwater + organic fertilizer, (3) Analemma Water, (4) Analemma Water + organic fertilizer (5) Water 2, (6) Frequency water. In treatments 2 and 4, approximately 250-300 g of organic fertilizer (Fa. Orgapower, Amersfoort, NL) was used per plant. From Day 15 onward, each plant received 400 mL of the assigned water daily at 7 a.m. CEST using the automated dosing system.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

Kwekerij Poot (Schiedam, The Netherlands)

Chemical composition of soil

ANALYSIS OF CHEMICAL COMPOSITION

On Day 2 of the experiment, a baseline sample of the soil used in this study was collected and delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). At the end of the growth period (Day 118), soil samples were collected from the 24 pots in which cherry tomato plants were grown under specific watering regimes. Soil samples were delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). Chemical composition was measured for all soil samples.

RESULTS

Since more than 20 parameters were measured using 7 different groups of soil samples (Baseline at Day 2 + 6 treatments at Day 118), a large dataset was obtained. For simplicity, the data shown here was reduced to only the most relevant information. The chemical parameters measured for three groups, Baseline, Analemma Water and Rainwater, are shown in different graphs in **Figure 1**. The measuring units of each parameter are listed in **Table 1**.

The majority of parameters remained largely unchanged between the Analemma Water and Rainwater group. However, several parameters pointed toward interesting changes ocurring in these two groups. Firstly, **iron content was higher in Analemma Water group compared to control**. Next, interesting differences were found in parameters linked to availability and utilization of soil nitrogen. These results are shown individually in **Figure 2**. **Total nitrogen and Nitrogen delivery capacity were significantly higher in the Analemma group compared to Rainwater.** Total nitrogen is the major indicator of soil fertility and quality in an agricultural ecosystem, while the nitrogen delivery capacity is the amount of nitrogen from organic matter that can be made available to plants over a longer period of time.

Nitrogen is an essential plant nutrient, but the majority of soil nitrogen appears in different organic forms which plants are unable to take up into their cells. However, plants can readily take up mineral forms of nitrogen, such as nitrates. Soil microorganisms convert organic forms of nitrogen to mineral forms through a process called mineralization, which involves decomposition of organic matter.

INSTITUTIONS

Groen Agro Control (Delfgauw, The Netherlands)

Normec Groen Agro Control

Chemical composition of soil

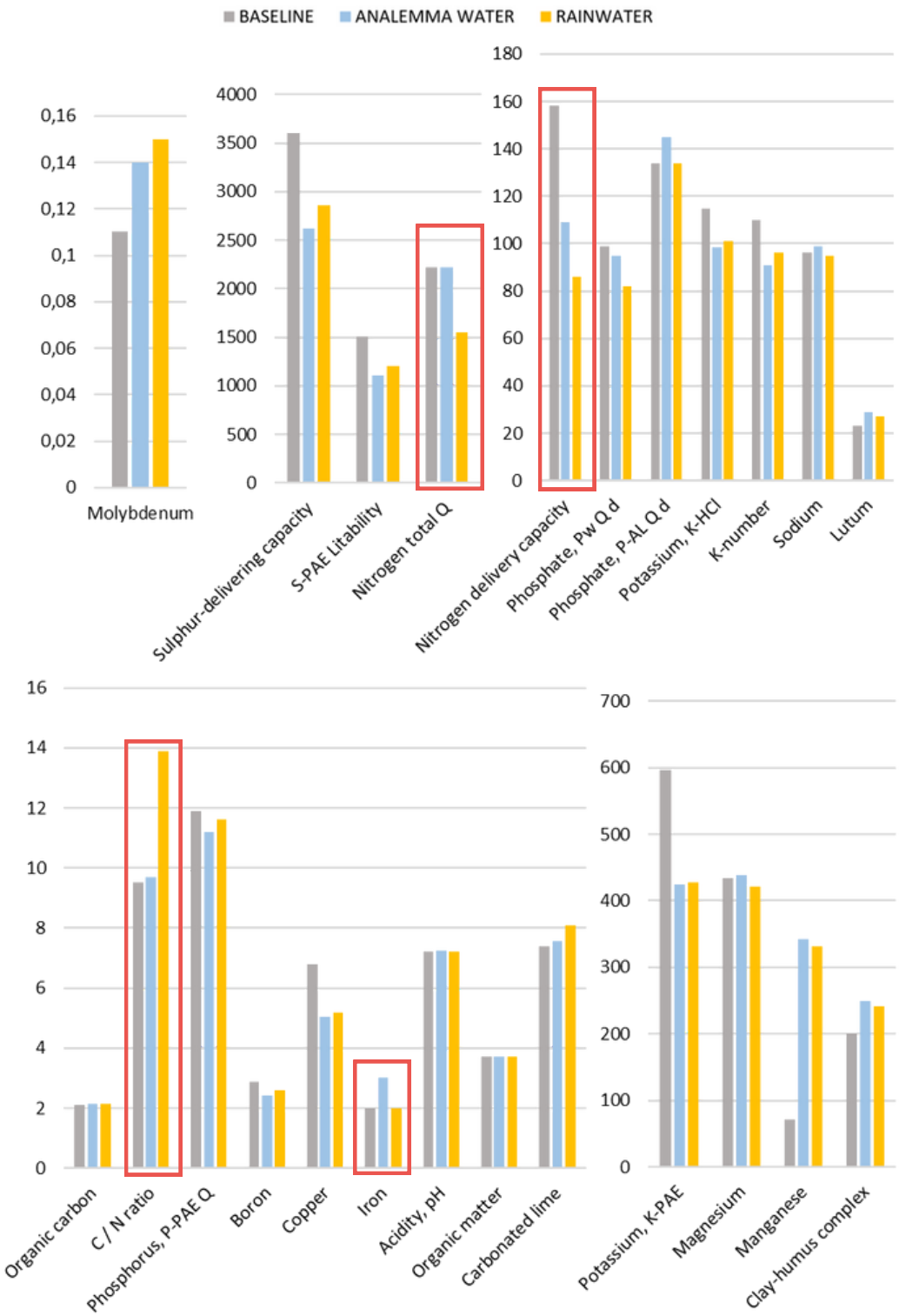


Figure 1. Mean values of chemical parameters measured for three groups of soil samples: Baseline, Analemma Water and Rainwater (Control). Baseline samples were obtained on Day 2, and treated samples on Day 118 of the experiment (n=4). The measuring units are listed in **Table 1**. The parameters are grouped according to similarity in value. Red boxes indicate parameters with differences between Analemma Water group and Rainwater group.

Chemical composition of soil

A parameter referred to as the carbon to nitrogen ratio (C/N ratio) provides information on how easily nitrogen can be released from organic matter. A low C/N ratio indicates high levels of nitrogen in organic matter, and a high level of decomposition and mineralization. **Here, the C/N value of soil treated with Analemma Water was significantly lower than in soil treated with Rainwater (Figure 2), indicating higher mineralization rates.**
Although these results are only preliminary, and a larger sample size is needed for more elaborate conclusions, they are already solid indicators that Analemma Water has a strong impact on the nitrogen cycles which underlie soil fertility. In the future, it will be exciting to broaden these findings with more in-depth research on the effects of Analemma Water on plant physiology and crop yield, as both are affected by changes in nitrogen availability.

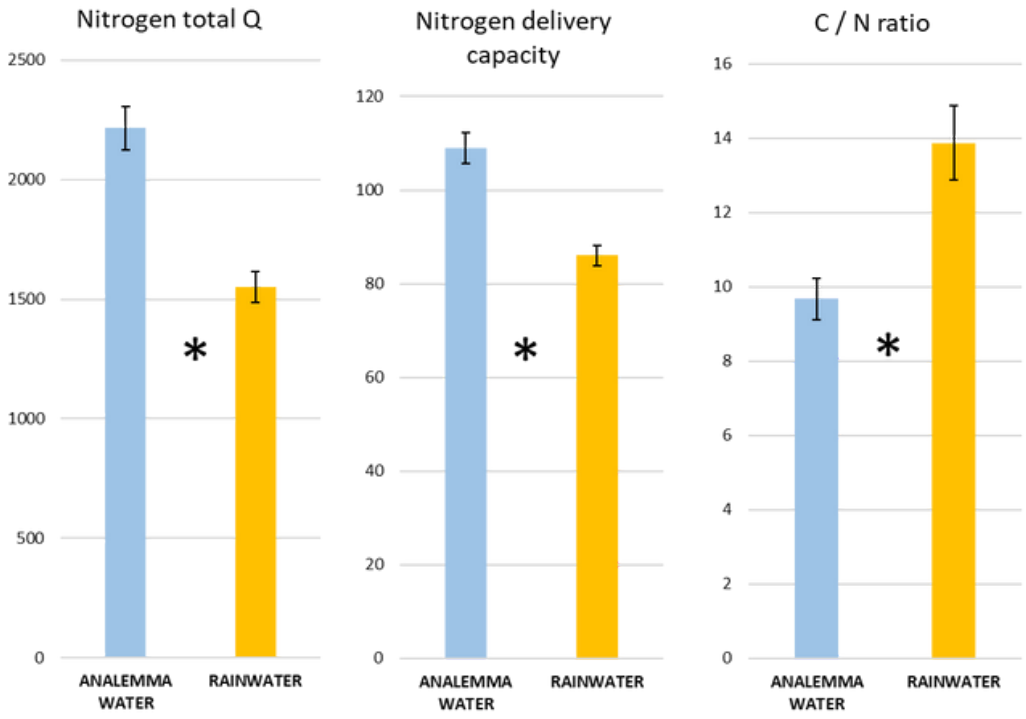


Figure 2. Differences in total nitrogen, nitrogen delivery capacity and carbon to nitrogen ratio (C/N ratio) between soil watered with Analemma Water and Rainwater (Day 118). The measuring units of each parameter are listed in **Table 1**. The bars show mean ± SD values (n=4). Asterisks indicate statistical significance (Student's T test, $p < 0,05$).

Chemical composition of soil

Table 1. The measuring units of 24 chemical parameters measured in the study, describing the values showed in **Figures 1** and **2**.

PARAMETER	UNIT
Organic carbon	% C
C / N ratio	-
Phosphorus, P-PAE Q	mg P/kg
Boron	mg B/kg
Copper	mg Cu/kg
Iron	mg Fe/kg
Molybdenum	mg Mo/kg
Acidity, pH	
Organic matter	%
Carbonated lime	%
Nitrogen delivery capacity	kg N/ha per year
Phosphate, Pw Q d	mg P2O5/L
Phosphate, P-AL Q d	mg P2O5/100 g
Potassium, K-HCl	mg K2O/100 g
K-number	-
Potassium, K-PAE	mg K/kg
Magnesium	mg MgO/kg
Sodium	mg Na/kg
Manganese	mg Mn/kg
Clay-humus complex (CEC)	mmol+/kg
Lutum	%
Nitrogen total Q	mg N/kg
S-PAE Litability	mg S/kg
Sulphur-delivering capacity	kg S/ha per year

Effects on fungal properties

ANALYSIS OF FUNGAL COMPOSITION

At the end of the growth period (Day 118), soil samples were collected from the 24 pots in which cherry tomato plants were grown under specific watering regimes. Soil samples were delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). Analysis of fungal properties of the soil consisted of measuring the value known as colony forming unit per gram of ground (CFU/g). The CFU value refers to the number of viable microbial cells in a sample.

RESULTS

The results of the analysis are presented in **Table 2**. While several groups of fungi were identified on the genus level (denoted by the name of the genus followed by "sp."), others were grouped together as "other molds". Both the total number of fungi and the total number of other molds followed a similar pattern, with highest number detected in soil treated with Rainwater and the lowest number in soil treated with Rainwater supplemented with fertilizer, followed by Analemma Water (**Figures 3** and **4**). Since fungal communities in the soil and their relationships with different plants are highly complex, these results do not offer immediate conclusions. However, by looking at individual genera (**Figure 5**), it appears that the fungi from the genus Cladosporium were less abundant in soil treated with Analemma Water than Rainwater. This is interesting with regards to the known pathogenic nature of different species from this genus. Cladosporium fungi can cause black point in cereal species, they cause scab in Cucurbita species, and brown leaf spots in tomato. In humans, they mainly cause allergic reactions which sometimes lead to asthma. **The reduction in soil Cladosporium after Analemma Water treatment points to a beneficial role of Analemma Water in maintaining a healthy soil microbiome**. Although these results were not statistically analyzed, they are an important step in the direction of further and more specific analyses of the effects of Analemma Water on the fungal properties of soil.

INSTITUTIONS

Groen Agro Control (Delfgauw, The Netherlands)

Normec Groen Agro Control

Effects on fungal properties

Table 2. Fungal CFU/g for all watering conditions and individual plants. Red text color indicates values above the upper detection limit (5000). Blue text color indicates values below the lower detection limit (100). For easier reading, different value categories were highlighted as follows: 10-1000 CFU/g representing a low amount of fungal cells (highlighted in yellow), 1000-100 000 CFU/g representing an average amount of fungal cells (highlighted in green), and more than 100 000 CFU/g representing a high amount of fungal cells (highlighted in red).

	CFU/g	total fungi	*Penicillium sp.*	*Cladosporium sp.*	*Trichoderma sp.*	*Fusarium sp.*	other molds	yeasts	*Pythium sp.*	*Phytoph-thora sp.*
Rain water	B1	160 000	10 8000	20 000	> 5 000	< 100	52 000	<100	+	-
	B2	70 000	1 000	5 000	3 000	2 000	59 000	<100	+	-
	B7	37 000	11 000	3 000	3 000	1 000	19 000	<100	+	-
	B8	31 300	300	< 100	3 000	300	2 500	<100	+	-
Rain water with organic fertilizer	B15	4 800	8 000	< 100	4000	5 000	3 100	<100	+	-
	B16	29 400	7000	< 100	400	3 000	19 000	<100	+	-
	B23	41 000	13 000	2 000	400	2 000	24 000	<100	+	-
	B24	21 000	8 000	3 000	1 000	1 000	8 000	<100	+	-
Water 1	T3	18 000	< 100	2 000	1 000	3 000	13 000	<100	+	-
	T4	27 200	8 000	2 000	2 000	200	15 000	<100	+	-
	T5	44 300	10 000	< 100	300	3 000	31 000	<100	+	-
	T6	40 000	6 000	< 100	> 5 000	3 000	31 000	<100	+	-
Water 1 with organic fertilizer	T9	48 300	4 000	2 000	300	3 000	39 000	<100	+	-
	T10	60 000	7 000	< 100	> 5 000	4 000	49 000	<100	+	-
	T17	42 000	11 000	4 000	2 000	3 000	22 000	<100	+	-
	T18	51 000	7 000	6 000	5 000	3 000	35 000	<100	+	-
Water 2	T11	26 300	8 000	1 000	300	1 000	16000	<100	+	-
	T12	40 200	16 000	5 000	200	3 000	16000	<100	+	-
	T21	42 000	8 000	2 000	6 000	2 000	24000	<100	+	-
	T22	40 000	10 000	3 000	> 5 000	6 000	21000	<100	+	-
Frequency water	T13	57 000	15 000	2 000	1 000	1 000	38 000	<100	+	-
	T14	47 000	6 000	6 000	> 5 000	6000	29 000	<100	+	-
	T19	45 000	5 000	15 000	2 000	3 000	2 0000	<100	+	-
	T20	95 000	23 000	16 000	> 5 000	2 000	54 000	<100	+	-

Effects on fungal properties

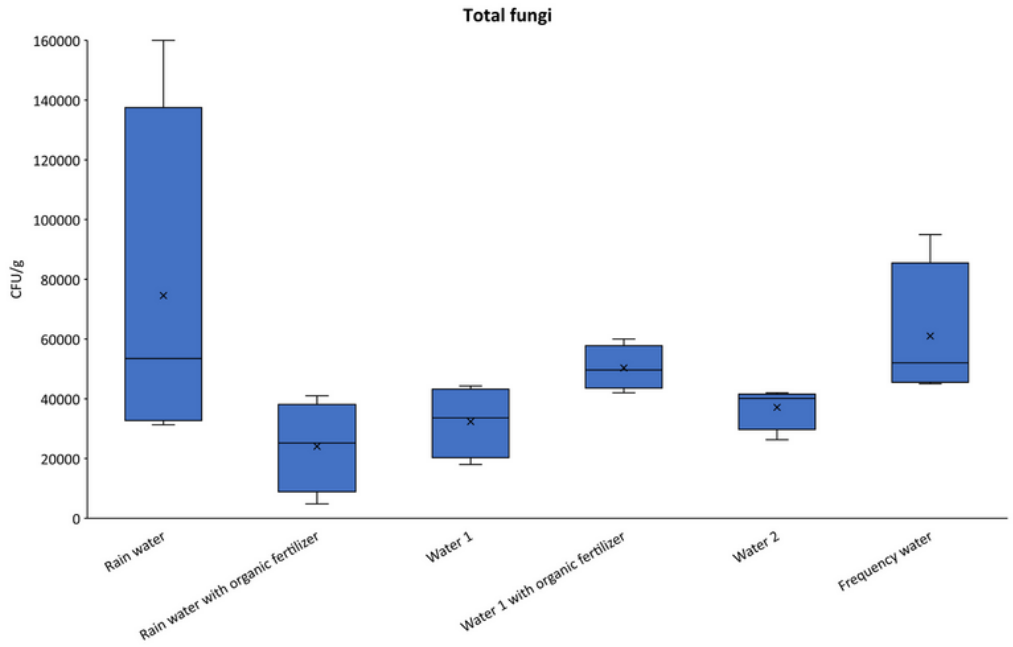


Figure 3. Total fungal community from soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. Each box plot summarizes CFU/g of four samples per watering condition.

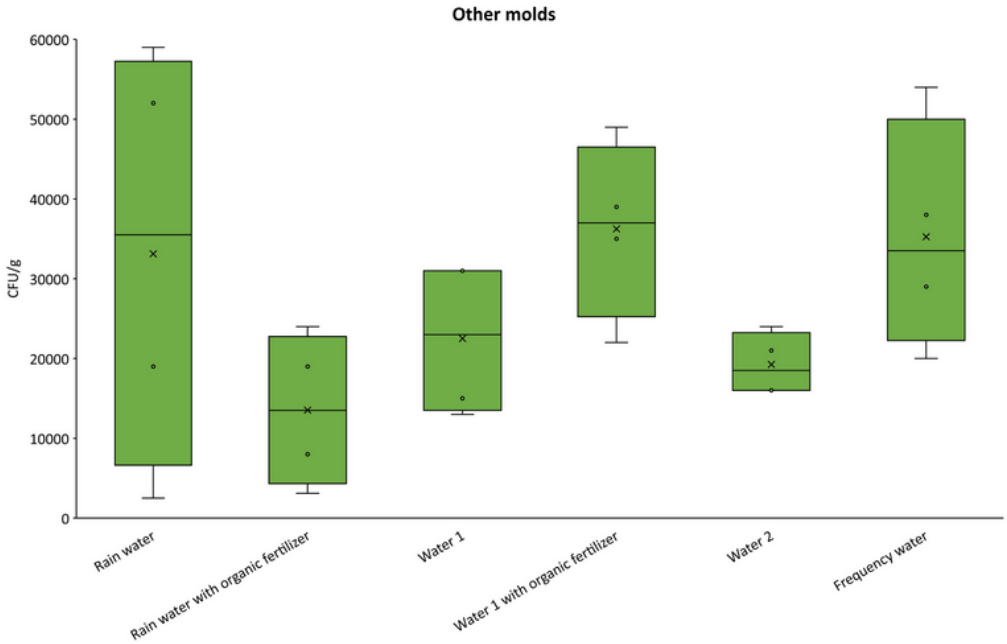


Figure 4. Total fungal community from soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. The graph shows fungal communities not characterized on the genus level but grouped together as a separate category of "other molds". Each box plot summarizes CFU/g of four samples per watering condition.

Effects on fungal properties

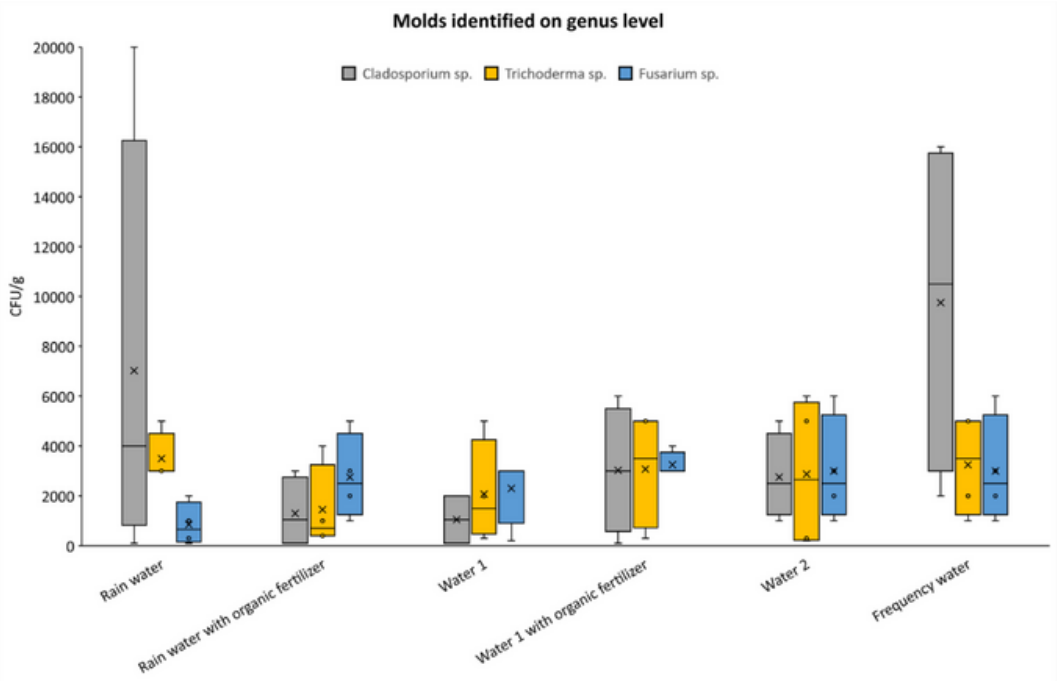


Figure 5. Fungal communities of three genera: Cladosporium sp. (grey), Trichoderma sp. (yellow), and Fusarium sp. (blue), measured in soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. Each box plot summarizes CFU/g of four samples per watering condition.

Analysis of bacterial composition and diversity

Next-generation sequencing (NGS) is the latest sequencing technology used to detect nucleotide sequences of entire genomes or targeted regions of DNA or RNA. The most commonly used marker for evaluation of bacterial composition of a sample is the 16S rRNA gene. Total DNA is first extracted from a sample such as soil, followed by amplification of a specific region in the 16S rRNA gene. Next, all amplified fragments are sequenced and these sequences are then compared with a vast database of 16S rRNA sequences from known species of bacteria, also known as reference sequences.
Using this method, the samples can be screened for presence of a large number of different bacterial species (or, more commonly, groups of similar species; taxa). Additionally, the change in bacterial diversity can be analyzed between different samples.

BACTERIAL DIVERSITY

NGS provides two common types of diversity indexes: **alpha diversity and beta diversity**.

Alpha diversity tells us how many different species are detected in each sample ("species richness") and also about how abundant each species is ("evenness"). For example, it can tell us that sample A contains 5 different species of bacteria, while sample B contains only 3. Alpha diversity is greater in sample A. Alpha diversity does not look into the identity of species observed in each sample, only the number.

Beta diversity measures the change in species diversity between two or more samples. It counts the total number of species that are unique to each of the samples being compared. Beta diversity provides information about species identity.

Analysis of bacterial composition and diversity

NEXT-GENERATION SEQUENCING

At the end of the growth period, soil samples were collected from 8 pots in which cherry tomato plants were grown under different watering regimes: Rainwater Control (n=4), and Rainwater treated with Analemma Water (n=4). Soil samples were delivered to the BaseClear laboratory (Leiden, The Netherlands) for analysis of bacterial composition and diversity. This analysis was performed by next-generation sequencing using the 16S rRNA marker. The measured parameters were as follows: **alpha diversity** (Observed richness, Shannon's index and Simpson's index), **beta diversity** (Redundancy analysis; RDA), **association testing** (Differential Abundance Analysis; DAA) and **Linear discriminant analysis Effect Size** (LEfSE). Besides providing statistical data, these analysis indicate existence of **Key Biomarker and Signature Species** in a given microbiome dataset.

RESULTS

The NGS bacterial profiling data showed that **alpha diversity significantly increased in soil treated with Analemma Water compared to Rainwater Control (Figure 6)**. This indicates that the number of different bacterial taxa and their abundance was larger in soil samples treated with Analemma Water. Beta diversity, measured by the RDA analysis, was not significantly different between water treatments.
Using machine learning methods (DAA and LEfSe), the two groups of samples were analyzed for differences in low abundant bacteria (i.e., bacterial genera whose relative abundance is less than 2%). Although these results did not reach statistical significance, they did indicate several genera which were differentially associated with the use of specific water treatments (Table 3). These results give insights into possible specific effects of Analemma Water treatment on soil bacteria. **For instance, bacteria of the genus Chelativorans, which was associated with the Analemma Water treatment, were recently shown to decrease the toxicity of antimony and its uptake into rice, thus serving an ecological role in remediation of soil contamination with toxic elements**. This finding would be very interesting to look into in the future.

INSTITUTIONS

BaseClear B.V. (Leiden, The Netherlands)

Analysis of bacterial composition and diversity

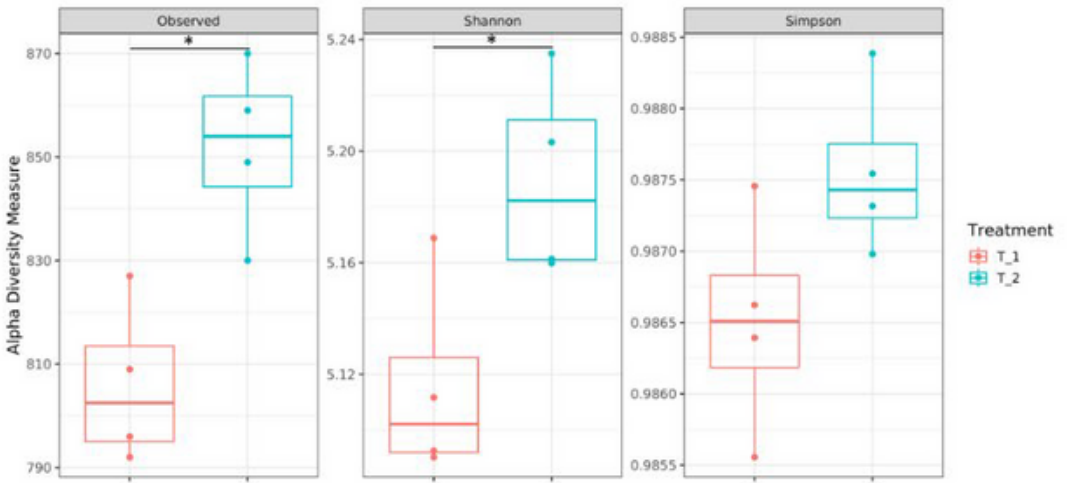


Figure 6. Results of 16S profiling. Alpha diversity was higher in soil treated with Analemma Water (blue boxplots on the right) compared to Rainwater (red boxplots on the left). Alpha diversity was measured using three indices. Statistical significance was measured in Observed richness (left panel) and Shannon's index (middle panel), $p < 0.05$.

Table 3. Results of 16S profiling. Listed in the table are results of alpha and beta diversity analysis, and Bacterial Biomarker Genera (low-abundant species) differentially found in the Analemma Water group using machine learning methods (DAA and LEfSe).

Compared groups	Alpha diversity, p<0.5	Beta diversity p<0.5	Bacterial Biomarker Genera
Control: Rainwater **Test Water**: Analemma Water	**YES - higher in Analemma Water group (Observed richness + Shannon's index)**	**NO**	Analemma Water: *Thermomarinilinea, Chelativorans, Hydrogenophaga, Pseudoxanthomonas*

BIOPHOTON EMISSION RESEARCH (2014-2018)

The following studies were based on biophoton emission analysis of plants treated with Analemma Water. Biological organisms continuously emit ultra-weak photon emission, also known as biophoton emission. Biophotons are generated via relaxation of electronic excited states in the course of oxidative metabolic processes and oxidative stress reactions which regularly occur in living organisms. This phenomenon has been observed in virtually all metabolically active systems, from the level of bacteria and fungi, across germinating seeds and whole plants, to animal tissue cultures and whole organisms, including human beings.

Because the phenomenon of biophoton emission reflects oxidative processes, either metabolic or stress-related, it can be widely used as a non-invasive tool for monitoring the physiological state of biological systems.

MEASURING BIOPHOTON EMISSION

Biophotons are emitted by organisms in the wavelength range from 200 to 800 nm. Biophoton emission analysis can be divided into two categories: **spontaneous emission** (SE), also called ultra weak photon emission (UPE), and **delayed luminescence** (DL).

Both of these properties, SE and DL, were analyzed in the following experiments using low-noise photomultiplier tubes and highly sensitive charge coupled device cameras.

Biophoton emission of tomato fruits (2018)

EXPERIMENTAL DESIGN

Plant treatment: Plants were grown in standard soil in climate-controlled greenhouse conditions. Plants were divided into two groups according to the watering treatment (**Figure 1**). One group was watered with untreated tap water (Control, n=3) and the other group with Analemma-treated tap water (Analemma Water; Treated, n=3). Plants were grown until the fruit ripening stage, producing red tomatoes.

Sampling and biophoton emission analysis: Fruit samples were harvested on 8 different days in the period between 16/07/2018 and 08/08/2018. On every harvest day, one fruit was harvested per plant according to a predefined protocol. The fruit was immediately used for biophoton emission analysis (DL and SE). After the DL measurement, the tomatoes were dark-adapted for more than 1.5 hours and subsequently their SE signals were recorded. The DL data was analyzed using 3 different models, and SE data using 2 different models. The models used for statistical analysis of biophoton emission data are discussed in **Appendix F**.

Figure 1. Plant watering treatment setup in a climate-controlled greenhouse. Tomato plants were grown until the fruit-ripening period and watered with either untreated tap water (Control, left) or Analemma-treated water (right). This was followed by tomato fruit harvesting and biophoton emission analysis.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

RESULTS

The DL and SE analyses showed that watering treatment strongly influenced the energy storage properties of tomato fruits.
Using three different statistical models each with its own set of parameters, it was shown that tomato fruits of plants watered with Analemma Water exhibited significantly different DL profiles than fruits of plants watered with untreated water. Statistical differences were found in the majority of parameters of all three models (Table 1). For easier visualization of these differences, two parameters, namely DL_Mean and T, are shown individually in Figure 2. To conclude, **higher average amount of delayed luminescence indicates higher energy storage capacity of fruits produced by plants treated with Analemma Water**.

Additionally, the two water treatments resulted in several differences in biophoton emission (SE). One of the parameters shown to be statistically different between treatments (SE Strength) is shown in Figure 3. Larger spontaneous biophoton emission measured in fruits of plants treated with Analemma Water indicate **increased mitochondrial activity and slower biological aging**.

FOR DETAILS, SEE **APPENDIX F**

Table 1. Differences in DL parameters between tomato fruits of plants treated with Analemma Water and Control. Change values (t) are shown for all parameters of the 3 models. Significant differences are highlighted in green ($p < 0.05$).

	Parameter	t-value	p-value
MODEL 1	DL_Mean	-2.69389	0.007701
	DL_SD	-2.67917	0.008035
	I0	-2.70251	0.007512
	Tau	-0.05415	0.956876
	Beta	-2.41271	0.016795
	T	1.98655	0.048425
	R1	-2.72293	0.007080
MODEL 2	Y0	-3.02229	0.002858
	A1	-2.70618	0.007433
	12	-2.62002	0.009511
	t1	0.92529	0.356001
	t2	1.01676	0.310574
	R2	-2.35394	0.019608
MODEL 3	A	-2.47811	0.014090
	B	1.07146	0.285338
	C	2.55766	0.011327
	R3	-2.68746	0.007846

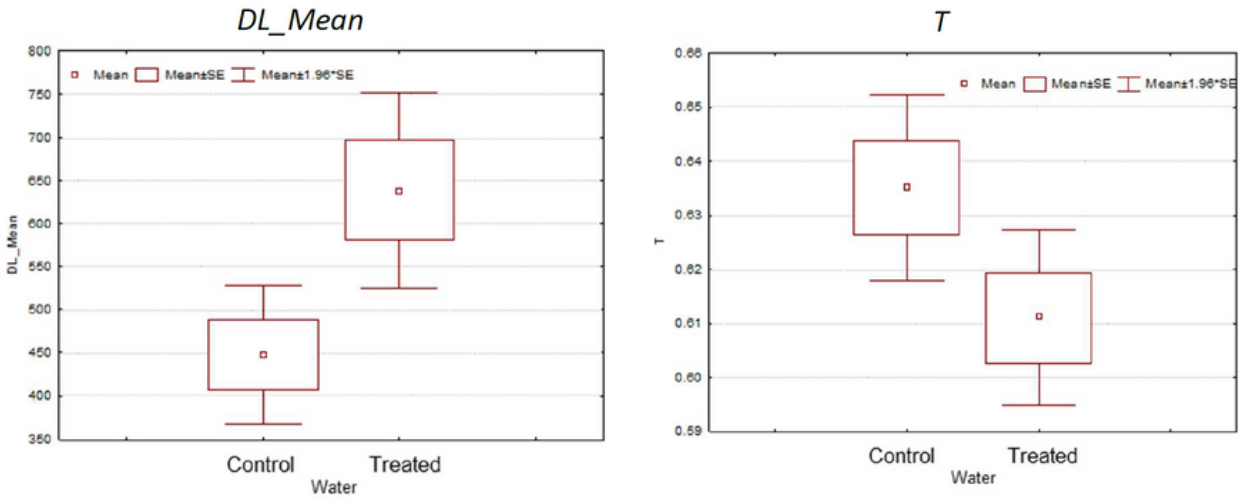


Figure 2. Means and variances for parameters DL_Mean and T of the hyperbolic decay model (Model 1) used for delayed luminescence (DL) analysis. The parameters show statistically significant differences in DL measured between tomato fruit samples of plants grown under two different watering treatments: non treated tap water (Control) and Analemma-treated tap water (Treated).

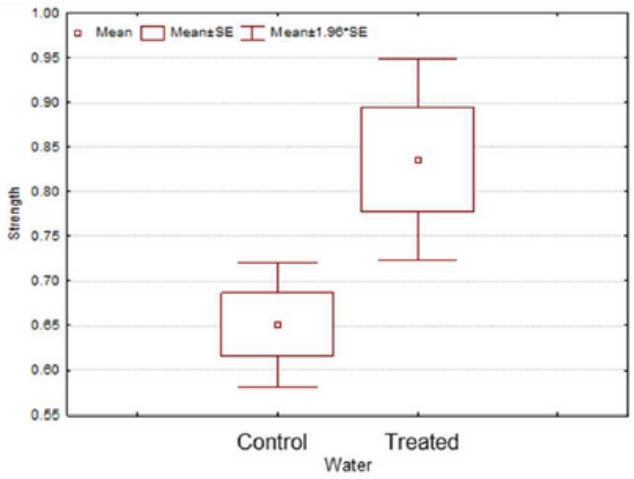


Figure 3. Means and variances for parameter Strength of the fractality model used for spontaneous emission (SE) analysis. This parameter shows statistically significant difference in SE intensity measured between tomato fruit samples of plants grown under two different watering treatments: non treated tap water (Control) and Analemma-treated tap water (Treated).

Biophoton emission of wheat seeds (2014)

EXPERIMENTAL DESIGN

Seed germination: Wheat seeds were placed in three square 12x12cm Petri dishes filled with 25 mL of either untreated tap water (Control), Analemma Water (Treated) or Test Water 2 (Treated2) and allowed to germinate.

Biophoton emission analysis: After two days of germination, wheat seedlings were transferred to 15 cuvettes (5 for each water type). One cuvette was left empty for background measurements. Delayed luminescence was measured using a photon counting system with a multiplier tube in the spectral range of 160-870 nm. In all experiments, samples were placed in random order in the carrousel. Delayed luminescence was measured during 2 days. In total, 9 experiments were performed from March to June 2014, using 25 seeds per experiment. DL50-100 values were measured for each sample and plotted on graphs. The DL50-100 value corresponds to the mean value between the 5th and 10th second after excitation.

RESULTS

In 7 out of 9 cases, the seedlings germinated in Analemma Water exhibited the highest average DL50-100 values (results of one exemplary experiment shown in **Figure 4**). This indicates that **seeds germinated in Analemma Water have the highest energy storage capacity**, which is directly linked to oxidative processes underlying these highly sensitive stages of early plant life.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

Biophoton emission of wheat seeds (2014)

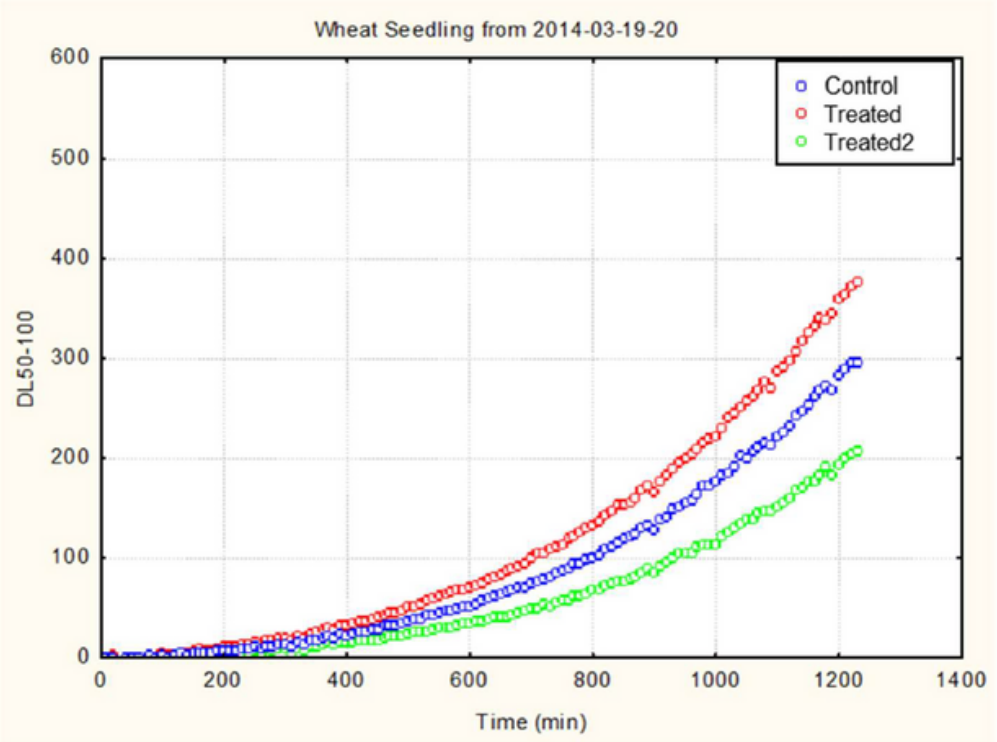


Figure 4. DL50-100 values of wheat seedlings germinated using untreated tap water (Control, blue circles), Analemma Water (Treated, red circles) or Test Water 2 (Treated2, green circles). Seeds treated with Analemma Water produced the highest DL50-100 values. This graph shows results of one representative experiment. A similar trend was observed in 7 out of 9 experiments performed during the course of the study.

Long-term biophoton emission of wheat (2014)

EXPERIMENTAL DESIGN

Plant treatment: Mature wheat seeds were harvested from adult plants grown in standard conditions. Seeds were distributed on soil and kept in standard growth conditions. Seeds were divided into two groups which differed only in the type of watering treatment. In the control group, seeds were watered with untreated tap water, and in the Analemma Water group, seeds were watered with Analemma-treated tap water. Germinating seeds and the resulting seedlings were grown and analyzed for biophoton emission.

Biophoton emission analysis: Ultra-weak photon (biophoton) emission was measured using low-noise photomultiplier tubes and highly sensitive charge coupled device cameras. Biophoton emission was measured at 10 different time-points over a period of 38 days. Both groups of seeds/growing plants were analyzed, using the software coupled to the measuring apparatus.

RESULTS

The spectral data obtained by biophoton emission analysis of control seeds and Analemma-treated seeds showed two distinct patterns (**Figure 5**). Control seeds did not exhibit a regular variation in biophoton emission relative to time of day (**Figure 5, red line**). On the other hand, Analemma-treated seeds showed a distinct semidiurnal pattern of biophoton emission (**Figure 5, blue line**). **These results indicate that treatment with Analemma water affects physiological processes which underlie biophoton emission during early plant growth (e.g. oxidative metabolic processes). Analemma renders these processes more periodic, following a distinct daily cycle, as evident by two peaks and then two drops in biophoton emission.** Although it is unclear how this affects the growing plant, the result strongly indicates that Analemma water affects oxidation in the plant, and that this happens with respect to the natural day/night cycle.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

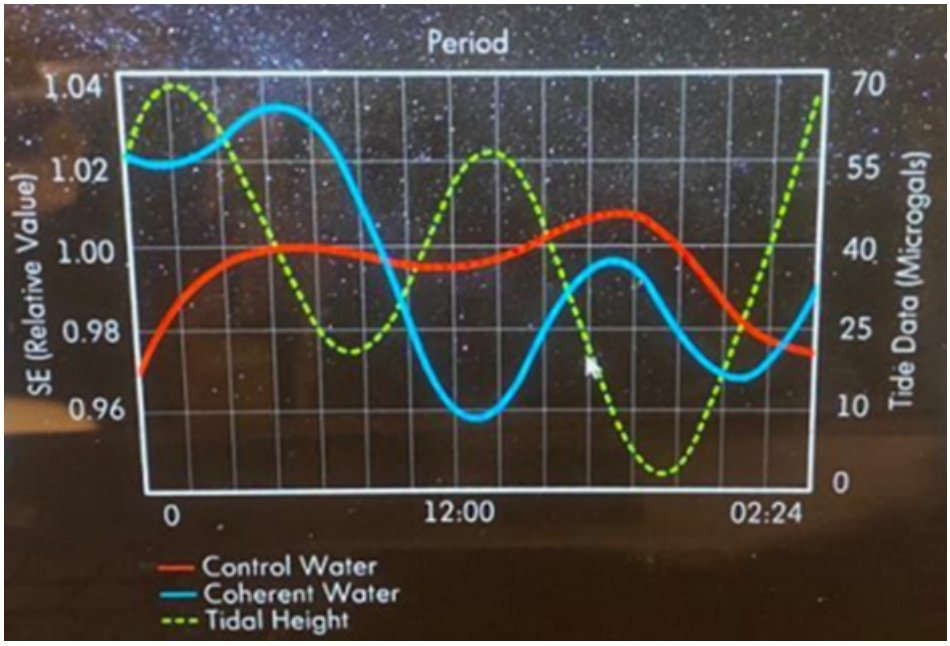


Figure 5. Biophoton emission of seeds treated with Analemma coherent water (blue line) vs control (red line). Analemma-treated seeds exhibit a semidiurnal pattern of biophoton emission synchronized with the Earth's tides (yellow dotted line).

When the researchers plotted the biophoton emission data against the Earth's tide data, it was evident that the two patterns were correlated (Figure 5, yellow dotted line), with both lines showing distinct bursts and drops relative to the daily cycle. Earth's tides are caused by the difference in gravitational forces from the Moon and the Sun on the different sides of Earth. The relative phase of the Sun and Moon cause an elastic deformation of the Earth, resulting in a diurnal and semi-diurnal periodic variation in the distance between the Earth's crust and center of mass. Corresponding to the variation in distance is the variation in the gravitational acceleration, which can be measured at any given location on Earth (referred to as tide data, expressed in microgals). **The apparent synchronized behavior of Analemma-treated plants with the Earth's tides indicates a strong connection between Analemma coherent water and the tidal forces of external planetary bodies in their natural cycles.** This correlation was, at the very least, striking, and had since then sparked numerous questions regarding the full potential of coherent water.

WHAT'S NEXT?

The results on biophoton emission were among the earliest findings on the effects of Analemma water on plants. The results of the long-term experiment performed on wheat are interesting with respects to the so-called circadian clock, an endogenous mechanism that plants use to adjust their growth and physiology to daily environmental cues, such as the daily rhythms of light and temperature. A healthy plant is a well-adjusted plant. The apparent periodicity in oxidative processes of young plants, together with a possible connection with the Earth's tidal forces, opens exciting questions for further research – **can coherent water help plants become more "in tune" with natural cycles, more primed to respond to changes in their environment? And since we as humans also possess our own intrinsic circadian clocks, can coherent water have a similar effect on us?**

The results obtained in these experiments, together with general insights from long-term usage of Analemma Water in greenhouse conditions, led to the conclusion that Analemma Water had an effect on plant physiology and overall health.

These findings have been of utmost importance in shaping our perspective and establishing current collaborations with laboratory groups researching plant physiology. **We are currently in the first stage of a research study** being performed in collaboration between the Faculty of Agronomy affiliated with University in Zagreb. This study will **explore the effects of Analemma Water on resistance of basil plants to conditions of drought via assessing multiple morphological, physiological and biochemical parameters**.

RESEARCH REPORT
JANUARY 2023

APPENDIX A

The study of ATP levels in humans (2022)

A1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study was performed in collaboration with Raptim Research Pvt. Ltd., (Navi Mumbai, India). The administrative structure of the study is described in **Table A1**.

Table A1. Administrative structure of the study

<table>
<tr><th colspan="2">Contract Research Organization</th></tr>
<tr><th>Name</th><th>Address</th></tr>
<tr><td>Clinical Investigator:
Dr. Yashvant Khaire, M.B.B.S., Diploma in Anesthesia</td><td rowspan="5">Raptim Research Pvt. Ltd.,
Clinical Pharmacology Unit (Clinic and Pathology): A-226;
Screening Facility (PAP-213, PAP-A-218 and PAP-A-219);
Bioanalytical and Biostatistical Unit: A-242;
T.T.C., Industrial Area, Mahape M.I.D.C., Navi Mumbai – 400 710, India.
Tel. No.: +912227781889: Ext. No. 112 (Clinic); 120 (Pathology); 159 (Bioanalytical and Statistical); Fax No.: +912227781884</td></tr>
<tr><td>Clinical Co-Investigator:
Dr. Nandkishor Gameti, M.B.B.S.
Dr. Raviraj Jagdhani, M.B.B.S. M.D. Pharmacology</td></tr>
<tr><td>Bioanalytical Investigator:
Dr. Milind Bagul, M. Pharm, Ph.D.</td></tr>
<tr><td>Biostatistician:
Mr. Veerababu Yegi, M.Sc. Statistics</td></tr>
<tr><td>Head of Quality Assurance:
Mrs. Usha Ramakrishnan, B. Pharm.</td></tr>
<tr><th colspan="2">Independent Ethics Committee</th></tr>
<tr><td colspan="2">HumanCare Independent Ethics Committee Shop. No. 16, Lodha Elite, Near Nilje Railway Station, Lodha Heaven, Dombivali (East), Thane 421204, India.
Tel. No.: +91 77383 60789
Email: humancareethics@gmail.com</td></tr>
<tr><th colspan="2">Sponsor's Representative</th></tr>
<tr><td colspan="2">Madhusudan Rajagopalan (Director)
Water and Light Applications India Private Limited 142, 6A, Kalpataru Estate. JV Link Road Andheri East Mumbai 400 093</td></tr>
<tr><th colspan="2">Clinical Pathology Laboratory</th></tr>
<tr><td colspan="2">In-house Clinical Pathology Laboratory,
Raptim Research Pvt. Ltd., A-226, T.T.C. Industrial Area, Mahape M.I.D.C., Navi Mumbai – 400710, India.
Tel. No.: +912227781889, Ext. no.: 120
Fax No.: +912227781884</td></tr>
</table>

A2. ETHICS

A2.1 INDEPENDENT ETHICS COMMITTEE (IEC)

The study protocol version 00, dated 07/07/22, Subject Information Sheet and Informed Consent Form (SIS-ICF) version 00 (dated 08/07/22; English, Hindi, and Marathi), Investigational Product (IP) Information and other protocol related documents were reviewed and approved by HumanCare Independent Ethics Committee in the meeting held on 17/07/22.
The study protocol amendment version 00, dated 18/07/22 to protocol version 00, dated 07/07/22, and other protocol related documents were reviewed and approved by HumanCare Independent Ethics Committee in the meeting held on 19/07/22.

A2.2 ETHICAL CONDUCT OF THE STUDY

The study was conducted in compliance and accordance with the ethical principles that have their origins in the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013)1, Good Clinical Practice (International Council for Harmonization – E6 (R2) Guidelines, Current Step 4 Version, dated 9 Nov 2016)2, New Drugs and Clinical Trials (Amendment) Rules, 2021 [Gazette notification G.S.R.227 (E), dated 19.03.2019 & G.S.R.605 (E), dated 31.08.2021] , Ministry of Health and Family Welfare, Government of India3, CDSCO Guidelines for Bioavailability & Bioequivalence Studies Mar 20054, Indian Council of Medical Research (Ethical Guidelines for Biomedical Research on Human Participants, 2017)5, and other applicable regulatory requirements. In order to achieve and comply with the ethical principles mentioned in above guidelines: clinical monitoring was performed; integrity of data was maintained during its generation and only quality assurance approved data were used for estimation of pharmacokinetic (PK) parameter and assessment of safety in the present study.

A2.3 SUBJECT INFORMATION AND CONSENT

All the subjects screened for the study received information both verbally and in written form in English language regarding the purpose and procedures involved in the screening. Screening procedures were performed only after obtaining screening consent from the subjects. At the time of enrollment in the study, the subjects were selected from those qualified during the screening process and received information both verbally and in written form in English language explaining the purpose and nature of the study and its procedures as well as potential risks and benefits (if any) associated with IPs as per the SIS-ICF. The subjects were provided enough time and opportunity to read the SIS-ICF. The subjects were encouraged to ask questions and clarify their doubts before signing the ICF in the presence of an investigator or qualified medical personnel of Raptim Research Pvt. Ltd., India prior to participation in the study. Subject's signature was obtained in the respective vernacular ICF. A copy of the signed and dated study specific SIS-ICF was provided to the individual subject.

A3. OVERALL STUDY DESIGN

This was a double blind, placebo controlled, randomized, parallel group clinical study conducted in 50 normal, healthy, adult, human subjects. Subjects were randomized into two treatment sequence groups. The study was conducted in following phases based on the activities performed.

A3.1 SCREENING PHASE

The following screening activities were preformed within 21 days prior to check-in: Registration/identification in the biometric system, obtaining written informed consent for screening, demographic parameters (gender, race, ethnicity, age, height, weight, body mass index), medical and medication history, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), well-being, 12-lead ECG recording, laboratory investigations (hematology, biochemistry, serology and urine analysis), urine pregnancy test, gynecological history (for female subjects) and evaluation of inclusion and exclusion criteria. Subjects were given awareness instructions for corona virus disease, consent was taken and assessment was performed for COVID-19.

A3.2 TREATMENT PHASE

The following activities were performed from the day of check-in until post-study safety assessments.

A3.2.1 Baseline Activities (Day -1)

One day prior to consumption of Analemma Water, Test Water 2 or Placebo drinking water multiple activities were performed, including biometric identification, obtaining study specific written informed consent, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), assessing general well-being since last visit and evaluation of inclusion and exclusion criteria.

Blood samples were obtained at Baseline (Day -1) for ATP level analysis.

Healthy subjects were randomized to receive either Analemma Water, Test Water 2 or Placebo for 90 consecutive days.

A3.2.2 Treatment

During the treatment phase (Day 1 - Day 90), subjects were instructed to consume a daily dose of at least 1.5 L of the water that was supplied to them according to the randomization schedule

A3.2.3 Compliance checks and safety assessments

Water consumption compliance and safety were assessed during Compliance Check Visits. The safety of subjects was assessed by monitoring for occurrence of any adverse effects as well as vital signs and general well-being during the in-house stay. Vital signs measurements (blood pressure, pulse rate, body temperature, and respiratory rate) and well-being were evaluated. All subjects visited the facility every 10 days up to day 80 for water consumption compliance check (i.e. on Day 10, Day 20, day 30, day 40, Day 50, Day 70, and Day 80; **Table A2**). Subjects were allowed to visit the facility ±2 days from above scheduled days. A post-study safety assessment was conducted at the end of the study (Day 91).

Table A2. Study activities organized by phase, day and visit number.

Phase	Day	Visit No.	Activity
Baseline Day	Day -01	01	Blood sample collection for ATP level analysis.
Compliance Check	Day 10	02	Water consumption compliance check
	Day 20	03	Water consumption compliance check
	Day 30	04	Water consumption compliance check
	Day 40	05	Water consumption compliance check
	Day 50	06	Water consumption compliance check
ATP levels of whole blood	Day 60	07	Blood sample collection for ATP levels of whole blood and Water consumption compliance check
Compliance Check	Day 70	08	Water consumption compliance check
	Day 80	09	Water consumption compliance check
Post Study Safety Assessment	Day 91	10	Post study safety assessment

A4. SELECTION OF STUDY POPULATION

The targeted study population was drawn from a local population database of Raptim Research Private Limited, Navi Mumbai, India. Clinical Investigator qualified only those volunteers for entry into the study who met the following inclusion criteria and none of the exclusion criteria at screening and during check-in procedures for each study period.

A4.1 INCLUSION CRITERIA

A subject who fulfilled the following criteria was included in the present study:

- Willing to provide written informed consent for participation in the study, and an ability to comprehend the nature and purpose of the study
- Willing to be available for the entire study period and to comply protocol requirements
- Normal, healthy, adult, human subject of 18-60 years (both inclusive) of age
- Body mass index in the range of 18.50 – 29.99 kg/m2 (both inclusive)
- Normal health status as determined by baseline medical and medication history, at the time of screening and vital signs measurements and physical examination at the time of screening as well as prior to baseline day visit
- Normal or clinically non-significant laboratory values as determined by hematological, biochemistry tests and urine analysis
- Normal or clinically non-significant 12-lead ECG recording
- Non-smokers
- Non-Alcoholic
- For female subjects
- Negative urine pregnancy test during screening visit

A4.2 EXCLUSION CRITERIA

A subject with the following criteria was excluded from the study:

- Any medical or surgical conditions, which might significantly interfere with the functioning of gastrointestinal tract and of blood forming organs
- Significant history or current evidence of malignancy or chronic - infectious, cardiovascular, renal, hepatic, ophthalmic, pulmonary, neurological, metabolic (endocrine), hematological, gastrointestinal, dermatological, immunological or psychiatric diseases, or organ dysfunction
- Any major illness or hospitalized within 90 days prior to the baseline day visit
- Requiring medication for any ailment having enzyme-modifying activity within one month prior to baseline day visit and throughout the study
- Use of any depot injection or an implant of any drug within 3 months prior to baseline day visit and throughout the study

- Use of any prescribed medication (including herbal medicines and vitamin supplements) or OTC products within 30 days prior to baseline day visit and throughout the study
- Vaccinated 7 days prior to baseline day visit and willing to get vaccinated throughout the study
- History or presence of significant gastric and/or duodenal ulceration
- Use of any recreational drug or history of drug addiction
- Participated in any clinical investigation requiring repeated blood sampling or have donated blood in past 90 days prior to baseline day visit
- Positive urine alcohol and urine drug of abuse tests during baseline day visit
- Reactive test for Human Immunodeficiency Virus (HIV) type I/II antibodies or Hepatitis B surface antigen (HBsAg) or Hepatitis C virus antibodies
- Lactating or nursing female subjects
- Female subjects using hormonal contraceptive (either oral/implants)
- History of difficulty in accessibility of veins in arms

A4.3 REMOVAL OF SUBJECTS FROM THE STUDY

Subjects were to be removed from the study or study evaluations for any of the following reasons:

- Withdrawal: Subject's decision to withdraw his/her voluntary participation, anytime during the study period
- Termination: The clinical investigator may terminate a subject from the study for any of the valid reasons, which is appropriate in view of the safety and well-being of subject, GCP principles or objectives of the study, in particular for but not limited to:
  - Any serious adverse event (SAE) during the study
  - Any illness requiring surgical procedures or administration of other medication(s) during the study, which could impact the PK profile of investigational product
  - Protocol violation or noncompliance to the study protocol by the subject
  - Further continuation in the study exposes the subject to potential AE that may prove harmful to the subject
  - Sponsor's decision to terminate the study based on safety issues related to the investigational product

Summary of demographic and baseline data for all the enrolled subjects in the study and subjects considered eligible for clinical assessment are presented in **Table A3**.

Table A3: Demographic and baseline data of all subjects enrolled in the study (n=50)

Parameter	Age (yrs)	Weight (kg)	Height (cm)	BMI (kg/m2)
Mean	30.22	67.34	163.84	25.03
SD	6.91	11.00	8.96	3.14
Median	28.50	67.90	164.05	25.20
Min	21.00	46.00	144.00	18.57
Max	51.00	92.00	180.00	29.81
% CV	22.87	16.33	5.47	12.56
Sex				
Male	36 (72%)			
Female	14 (28%)			
Race				
Asian	50 (100%)			

A5. RANDOMIZATION AND BLINDING

A5.1 SUBJECT RANDOMIZATION

As per the study design, (A and B) randomization schedule was generated by a Biostatistician at Raptim Research Pvt. Ltd., India by PROC PLAN procedure (such that the design being balanced over the period and sequence combination) using Statistical Analysis Software SAS® Version 9.4 (SAS Institute Inc., U.S.A.). Subjects were allocated sequential numbers starting from 01 on the day of check-in for period I and were assigned randomization sequence as per their number stated in randomization schedule. The numbers assigned to subjects were as follows:

Water-1 dosed Subjects: 2, 3, 6, 7, 9, 11, 13, 16, 18, 19, 21, 23 and 25.

Water-2 dosed Subjects: 28, 29, 31, 34, 36, 38, 40, 42, 44, 46, 48 and 49.

Placebo dosed Subjects: 1, 4, 5, 8, 10, 12, 14, 15, 17, 20, 22, 24, 26, 27, 30, 32, 33, 35, 37, 39, 41, 43, 45, 47 and 50.

A5.2 BLINDING

The present study was designed as a blinded. The study subjects, the Clinical Investigator, the study staff involved in study activities, bio-statistician, bio-analyst and the sponsor were blinded for the treatment administered to subject. Only Pharmacist, the assistant pharmacist who are responsible for dispensing of investigational products and the QA auditor monitoring the dispensing activity had access to the randomization schedule and they were not be involved in any other study related activities until completion of the analysis.
The pharmacist assigned the treatment code (which is an alphabetic code i.e. X or Y or any other notation) for the randomization notation (i.e. if A/B, T/R or any other notation) given in the Randomization schedule generated by the Bio-statistician for test or placebo treatment. The treatment code assigned for test and placebo was recorded in the 'Assignment of treatment code for blinded study design' format which was kept in a sealed envelope in the pharmacy. The photocopy of this format was provided to biostatistician after completion of clinical and after completion of ATP level analysis of the study for statistical analysis.
For code breaking procedure (in case of occurrence of any SAE or in any emergency condition citing safety concern of the subject) individual envelope containing the label with the details of subject number and the randomization sequence of the subject was prepared by the Pharmacist prior to baseline day visit. These labels were verified with the randomization schedule by the assistant pharmacist and the QA auditor. The sealed envelopes with subject number written on were kept in a secure place in Pharmacy or with Clinical Investigator.

A6. INVESTIGATIONAL PRODUCTS

Investigational products (IPs) were supplied by the Sponsor and received by the pharmacist at Raptim Research Pvt. Ltd., India. IPs received were verified intact condition of packaging. The identities of the IPs are provided in **Table A4**. On study completion, the quantity of investigational product received (test product and reference product) from the Sponsor, dispensed, undispensed quantity, and unused dispensed quantity were reconciled and retained as per in-house SOP for the estimation of the balance quantity of IP.

All the unused IPs were returned to the pharmacy and stored in the same formulation cabinet with that of the undispensed IPs.

A6.1 PREPARATION OF COHERENT WATER

The individual who prepared the coherent water was instructed to use the Water Tube exclusively to produce coherent drinking water for human consumption: water of good quality that has already been filtered and purified and that is free from chemical and/or biological pollution.

Table A4. Investigational product details.

Parameter	Test product (A)	Reference product (B)
Product name	Analemma Water, Test Water 2	Placebo
Manufacturer	Water and Light Applications India Private Limited	Water and Light Applications India Private Limited

A7. TREATMENTS

A7.1 TREATMENT ADMINISTRATION

From Day 1 to Day 90, sufficient volumes of Analemma Water, Test Water 2 or Placebo drinking water were provided to all subjects according to the randomization schedule. Subjects were instructed to consume a minimum of 1.5 L of water daily. Subjects were instructed to record the details (quantity, date and time) of water consumption in the subject diary from day 1 to day 90.Details of water treatment administration are provided in **Table A5**.

Table A5. Treatment administration details.

Parameter	Test Product (A)	Placebo (B)
Dosage form	Water (Analemma Water and Test Water 2)	Water
Route	Oral	Oral
Dose and Mode of administration	Analemma Water or Test Water 2 was provided to subjects, with instruction to consume a minimum of 1.5 L of water daily from day 1 to day 90 at ambient temperature	Placebo drinking water was provided to subjects, with instruction to consume a minimum of 1.5 L of water daily from day 1 to day 90 at ambient temperature
Baseline Day Date (Day 01, Group 1)	22/07/22	
Treatment Start Date (Day 01, Group 2)	24/07/22	
Post Study Date (Day 91, Group 1)	20/10/22	
Post Study Date (Day 91, Group 2)	22/10/22	

A7.1.1 Restrictions of fluid and other substances

Consumption of normal drinking water (i.e. water not supplied through the study) was restricted during the duration of the study. Subjects abstained from smoking or chewing tobacco products, alcohol or alcoholic products, xanthine or its derivative containing food or beverages and grapefruit or its juice.

APPENDIX A

A8. SAMPLING, MEASUREMENTS AND ANALYSIS

A8.1 BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples (15.0 mL) were collected during Baseline day -1 (Visit 01) and during post study safety assessment on Day 60 (Visit 7). Blood samples were collected in pre-labeled vacutainers containing K3EDTA as an anticoagulant. A total of two blood samples were collected for ATP level analysis. After sample collection, vacutainers were stored in the refrigerator (at 2˚C to 8˚C). After collection of samples from all subjects, samples were shipped at 2˚C to 8˚C to the bioanalytical facility of Raptim Research Pvt. Ltd. (A-242).

A8.1.1 Blood loss

Total blood loss for a subject during the study did not exceed 55.0 mL (for male subjects) or 57.0 mL (for female subjects).

A8.2 BIOANALYSIS

A8.2.1 ATP measurement

Blood ATP levels were measured by the firefly bioluminescence assay kit (AMERIC-ATP kit; Wako Pure Chemical Industries, Osaka, Japan) according to the protocol supplied by the manufacturer. ATP levels were measured from whole blood samples of all participants obtained on Day -1 (baseline) and on Day 60 (after water consumption).

A8.3 STATISTICAL ANALYSIS

Statistical analysis was performed using SAS® Version 9.4 or higher (SAS Institute Inc., USA). The individual and descriptive summary statistics was performed for ATP levels of whole blood before consumption of water (Day -1) and after consumption of water (Day 60). Measurements obtained on Day 60 were compared with Baseline measurements (Day -1) for each participant and the percentage of change was calculated (**Table A6**). A one-way analysis of variance (ANOVA) was performed on ATP levels data to test significance of change values (before and after consumption of water) between Analemma Water group and Placebo group (**Table A7**). Mean change values in ATP levels calculated for Test Water 2 group are presented in **Table A8**. Analysis of variance was not performed for the Test Water 2 group relative to Placebo.

A8.4 RESULTS

The mean change in ATP levels calculated for the Analemma Water group was 26.8414705 ± 24.9047948 (mean ± standard deviation). The mean change in ATP levels calculated for the Placebo group was 7.3233438 ± 26.6464522 (mean ± standard deviation). According to ANOVA, the change in ATP levels measured before and after consumption of water was significantly higher in the group consuming Analemma Water (Water-1), than Placebo, with the probability value of 0.0352 (**Table A7**).

APPENDIX A

Table A6. ATP levels were measured before (Day -1) and after consumption of water (Day 60). Mean change in ATP levels was calculated for Analemma Water group (n=13) and Placebo group (n=25). Mean values and standard deviations (SD) are listed.

Group	Analemma Water (n=13)	Placebo (n=25)
Change in ATP level (mean ± SD)	26.8414705 ± 24.9047948	7.3233438 ± 26.6464522

Table A7. Analysis of variance (ANOVA) performed for values of change in ATP levels (before and after consumption of water) between Analemma Water group and Placebo group. The main ANOVA parameters (sum of squares, mean square, F-value and p-value) are presented.

Sum of Squares	**Mean square**	**F Value**	**p-value**	**Result**
3258.187159	3258.187159	4.79	0.0352	Significant

f P ≤ 0.05, the result is Significant;
If P > 0.05, the result is Non-Significant.

Table A8. ATP levels were measured before (Day -1) and after consumption of water (Day 60). Mean change in ATP levels was calculated for Test Water 2 group (n=12) and Placebo group (n=25). Mean values and standard deviations (SD) are listed.

Group	Test Water 2 (n=12)	Placebo (n=25)
Change in ATP level (mean ± SD)	5.87461351 ± 19.0046343	7.3233438 ± 26.6464522

A9. POST-STUDY SAFETY ASSESSMENT

Post-study safety evaluations were performed on day 91. During post-study safety assessments, physical examination, vital signs measurements (blood pressure, pulse rate, body temperature and respiratory rate), well-being, and blood sample collection for laboratory analysis [hematology and biochemistry (Serum creatinine, SGOT/AST, SGPT /ALT, serum bilirubin–Total, serum blood urea nitrogen) were performed. The schedule of study events (**Table A9**) and a list of laboratory tests performed (**Table A10**) as part of the screening and post-study safety examinations are presented below.

Table A9. The complete schedule of study events

Procedure	Screening (within 21 days prior to baseline day -1)	Treatment period		Post Study (Day 91) of a subject
		Baseline Day (Day -1)	Compliance Check Day 10, Day 20, Day 30, Day 40, Day 50, Day 60, Day 70, and Day 80	
Screening consent form	X			
Study specific informed consent		X[#]		
Demographics	X			
Medical and medication History	X	X		
Physical examination	X	X		
Vital signs	X	X	X	
Well-being		X	X	
Hematology	X			X
Biochemistry	X			X
Serology	X			
Urine analysis (Routine/Microscopic)	X			
Urine pregnancy test (for female subject)	X			
12 Lead ECG recording	X			
Applicable Inclusion-Exclusion criteria check	X	X		
Compliance Check		X[$$]	X	
Urine screen for drugs of abuse		X		
Urine alcohol test		X		
Water Consumption[%]			X	
Blood samples		X*	X*	X
Safety monitoring		X	X	X

[#]Only on baseline day -1
* Blood samples were collected for ATP levels of whole blood (Day -1 and Day 60)
[$$]Compliance check was performed on Day 10, Day 20, Day 30, Day 40, Day 50, Day 60, Day 70, and Day 80
[%] From Day 1 to Day 90.

APPENDIX A

Table A10. Laboratory tests performed during the study

Hematology	Biochemistry	#Urine analysis	
		Routine	Microscopy
• Erythrocyte Count • Hemoglobin • WBC Count • Platelet Count • Neutrophils • Eosinophils • Lymphocytes • Basophils • Monocytes	• Serum Alkaline Phosphatase • Serum Creatinine • Serum SGOT/AST • Serum SGPT/ALT • Serum Uric Acid • Serum Blood Urea Nitrogen • Plasma Glucose (Random) • Serum Bilirubin - Total	• Color • Appearance • Reaction pH • Protein (Albumin) • Ketone bodies • Sugar (Glucose) • Occult Blood • Urobilinogen	• Red Blood Cells • Pus Cells • Epithelial Cells • Casts • Crystals • Bacteria
#Serology		*Urine Examination For Drug Abuse	
• HIV antibodies (I &II) • HBsAg • HCV		• Benzodiazepines (BZD) • Barbiturates (BAR) • Tetrahydrocannabinol (THC)	• Opiate (OPI) • Cocaine (COC) • Amphetamine (AMP)

*Activities at baseline (Day -1).
#At screening only

A9.1 SAFETY ASSESSMENT CRITERIA

Safety and tolerability was assessed in terms of adverse events (AEs), serious adverse event (SAE) if any, or any illness requiring administration of other medication(s) during the study, vital signs and laboratory assessments were performed during the entire course of the study. Adverse events were evaluated based on frequency, severity grades, causality and outcome.

A9.2 SAFETY ASSESSMENT RESULTS

- No adverse events were reported during study periods.
- No adverse events were reported during post-study safety assessments.
- There were no SAEs reported during the study.

A10. DATASETS

- Bioanalysis dataset: Samples of all 50 subjects were analyzed for ATP levels.
- Overall statistics dataset: As per the study protocol, data (ATP levels) of 50 subjects completing study period was considered for statistical analysis.
- Assessment dataset: Data of 38 subjects for Water-1 (13 subjects) and Placebo (25 subjects) were considered for statistical assessment. However, data of 37 subjects for Water-2 (12 subjects) and Placebo (25 subjects) were evaluated and reported as additional information.
- Safety dataset: Subjects (N=25 for test product and N=25 for placebo) who received at least one dose of either of the IPs were evaluated for safety.

A11. DATA QUALITY ASSURANCE

The clinical Investigator/designee ensured that the data entered in the CRFs were as per the current in-house SOPs and in compliance with the study protocol. Quality control personnel checked 100% data of all CRFs as well as pre-study and post-study documents for correctness, completeness, and legibility of the entries. During the study, the Quality Assurance personnel performed the quality audits of involved departments (clinical, pathology, bioanalytical, and biostatistics) and confirmed that the study conduct, bioanalysis, procedures, and the documentation were performed in compliance with the ICH-GCP guidelines, study protocol and the respective in-house SOPs for each activity.

A12. REFERENCES

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab. 21(10): 1133-1145.

Barclay CJ (2015) Energetics of contraction. Compr Physiol. 5(2): 961-995.

Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Published: November 27, 2017, U.S. Department of Health And Human Services;

Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, World Medical Association – General Assembly, Fortaleza, Brazil, October 2013.

Good Clinical Practice (International Council for Harmonisation – E6 (R2) Guidelines, Current Step 4 version dated 9 November 2016.

Guidelines for Bioavailability and Bioequivalence Studies, CDSCO, Central Drug Standard Control Organization, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India, New Delhi, March 2005.

Ethical Guidelines for Biomedical Research on Human Participants, Indian Council of Medical Research, 2017.

New Drugs and Clinical Trials (Amendment) Rules, 2021 [Gazette notification G.S.R.227 (E), dated 19.03.2019 & G.S.R.605 (E), dated 31.08.2021], Ministry of Health and Family Welfare, Government of India.

Structure and Content of Clinical Study Reports (International Conference on Harmonization – E3 Current step 4 version – 1995.

APPENDIX B

The human microbiome study (2022)

Data will be available by February 15th, 2023.

APPENDIX C

GlycanAge study (2021)

C1. ADDITIONAL LITERATURE BACKGROUND

Glycans are sugar molecules that surround and modify proteins in the human body. The glycome has also been shown to have a contribution as a major regulator of the immune system: it regulates key pathophysiological steps within T cell biology such as T cell development and thymocyte selection, T cell activity and signalling as well as T cell differentiation and proliferation. In this context, glycans are critical determinants in autoreactive responses both by directly regulating T cell activity and through the creation of abnormal glycoantigens that can activate an autoreactive immune response. Particularly, the dysregulation of the N-glycosylation pathway has been associated with autoimmune-like phenotypes. Evidence addressing the relationship between the dysregulation of N-glycosylation and human autoimmunity was observed in multiple sclerosis (MS) patients.

The glycoprotein immunoglobulin G (IgG) is a major effector molecule of the human humoral immune response. Antibodies of the IgG class express their predominant activity during a secondary antibody response. In the serum more than 30 different IgG glycoforms can be identified, and the pattern of IgG glycosylation promote binding of IgG to different receptors, in this way modulating action of the immune system by changing the IgG function from pro- to anti-inflammatory and vice versa. The type of glycans attached to IgG are well researched and its glycosylation has been well defined.

Galactosylation, addition of a galactose molecule to a glycan, strongly decreases the proinflammatory function of IgG. A decrease in IgG galactosylation is seen in aging and triggered by factors like excessive or unsuitable diet or fitness regimen, hormonal changes, ethnic background, and toxic environmental factors. This causes chronic, sterile, low-grade inflammations in the body and contributes to the pathogenesis of age-related diseases (process called inflammaging). Considering the important role of IgG glycans in inflammation and because the increase in age promotes inflammation, changes in IgG glycosylation seem to represent a factor contributing to aging.

C2. METHOD

The GlycanAge test consists of three different components: 1. The ‘glycan mature index’, which is determined by the appearance of G0 glycans on IgG, 2. ‘Glycan health’, which is determined by the appearance of G2 glycans on IgG and 3. ‘Glycan youth’, which is determined by the appearance of S glycans on IgG.

APPENDIX C

G0 glycans (glycans without galactose) are known to promote inflammation (so all diseases that have an inflammatory background – this is actually way more diseases than we may think). These glycans do that by activating the complement system through the lectin pathway, thus low levels of G0 are generally considered to be beneficial for general health. On average, the older a person is, the lower level of G2 and the higher level of G0 so the lower result you have - the better.

In a way, the opposite of G0. G2 glycans acts anti-inflammatory, i.e. protective vs inflammation. As we age, our levels of G2 decrease. Decreased levels of G2 are associated with different autoimmune and inflammatory diseases, thus high levels of G2 are considered to be biomarkers of youthfulness. Also, before menopause women tend to have higher levels of G2, thus in a way being protected from inflammation. During menopause there is usually a sharp drop in G2, meaning that their body "ages" significantly during this period and is less protected against inflammation after menopause.

Youth glycans or S glycans are the most immunosuppressive members of the IgG glycome and are believed to be essential for the anti-inflammatory activity of intravenous immunoglobulin preparations. High levels of S glycans are associated with the absence of different autoimmune and inflammatory diseases and are generally considered to be biomarkers of good health. It is thought that some drugs that are actually pooled immunoglobulins from many donors owe their anti-inflammatory activity to this glycan component.

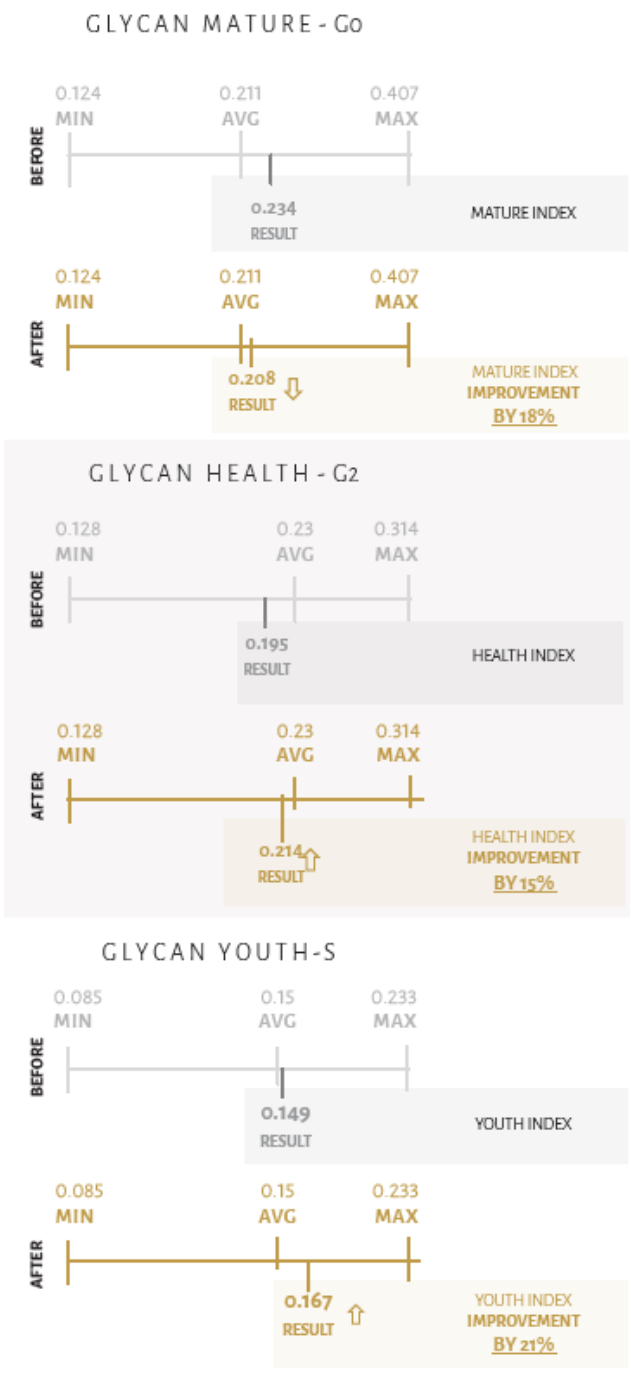


Figure C1. Example of changes in different glycan indices, measured in a person whose biological age reversal value was -12 years after three months of Analemma Water consumption.

APPENDIX C

C3. REFERENCES

Pereira MS, Alves I, Vicente M, Campar A (2018) Glycans as Key Checkpoints of T Cell Activity and Function. Frontiers in Immunology 9: 2754.

Pu M, Novokmet M, Mangino M, Thaqi K, Rudan P, Novokmet N, et al. (2014) Glycans Are a Novel Biomarker of Chronological and Biological Ages. J Gerontol A Biol Sci Med Sci 69(7): 779–89.

Franceschi, C., Garagnani, P., Parini P et al. (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14: 576–590.

APPENDIX D

Preliminary research on brainwaves (2019)

D1. GENERAL EFFECTS ON SPECTRA AND COHERENCE

D1.1. PARTICIPANTS

Age, gender and received treatment of the participants are summarized in **Table D1**.

Table D1. Study participants, with listed initials, sex, age and type of treatment received.

INITIALS	GENDER	AGE	TREATMENT
JC	F	68	ANALEMMA
IB	F	60	ANALEMMA
NH	F	27	PLACEBO
IF	F	64	PLACEBO
JK	M	71	ANALEMMA
BS	F	73	PLACEBO

D1.2. qEEG MEASUREMENTS

The measurements consisted of 3 full consecutive qEEG measurements conducted with 10 minute intervals:
1. qEEG base measurement (M 3)
2. qEEG measurement after 2 minute cell phone call (M 4)
3. qEEG measurement 10 minutes after drinking the designated water (M5)

All data are interpreted with reference to Z-scores, i.e. deviations from the expected value for healthy individuals of a certain age group; the reference values used are from the internationally accepted Thatcher database for qEEG.

APPENDIX D

For each condition 5 minute measurements were taken at a sampling rate of 256 samples per second. 20% of the data were used after artefact removal, thus rendering 1 minute averages per condition (based on about 15.000 samples per location).

D1.3. DATA EVALUATION

In order to simplify the overall evaluation of so many data originating from qEEG measurements, a value between 0 and 5 was allocated to the following 32 aspects, indicating how much they differed (upon visual inspection) from the qEEG with which they were being compared :

Spectra (EC, EO,EOR,EOM) (max total value 20)
Frequency bands x Coherence (EC, EO,EOR,EOM) (max total value 140)

Delta (1-3.5Hz) x Coherence (EC, EO,EOR,EOM)
Theta (4-7.5 Hz) x Coherence (EC, EO,EOR,EOM)
Alpha (8-12 Hz) x Coherence (EC, EO,EOR,EOM)
Beta 1 (12-15 Hz) x Coherence (EC, EO,EOR,EOM)
Beta2 I (15- 17.5 Hz) x Coherence (EC, EO,EOR,EOM)
Beta2 II (18-25 Hz) x Coherence (EC, EO,EOR,EOM)
Beta 3 (25.5-30 Hz) x Coherence (EC, EO,EOR,EOM)

Each aspect was evaluated for the four different conditions (eyes closed EC, eyes open EO, reading EOR, and watching a movie EOM). Evaluation was not conducted in the sense of positive or negative tendency, as change in itself was considered to be the important aspect. The reason was that on the long run positive tendencies are usually precursed by disintegrating dysfunctional circuits before more functional circuits are formed, so “chaos” may temporarily seem to occur.
Therefore, when total values are presented in a graph, these values are derived from adding the allocated change values (between 0 and 5).

APPENDIX D

D2. EFFECTS ON DELTA BRAINWAVES

To further assess the effect of Analemma Water on delta brainwaves, additional spectral graphs were obtained for a specific centrally located brain location (Pz), which is not very susceptible to artefacts. The results are shown in **Figures D1** and **D2**. Similar tendencies have also been found for other brain locations (not shown).

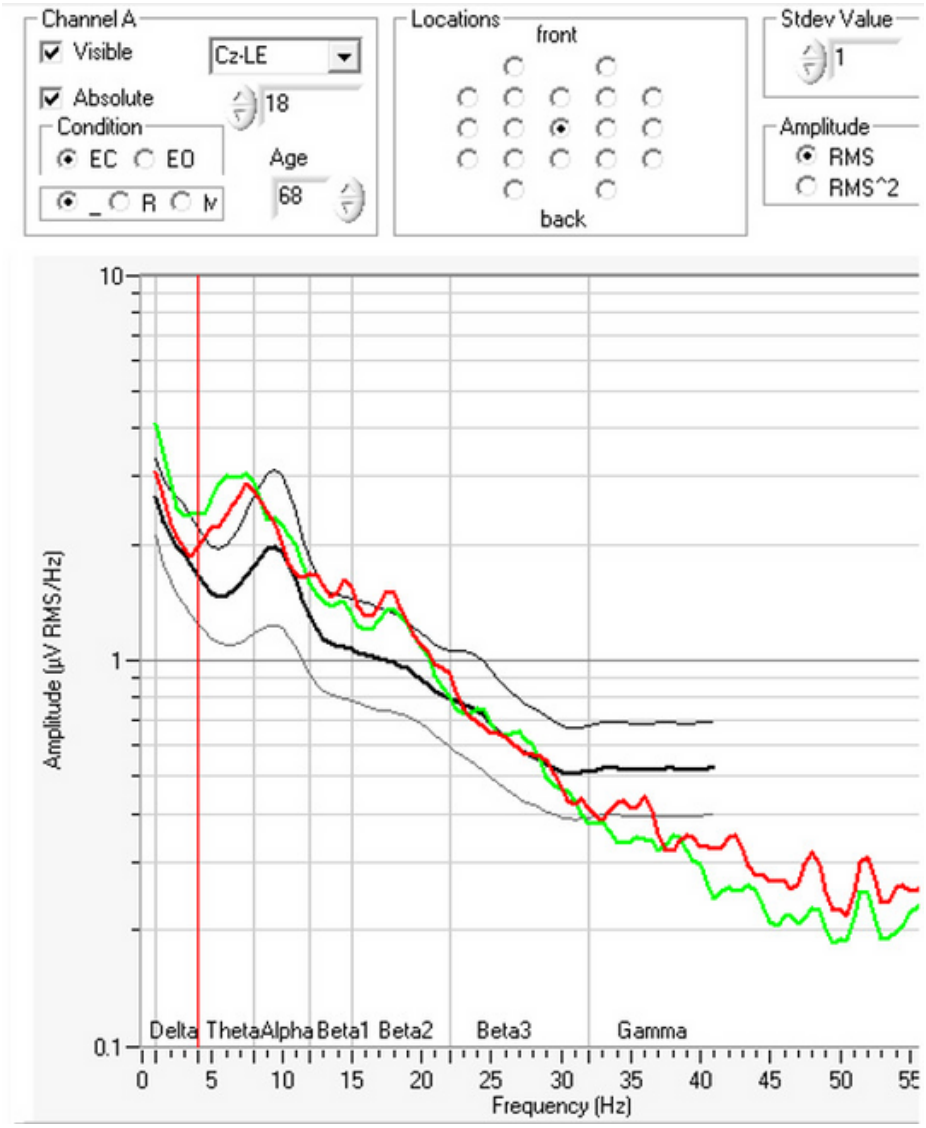


Figure D1. EC Spectrum of location Pz : baseline measurement (green line) and measurement obtained immediately after consuming Analemma Water (red line). Black lines depict the reference value for healthy individuals (+- 1 std dev.)

APPENDIX D

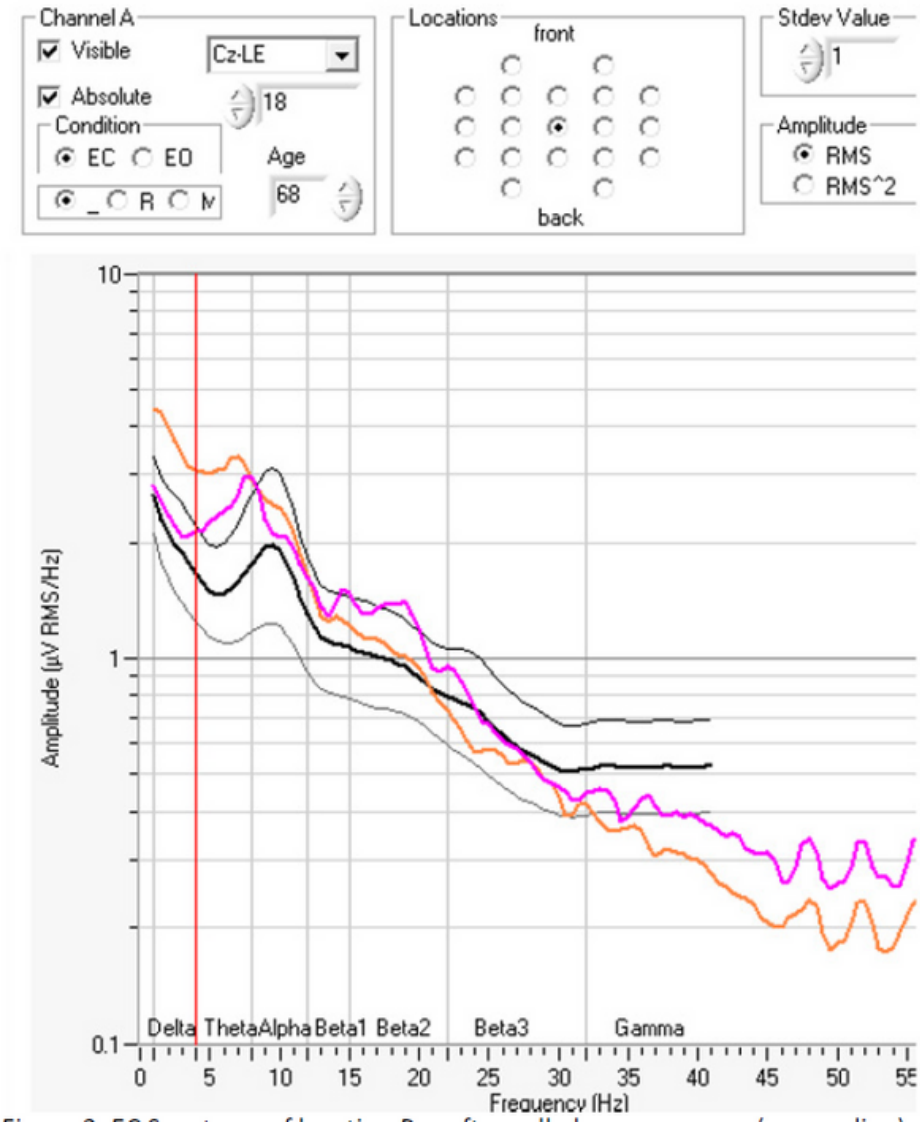


Figure D2. EC Spectrum of location Pz: measurement after cell phone exposure (orange line) and measurement obtained immediately after consuming Analemma Water (pink line).. Black lines depict the reference value for healthy individuals (+- 1 std dev.).

APPENDIX D

D3. REFERENCES

Bennys K, Rondouin G, Vergnes C, Touchon J (2001) Diagnostic value of quantitative EEG in Alzheimer's disease. Neurophysiol Clin 31(3): 153–60.

Lustenberger C, Murbach M, Dürr R, Schmid MR, Kuster N, Achermann P, Huber R (2013) Stimulation of the brain with radiofrequency electromagnetic field pulses affects sleep-dependent performance improvement. Brain Stimul 6(5): 805-11.

Perentos N, Croft RJ, McKenzie RJ, Cosic I (2013) The alpha band of the resting electroencephalogram under pulsed and continuous radio frequency exposures. IEEE Trans Biomed Eng 60: 1702–1720.

Sankari Z, Adeli H, Adeli A (2011) Intrahemispheric, interhemispheric, and distal EEG coherence in Alzheimer's disease. Clin Neurophysiol 122(5): 897-906.

APPENDIX E

E1. PLANT GROWTH CONDITIONS AND TREATMENT

From Day 1 to Day 15, plants were kept in an isolated part of a climate-controlled greenhouse in similar conditions (**Figure E1**). From Day 15 until the end of the study, plants were distributed according to their designated groups to receive the specified water treatment using an automated dosing system, The exact distribution is shown in the schematic representation in **Figure E2**.

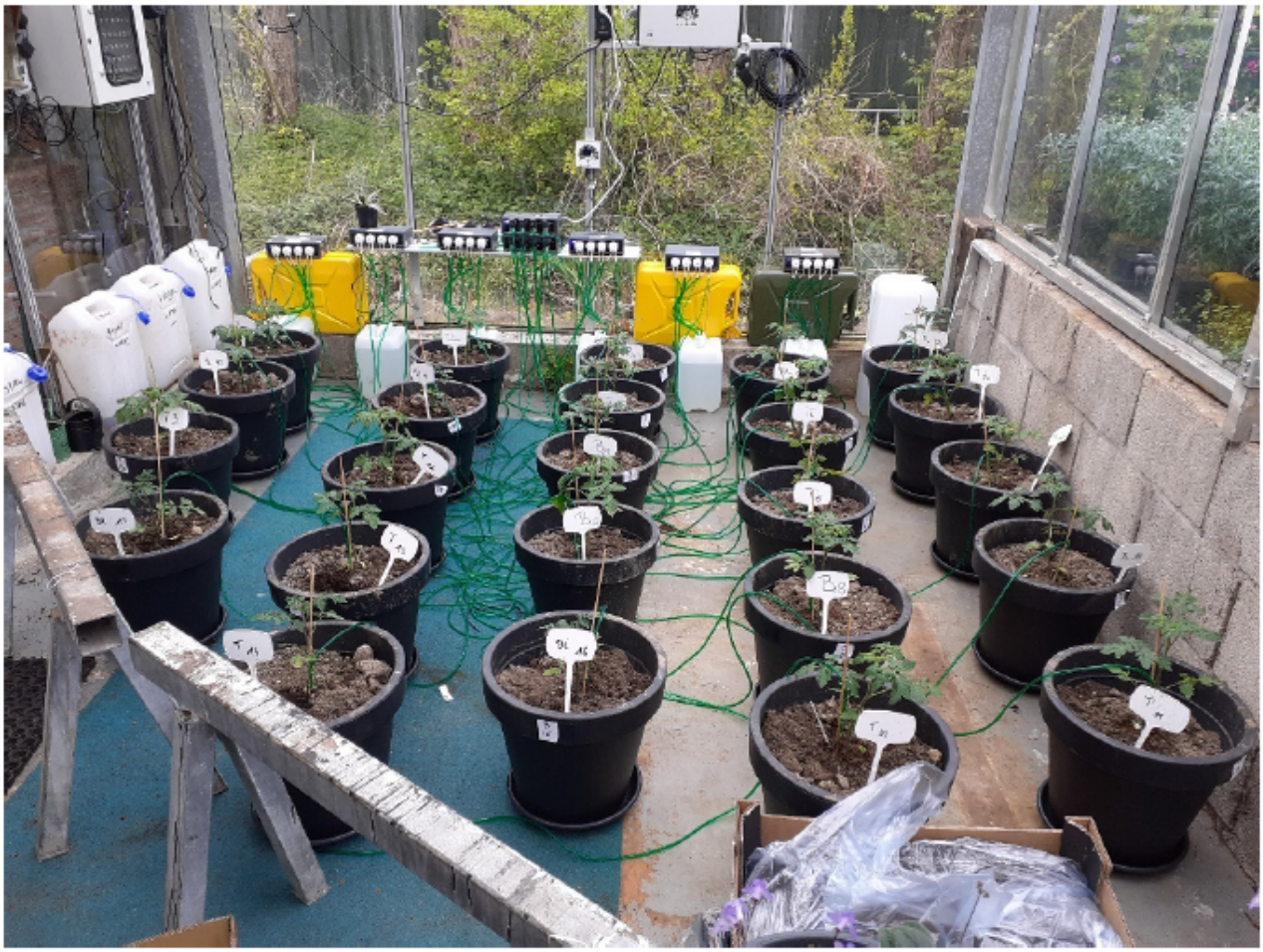


Figure E1. Plant growth setup from Day 1 to Day 15. All plants were grouped together in a climate-controlled greenhouse and watered with Rainwater using an automated dosing system.

APPENDIX E

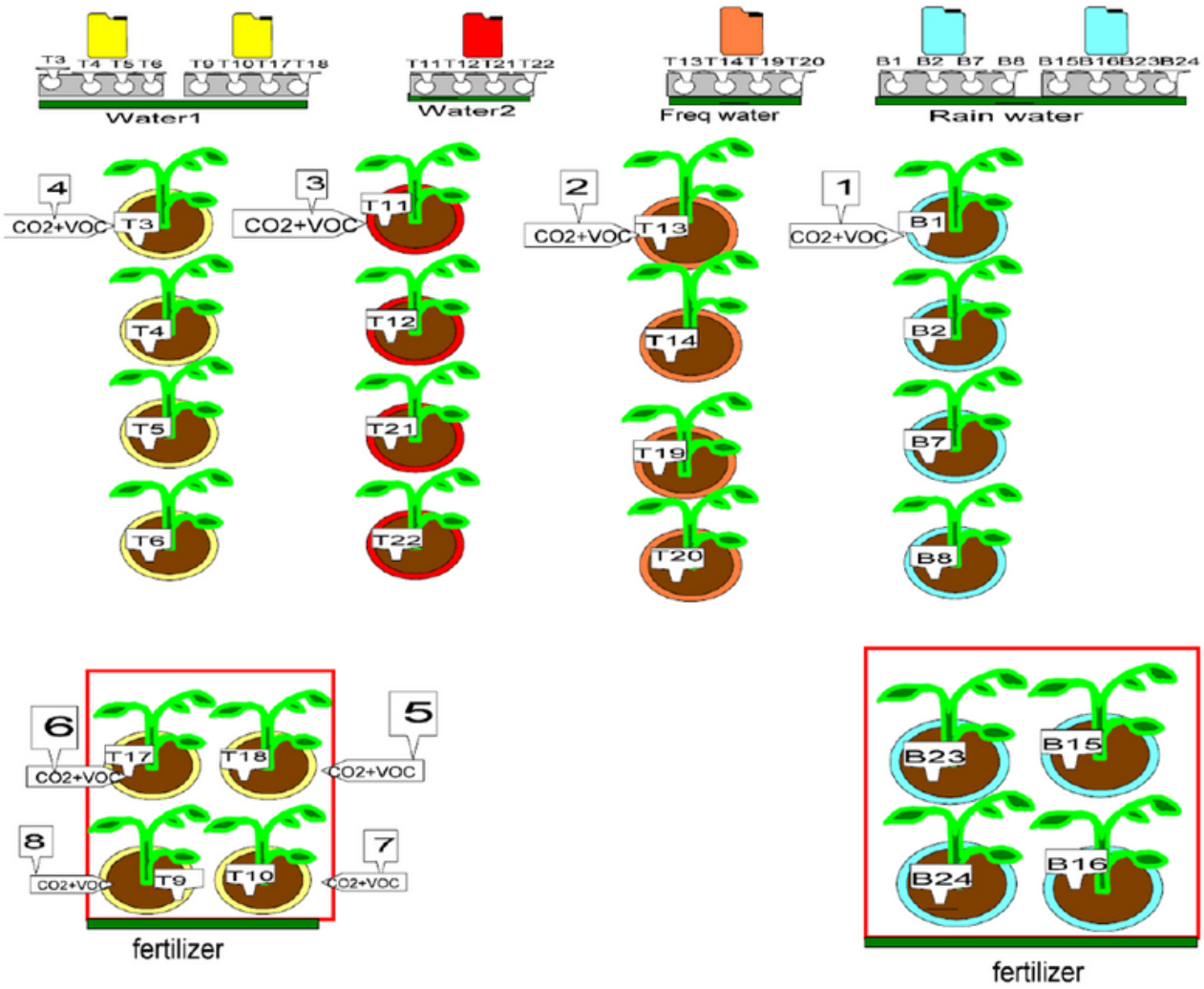


Figure E2. A schematic representation of the plant growth setup from Day 15 to the end of the study Plants were grouped according to their designated watering treatment in a climate-controlled greenhouse. Plants were watered with the specified water type using an automated dosing system.

APPENDIX E

E2. CHEMICAL COMPOSITION OF SOIL

Mean values of all 26 parameters measured in the chemical composition analysis of soils treated with different types of water are listed in **Table E1**.

Table E1. Overview of the chemical analysis showing the mean values per watering condition.

Parameter in dry soil	*Unit for condition*	*Base line measure ment*	*Water I*	*Water II*	*Frequency water*	*Rain water*	*Water I + organic fertilizer*	*Water I + organic fertilizer*	*Rain water + organic fertilizer*
Organic carbon	% C	*2,1*	*2,15*	*2,23*	*2,23*	*2,15*	*2,60*	*2,50*	*2,55*
C / N ratio	-	*9,5*	*9,68*	*11,65*	*15,45*	*13,88*	*11,95*	*11,65*	*15,63*
Phosphorus, P-PAE Q	mg P/kg	*11,9*	*11,18*	*10,20*	*10,65*	*11,63*	*10,30*	*11,80*	*9,85*
Boron	mg B/kg	*2,88*	*2,43*	*2,42*	*2,30*	*2,58*	*2,25*	*2,60*	*2,58*
Copper	mg Cu/kg	*6,8*	*5,03*	*4,40*	*4,40*	*5,18*	*4,80*	*5,80*	*4,88*
Zinc	mg Zn/kg	*<0,15*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Iron	mg Fe/kg	*2*	*3*	*2*	*3*	*2*	*3*	*4*	*2*
Molybdenum	mg Mo/kg	*0,11*	*0,14*	*0,12*	*0,13*	*0,15*	*0,14*	*0,15*	*0,13*
Acidity, pH	-	*7,2*	*7,24*	*7,20*	*7,21*	*7,20*	*7,17*	*7,20*	*7,20*
Organic matter	%	*3,7*	*3,70*	*3,83*	*3,83*	*3,73*	*4,45*	*4,30*	*4,40*
Carbonated lime	%	*7,4*	*7,57*	*7,78*	*7,53*	*8,08*	*7,15*	*7,75*	*7,53*
Nitrogen delivery capacity	kg N/ha per year	*158*	*109*	*101*	*82*	*86*	*108*	*107*	*89*
Phosphate, Pw Q d	mg P_2O_5/L	*99*	*95*	*84*	*88*	*82*	*85*	*90*	*87*
Phosphate, P-AL Q d	mg P_2O_5 /100 g	*134*	*145*	*136*	*143*	*134*	*141*	*141*	*141*
Potassium, K-HCl	mg K_2O /100 g	*115*	*98,35*	*92,43*	*87,75*	*101,13*	*110,85*	*116,15*	*104,03*
K-number	-	*110*	*91*	*87*	*82*	*96*	*105*	*111*	*98*
Potassium, K-PAE	mg K/kg	*596*	*425*	*395*	*378*	*427*	*429*	*550*	*463*
Magnesium	mg MgO/kg	*433*	*438*	*394*	*394*	*421*	*428*	*502*	*412*
Sodium	mg Na/kg	*96*	*99*	*78*	*71*	*95*	*103*	*130*	*87*
Manganese	mg Mn/kg	*70,9*	*342,75*	*300,40*	*322,50*	*331,00*	*305,00*	*328,00*	*319,73*
Clay-humus complex (CEC)	mmol+/kg	*200*	*250*	*232*	*237*	*241*	*237*	*245*	*236*
Litability	%	*38,5*	*43,35*	*42,73*	*42,63*	*40,70*	*40,85*	*39,85*	*42,18*
Lutum	%	*23*	*29*	*29*	*29*	*27*	*28*	*27*	*28*
Nitrogen total Q	mg N/kg	*2220*	*2215*	*1963*	*1440*	*1550*	*2170*	*2150*	*1643*
S-PAE Litability	mg S/kg	*1511*	*1103*	*1220*	*1069*	*1200*	*1405*	*1496*	*1095*
Sulphur-delivering capacity	kg S/ha per year	*3598*	*2620*	*2887*	*2535*	*2857*	*3264*	*3489*	*2557*

APPENDIX E

E3. EFFECTS ON FUNGAL PROPERTIES

E3.1. Generation of graphs

Graphs showing the total number of fungi (either all fungi, other molds or individual genera) were generated based on CFU/g values. When these values were higher than the detection threshold (> 5000) or lower than the detection limit (< 100), they were entered as 5000 and 100, respectively, into the calculations for the graphical representation. Although these values are not exact representations of the soil fungal composition due to detection limits, they do show trends in abundance of different groups of fungi.

E4. ANALYSIS OF BACTERIAL COMPOSITION AND DIVERSITY

E4.1. Methods

The following descriptions were provided in the scientific report delivered by BaseClear B.V..

Alpha diversity - Alpha diversity refers to the average species diversity in a habitat or specific area. Alpha diversity is a local measure. We measure alpha-diversity as the observed richness (number of taxa) or evenness (the relative abundances of those taxa) of an average sample within a habitat type.

Beta diversity - We quantify beta-diversity as the variability in community composition (the identity of taxa observed) among samples within a habitat.

RDA - Redundancy analysis (also called principal components analysis of instrumental variables) is a technique for two sets of variables, one set being dependent of the other. Its aim is maximization of the explained variance of the dependent variables by a linear combination of the explanatory variables. The principal components of a collection of points in a real coordinate space are a sequence of p unit vectors, where the i*" vector is the direction of a line that best fits the data while being orthogonaL to the first i-1 vectors. Here, a best-fitting line is defined as one that minimizes the average squared distance from the points to the line. These directions constitute an orthonormal basis in which different individual dimensions of the data are linearly uncorrelated.

Association Testing (DAA) - This section aims at detecting differentially abundant microbiome features (species/OTUs) between two predefined classes of samples, where a microbiome feature is considered differentially abundant if its mean proportion is significantly different between two conditions.

APPENDIX E

E5. REFERENCES

Al-Kaisi MM, Yin X, Licht MAT (2005) Soil Carbon and Nitrogen Changes as Influenced by Tillage and Cropping Systems in Some Iowa Soils. Agric. Ecosyst. Environ. 105: 635–647.

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci. 17: 478–486.

Dawson W, Hör J, Egert M, van Kleunen M, Pester M (2017) A Small Number of Low-abundance Bacteria Dominate Plant Species-specific Responses during Rhizosphere Colonization. Frontiers in Microbiology 8: 975.

Del Chierico F, Ancora M, Marcacci M, Cammà C, Putignani L, Conti S (2015) Choice of Next-Generation Sequencing Pipelines, In: Bacterial Pangenomics: Methods and Protocols (eds. Mengoni A, Galardini M, Fondi M) 31–47. Springer, New York.

Gupta V, Sivasithamparam K (2007) Relevance of Plant Root Pathogens to Soil Biological Fertility. In: Abbott LK, Murphy DV (eds.) Soil Biological Fertility. Springer, Dordrecht.

Nielsen MN, Winding A (2002) Microorganisms as indicators of soil health. National Environmental Research Institute, Denmark, Technical report No. 388.

Ogórek R, Lejman A, Pusz W, et al. (2012) Characteristics and taxonomy of Cladosporium fungi, Mikologia Lekarska 19 (2): 80-85.

Zhang M, Li Z, Häggblom MM, Young L, Li F, He Z, Lu G, Xu R, Sun X, Qiu L, Sun W (2021) Bacteria responsible for nitrate-dependent antimonite oxidation in antimony-contaminated paddy soil revealed by the combination of DNA-SIP and metagenomics, Soil Biology and Biochemistry 156: 108194,

APPENDIX F

Biophoton emission research (2014-2018)

F1. BIOPHOTON EMISSION OF TOMATO FRUITS (2018)

G1.1 .TECHNOLOGY

Delayed luminescence (DL) and spontaneous ultra-weak photon emission (SE) of intact tomato fruits were measured using a photomultiplier device. The device includes a dark sample chamber (9.5 cm × 15 cm × 16 cm) with a vertically positioned photomultiplier tube (PMT) (Electron Tubes Enterprises Ltd., Ruislip, UK, type 9558QB). The sample chamber was kept at 22 ˚C (**Figure F1**). The window opening to the PMT has a diameter of 44mm. The sensitivity of the PMT is in the range between 160 and 870 nm. The PMT was cooled to -25 ˚C to reduce the dark count rate to 10 counts per second. A fast preamplifier (ORTEC, USA, type 9301) was used to enlarge the photon emission signal. A PC with a counting card (National Instruments, USA, type 6602) was used for data acquisition.

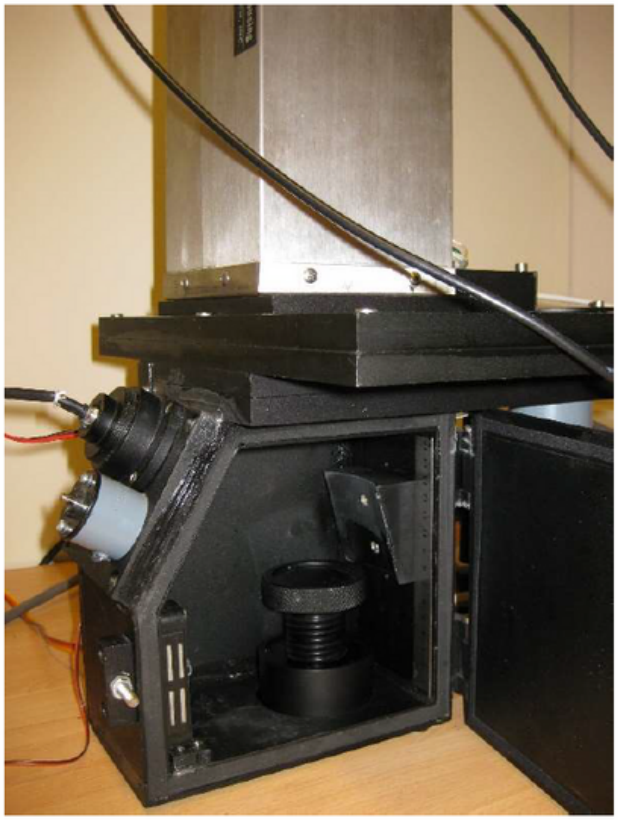


Figure F1. Photomultiplier tube and a dark chamber used for biophoton emission measurement.

F1.2. ANALYSIS

The delayed luminescence was recorded in consecutive 0.2-second time intervals for a 2-minute period, yielding a total of 600 data points. The decay curve showing light intensity decrease in time was analyzed according to three common mathematical models used by other researchers. Each model uses its own original variables (parameters).

APPENDIX F

Model 1 (hyperbolic decay model) contains the parameters Mean DL (DL_Mean), Initial intensity (I0), decay properties (Tau, Beta and T), and the goodness of fit (R).

Model 2 (double exponential model) contains the parameters: Initial intensity (y0), the properties two decays (A1, t1 and A2, t2), and the goodness of fit (R).

Model 3 (hyperbolic cosine model) contains the parameters of a decay curve that follows a hyperbolic and a shift including the parameters A, B, C, and the goodness of fit (R).

Steady state spontaneous emission of light energy (SE) was recorded in consecutive 0.05-second periods for a total of 5 minutes, yielding a total of 6000 data points. The fluctuations in photon numbers in time were then analyzed according to two models. Each model uses its own original variables (parameters).

Fractality model contains the parameters: Strength, Fano factor time curve variables (intercept and slope) and a fixed time Fano Factor (FF10).

Quantum squeezed state model contains the parameters: ABS_a, r, Theta, Phi, SSR, SSI, S.

All parameters are described in the book: Roeland Van Wijk, Yu Yan, Eduard Van Wijk (2017). Biophoton technology in energy and vitality diagnostics. A multi-disciplinary, systems biology, and biotechnology approach. Meluna, Wageningen, The Netherlands

F1.3. RESULTS

Statistical data describing the SE parameters of tomato fruits of plants treated with Analemma Water or Control are shown in **Table F1**.

Table F1. Differences between SE parametres of tomato fruits of plants treated with Analemma Water or Control. Change values (t) are shown for all parametres of the 2 models. Significant differences are indicated in red ($p < 0.05$).

Variable	t-value	p
Strength	-2.74058	0.006721
Intercept	0.22838	0.819597
Slope	-1.10491	0.270602
FF	0.42302	0.672762
ABS_a	-2.58665	0.010443
r	0.93003	0.353544
Theta	-0.03444	0.972566
Phi	0.03449	0.972520
SSR	-0.05630	0.955163
SSI	1.67070	0.096437
S	-2.05686	0.041074
FF10	-0.07624	0.939307

APPENDIX F

F2. REFERENCES

Agnew DC (2007) Earth tides, In: Herring, T (Ed.) Treatise on Geophysics, Volume 3: Geodesy, Elsevier B.V., Amsterdam

Bradford KJ (1995) Water relations in seed germination, In: Kigel, J (Ed.) Seed development and germination, Routledge, New York

Cifra M, Pospíšil P (2014) Ultra-weak photon emission from biological samples: Definition, mechanisms, properties, detection and applications. Journal of Photochemistry and Photobiology B: Biology 139, 2-10.

Gallep CM, dos Santos SR (2007) Photon-counts during germination of wheat (Triticum aestivum) in wastewater sediment solutions correlated with seedling growth. Seed Science and Technology 35, 607–614.

Mason MG, Nicholls P, Cooper CE (2014) Re-evaluation of the near infrared spectra of mitochondrial cytochrome c oxidase: Implications for non invasive in vivo monitoring of tissues, Biochimica et Biophysica Acta - Bioenergetics, 1837(11): 1882-1891.

Nozue K, Maloof JN (2006) Diurnal regulation of plant growth. Plant, Cell & Environment 29, 396-408.

Pang J, Fu J, Yang M, Zhao X, van Wijk E, Wang M, Fan H, Han J (2016) Correlation between different therapeutic properties of Chinese medicinal herbs with delayed luminescence. Luminescence. 31(2): 323-327.

Steinbrecher T, Leubner-Metzger G (2017) The biomechanics of seed germination, Journal of Experimental Botany 68, 765–783.

Sun M, Li L, Wang M, van Wijk E, He M, van Wijk R, Koval S, Hankemeier T, van der Greef J, Wei S (2016) Effects of growth altitude on chemical constituents and delayed luminescence properties in medicinal rhubarb. J Photochem Photobiol B 162: 24-33.

Sun M, van Wijk R, van Wijk E, Wang M, van Wietmarschen, H, Hankemeier T, van der Greef J (2016) Delayed luminescence: an experimental protocol for Chinese herbal medicines. Luminescence. doi: 10.1002/bio.3094

van Wijk R, Yan Y, van Wijk E (2017). Biophoton technology in energy and vitality diagnostics. A multi-disciplinary, systems biology, and biotechnology approach. Meluna, Wageningen, The Netherlands