UNIVERSITÀ DEGLI STUDI DI PADOVA
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Master Thesis

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CLOSING THE LOOP FOR A SUSTAINABLE LANDFILL LEACHATE TREATMENT: AN EXPERIMENTAL TRIAL WITH HORIZONTAL AND VERTICAL PHYTOTREATMENT USING SUNFLOWERS

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Ringraziamenti

Ringrazio la mia famiglia per il supporto che mi ha sempre dato

Ringrazio i miei cugini e i miei amici di sempre con cui ho condiviso un sacco di esperienze divertenti

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SECTION I:

My thesis and me

1. THE BEGINNING

I started thinking about my master thesis in January 2014. I already knew the topics proposed by my professors because I had already talked with them during the previous years. In particular, I knew in details the researches proposed by Professor Maria Cristina Lavagnolo, who already helped me during the Bachelor Degree. Among others, she is working on projects of integrated wastewater management systems involving phytotreatments.

I have been always attracted by plants because I live in the countryside, so they are an indissoluble part of my life. During my Bachelor Degree I already attended a seminar on phytotreatment techniques held by her, which further increased my interest on the theme.

When I was young, I wanted to become a mechanist, a farmer, a train driver, but mainly a gardener…now I am a student of Environmental Engineering! Great change, eh?

No, I still have the passion for plants and flowers! I spend a lot of time in my garden in which I cultivate magnificent sunflowers.

So the possibility to use sunflowers to detoxify municipal solid waste landfill leachate represented the best compromise between actual studies and old passions!

I contacted Professor Lavagnolo and we fixed a meeting in my future working place, the LISA laboratory of Voltabarozzo (Padova). The result of the meeting?

Well…I officially started my thesis!

2. THE BIBLIOGRAPHIC RESEARCH

The starting point of any scientific experiment is the evaluation of the state-of-the-art of the topic.

Articles available in literature are examined to gain a better knowledge of the theme and to avoid the arising of problems which have already been solved by other researchers.

I used to visit the ScienceDirect website, one of the most important scientific database containing thousands of scientific articles.

I found articles referring experiences on phytotreatment of raw municipal wastewater, sewage sludge and treated effluent carried out successfully on cultivations of *Cynara cardunculus*, *Typha latifolia*, *Arundo donax* and *Phragmites australis* (Manãs *et al*., 2014; Zema *et al*., 2012).

Treated municipal wastewater, indeed, may improve biodiesel quality obtained from sunflower seeds (Tsoutsos *et al*., 2013).

Researchers also tried to irrigate poplar and willows coppices with landfill leachate, identifying the irrigation rate as the controlling parameter to be monitored (Dimitriou *et al.*, 2010; Zalesny *et al.*, 2009).

The concept of landfill leachate phytotreatment with oleaginous plants, proposed for the first time by the researchers of the LISA laboratory, is recent and innovative, therefore there are only few publications available.

In fact I obtained most of the information needed from thesis of students which already worked on the topic (Leigue Fernàndez, 2014; Nicoletti, 2012).

3. PHYTOTREATMENT

Phytotreatment is based on the use of plants and their associated microbes to extract, sequester and/or detoxify contaminants from wastewater.

The processes involved are represented in Figure 1.

Plants can promote biodegradation of organic pollutants by microbes in their rhizosphere (phytostimulation or rhizodegradation). Plants can also degrade organic pollutants directly via their own enzymatic activities (phytodegradation). After uptake in plant tissues, certain organic pollutants can leave the plant in volatile form (phytovolatilization); other kinds of pollutants are accumulated in the biological tissues (sequestration).

Inorganic contaminants cannot be degraded, but they can be phytoremediated via stabilization or sequestration in harvestable plant tissues. Inorganic pollutants include macronutrients such as nitrate and phosphate (Pilon-Smits, 2005).

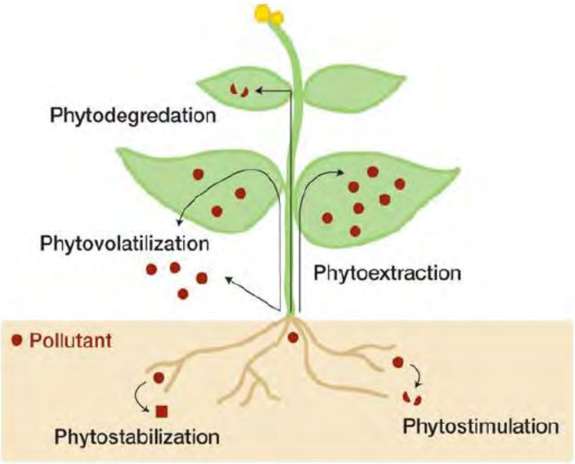

Figure 1. Fate of pollutants during phytoremediation (Pilon-Smits, 2005)

Sunflowers, widely branched annual terrestrial plants with large roots and multiple yellow flower heads are considered to be good accumulators of inorganic compounds (Pilon-Smits, 2005).

The use of plants for the removal of contaminants from wastewater has both advantages and disadvantages. The main advantages are cost-effectiveness of the process and acceptability of citizens. The relevant disadvantages are the long time required to achieve the remediation limits and the potential non-bioavailability of contaminants to be removed (Nagendran *et al*., 2006).

Phytotreatment of contaminated wastewater is traditionally carried out in "constructed wetlands" in which a combination of chemical (e.g. precipitation), physical (e.g. filtration, sedimentation, adsorption) and biological processes are exploited.

They are designed to simulate natural processes occurring in real wetlands. Incoming nutrients support the growth of vegetation and biological activities, therefore the primary productivity of wetland ecosystems are typically high (Brix, 1993)

Constructed wetlands, however, are less reliable than conventional wastewater treatment methods because biological components are sensitive to toxic compounds and environmental change (Massoud *et al*., 2009).

The role of plants is essential for the microbes living in symbiosis: they transfer oxygen and organic compounds to the root zone thus enhancing biological removal of pollutants.

Constructed wetlands can be divided into free water surface similar to marshes; horizontal subsurface flow (Figure 2), implying the presence of a soil bed planted with vegetation in which water flows horizontally from the inlet to the outlet; vertical flow (Figure 3), implying distribution of water across the surface of a sand or gravel bed planted with vegetation to promote vertical flow (Kadlec, 2009).

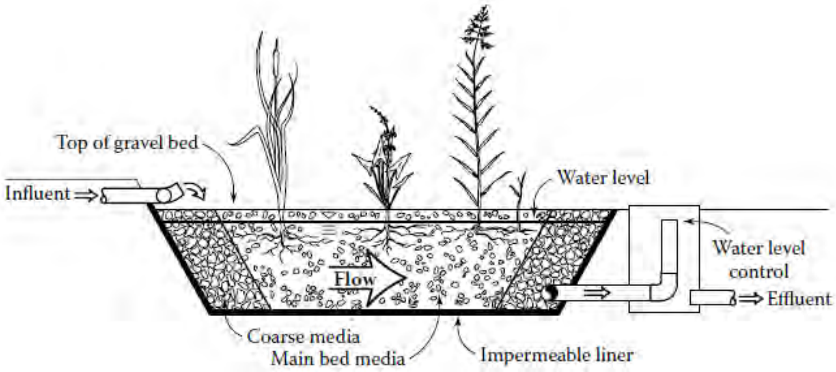

Figure 2. Typical cross section of horizontal subsurface flow wetlands (Kadlec, 2009)

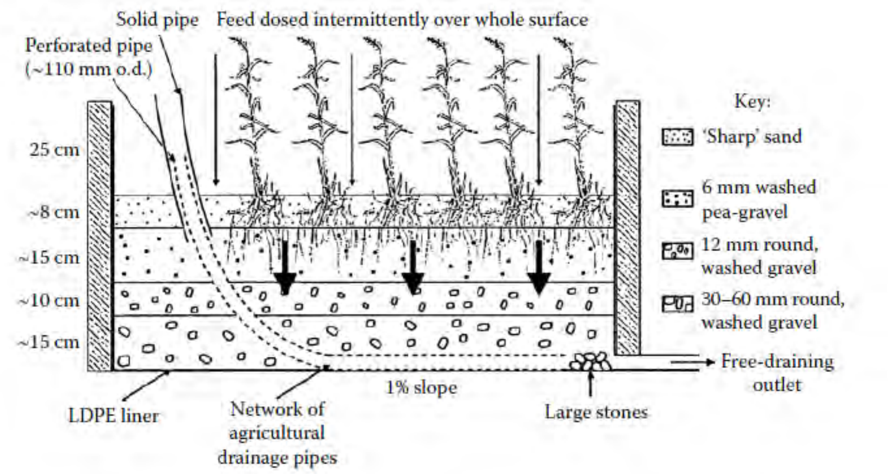

Figure 3.Typical cross section of vertical flow wetlands (Kadlec, 2009)

4. THE RESEARCH PROGRAM

Professor Lavagnolo and her students already tried to use oleaginous plants to remediate wastewater with the aim of closing the loop of matter by producing biofuels. They tested plants which are traditionally cultivated in the northern part of Italy, such as *Helianthus annuus* (sunflower), *Glycine max* (soybean) and *Brassica napus* (rapeseed). Sunflowers demonstrated to have the greatest potential in terms of decontamination capacity and biomass growth. Moreover, they are more easily accepted by the population, even if they are used to treat "dirty" water, because of their pleasant presence. This is the main reason why they have been proposed to treat landfill leachate: they might be planted on the top of closed landfills, allowing a cost-effective treatment of contaminated water and a pleasant view of the site itself.

So we decided to focus exclusively on sunflowers fed with diluted municipal solid waste landfill leachate.

The previous experiments have been carried out on large tanks made up of rigid, dark plastic which did not allow the possibility to control the water level inside (Figure 4).

Figure 4. Tanks used during previous experiments

The knowledge of the effects of the water table level on plants growth is fundamental to adjust the irrigation rate. So after several discussions (mainly on the ratio between costs and benefits) we have chosen to build new tanks, transparent and equipped with a drainage systems which allows the extraction of the outlet for chemical analysis.

Besides the traditional vertical flow, we have hypothesized to test an innovative horizontal subsurface flow system which has never been applied to oleaginous plants.

In order to obtain as much information as possible, two equal lines (corresponding to four different tanks) have been applied: vertical flow fed with landfill leachate and horizontal subsurface flow fed with landfill leachate.

The tanks were called V1R, V2, H1R, H2 in which "V" stands for vertical, "H" stands for horizontal.

When I say "fed with landfill leachate", I do not mean pure leachate but a mixture leachate-tap water: we started from a mixture containing 10 % (v/v) up to 30 % (v/v), even more than the maximum amount typically admitted by plants (Leigue Fernàndez, 2014).

Two additional tanks, called VC and HC, characterized by vertical and horizontal flow respectively, fed with tap water, were used as control to compare the effects of leachate on plants growth.

Therefore six tanks, three characterized by vertical flow, the others characterized by horizontal subsurface flow, were hosted in a special greenhouse, located in the LISA laboratory.

Initially the experiment included the complete closure of the drainage system in order to record the effects of the water table level (the tap was opened once a week just to empty the tanks and collect samples for chemical analysis); we were forced to change this practice, maintaining the drainage continuously opened, when plants started to suffer the phenomenon known as "root asphyxia".

In fact, when you are working with living organisms, the research program cannot be defined a priori; it must be adjusted according to their response.

Despite it, we decided to take advantage of the different situation introducing a new feeding mood: in two tanks (V1R and H1R) I started to re-circulate the outlet collected every day from the same tanks. Most of times it was not enough; therefore I topped up with diluted leachate.

5. THE CONSTRUCTION OF THE TANKS

The construction of the tanks required almost one month.

The Ph. D. student Luca Morello helped me in this long and hard phase. We tested tanks having different volumes, properties and drainage systems. Finally, together with Professor Lavagnolo, we realized that 130 liters tanks were sufficient to contain four or six sunflowers.

Then the problem of the realization of the drainage system came up: tests carried out by previous students, in fact, have been limited by incomplete drainage of the treated water. In order to solve it, we decided to simulate the drainage systems used in landfills.

5.1 Vertical flow tanks

Irrigation water flows vertically, from the top to the bottom, driven by gravity. The tanks have been constructed to maximize the possibility to collect and drain effluent water: the 20 cm thick seedbed lays on a 10 cm thick layer of gravels (with a diameter of 2-3 cm) within which a holed PVC pipe (with a diameter of 3 cm), connected to the final tap, promotes the removal of the fluid (Figure 5). Between the layers, an horizontal plastic net has been inserted to limit soil intrusion into the gravels voids. A vertical plastic cylinder, 3 cm in diameter, was infixed vertically in the seedbed to allow the measurement of the water table level by means of the insertion of a shaft. Six plants, arranged in two equal lines composed of three sunflowers each, were hosted in each reactor (Figure 6).

Construction details are reported in Figures 7, 8, 9, 10, 11, 12.

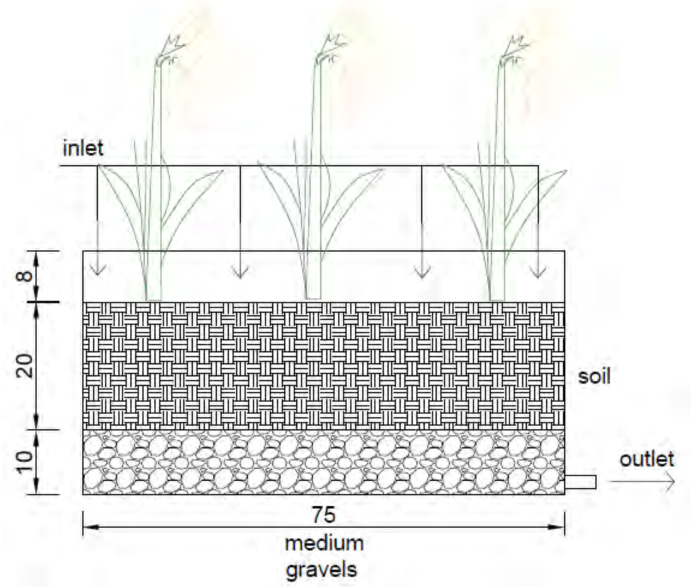

Figure 5. Sketch of a vertical flow tank (measures expressed in centimetres)

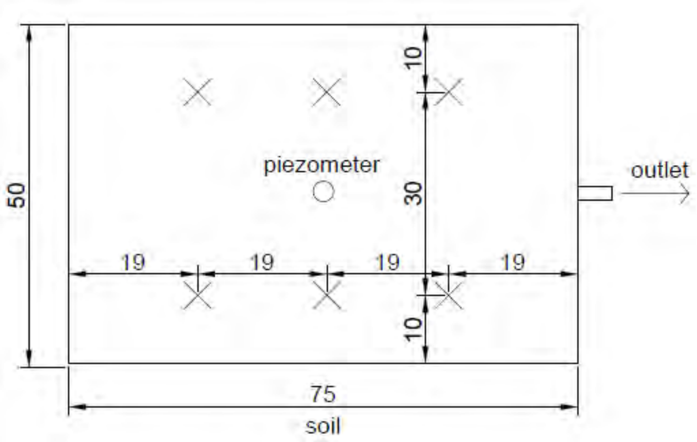

Figure 6. Position of sunflowers in vertical flow tanks (measures expressed in centimetres)

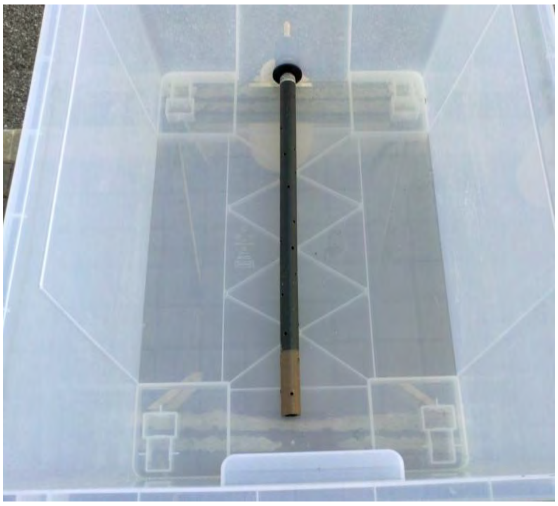

Figure 7. Holed PVC pipe inserted within the bottom gravel layer, used to promote the drainage of the effluent

Figure 8. Gravel drainage layer on the bottom of vertical flow tanks

Figure 9. Tap system used to drain effluent water

Figure 10. Plastic net used to separate the layers

Figure 11. Vertical plastic cylinder inserted in the seedbed

Figure 12. Cross-section of a vertical flow tank

5.2 Horizontal subsurface flow tanks

Irrigation water flows horizontally, driven by the hydraulic gradient between inlet and outlet zones. The tank contains three vertical layers: a 10 cm thick layer composed of large gravels (4-6 cm) in the load zone; a 55 cm thick layer of seedbed, a 10 cm thick layer composed of medium/small gravels (1-2 cm) in the outlet zone (Figure 13). Between the layers, a vertical plastic net has been inserted to limit particles movement. A vertical plastic cylinder, 3 cm in diameter, was infixed vertically in the seedbed to allow the measurement of the water table level by means of the insertion of a shaft (Figure 14).

In the outlet zone a holed PVC pipe, transversal to the movement of water, has been inserted within the gravel layer to promote liquid drainage.

Four plants, arranged in order to produce a rectangular pattern in the central portion, were hosted in each reactor.

Construction details are reported in Figures 15, 16, 17, 18, 19.

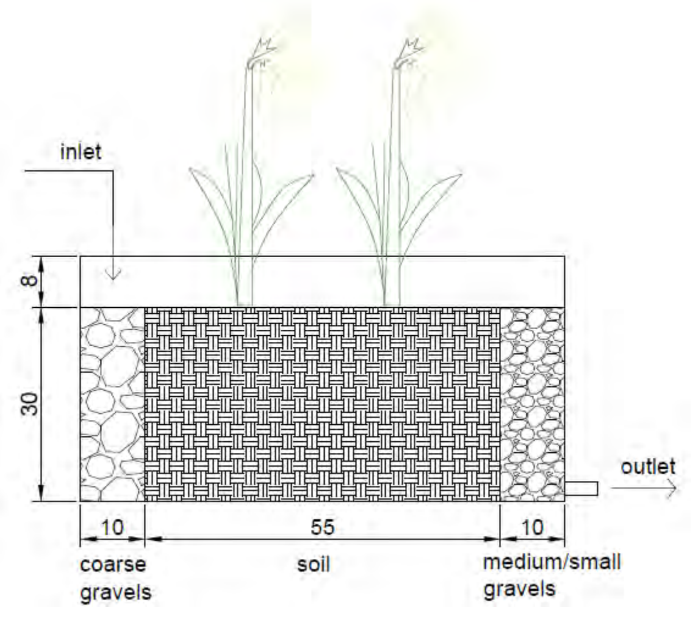

Figure 13. Sketch of a horizontal subsurface flow tank (measures expressed in centimetres)

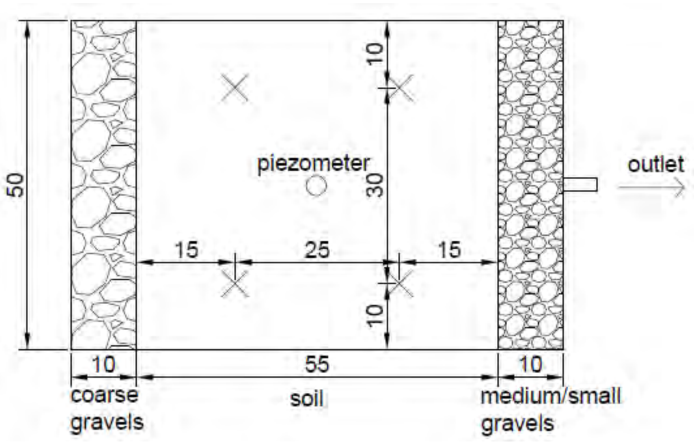

Figure 14. Position of sunflowers in horizontal subsurface flow tanks (measures expressed in centimetres)

Figure 15. Holed PVC pipe, transversal to the flow pattern, used to promote the drainage of the effluent

Figure 16. Tap system installed on horizontal subsurface flow tanks

Figure 17. Vertical plastic net used to separate the three layers

Figure 18. Vertical plastic cylinder inserted in central layer

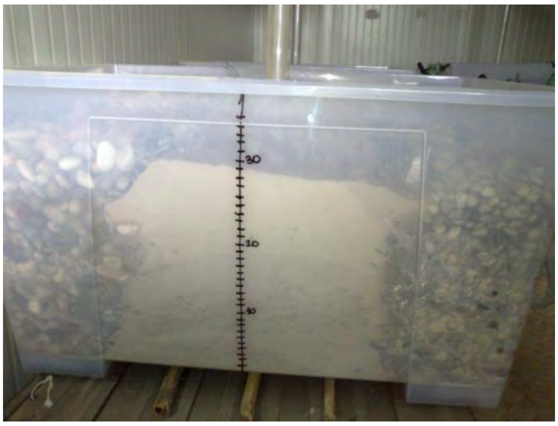

Figure 19. Cross-section of a horizontal subsurface flow tank

6. THE GREENHOUSE

The greenhouse, a prefabricated commercial container sized 6.00 x 2.20 m (Figure 20), allowed the control of the parameters essential for plants growth: light intensity, temperature and irrigation rate.

It contains 9 lamps with a nominal power of 400 W each, whose functioning is regulated by an automatic switcher. It has been decided to simulate typical summer conditions (14 hours of sunlight every day): lamps switched on at 6.00 a.m. and switched off at 8.00 p.m. automatically. The windows have been covered with black plastic sheets in order to completely eliminate the influence of sunlight on the test. Therefore, 10 hours of dark have been ensured inside the facility.

An air conditioner, working continuously, maintained acceptable temperatures inside it, which ranged between 15-17 °C during the night and 35-40 °C during the day.

Temperature was measured by means of a special thermometer, capable of recording the minimum and maximum values occurred (Figure 21).

The main components of the greenhouse are reported in Figures 22, and 23. Greenhouse layout and tanks arrangement are shown in Figures 24, 25 and 26.

Figure 20. Greenhouse used for the experiment

Figure 21. Thermometer capable of measuring minimum and maximum temperature

Figure 22. Air conditioner system installed in the greenhouse

Figure 23. Automatic switcher installed in the greenhouse

Figure 24. Tanks arrangement inside the greenhouse just after having constructed them

Figure 25. Tanks arrangement inside the greenhouse after 2 weeks from transplantation

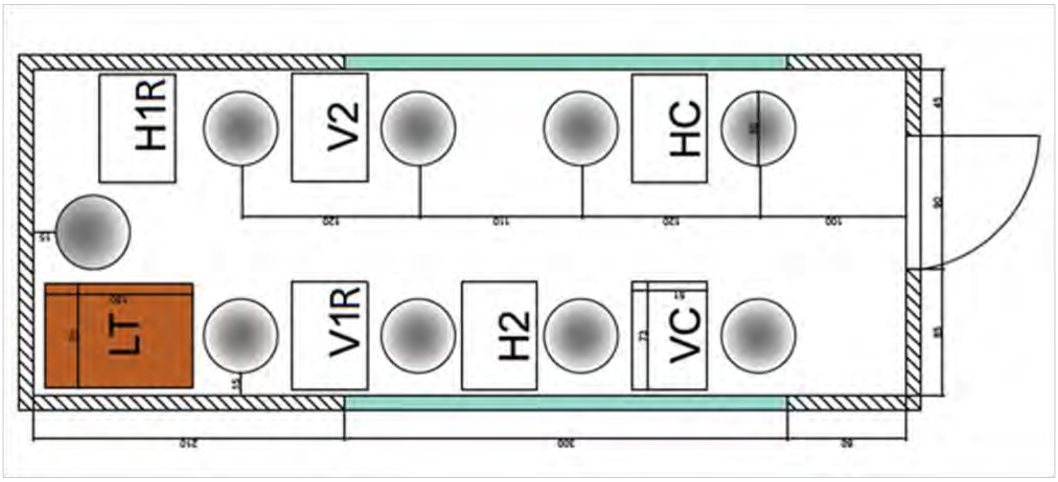

Figure 26. Layout of the greenhouse in operation

7. THE SEEDBED

The seedbed is the supporting material for plants and root microbes. Long-term studies on the hydraulics of constructed wetlands with different soil parameters indicate that mixtures of soil and sand produces the best results in terms of both hydraulic condition and removal of contaminants. Sand is a poor substrate with no nutrients, agricultural soil is rich in organics and nutrients essential to plants growth; an optimal mixture should be used to guarantee an acceptable compromise between the needs for macroporosity, air circulation and root development while avoiding the presence of stagnant water (Jones et al., 2006).

Professor Lavagnolo contacted her colleague, Professor Mario Malagoli, for finding the materials. Professor Malagoli belongs to the Dafnae department of the University of Padova; he is an expert of phytotreatment techniques.

In few days he obtained the materials needed: agricultural soil from Legnaro (Padova) and river sand.

I have been helped by the Ph. D. student Luca Morello and my colleague Matteo Costa to mix soil and sand by using a small electric cement mixer.

Two mixtures have been prepared, whose textural classes are shown in Table 1. Soil taxonomy, proposed by USDA (USDA-NRCS, 1999) is reported in Figures 27 and 28.

Table 1. Composition of the soil mixtures

Tanks	***Clay (w/w %)***	**Silt (w/w %)**	***Sand (w/w %)***
Vertical flow	*12*	16	*72*
Horizontal flow	*12*	12	*76*

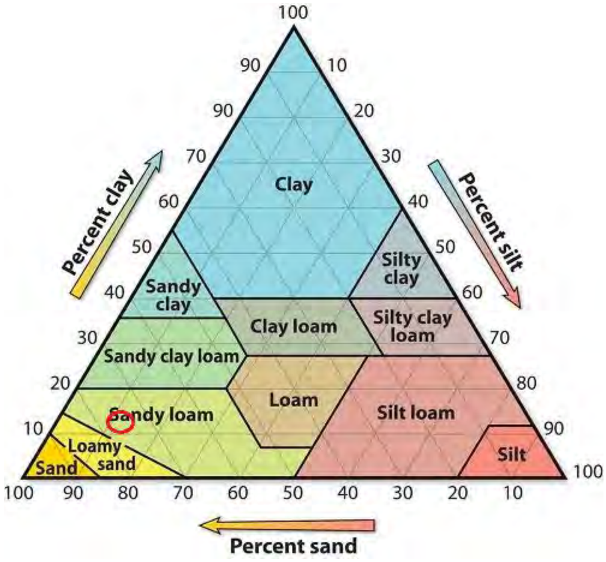

Figure 27. Seedbed classification of vertical flow subsurface tanks (USDA-NRCS, 1999)

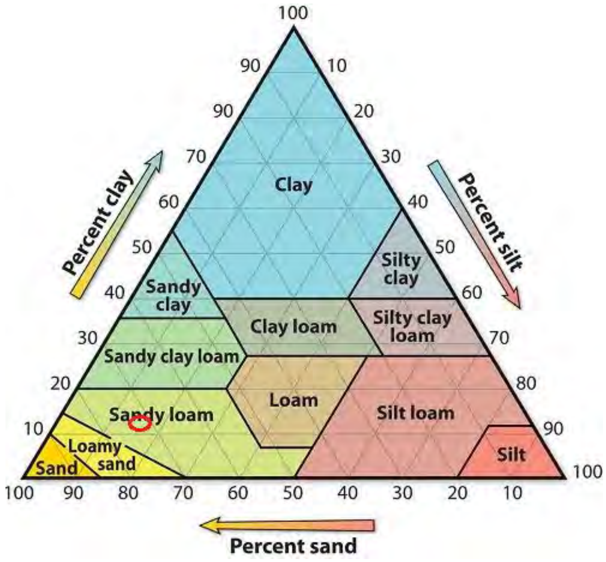

Figure 28. Seedbed classification of horizontal flow sub-superficial tanks (USDA-NRCS, 1999)

The mixtures have been prepared with the aim of having a higher amount of sand in the horizontal flow vessels to favor the horizontal movement of water: an excessive amount of clay, in fact, may have limited it due to the presence of impermeable layers of cohesive material.

8. LANDFILL LEACHATE

Leachate is a wastewater generated by excess rainwater percolating through the waste layers in a landfill (Christensen *et al*., 1992).

The pollutants are transferred from the waste material to the percolating water by a variety of physical, chemical and biological processes.

The quality of leachate depends on:

1. quality and type of waste
2. conditions in the landfill (aerobic, anaerobic or semi-aerobic)
3. the age of the landfill

The quantity depends on:

1. climatic and meteorological conditions
2. physical characteristics of waste
3. characteristic of the barrier system

We have decided to use old landfill leachate coming from the closed municipal solid waste landfill of Mazzano (Brescia). The disposal of waste in the landfill was officially stopped on the 1st December 1990 by the local authorities.

The characterization is included in Table 2.

Values of COD and BOD are lower than those typically found in young municipal solid waste landfills, indicating the absence of readily biodegradable organic substances such as volatile fatty acids and the presence of refractory substances, such as humic and fulvic compounds.

Table 2. Chemical characterization of Mazzano leachate

MAZZANO LEACHATE		
Collection date	09/05/2014	
Chemical analysis		
Parameter	**Value**	**Unit**
pH	8	-
TKN	1240	mgN/l
NH_4^+	1221	mgN/l
P_{TOT}	11	mgP/l
PO_4^{3-}	11	mgP/l
TS	4277	mg/l
VS	1102	mg/l
COD	1325	mgO$_2$/l
BOD	50	mgO$_2$/l
Cl^-	1138	mgCl/l
NO_3^-	0	mgNO$_3$-N/l
SO_4^{2-}	0	mgSO$_4$/l
Na	2700	mgNa/l
Ca	85	mgCa/l
K	1050	mgK/l
Mg	97	mgMg/l
Cd	10	µgCd/l
Cr	199	µgCr/l
Cu	138	µgCu/l
Fe	8358	µgFe/l
Mn	131	µgMn/l
Ni	179	µgNi/l
Pb	64	µgPb/l
Zn	946	µgZn/l

9. THE SEEDLINGS

Seeds of sunflowers were planted by Professor Malagoli in the structures of Agripolis, the agricultural university campus of the University of Padova.

They sprang up rapidly, after one week I transplanted them in the reactors (on the 23rd of May).

During the first week (called also “Week 0”) they were all fed with pure tap water to promote their rooting.

Figure 29 shows the seedlings just after having been transplanted.

Figure 29. Seedlings just after having been transplanted

10. CHEMICAL ANALYSIS

We have decided to carry out a series of chemical analysis on the outlet of each tank, completed with weekly frequency, to evaluate contaminants removal efficiencies. We have chosen analysis known and used worldwide.

The monitored parameters are:

- COD
- TKN
- ammonia
- nitrate
- total phosphorous
- chloride
- sulphate
- total solids

- volatile solids
- metals

From the beginning of the test (23rd of May) up to the 29th of June, the drainage system of each tank was opened only once a week, every Friday, and the collected effluent stored in the fridge during the weekend. The analysis train, in fact, started the subsequent Monday.

From the 30th of June to the 5th of August (end of the trial), the drainage system remained continuously opened: despite the effluent was collected every day, only those drained on Friday, assumed to be representative of the entire week, was stored in the fridge and analyzed the subsequent week.

Chemical analysis have been carried out in the LISA laboratory in Voltabarozzo; I would like to thank Dr. Annalisa Sandon for the time she spent teaching me the procedures to be followed.

11. DAILY ROUTINE

I used to arrive at the laboratory at 9.00 a.m.. After the greetings to my colleagues and to the professors, I entered the greenhouse to check the correct functioning of lamps and air conditioner. If everything was working correctly, I started with the daily procedure which included:

1. measurement of the water table inside the tanks
2. recording of minimum and maximum temperature registered by the thermometer
3. addition of inlet according to the needs of plants

Once a week (every Friday) I opened the drainage system and collected the effluent, measured the weekly evaporation and plants height.

From the 30th of June we were forced to keep the tap opened, therefore I did not measure the water level anymore but I measured every day the drained water and, in tanks V1R and H1R, I started to re-circulate the effluent.

The entire operations lasted for almost two hours.

So at 11 a.m. I used to start the chemical analysis: I did not follow a precise order to analyze the parameters because I had to share the laboratory with other 4-5 students so the instruments were not always available as expected.

At 13.30-14 a volunteer prepared lunch for all people present, I usually cooked pasta but I always asked my friends Nicoletta or Francesca to prepare some condiment because I am hopeless at cooking!!

In the afternoon I continued with chemical analysis: most of times, in fact, a single chemical analysis requires the entire working day.

I went home at 18-18.30, after having closed the greenhouse with the key! In reality, before going home, I used to go to the café near the laboratory with my colleagues for a beer.

12. CHANGES ON THE ROAD

When you are working on an experimental thesis, you should always consider the possibility to face problems which may change the programs.

I have already talked, for instance, about the need to keep the drainage opened to avoid the phenomenon of root asphyxia.

The choice of transparent vessels showed both advantages and disadvantages: I could monitor the water table level inside the tanks, but the light beams promoted the formation of unwanted green microalgae along the internal walls and on the surface of the seedbed.

Their presence could affect the results of the tests because, as living microorganisms, consume substances present in the leachate and produce metabolic residues.

Therefore I was forced to “wrap” laterally the reactors with black plastic sheets and to cover the surface with a thin layer of expanded granular clay to avoid light penetration and consequent development of microscopic vegetation.

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SECTION II:

Scientific paper

Closing the loop for a sustainable landfill leachate treatment: an experimental trial with horizontal and vertical phytotreatment using sunflowers

Francesco Garbo

Abstract

The use of energy crops for the decontamination of wastewater is gaining interest due to water scarcity in many countries and the possibility of obtaining alternative fuels of vegetable origin. The aim of this work is to evaluate the feasibility of landfill leachate phytotreatment by means of sunflowers, as seeds may be used in biodiesel production. Two different irrigation systems were tested: vertical flow and horizontal subsurface flow, with or without effluent recirculation. Plants were arranged in a special greenhouse in 130 L rectangular tanks. Leachate irrigated reactors were submitted to increasing nitrogen loads (5000 - 35000 mgN/week/m^2). The experiment showed good removal efficiencies for COD ($\eta > 50\%$), nitrogen ($\eta > 60\%$) and phosphorous ($\eta > 90\%$). Leachate was successfully tested as an alternative fertilizer for plants, it did not inhibit biomass development. Vertical flow tanks proved more suitable to host sunflowers: plants developed larger biomasses and the N:P ratio indicates a balanced nutrients supply.

1. INTRODUCTION

The use of energy crops for the decontamination of wastewater is gaining increasing interest due to water scarcity in many countries worldwide and the possibility to obtain alternative fuels of vegetable origin.

Energy crops can be defined as low-cost and fast-growing plants used to produce biofuels (such as ethanol or biodiesel) or combusted to generate electricity or heat.

Recent developments on energy crops cultivation are driven by the need of advanced industrial societies to reduce their energetic dependence on fossil fuels and emissions of greenhouse gases (Zema *et al*., 2012).

Improvements on renewable energy production are encouraged and required by the European Union: Directive 2009/28/EC sets targets for each Member State in order to reach the objective of 20 % of renewable energy by 2020 (Manãs *et al.*, 2014). The Directive, moreover, specifies a 10 % mandatory target for biofuel consumption (Spugnoli *et al.*, 2012).

Nowadays about 86 % of current water use is dedicated to irrigation for agricultural cultivations (Tsoutsos *et al.*, 2013). The increasing bioenergy use, however, requires high irrigation rates to produce relevant amounts of biomass which may shorten fresh water availability, especially in dry areas.

The use of non-conventional water resources (raw or treated urban or industrial wastewater, landfill leachate) seems to be an optimal compromise between the needs of biofuel production and conservation of water storages, guarantying at the same time acceptable levels of contaminants in the discharged treated flows (Zema *et al.*, 2012).

Applications of raw municipal wastewater, sewage sludge and treated effluent have been tested successfully on cultivations of *Cynara cardunculus, Typha latifolia, Arundo donax* and *Phragmites australis;* showing increased biomass yields and limited percolation of contaminants into the groundwater (Manãs *et al.*, 2014; Zema *et al.*, 2012).

Detailed researches demonstrated the possibility to improve biodiesel quality obtained by *Helianthus annuus* (sunflower) and *Ricinus communis* (castor) fed with treated municipal wastewater (Tsoutsos *et al.*, 2013).

Landfill leachate might be used to irrigate poplar and willows coppices, even if infiltration rates must be adjusted properly to minimize groundwater disturbances (Dimitriou and Aronsson, 2010; Zalesny *et al.*, 2009).

Focusing on oleaginous plants treated with municipal solid waste landfill leachate, those having the highest market value have been investigated in order to evaluate the ability of detoxify contaminants in combination with the possibility to produce high quality biodiesel. *Helianthus annuus* (sunflower), *Glycine max* (soybean) and *Brassica napus* (rapeseed) represent an optimal solution for Mediterranean and Continental areas: they combine good removal efficiencies and significant oil productions and can be easily transferred to full-scale applications (Marchiol *et al.*, 2007; Singh and Singh, 2010; Lavagnolo *et al.*, 2011).

This paper examines the effects of leachate irrigation on *Helianthus annuus* grown on tanks characterized by four different feeding practices: vertical and horizontal sub-superficial flow with partial recirculation of outlet; vertical and horizontal sub-superficial flow without recirculation of outlet.

The main goal of the study was to collect information concerning pollutants abatement and contaminants fate according to different flow patterns. An additional objective of the experiment was the identification of potential effects of landfill leachate irrigation on plants growth compared with control irrigation with tap water.

2. MATERIALS AND METHODS

2.1 Research program

The experiments were performed at the Laboratory of Environmental Engineering (LISA), ICEA Department of the University of Padua, located in Voltabarozzo.

The entire research lasted for almost three months. Six tanks were constructed: three characterized by horizontal subsurface flow (H1R, H2 and HC); three characterized by vertical flow (V1R, V2 and VC). H1R, H2, V1R and V2 represent the tanks irrigated with diluted leachate; HC and VC represent the control vessels, fed exclusively with tap water. They were all hosted in a special greenhouse.

The entire research program is summarized in Table 1.

Reactors irrigated with diluted leachate were periodically submitted to increasing leachate concentrations: detailed antecedent researches, conducted at the LISA laboratory, revealed toxic effects on plants produced by excessive concentration of nitrogen compounds into the feeding water; in particular sunflowers may suffer TKN concentrations exceeding 400 – 450 mgN/l (Leigue Fernàndez, 2014). The nitrogen load, therefore, was used as the reference parameter to adjust leachate dosage.

The use of a single diluted leachate storage tank ensured the possibility to irrigate all sunflowers with feed characterized by the same quality.

During Phase 1, Phase 2 and Phase 3A the effluent, drained once a week, was analyzed after having determined the volume. In this period of the research, all vertical and horizontal tanks were managed in the same way respectively. The experiment included two replicas of vertical reactors and two replicas of horizontal tanks fed with diluted leachate: therefore results obtained from Phase 1 to Phase 3A can be compared and discussed without conceptual and methodological limitations.

During Phase 3A sunflowers showed signs of stress caused by root asphyxia: old leaves desiccation and formation of black spots on new leaves. Subsequently, tanks were completely drained and a new feeding mode, characterized by drainage system continuously opened, was applied (Phase 3B). Contemporarily, recirculation of outlet started in vessels V1R and H1R: if the volume drained was not sufficient to irrigate sunflowers, diluted leachate was added.

During Phase 3B the effluent was collected every day: that drained on Friday, assumed to be representative of the entire week, was analyzed.

The parameters monitored in the effluent were: COD, TKN, ammonia, nitrate, total phosphorous, chloride, sulfate, total solids (TS), volatile solids (VS) and metals.

Analysis were carried out according to the Italian analytical standards for water and wastewater samples (CNR-IRSA, 29/2003).

At the end of the trial, nutrient content of soil samples, collected randomly in each tank, was detected; plants were harvested, dried at 60 °C in an oven, weighed and analyzed: dry mass, total phosphorous and TKN of leaves, roots and stems were determined. Analysis on sunflowers and soil followed the IRSA-CNR guidelines for solid samples (CNR-IRSA, 64/1986).

Table 1. Research program of the entire experiment on tanks H1, H2, V1, V2

PHASE	FEEDING QUALITY	*NOTES ON OPERATION*	FEEDING REGIME
Acclimation Phase *23/05 – 29/05*	100 % tap water	*Water provided to sustain initial growth*	1 - 2 l/tank/d
Phase 1 *30/05 – 08/06* *(week 1)*	90 % tap water + 10 % leachate	*Drainage system opened once a week to empty the tanks and drain the effluent*	1 – 5 l/tank/d
Phase 2 *09/06 – 15/06* *(week 2)*	80 % tap water + 20 % leachate	*Drainage system opened once a week to empty the tanks and drain the effluent*	1 – 6 l/tank/d
Phase 3A *16/06-29/06* *(weeks 3-4)*	70 % tap water + 30 % leachate	*Drainage system opened once a week to empty the tanks and drain the effluent*	2 – 6 l/tank/d
Phase 3B *30/06 – 05/08* *(weeks 5-9)*	70 % tap water + 30 % leachate	*Drainage system continuously opened to promote oxygen intrusion into the soil; outlet recirculation on tanks V1R and H1R (and eventual top up with leachate)*	2 – 8 l/tank/d

2.2 Experimental setup

Transparent tanks were used to monitor the water table level, which has been chosen to be the reference parameter to adjust the irrigation rate.

Seedlings, grown in a nourishing seedbed at the DAFNAE department of the University of Padua, were transplanted in the tanks one week after germination.

Vertical flow tanks contained six sunflowers each, horizontal flow tanks four plants each.

Plants were arranged in the reactors in order to ensure a minimum interfile distance of 15 cm.

2.2.1 Vertical flow tanks

The transparent tanks, sized 75 x 50 x 38 cm, were holed at the bottom and connected to a flexible pipe, 1.5 cm in diameter.

The 20 cm thick seedbed lay on a 10 cm thick layer of gravels (with a diameter of 2-3 cm) within which a holed PVC pipe (with a diameter of 3 cm), connected to the final tap, promoted the removal of fluid (Figure 1). Between the layers, a plastic net has been inserted horizontally to limit soil intrusion into the gravels voids.

A vertical plastic cylinder, 3 cm in diameter, was infixed in the seedbed to allow the measurement of the water table level by means of the insertion of a shaft, as a piezometer.

Feed was added manually with a watering can; it was distributed uniformly on the entire surface: the formation of a water table of almost 1 – 1.5 cm on the surface was used as the main evidence to indicate the saturation achievement in the tank..

Six plants, arranged in two equal lines composed of three sunflowers each, were hosted in each reactor (Figure 2). Plant density (16 plants/m^2) matched the values suggested in literature for Mediterranean areas (Ibrahim, 2012; Barros *et al*., 2004).

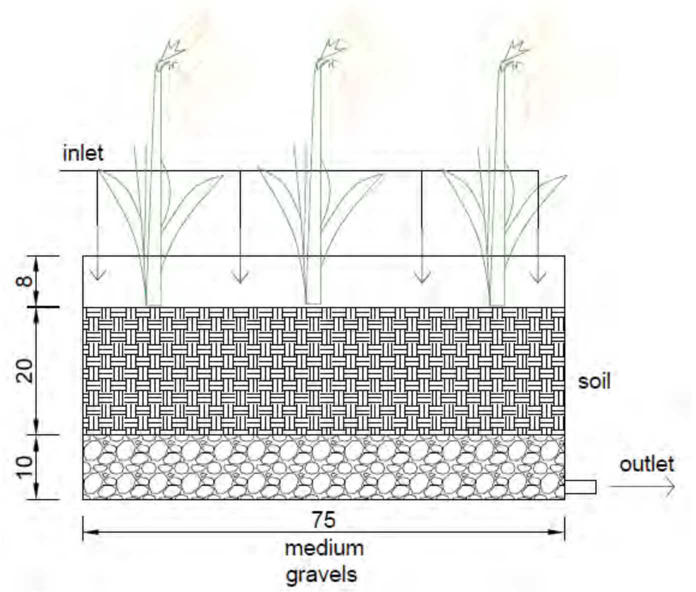

Figure 1. Cross section of a vertical flow tank (measures expressed in centimetres)

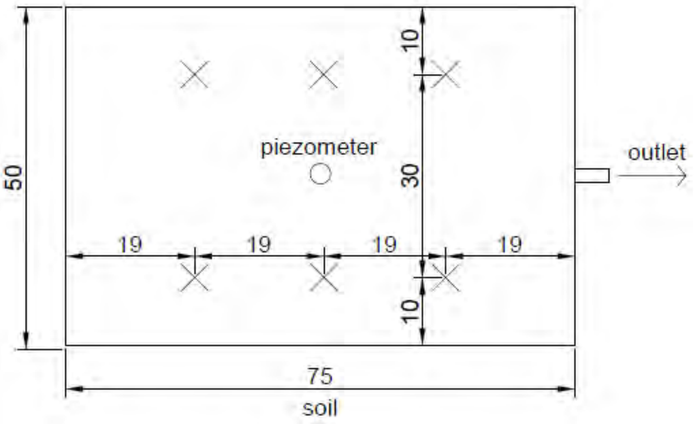

Figure 2. Position of sunflowers in vertical flow tanks (measures expressed in centimetres)

2.2.2 Horizontal subsurface flow tanks

The transparent tanks, sized 75 x 50 x 38 cm, were holed at the bottom and connected to a flexible pipe, 1.5 cm in diameter.

Each tank contained three vertical layers: a 10 cm thick layer composed of large gravels (4-6 cm) in the inlet zone; a 55 cm thick layer of seedbed, a 10 cm thick layer composed of medium/small gravels (1-2 cm) in the outlet zone (Figure 3). Between the layers, a plastic net has been inserted vertically to limit particles movement.

In the outlet zone a holed PVC pipe (with a diameter of 3 cm), transversal to the movement of water, was inserted within the gravel layer to promote liquid drainage.

A vertical plastic cylinder, 3 cm in diameter, was infixed in the seedbed to allow the measurement of the water table level by means of the insertion of a shaft, as a piezometer.

Feed was added exclusively on the inlet zone in order to reach its saturation (visually detectable: the amount added, therefore, was not constant; it changed proportionally to the quantity which crossed the central layer during the antecedent day).

Four plants, arranged in order to produce a rectangular pattern, were hosted in the central portion of each reactor (Figure 4). Plant density (23 plants/m^2) exceeded the maximum suggested value (19 plants/m^2) for Mediterranean Areas (Ibrahim, 2012; Barros *et al.*, 2004). It has been chosen to submit sunflowers to non optimal living conditions in order to maintain a precautionary approach on the test: if plants can successfully detoxify contaminants even if space available is limited, probably they may show better performances if optimal conditions are allowed.

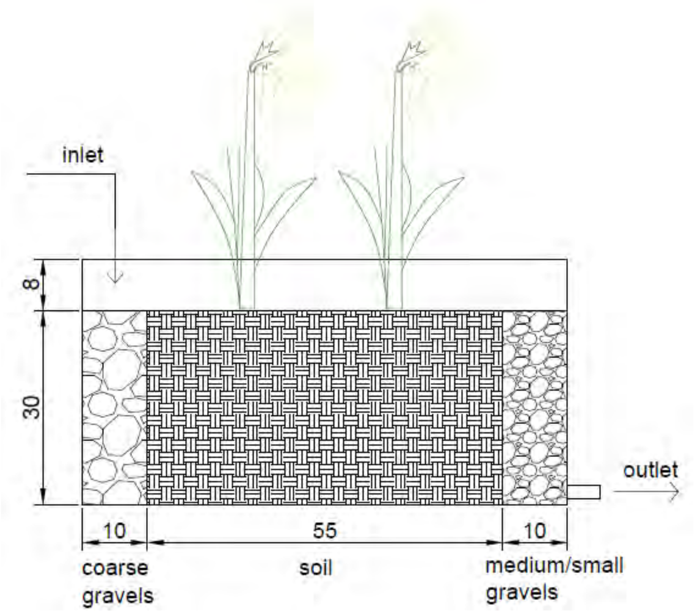

Figure 3. Cross section of a horizontal flow tank (measures expressed in centimetres)

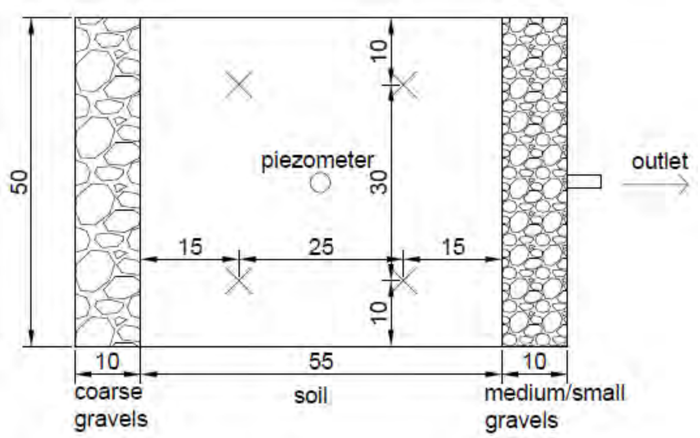

Figure 4. Position of sunflowers in horizontal flow tanks (measures expressed in centimetres)

2.3 The greenhouse

The greenhouse (Figure 5), a prefabricated commercial container, sized 6.00 x 2.20 m, ensured environmental conditions compatible with sunflowers farming: 14 hours of photoperiod allowed by the presence of nine lamps, temperature (regulated by means of an air conditioner) and frequency of irrigation: the feeding water was supplied manually, approximately 5 days per week.

Figure 5. Greenhouse used for the experiments

2.4 The soil

Two different seedbeds were used for horizontal and vertical flow respectively.

Long-term studies on the hydraulics of constructed wetlands with different soil parameters indicate that mixtures of soil and sand produce the best results in terms of both hydraulic condition and removal of contaminants. A suitable substrate should ensure an acceptable compromise between the needs for macroporosity, air circulation and root development while avoiding the presence of stagnant water (Jones et al., 2006; Leigue Fernàndez, 2014).

Soil textures, determined with the Bouyoucos Methods, are reported in Table 2.

According to the soil taxonomy proposed by USDA (USDA-NRCS, 1999), they were both classified as sandy loam.

Horizontal flow soil showed a higher sand content to favor water flow in the horizontal direction.

The main characteristics of the substrates are reported in Table 3, all the parameters were determined according to the Italian Analytical Standards (CNR-IRSA, 64/1986).

Table 2. Texture of soils used for the experiment

Tanks	**Clay (w/w %)**	***Silt (w/w %)***	**Sand (w/w %)**
Vertical flow	12	*16*	72
Horizontal flow	12	*12*	76

Table 3. Soils composition before the trial

Parameter	***Unit***	**Horizontal subsurface flow**	**Vertical flow**
TS	*w/w % (on raw sample)*	99.08	99.23
VS	*mg/kg*	1.53	2.03
Total carbon	*mg/kg*	31080	27500
Total organic carbon (TOC)	*mg/kg*	4530	2770
Total Nitrogen	*mg/kg*	420	320
Total Sulphur	*mg/kg*	450	470
Ca^{2+}	*mg/kg*	77.2	76.6
Mg^{2+}	*mg/kg*	1.9	2.0
Na^{+}	*mg/kg*	5.5	5.5
K^{+}	*mg/kg*	3.26	3.64

2.5 Chemical analysis

Landfill leachate and the outlet of each tank were analyzed according to the Italian analytical standards for water and wastewater samples (CNR-IRSA, 29/2003):

- pH (IRSA-CNR 29/2003 vol. 1 n. 2060)
- Total Kjeldahl Nitrogen, TKN (IRSA-CNR 29/2003 vol. 2 n. 5030)
- Ammonium nitrogen, NH_4^+ (IRSA-CNR 29/2003 vol. 2 n. 4030 A2, C)
- Total phosphorous, P_{TOT} (IRSA-CNR 29/2003 vol. 2 n. 4110 A2)
- Phosphates, PO_4^{3-} (IRSA-CNR 29/2003 vol. 2 n. 4110 A1)
- Total solids, TS (IRSA-CNR 29/2003 vol. 1 n. 2090 A)
- Volatile solids, VS (IRSA-CNR 29/2003 vol. 1 n. 2090 D)
- Chemical Oxygen Demand, COD (IRSA-CNR 29/2003 vol. 2 n. 5130)
- Biological Oxygen Demand, BOD (IRSA-CNR 29/2003 vol. 2 n. 5120 A, B, B2)
- Cloride, Cl^- (IRSA-CNR 29/2003 vol. 2 n. 4090 A1)
- Nitrate, NO_3^- (IRSA-CNR 29/2003 vol. 2 n. 4040 A1)
- Sulphate, SO_4^{2-} (IRSA-CNR 29/2003 vol. 2 n. 4140 B)
- Metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Na, Ca, K, Mg), (IRSA-CNR 29/2003 vol. 1 n. 3020)

2. 6 Oleaginous plants

Sunflowers (*Heliantus Annus*) can grow on lands characterized by low fertility and limited water availability; cultivations extend on 23 European countries, including Italy (Skolou et al., 2011).

Sunflower is a an annual plant which can reach 1 – 3.5 m in height. It has a thick, hairy, erect stem which gives rise to a large flower head. The plant has large, broad lower leaves which are oval and arranged alternately on the stem and smaller, narrower upper leaves which are attached individually to the stem. The flower head is a large disc reaching 10–30 cm in diameter which is made up of 16–30 individual florets which are yellow-gold in colour.

Sunflower is used to produce edible vegetable oil, which is extracted from its seeds. The residues, rich in nitrogen compounds, are used for animal feed in the form of oilseed cakes (Skolou et al., 2011).

Seeds of sunflowers grown in contaminated areas, or irrigated with landfill leachate or municipal/industrial wastewater, might be used to produce biodiesel (Lavagnolo *et al.*, 2011).

2.7 Landfill leachate

Leachate is a wastewater generated by excess rainwater percolating through the waste layers in a landfill (Christensen *et al*., 1992).

Landfilled waste is comprised of a wide range of inorganic, natural and xenobiotic compounds, the mixture of which in turn affects the composition and polluting potential of the leachate (Kjeldsen *et al*., 2002).

The pollutants are transferred from the waste material to the percolating water by a variety of physical, chemical and biological processes.

The quality of leachate depends on:

1. quality and type of waste
2. conditions in the landfill (aerobic, anaerobic or semi-aerobic)
3. the age of the landfill

The quantity depends on:

1. climatic and meteorological conditions
2. physical characteristics of waste
3. characteristic of the barrier system

Samples were collected in a closed anaerobic MSW landfill located in Mazzano (Brescia), North of Italy, in which untreated refuse municipal solid waste have been disposed of between 1983 and 1990.

Chemical characterization of leachate is reported in Table 4.

Values of TKN, ammonium nitrogen and the BOD to COD ratio (BOD/COD equal to 0.04) are typical of a leachate produced during the stable methanogenic phase (Stegmann *et al*., 2005; Jones *et al*., 2006).

Table 4. Mazzano leachate chemical characterization

Chemical analysis		
Parameter	**Value**	***Unit***
pH	8	-
TKN	1240	*mgN/l*
NH_4^+	1221	*mgN/l*
P_{TOT}	11	*mgP/l*
PO_4^{3-}	11	*mgP/l*
TS	4277	*mg/l*
VS	1102	*mg/l*
COD	1325	mgO_2/l
BOD	50	mgO_2/l
Cl^-	1138	*mgCl/l*
NO_3^-	0	*mgN/l*
SO42-	0	$mgSO4^{2-}/l$
Na	2700	*mgNa/l*
Ca	85	*mgCa/l*
K	1050	*mgK/l*
Mg	97	*mgMg/l*
Cd	10	*μgCd/l*
Cr	199	*μgCr/l*
Cu	138	*μgCu/l*
Fe	8358	*μgFe/l*
Mn	131	*μgMn/l*
Ni	179	*μgNi/l*
Pb	64	*μgPb/l*
Zn	946	*μgZn/l*

2. 8 Feeding mode

Plants were submitted to increasing leachate concentrations up to 30 % (Table 5).

TKN represents the main parameter monitored during the experiments to adjust the mixture between tap water and leachate: the reference value of 400 mgN/l, considered the upper limit of tolerance for plants (Leigue Fernàndez, 2014; Nicoletti, 2012), was not exceeded.

Average contaminants weekly loads are reported in Table 6. It should be noticed that the average weekly loads increased gradually over time to adapt plants to the presence of contaminants in the feeding water. The only exception is represented by tank V1R in which the loads applied were slightly reduced from Phase 2 to Phase 3A because plants were suffering, thus requiring the addition of a lower amount of water.

Table 5. Feeding trend of the tanks irrigated with diluted leachate

Phase	**Lasting days**	***Feed water composition (%)***		**Pollutants concentration (mg/l)**		
		Tap water	**Leachate**	***COD***	**P_{TOT}**	***TKN***
Acclimation	7	*100*	0	*10*	0.04	*0.1*
1	10	*90*	10	*142*	1.1	*124*
2	7	*80*	20	*273*	2.2	*248*
3A	14	*70*	30	*405*	3.3	*372*
3B	37	*70*	30	*405*	3.3	*372*

Table 6. Average contaminants weekly loads provided to the tanks, considering a week standard duration of seven days

Phase	**COD load (mgO_2/week)**	**P_{TOT} load (mgP/week)**	**TKN load (mgN/week)**	**COD load (mgO_2/week)**	**P_{TOT} load (mgP/week)**	**TKN load (mgN/week)**
1		**H1R**			**V1R**	
	2154	17.3	1937	1981	15.9	1781
		H2			**V2**	
	2526	20.2	1981	1981	15.9	1781
2		**H1R**			**V1R**	
	2457	20.1	2257	4095	33.5	3763
		H2			**V2**	
	4163	34.0	4095	4095	33.5	3763
3A		**H1R**			**V1R**	
	2882	23.7	2668	3742	30.8	3464
		H2			**V2**	
	4146	34.1	4955	4955	40.7	4588
3B		**H1R**			**V1R**	
	3692	30.4	3418	4132	34.0	3826
		H2			**V2**	
	12378	101.8	8379	8379	68.9	7759

3. RESULTS AND DISCUSSION

3.1 Sunflowers growth

During Acclimation Phase and Phase 1, seedlings grew uniformly in all reactors.

From Phase 2, the formation of visible superficial roots and yellow leaves, however, was an indication that sunflowers started to suffer.

During Phase 3A all plants showed old leaves desiccation and formation of black spots on new leaves, typical symptoms of root asphyxia. The problem was not associated to the presence of toxic compounds in the feed because even controls showed signs of stress; sunflowers were suffering the hydraulic regime to which they were submitted: the water table in the tanks was excessive and roots were not able to receive oxygen (Vartapetian and Jackson, 1997).

The complete drainage was necessary to avoid further damages to plants: total emptying of reactors allowed water expulsion and air intrusion within soil voids.

As already explained in the Research Program paragraph, Phase 3B was characterized by opened drainage to guarantee proper oxygen intrusion into the soil.

Bloom of plants occurred in the middle of Phase 3B (week 7), followed by a sudden senescence.

At the end of week 9 complete senescence was achieved so plants were harvested and dried in the oven. The total dry biomass was determined to evaluate vegetation growth (Table 7).

In horizontal flow tanks, leachate did not limit vegetation development: the average dry weight of plants grown in reactors H1R (12.40 g/plant) and H2 (14.08 g/plant) almost matches the values of the corresponding control HC (12.19 g/plant).

In reactor V2, sunflowers did not suffer the presence of landfill leachate in the feeding water because the average weight (20.64 g/plant) is almost equal to the control VC (20.10 g/plant). Plants of reactor V1R, on the contrary, displayed an average dry weight almost half of those grown on VC. Stress of sunflowers planted on this tank was more evident than the other plants during Phase 3A: probably the subsequent development was still affected by the period of stress, resulting in a limited biomass growth. The removal of contaminants, anyway, was excellent and aligned with the other tanks, as discussed in the following chapters.

Vegetation growth is determined by nitrogen and phosphorous bioavailability. In general, N:P ratios in plant tissues <10 and >20 correspond to nitrogen and phosphorous limited biomass growth respectively (Gusewell S., 2004).

Values detected in sunflowers after harvesting are reported in Table 8.

Table 7. Sunflowers total and average dry weights. [Average weights based on 4 plants on horizontal tanks, 6 plants on vertical tanks]

	Total dry weight (g)	***Average dry weight (g/plant)***
Leaves H1R	15.84	*3.96*
Stems H1R	31.35	*7.84*
Roots H1R	2.39	*0.60*
TOTAL H1R	**49.58**	***12.40***
Leaves H2	19	*4.75*
Stems H2	35.49	*8.87*
Roots H2	1.84	*0.46*
TOTAL H2	**56.33**	***14.08***
Leaves HC	12.84	*3.21*
Stems HC	34.41	*8.60*
Roots HC	1.51	*0.38*
TOTAL HC	**48.76**	***12.19***
Leaves V1R	21.04	*3.51*
Stems V1R	37.1	*6.18*
Roots V1R	3.07	*0.51*
TOTAL V1R	**61.21**	***10.20***
Leaves V2	43.8	*7.30*
Stems V2	75.49	*12.58*
Roots V2	4.57	*0.76*
TOTAL V2	**123.86**	***20.64***
Leaves VC	40.49	*6.75*
Stems VC	75.4	*12.57*
Roots VC	4.65	*0.78*
TOTAL VC	**120.54**	***20.10***

Table 8. N:P ratios detected in sunflowers

Tank	**N : P ratios (mgN/mgP)**
H1R	27.50
H2	28.31
HC	21.75
V1R	19.81
V2	16.17
VC	19.67

Plants grew in horizontal flow tanks, even controls, are characterized by N:P ratios above 20. Since sunflowers density exceeded the maximum values described in literature, probably the amount of phosphorous added was not sufficient to sustain properly the biomass development. Sunflowers grown in vertical flow tanks and fed with diluted leachate, on the contrary, displayed values ranging between10 and 20, indicating optimal nitrogen and phosphorous supply and a balanced development (Gusewell S., 2004).

Analysis of values reported in Table 8 demonstrates that vertical flow tanks are more suitable to host plants and that sunflowers can grow with an optimal nutrients balance.

The knowledge of seeds production (Table 9) is fundamental to estimate oil yield, which is the basis for evaluating the feasibility of the systems as source of biodiesel production.

The amount of seeds produced is directly proportional to the biomass development, as shown by a comparison between Tables 7 and 9. Plants grown in tanks V2 and VC, characterized by the highest average dry mass values, produced the highest amounts of seeds.

Table 9. Dry weights of seeds produced by sunflowers [Average weights based on 4 plants on horizontal tanks, 6 plants on vertical tanks]

Tank	**Total seeds production (g)**	**Average seeds production (g)**
H1R	8.57	2.14
H2	9.43	2.35
HC	8.87	2.21
V1R	12.00	2.00
V2	24.01	4.00
VC	23.16	3.86

3.2 Influent and effluent volumes

Analysis of influent (V_{IN}) and outlet volumes (V_{OUT}) allows to estimate the weekly evapo-transpiration (ET), which results from the combined effect of plants requirements, evaporation from soil and transpiration from leaves (Figure 6):

$$\boldsymbol{ET = V_{IN} - V_{OUT} \quad (L/week)}$$

In horizontal subsurface flow tanks, feed was added to saturate the inlet zone and promote water flow across the central soil layer.

In vertical flow tanks, water addition aimed to produce a water head of 1-1.5 cm on the surface, indicating the achievement of the saturation.

During the entire experimental period, the average temperature was 27 °C, with a minimum of 24 °C and a maximum of 32 °C.

From Phase 1 (week 1) to Phase 3A (week 4) the weekly feed addition was gradually reduced in all tanks from almost 20 L/week (corresponding to 53 L/week/m^2 in vertical flow tanks, 114 L/week/m^2 in horizontal flow tanks) to almost 5 L/week (corresponding to 13.25 L/week/m^2 in vertical flow tanks, 28.5 L/week/m^2 in horizontal flow tanks) to balance the increasing amount of added contaminants due to the increasing leachate fraction present in the feed.

Throughout Phase 3B, the volume added increased gradually in all tanks up to 30 L/week in week 7 (characterized by plants bloom) and reached a peak of almost 35 L/week in H2 (corresponding to 200 L/week/m^2). During week 8 the volume added decreased because the temperature reached the minimum value in the greenhouse and evapo-transpiration process was limited. The amount increased again during week 9 reaching the same values detected in week 7. From Phase 1 to Phase 3A all tanks showed different performances in terms of evapo-transpiration capacity, with vertical flow reactors displaying the best potential (more than 10 L/week). In Phase 3B evapo-transpiration was comparable in all horizontal flow reactors and equal to almost 10 L/week (57 L/week/m^2), while it was relevant in tanks V2 and VC (almost 20-25 L/week; 53-66 L/week/m^2). V2 and VC were characterized by high biomass development, therefore the phenomenon was mainly due to transpiration from leaves.

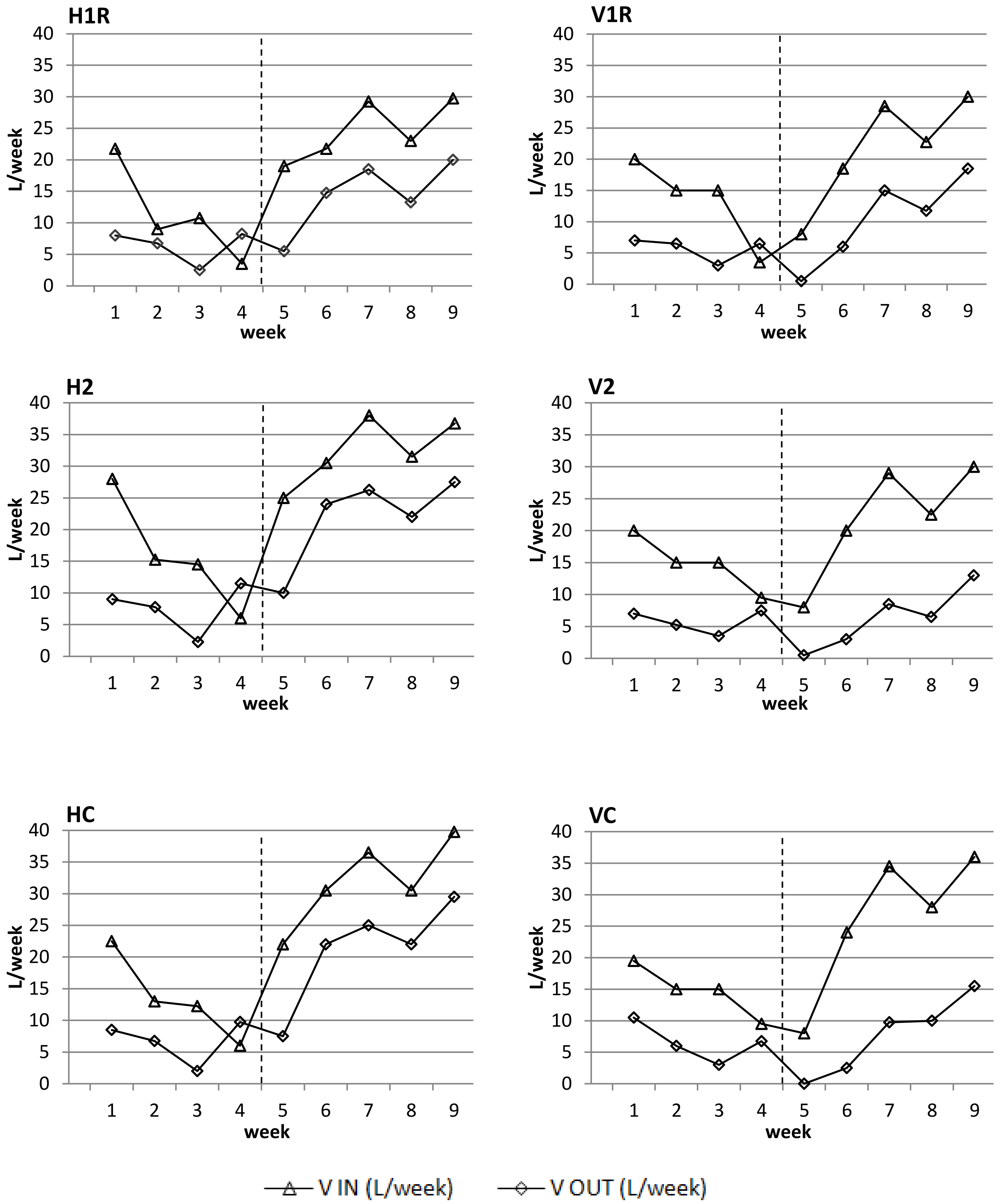

Figure 6. Volumes added (L/week) and volumes drained (L/week) over the whole experimental period. The dotted vertical lines indicate the beginning of Phase 3B

3.3 Water table levels

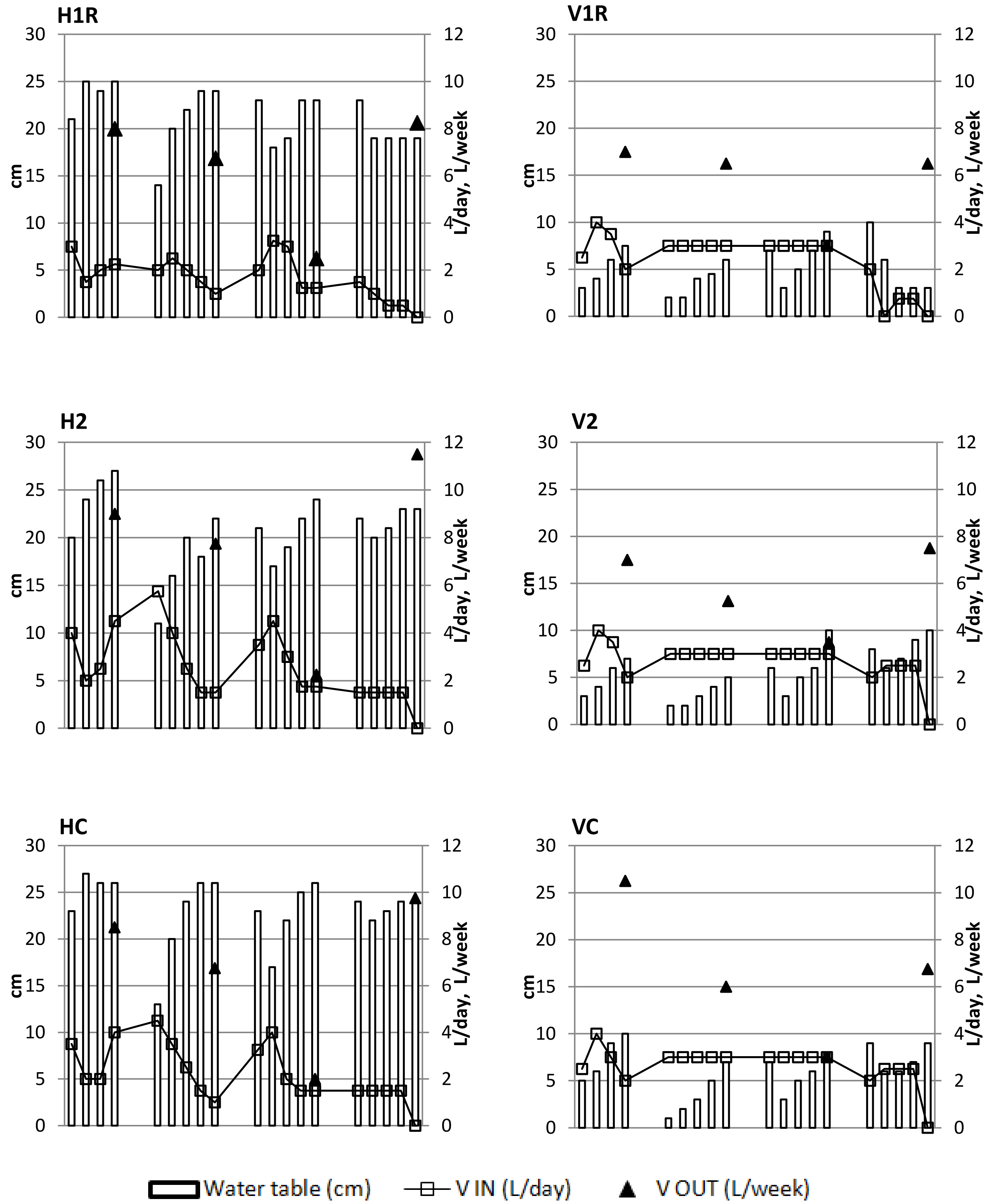

Figure 7. Volume added (L/day), volume drained (L/week) and water level inside the tanks (cm) throughout Phases 1, 2 and 3A (weeks 1-4). In horizontal flow tanks the level is referred to the central layer

During Phases 1, 2 and 3A (weeks 1 - 4) the water table level was measured and recorded every day, from Monday to Friday, through the insertion of a shaft in the piezometers (Figure 7).

Horizontal tanks displayed a similar trend in terms of water level throughout the entire period of observation, even if volumes added and drained were different. The level used to increase from Monday to Friday due to water addition and used to drop the subsequent Monday because feed was not added during the weekend.

Vertical flow reactors behaved in the same way throughout weeks 1-3; during week 4 the level lowered drastically in tank V1R because the volume introduced was significantly reduced, since plants stress was more evident than those hosted in the other reactors.

It should be noticed that levels were completely different in the systems: in vertical tanks the levels never exceeded 10 cm. In vertical flow reactors, in fact, water flows driven by gravity and tends to accumulate on the bottom filled with gravels; in horizontal one, on the contrary, the water level is determined by the amount of water present in the load zone. Since it was kept saturated, in the central layer the water table remained very close to the surface throughout the test.

The transparency of tanks allowed the possibility to monitor the root apparatus development: from Phase 2 it was clear that roots tended to exit the soil and reach the gravel layers. In vertical flow tanks, in fact, several visible roots reached the bottom gravel layer; in horizontal subsurface reactors roots slotted in the lateral gravel layers. Probably they were already suffering the phenomenon of root asphyxia so they were looking for oxygen in layers characterized by high porosity and presence of voids filled with air.

3.4 Outlet recirculation in tanks V1R and H1R

As already discussed in the Research Program paragraph, from Phase 3B outlet recirculation procedure started in tanks V1R and H1R. If the effluent was not sufficient, diluted leachate was added to reach the saturation of the soil or of the load zone respectively.

Volumes used are reported in Table 10.

In tank H1R the fraction re-circulated decreased progressively, displaying a reduced variability; in tank V1R it increased up to week 7, after that it remained constant.

Throughout the entire period of recirculation, in tank H1R most of the feed added was constituted by the outlet (61 %), contrary to V1R in which diluted leachate was preeminent.

A direct comparison of the systems is not possible because the hydraulic processes occurring are completely different. Despite that, even if sunflowers of tank V1R were slightly less developed than those grown on H1R, as shown by the average dry weights, the evapo-transpiration per dry biomass unit was higher: 1.30 L/g in V1R, 1.27 in H1R. Therefore V1R generated less effluent, resulting in a higher capacity to treat leachate.

Table 10. Volumes re-circulated and diluted leachate added in tanks V1R and H1R from week 5 to week 9

Week	***Unit***	**H1R**		**V1R**	
		Outlet recirculated	***Diluted leachate added***	**Outlet recirculated**	***Diluted leachate added***
Week 5	*L/week*	13	*6*	1	*7*
	%	68	*32*	13	*87*
Week 6	*L/week*	14.25	*7.50*	6	*12.50*
	%	66	*34*	32	*68*
Week 7	*L/week*	18.50	*10.75*	15	*13.50*
	%	63	*37*	53	*47*
Week 8	*L/week*	12.75	*10.25*	11.75	*11*
	%	55	*45*	52	*48*
Week 9	*L/week*	14.50	*10.25*	13	*12*
	%	59	*41*	52	*48*
Total	***L/week***	**75.50**	***48.25***	**52.75**	***59.50***
	%	**61**	***39***	**47**	***53***

3.5 Hydraulic retention time

Hydraulic retention time (HRT) affects phytotreatment performances. The effect of HRT may differ between constructed wetlands depending on the wastewater treated, plant species and temperature (Wu *et al.*, 2014). Huang et al. (2000) reported that ammonium and total nitrogen concentrations in treated effluent decreased with increasing HRT. Scientific literature recommends a minimum HRT of 7 days for landfill leachate phytotreatment applications (Kylefors, 1997); but a minimum value of 15 days, resulting from previous experiences with sunflowers, is strongly recommended (Nicoletti, 2012).

In the case under examination, the HRT has been calculated as:

$$HRT = \frac{V_{SATURATION}}{Q_{IN} - Q_{OUT}}$$

in which:

- $V_{SATURATION}$ is the volume used to saturate the soil in the reactors (L), maintained constant over the whole experimental period
- Q_{IN} is the weekly influent flow rate (L/week)
- Q_{OUT} is the weekly effluent flow rate (L/week)

The HRTs applied during the experiment is summarized in Table 11.
They exceeded the minimum literature recommended value in all tanks in all Phases.

Table 11. HRT applied during the trial in the tanks irrigated with diluted leachate

Phase	***Unit***	**H1R**	***V1R***	**V2**	***H2***
1	*Week*	2.2	*2.7*	1.8	*3.0*
2	*Week*	3.3	*4.2*	4.6	*3.9*
3A	*Week*	3.6	*3.0*	2.8	*3.3*
3B	*Week*	-	-	-	-
3C	*Week*	3.7	*3.8*	3.6	*2.8*

3.6 COD removal

COD is the measurement of oxygen needed for chemical oxidation of organic matter; therefore it provides indirect information on the organic content of the sample.
COD was supplied at progressively increasing loads up to week 3 (first week of Phase 3A), ranging from almost 2000 mgO_2/week (corresponding to 5333 $mgO_2/week/m^2$ for vertical flow, 11428 $mgO_2/week/m^2$ for horizontal flow) to 6000 mgO_2/week (corresponding to 16000 $mgO_2/week/m^2$ for vertical flow, 34284 $mgO_2/week/m^2$ for horizontal flow) (Figure 8). The removal efficiency should take into account the role of HRT; in this case, however, it was calculated considering exclusively the weekly loads because data available were based on weekly units:

$$\eta\,(\%) = \frac{(Q_{IN} * C_{IN} - Q_{OUT} * C_{OUT}) * 100}{Q_{IN} * C_{IN}}$$

in which:

- Q_{IN} is the weekly influent flow rate (L/week)
- Q_{OUT} is the weekly effluent flow rate (L/week)

- C_{IN} is the inlet concentration (mgO_2/L)
- C_{OUT} is the outlet concentration (mgO_2/L)

Performances of reactors, in the initial period, can be compared because they were managed in the same way: they all showed removal efficiencies ranging from 80% to over 90%. During week 4 (second week of Phase 3A) the volume added was lowered to overcome the stress phase of plants, thus reducing the COD load provided. Subsequently it increased again reaching the maximum during week 7 of Phase 3B (except for H1R, in which the maximum was reached during week 9 of Phase 3B).

H1R and V1R never exceeded the value of 6000 mgO_2/week due to the introduction of the outlet recirculation procedure while in H2 and V2 peaks of 14000 and 12000 mgO_2/week (80000 and 32000 $mgO_2/week/m^2$) were detected respectively.

Throughout Phase 3B, removal efficiencies decreased progressively to 50 – 60 % in tanks H2 and V2, while they remained always above 90% on tanks with outlet recirculation.

Figure 8 demonstrates that even if removal efficiencies of H2 and V2 were lower, the amount of organics removed was greater than H1R and V1R, indicating better decontamination properties.

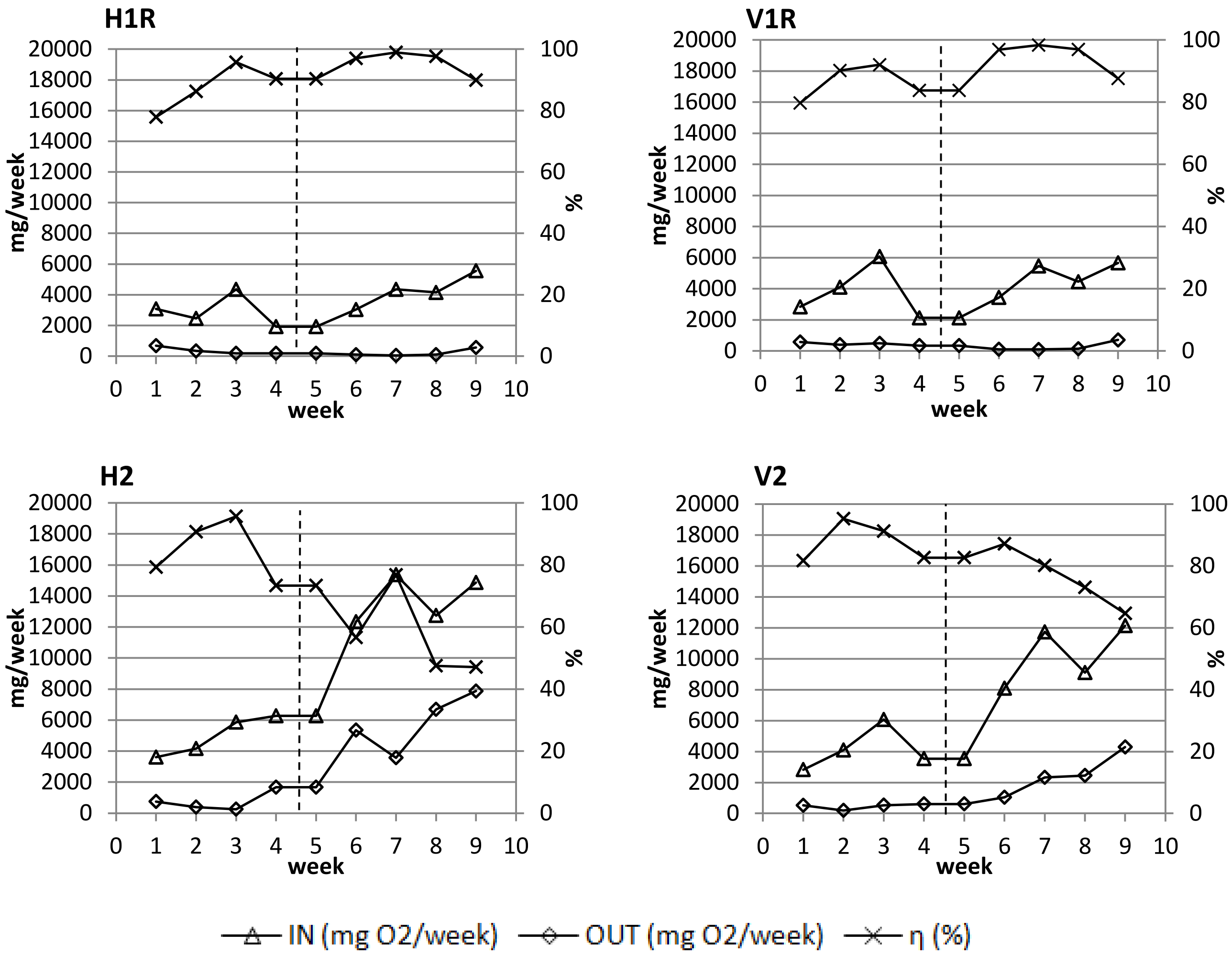

Figure 8. COD weekly input load (mgO_2/week), COD weekly output load (mgO_2/week) and COD removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

Focusing on effluent concentrations (Figure 9), during Phases 1, 2 and 3A all tanks respected the Italian discharge limit for surface water of 160 mgO_2/l (D. Lgs., 152/2006), in particular those characterized by horizontal subsurface flow remained always below 100 mgO_2/l. During Phase 3B, on the contrary, the law limit was not fulfilled several times by all reactors, even if loads applied were comparable to those applied in the first part of the test, as shown by the behavior of tanks H1R and V1R.

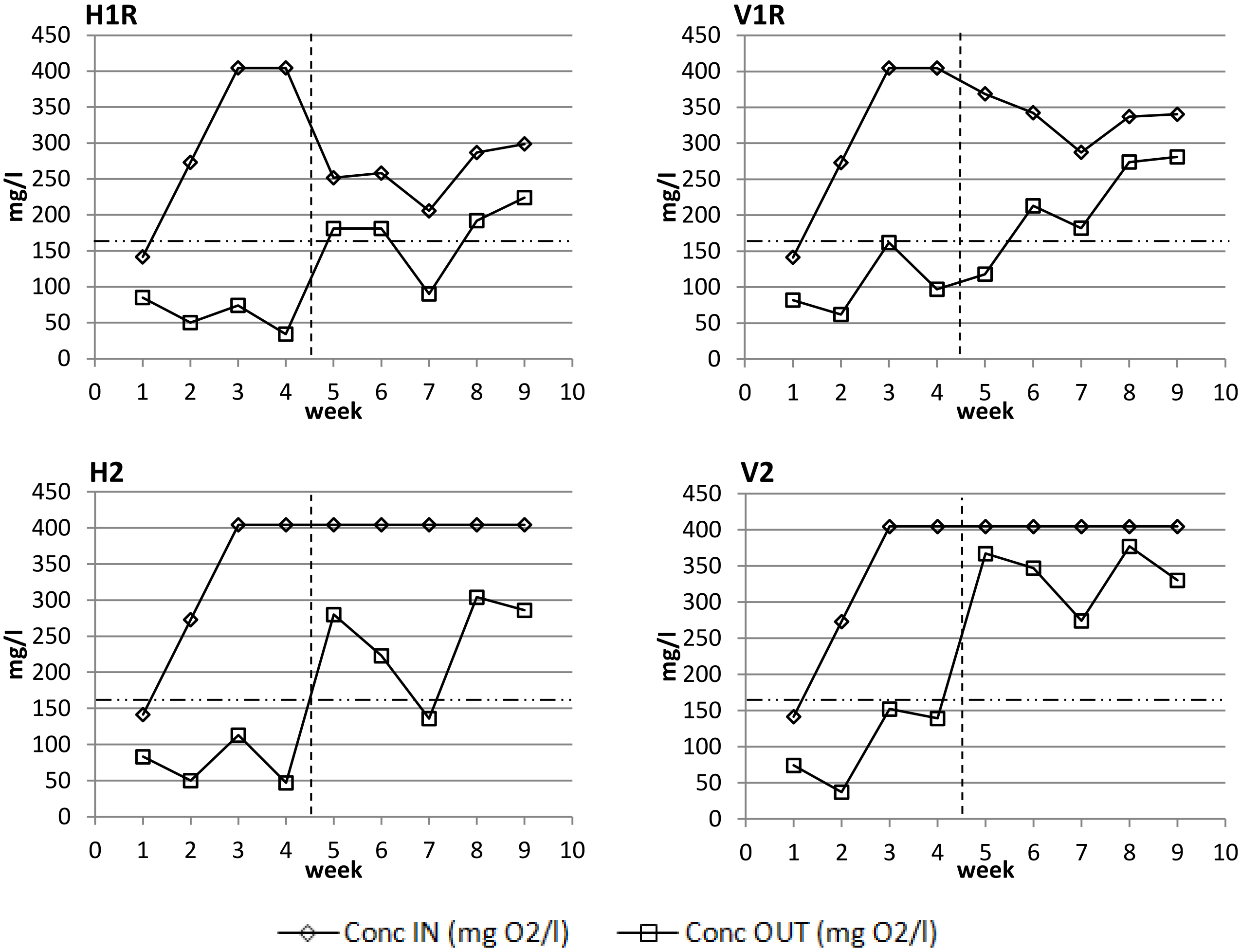

Figure 9. COD input concentration (mgO_2/l) and COD output concentration (mgO_2/l). The dotted vertical lines indicate the beginning of Phase 3B, the dotted horizontal lines indicate the discharge law limit

3.7 Nitrogen removal

From week 1 (Phase 1) to week 3 (first week of Phase 3A), all tanks were submitted to increasing nitrogen loads (Figure 10), starting from approximately 2000 mgN/week (corresponding to 5333 mgN/week/m^2 for vertical flow, 11428 mgN/week/m^2 for horizontal flow) and reaching values of almost 6000 mgN/week (corresponding to 16000 mgN/week/m^2 for vertical flow, 34284 mgN/week/m^2 for horizontal flow).

The removal efficiency, based on weekly loads (as already seen for the COD) was above 90 % in all cases and remained always close to 100 % in H1R and H2.

When the hydraulic regime changed (Phase 3B), the load was increased again reaching the maximum during week 7, which corresponded to plants bloom.

In tanks V1R and H1R it stayed always below 6000 mgN/week because of the recirculation of the already phytoremediated outlet, while in reactors H2 and V2 reached a peak of 14000 mgN/week and 11000 mgN/week respectively (80000 mgN/week/m^2 and 29333 mgN/week/m^2 respectively).

Removal efficiency remained above 90% in tanks V1R and H1, while it decreased progressively in the other reactors to 60 – 70 %.

Figure 10 demonstrates that even if removal efficiencies of H2 and V2 were lower, the amount of nitrogen removed was greater than H1R and V1R, indicating better decontamination properties.

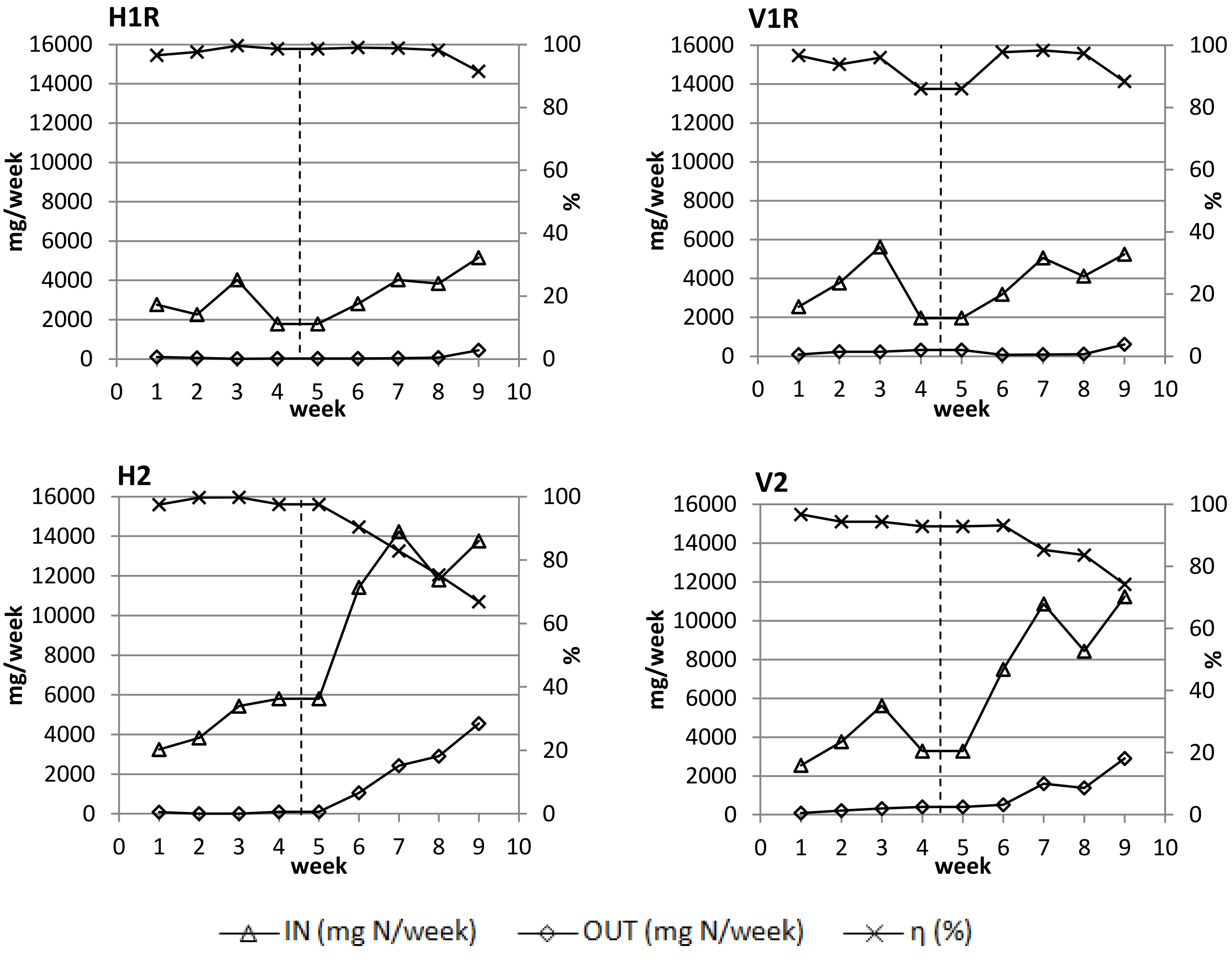

Figure 10. Total nitrogen weekly input load (mgN/week), total nitrogen weekly output load (mgN/week) and nitrogen removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

In Phases 1, 2 and 3A the output concentration remained below 100 mgN/l in all reactors (Figure 11). The results are aligned with the best performances described in literature: Cheng and Chu, in a similar test with vertical flow reactors, (Cheng and Chu, 2011) demonstrated that nitrogen concentration in the effluent water stayed below 90 mgN/l with an input load of 19600 mgN/week/m^2. In horizontal flow tanks, however, even if the weekly load per unit area was almost double than vertical flow, the effluent concentrations of ammonium ion and nitrate fulfilled the Italian discharge law limit (15 mgNH_4^+/L for ammonia, 20 mgN/L for nitrate). Therefore horizontal subsurface flow applications may be suggested to treat high strength wastewater when the discharge limits are restrictive.

The same consideration is not valid if the drainage system is maintained opened (Phase 3B). H1R and V1R demonstrated that even if loads applied were similar throughout the entire trial, the outlet concentration increased significantly.

Nitrification (ammonia oxidation to nitrate) was evident in tanks with outlet recirculation: recirculation promoted water oxygenation and thus the oxidation of ammonium nitrogen. Almost all the effluent nitrogen, in fact, exited the system as nitrate.

Focusing on tanks without recirculation, nitrification was more present in tank V2 than H2 since most of the influent ammonia has been converted to nitrate (Figure 11), proving that vertical flow reactors are more indicated for nitrification processes.

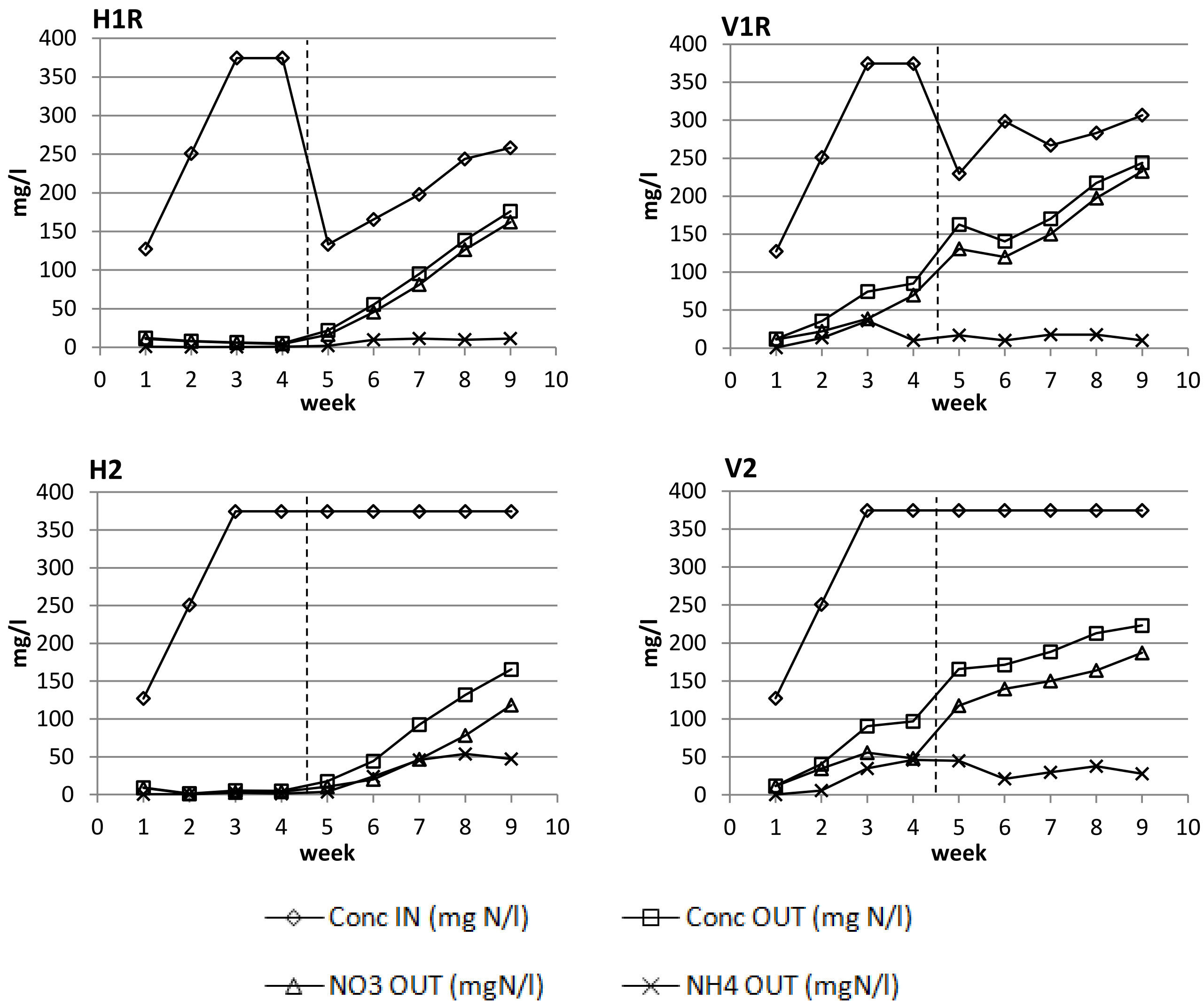

Figure 11. Total nitrogen input concentration (mgN/l), total nitrogen output concentration (mgN/l), nitrate output concentration (mgNO3-N/l), ammonium nitrogen output concentration (mgNH4-N/l). The dotted vertical lines indicate the beginning of Phase 3B

3.8 Phosphorous removal

Phosphorous removal capacity showed excellent performances for the whole experimental period (Figure 12). The removal efficiency, based on weekly loads, was always above 90 % in all reactors, even when input loads applied were remarkable (Phase 3B): 120 mgP/week in tank H2 (equal to 685 mgP/week/m^2), 100 mgP/week in tank V2 (corresponding to 267 mgP/week/m^2).

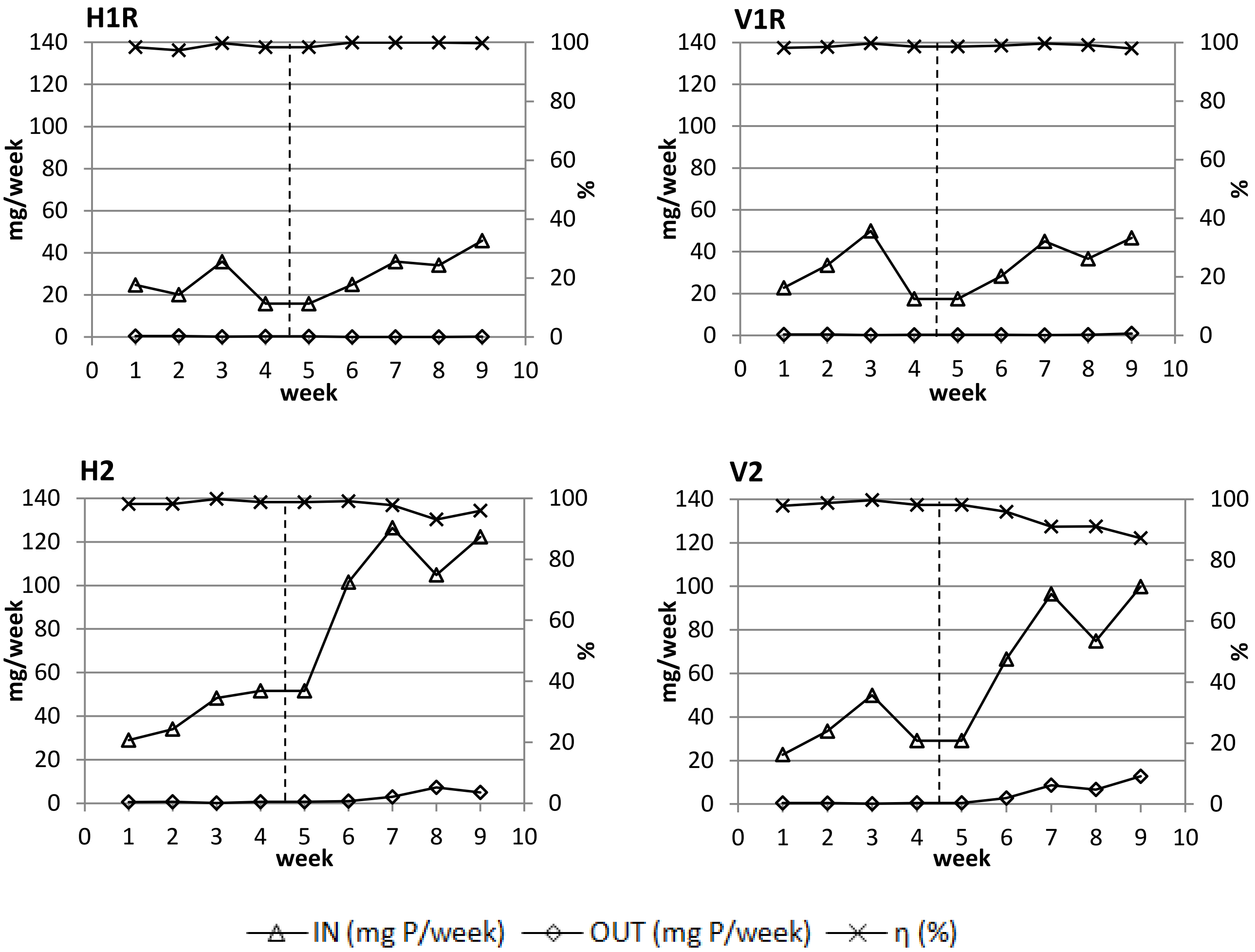

Figure 12. Total phosphorous weekly input load (mgP/week), total phosphorous weekly output load (mgP/week) and phosphorous removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

Phosphorous in the effluent stayed always below 10 mgP/l (Figure 13), the Italian discharge limit for surface water (D. Lgs., 152/2006).

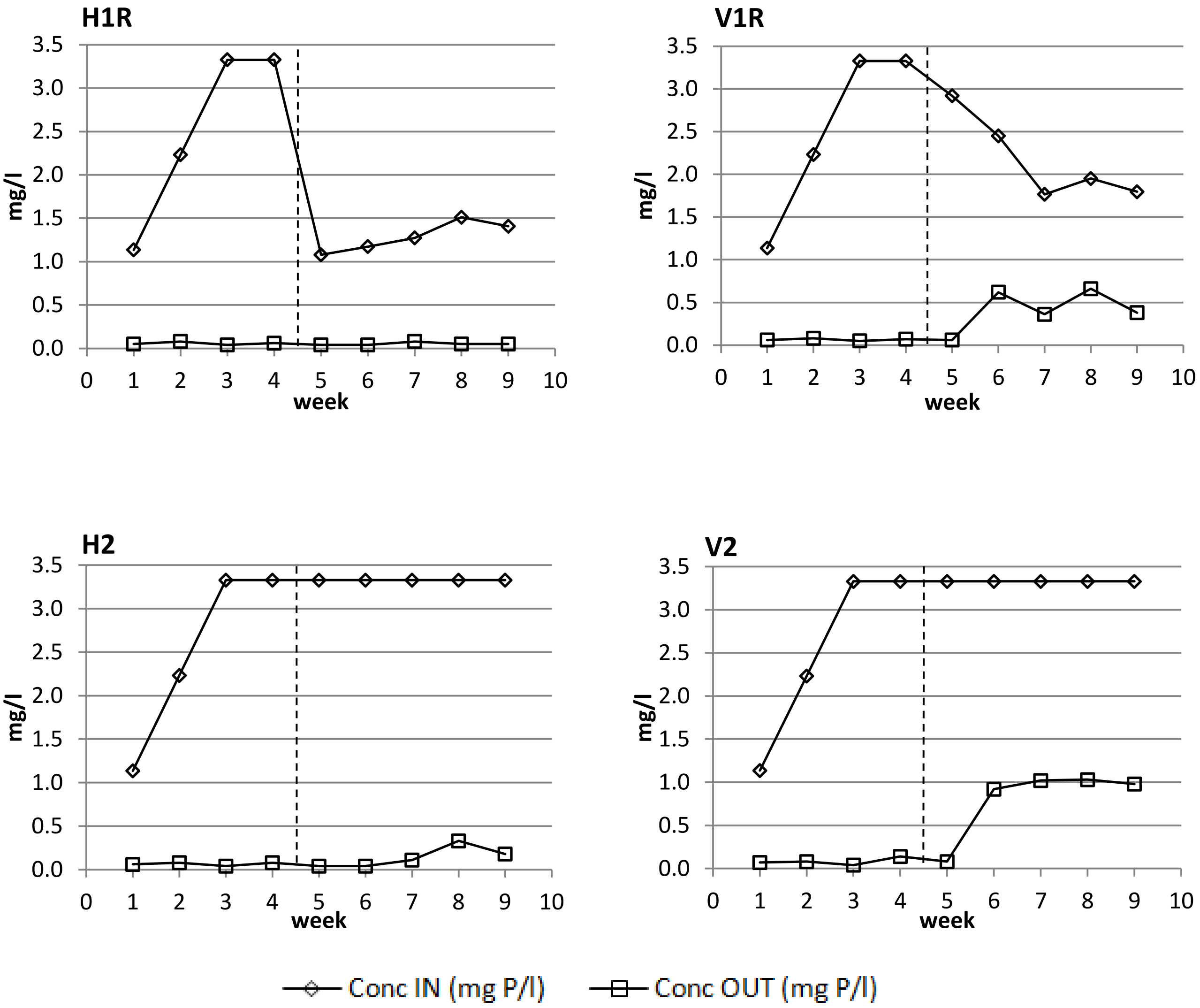

Figure 13. Total phosphorous input concentration (mgP/l) and total phosphorous output concentration (mgP/l). The dotted vertical lines indicate the beginning of Phase 3B

3.9 Nitrogen mass balance

Nitrogen mass balance was performed on all reactors to evaluate distribution among the main system components: water, soil and plants.

Most of nitrogen entered the system in form of ammonium ion (Tables 4 and 5): a small fraction was detected in the outlet, a portion was adsorbed by the soil matrix as organic nitrogen (Table 12), another portion was converted to nitrate (Figure 11). Subsequently nitrate was taken up by sunflowers or released in the effluent water.

Nitrogen inputs always exceeded the corresponding outputs, suggesting the occurrence of denitrification processes, as already observed by Cheng and Chu (Cheng and Chu, 2011).

The high temperatures present in the greenhouse and the pH of water (almost 8), indeed, might had promoted ammonia volatilization, as reported by Freney and Simpson (Freney and Simpson, 1983).

In tanks H2 and V2 the nitrogen loss was on average 39 %; in tanks with outlet recirculation was on average 19 %.

Nitrification was more evident in tanks V1R and H1R due to re-aeration of the feeding water: probably this practice negatively influenced the denitrification activity, which was inhibited by oxygen availability. The complete nitrogen removal in atmospheric form could be achieved by setting up a treatment train composed of a tank with outlet recirculation, whose effluent, rich in nitrate, should be treated in a tank without recirculation to simulate nitrification-denitrification processes.

Mass balances proved that sunflowers had a limited role in the removal of nitrogen, while showed the importance of soil, which acted as a filter. It demonstrates that phytoremediation consists of a series of different phenomena, which cannot be separated and discussed individually.

Controls displayed reduced soil nitrogen content at the end of the trial, proving that plants were forced to use the nitrogen stored in the seedbed to sustain their own growth.

Table 12. Nitrogen mass balance of reactors. Units expressed as mgN.

	H1R		**V1R**		**H2**		**V2**		**HC**		**VC**	
	IN	***OUT***	**IN**	***OUT***	**IN**	***OUT***	**IN**	***OUT***	**IN**	***OUT***	**IN**	***OUT***
WATER	28186	*5669*	33178	*10304*	72153	*12514*	56060	*15019*	767	*2725*	682	*986*
SOIL	31311	*38095*	33120	*46472*	31311	*34964*	33120	*49473*	31311	*18190*	33120	*23495*
PLANT		*1057*		*1507*		*1597*		*2008*		*1145*		*2607*
TOTAL	**59497**	***44821***	**66298**	***58283***	**103464**	***49075***	**89180**	***66500***	**32078**	***22060***	**33802**	***27088***

4.0 Metals outlet concentration

Metals outlet concentration measured during Phase 3B is reported in Table 13.

The values fulfilled the Italian discharge limits in surface water (D. Lgs. 152/2006), except for copper and zinc.

Further researches are required to identify operational strategies which allow the possibility to fulfill all emission limits.

Table 13. Metals concentrations measured during Phase 3B

	Cd	Cr	***Cu***	**Fe**	***Mn***	**Ni**	***Pb***	**Zn**
	µg/l	µg/l	*µg/l*	µg/l	*µg/l*	µg/l	*µg/l*	µg/l
H1R	*<10*	<20	*149*±9	316±46.9	*53.7*±22.9	79.4±14.2	*<20*	168±6
H2	*<10*	33±8.5	*183.5*±5.5	403±14	*314*±16	118.5±7.5	*<20*	191±30
HC	*<10*	<10	*<20*	<10	*<10*	<10	*<10*	<10
V1R	*<10*	25.8±5.4	*196*±15	607±70	*91.5*±31.5	71.2±26	*<20*	581.5±41.5
V2	*<10*	40.5±1.5	*197*±6	852.5±104.5	*204*±56	108±4.5	*<20*	595±18
VC	*<10*	<10	*<10*	<10	*<10*	<10	*<10*	<10

4. CONCLUSIONS

Leachate decontamination via sunflowers phytotreatment proved to be feasible under lab-scale conditions. Sunflowers were hosted in six reactors, four of them fed with diluted leachate, the others used as controls. Two treatment systems were tested: vertical and horizontal subsurface flow. The trial included different hydraulic practices: drainage system opened once a week during Phases 1, 2 and 3A; continuously opened during Phase 3B. From Phase 3B, indeed, recirculation of outlet started in two tanks: one characterized by vertical flow (V1R), the other by horizontal subsurface flow (H1R).

From Phase 1 to Phase 3A all reactors were submitted to increasing pollutants concentrations in the feeding water which resulted in increasing contaminants loads. After that, pollutants concentrations remained constant. TKN, used as the reference parameter to adjust the leachate dosage, was increased from 124 mgN/L of Phase 1 up to 372 mgN/L of Phase 3A and subsequent.

Throughout Phases 1-3A, COD and TKN loads increased from 5333 $mgO_2/week/m^2$ to 16000 $mgO_2/week/m^2$ in vertical reactors, from 11428 $mgO_2/week/m^2$ to 34284 $mgO_2/week/m^2$ in horizontal reactors.

In this period all tanks displayed excellent removal performances: above 80 % for COD; above 90 % for nitrogen. Phosphorous represented the contaminant of less concern: removal efficiency remained close to 100 %.

COD and phosphorous outlet concentrations stayed below the discharge limits set by the Italian legislation (D. Lgs. 152/2006), while nitrogen effluent concentration displayed a certain dependence on feed water composition, especially in vertical flow reactors.

The water table level remained very close to the surface in horizontal flow reactors, while it never exceeded the value of 10 cm from the bottom in vertical tanks.

At the end of Phase 3A stress of sunflowers was evident: they were suffering the hydraulic regime to which they were submitted and the phenomenon of root asphyxia was occurring.

From Phase 3B the drainage system was maintained continuously opened to promote air intrusion into the soil; contemporarily outlet recirculation started in V1R and H1R. Pollutants loads was further increased in tanks H2 and V2 reaching peaks of 80000 and 32000 $mgO_2/week/m^2$ respectively; 80000 and 29333 $mgN/week/m^2$ respectively; 685 $mgP/week/m^2$ and 267 $mgP/week/m^2$ respectively.

In tanks with outlet recirculation removal efficiencies of the main pollutants remained above 80%; in tanks H2 and V2 phosphorous removal efficiency stayed above 90% while TKN and COD showed performances decreasing over time up to 60%: the amount of contaminants removed, however, was greater than H1R and V1R.

During this Phase only the Italian emission limits for phosphorous have been always fulfilled by all tanks; COD and nitrogen outlet concentrations, on the contrary, stayed often above the maximum admitted values in all reactors.

Vertical flow tanks seemed to be more suitable to host sunflowers: plants grown on tanks V2 and VC showed a biomass development almost double than those planted on horizontal reactors; the only exception was represented by sunflowers of V1R; in all cases, however, the N:P ratio ranged between 10 and 20, indicating balanced growth (Gusewell, 2004) and proving leachate suitability as non edible crop fertilizer.

A well developed biomass allowed to treat a higher amount of contaminated wastewater, since the evapo-transpiration processes were significant: up to 66 $L/week/m^2$ in vertical reactors.

Nitrogen mass balance confirmed the occurrence of nitrification-denitrification phenomena, ammonia volatilization and the role of the soil acting as a filter for nutrients. Phytotreatment, in fact, is due to a combination of several processes, and not the result of the mere activity of sunflowers.

The complete nitrogen removal in atmospheric form could be achieved by setting up a treatment train composed of a tank with outlet recirculation, whose effluent, rich in nitrate, should be treated in a tank without recirculation to simulate nitrification-denitrification processes.

A field test is strongly recommended to evaluate the underlying phenomena under real operating conditions. Exposure to weathering conditions is fundamental to adjust design parameters and to establish the optimal leachate load.

Further investigations are required to identify the potential energetic use of plants residues which are not used to produce biodiesel: leaves and stems.

Other wastewater streams could be phytoremediated with the technique presented here (e. g. septic tank effluents) in order to ensure a sustainable and safe treatment; detailed researches are needed to evaluate feasibility and possibility to fulfill the discharge law limits in all operating conditions.

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SECTION III:

Annexes

ANNEX 1

Cloride input and output weekly loads; removal efficiency based on weekly loads; inlet and outlet concentrations.

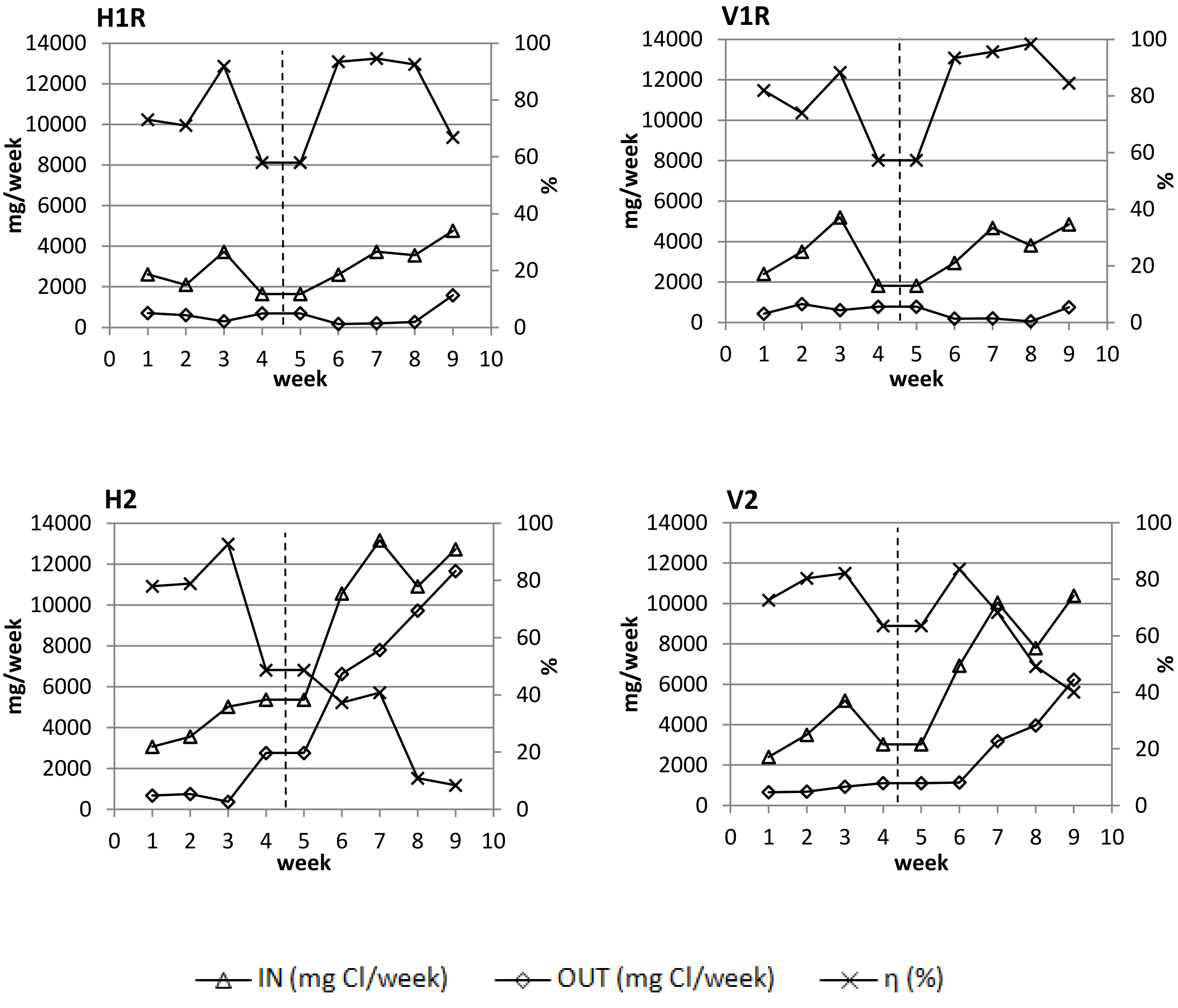

Figure 1. Cloride weekly input load (mgCl/week), cloride weekly output load (mgCl/week) and cloride removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

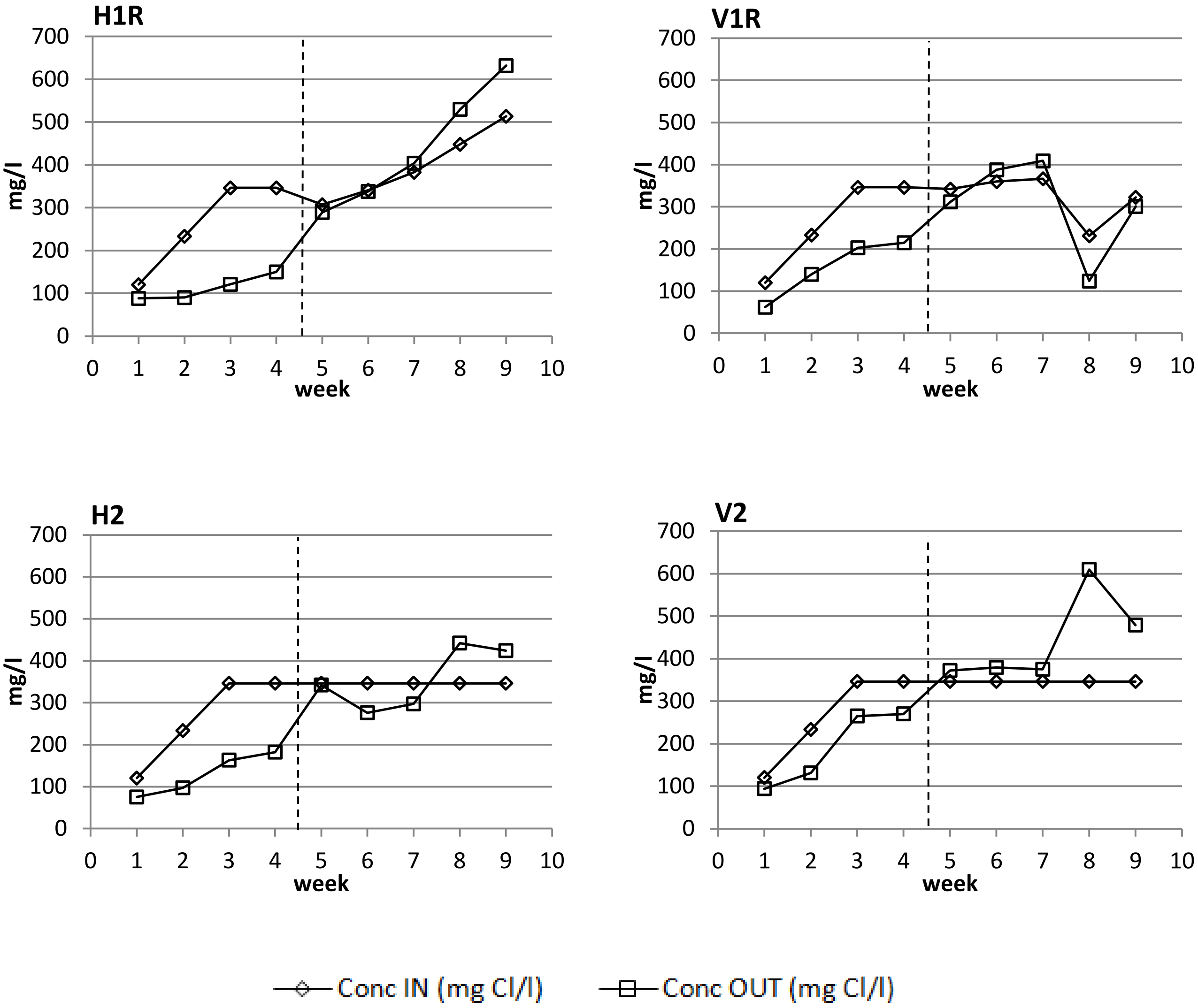

Figure 2. Cloride input concentration (mgCl/l) and cloride output concentration (mgCl/l). The dotted vertical lines indicate the beginning of Phase 3B

Total and volatile solids input and output weekly loads; removal efficiency based on weekly loads; inlet and outlet concentrations.

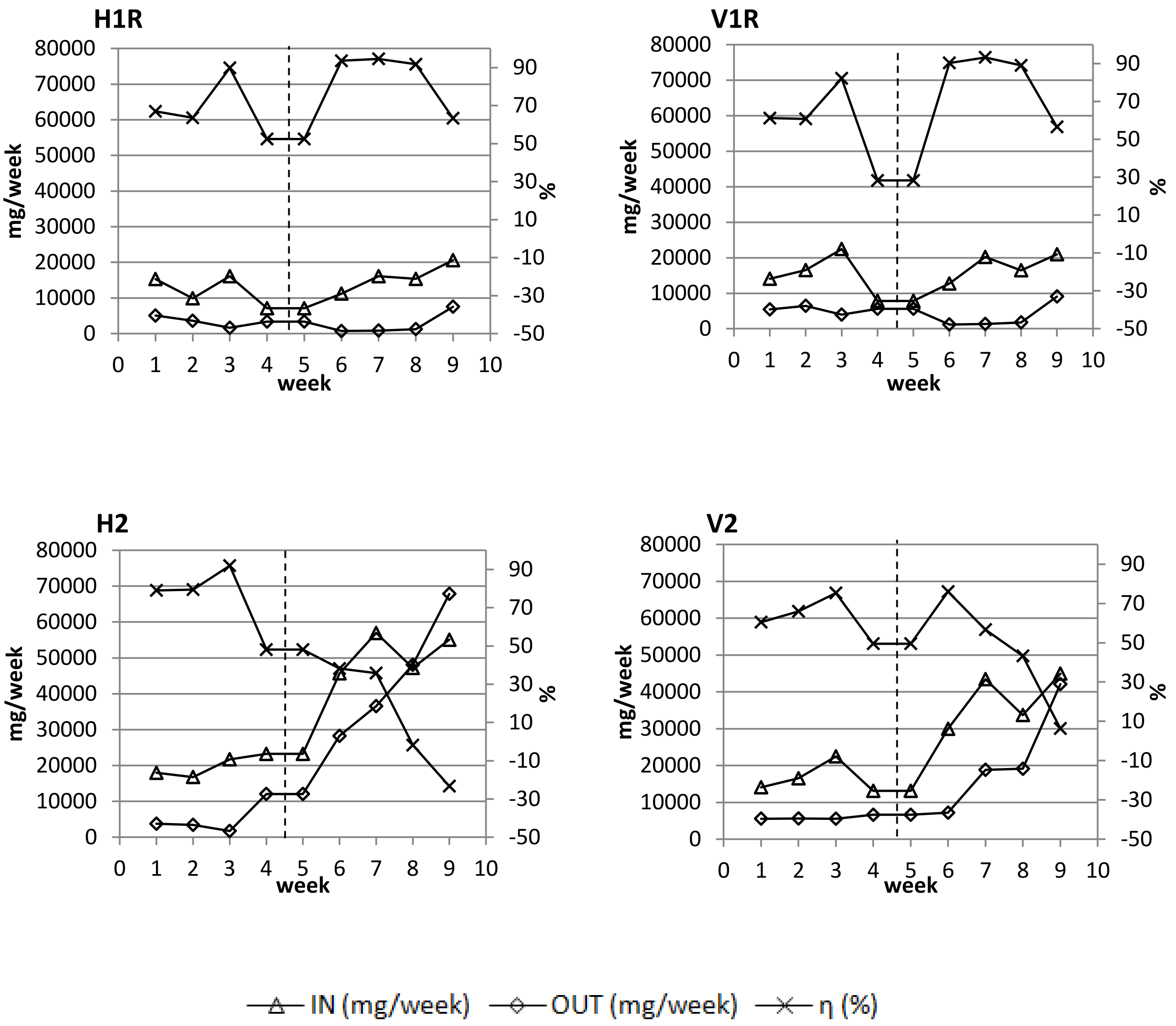

Figure 3. Total solids weekly input load (mgTS/week), total solids weekly output load (mgTS/week) and total solids removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

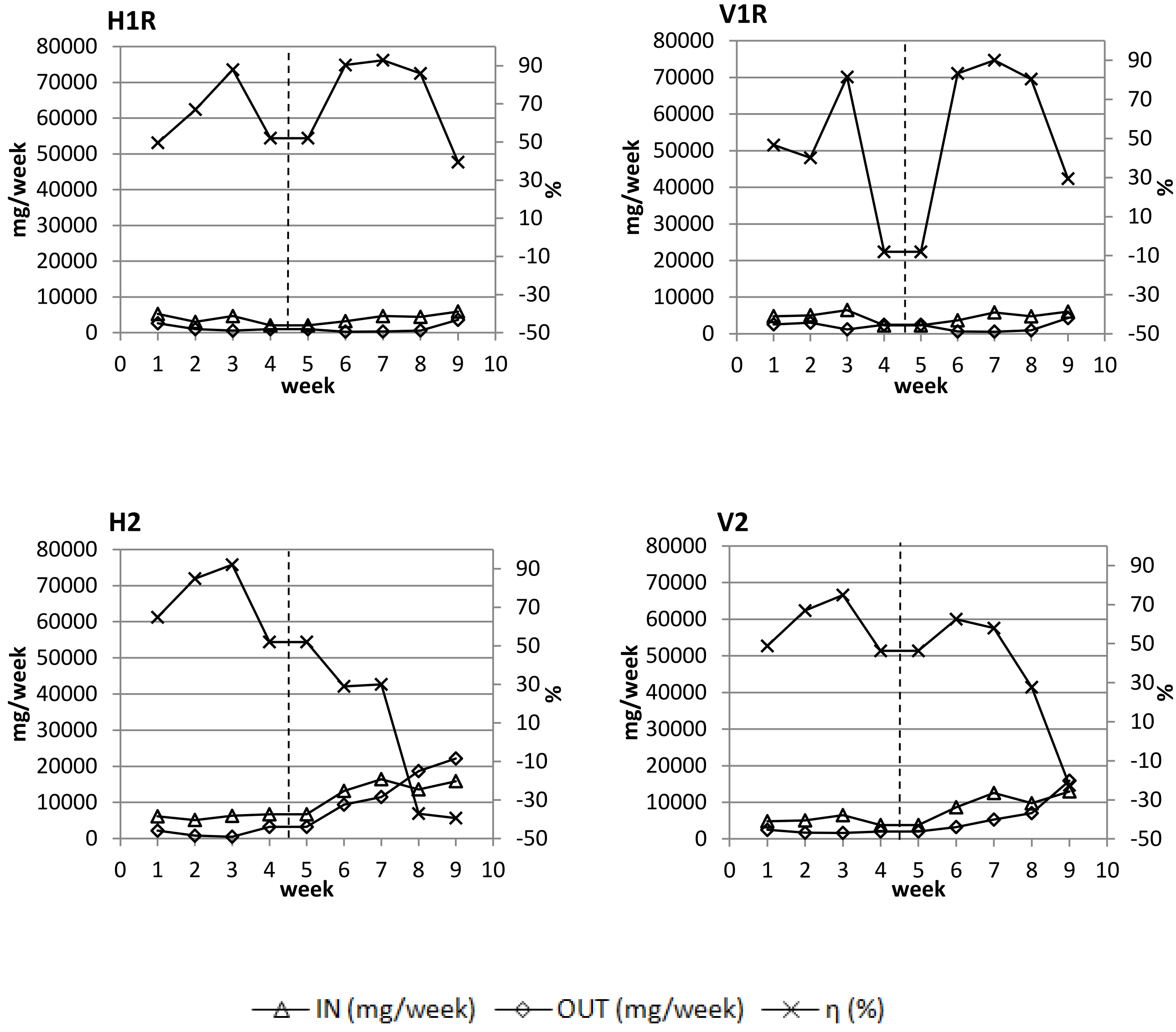

Figure 4. Volatile solids weekly input load (mgVS/week), volatile solids weekly output load (mgVS/week) and volatile solids removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

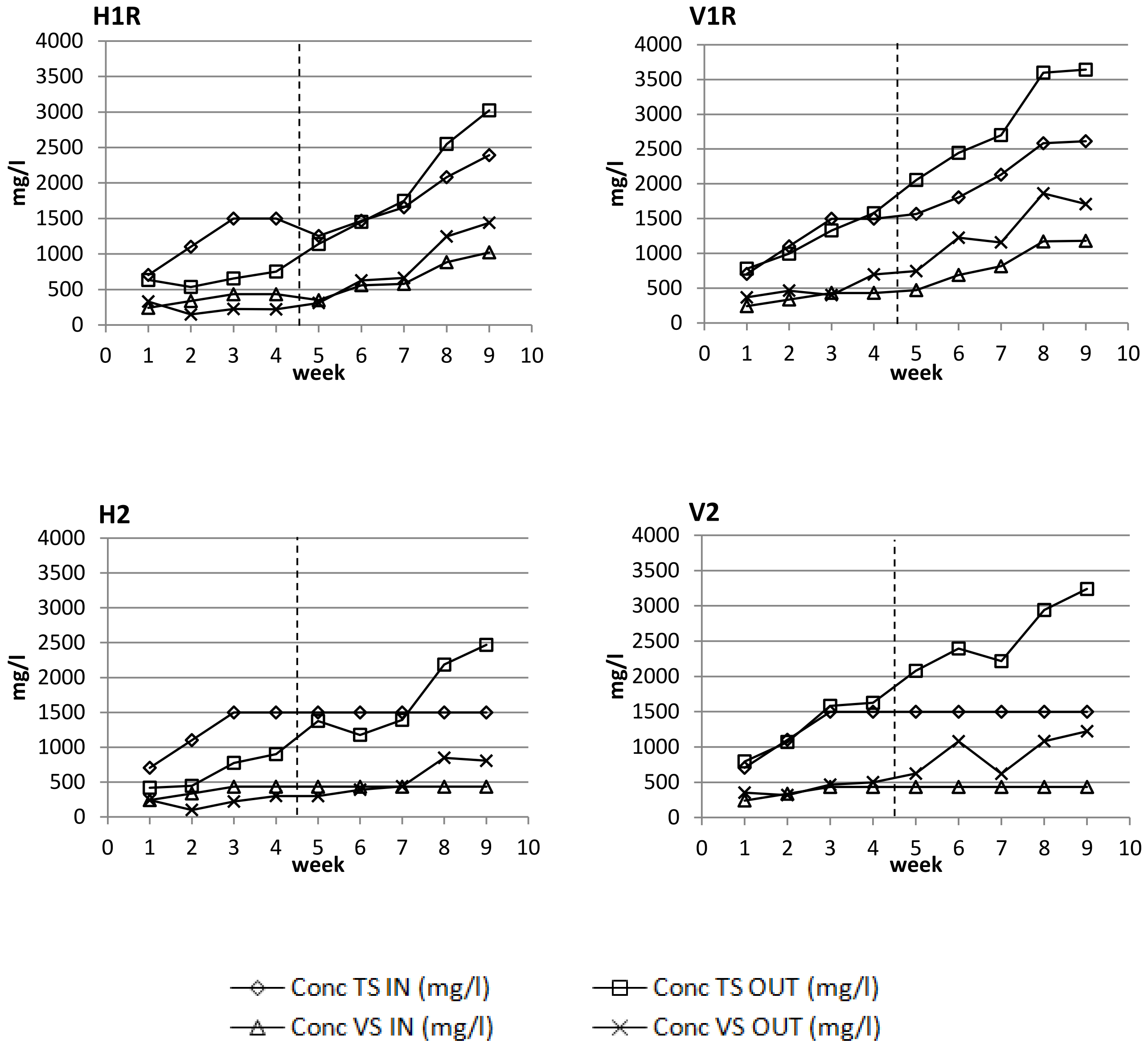

Figure 5. Total and volatile solids input concentration (mg/l); total and volatile solids output concentration (mg/l). The dotted vertical lines indicate the beginning of Phase 3B

Sulphate input and output weekly loads; removal efficiency based on weekly loads; inlet and outlet concentrations.

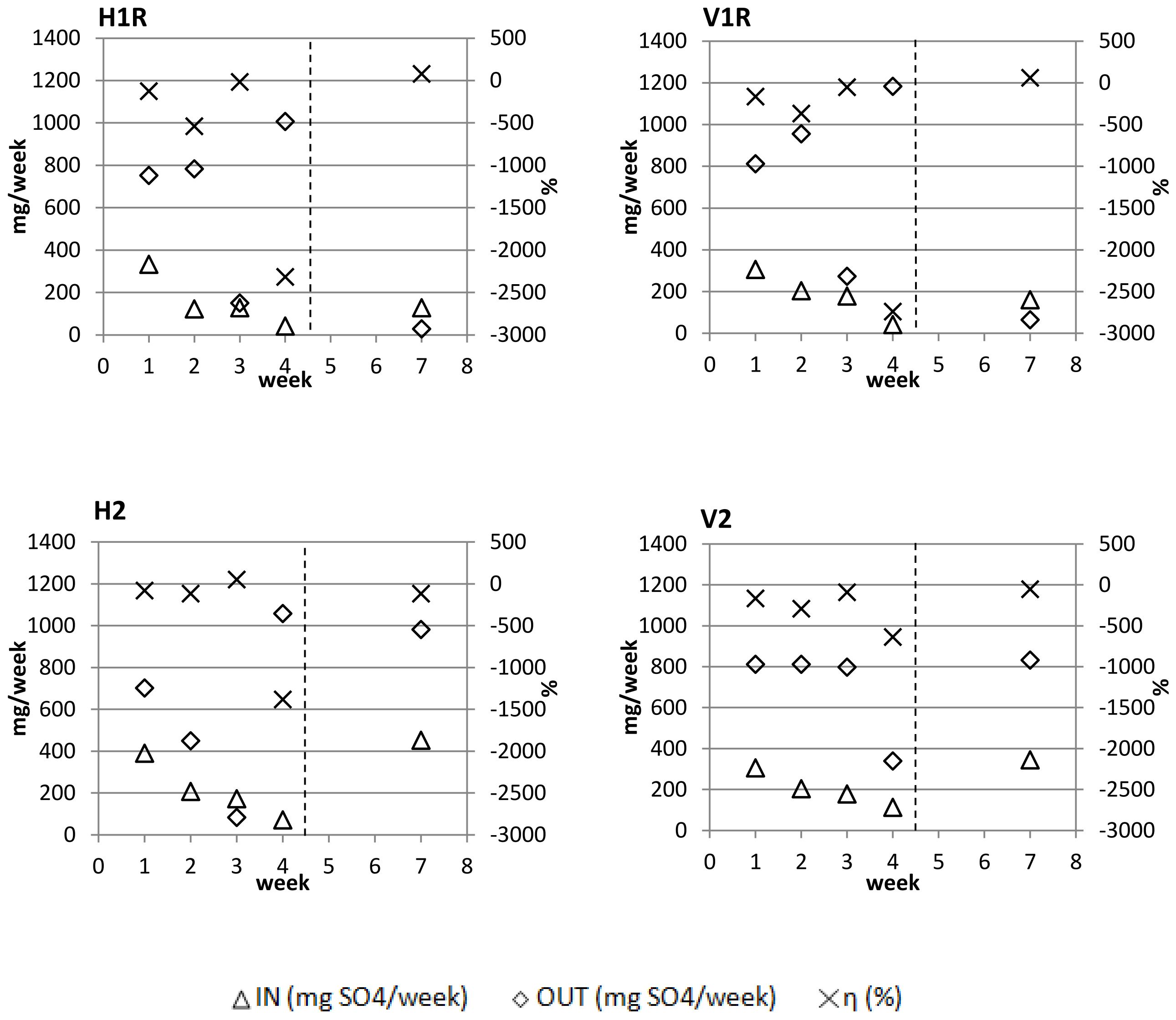

Figure 6. Sulphate weekly input load (mgSO4/week), sulphate weekly output load (mg SO4/week) and sulphate removal efficiency (%). The dotted vertical lines indicate the beginning of Phase 3B

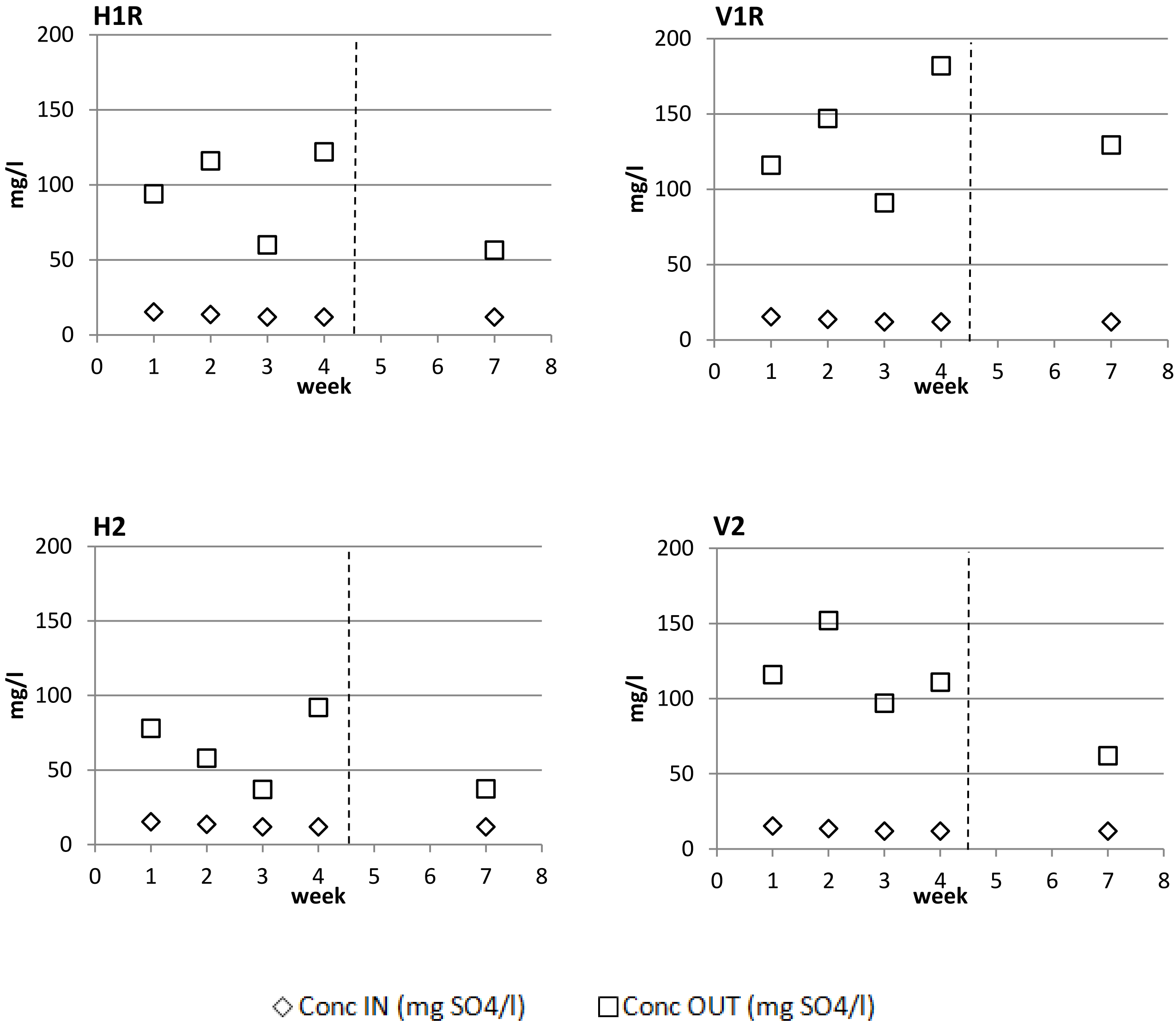

Figure 7. Sulphate input concentration (mg/l) and sulphate output concentration (mg/l). The dotted vertical lines indicate the beginning of Phase 3B

ANNEX 2

Contaminants concentrations detected in the output of each tank.

Table 1. Cloride concentrations detected in the output of each tank throughout the entire trial (mgCl/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	167	52	75	94	88	62
2	**2**	66	88	97	131	90	140
3A	**3**	73	39	163	265	121	203
	4	70	41	182	270	150	215
3B	**5**	60	-	342	372	289	312
	6	10	30	276	379	338	388
	7	6	18	297	375	404	409
	8	6	21	442	610	530	124
	9	7	8	424	479	632	301

Table 2. Phosphorous concentrations detected in the output of each tank throughout the entire trial (mgP/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	0.08	0.06	0.06	0.07	0.05	0.06
2	**2**	0.04	0.08	0.08	0.08	0.08	0.08
3A	**3**	0.04	0.04	0.04	0.04	0.04	0.05
	4	0.06	0.04	0.08	0.14	0.06	0.07
3B	**5**	0.01	-	0.04	0.08	0.04	0.06
	6	0.01	0.04	0.04	0.92	0.04	0.62
	7	0.04	0.04	0.11	1.02	0.08	0.36
	8	0.04	0.04	0.33	1.03	0.05	0.66
	9	0.02	0.02	0.18	0.98	0.05	0.38

Table 3. TKN concentrations detected in the output of each tank throughout the entire trial (mgN/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	0.72	1.43	1.00	0.51	2.52	0.75
2	**2**	0.43	0.86	1.00	6.02	1.01	20.25
3A	**3**	0.58	0.71	5.00	35.60	1.51	53.85
	4	0.65	0.74	2.30	48.70	1.92	15.20
3B	**5**	2.03	-	7.03	48.00	5.37	32.00
	6	7.41	12.82	25.40	31.50	9.95	20.90
	7	4.74	12.89	46.19	38.43	14.03	20.14
	8	6.44	12.32	54.15	48.77	12.19	20.01
	9	1.46	1.95	47.49	35.99	13.97	11.37

Table 4. Ammonia concentrations detected in the output of each tank throughout the entire trial (mgNH_4-N/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	0.5	0.5	0.5	0.5	1.0	0.5
2	**2**	0.3	0.3	0.5	5.9	0.4	13.5
3A	**3**	0.4	0.25	2.5	34.9	0.6	35.9
	4	0.45	0.26	1.15	46	0.76	10.14
3B	**5**	0.71	-	3.5	45	2.13	16.95
	6	2.6	4.5	24	21.3	9.8	10.1
	7	2	4.54	45.85	29.83	11.41	17.63
	8	2.72	4.34	53.76	37.86	9.92	17.52
	9	0.62	0.69	47.15	27.94	11.37	9.96

Table 5. Nitrate concentrations detected in the output of each tank throughout the entire trial (mgNO_3-N/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	11.5	8.11	8.68	11.5	11	11.5
2	**2**	2.88	4.31	0.95	34.5	7.65	22.1
3A	**3**	0.34	3.45	2.86	55.7	5.8	38.5
	4	0.62	2.63	3.7	48.2	4.4	69.73
3B	**5**	1.55	-	10.4	117.6	16.52	130.6
	6	2.11	19.34	20.16	139.62	45.51	119.7
	7	2.4	21.03	46.72	150	81.22	150
	8	2.99	29.39	78.25	163.95	126.43	197.37
	9	2.72	3.53	118.38	187	162.15	232.61

Table 6. COD concentrations detected in the output of each tank throughout the entire trial (mgO_2/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	1	118	79	83	74	85	82
2	2	32	44	50	37	50	62
3A	3	83	94	113	152	74	162
	4	10	12	47	139	34	97
3B	5	77	-	280	367	181	118
	6	56	55	223	347	181	213
	7	64	58	136	274	90	182
	8	13	88	304	377	192	274
	9	10	90	286	330	224	281

Table 7. TS concentrations detected in the output of each tank throughout the entire trial (mgTS/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	1	1195	610	418	795	633	780
2	2	525	570	445	1070	535	995
3A	3	562	522	775	1582	655	1330
	4	480	557	900	1625	752	1577
3B	5	375	-	1377	2078	1140	2055
	6	297	512	1177	2395	1452	2445
	7	420	512	1392	2215	1748	2700
	8	325	560	2185	2940	2550	3598
	9	340	313	2468	3238	3023	3640

Table 8. VS concentrations detected in the output of each tank throughout the entire trial (mgVS/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	1	480	180	240	352	330	367
2	2	113	120	100	318	148	465
3A	3	340	110	220	465	225	400
	4	177	122	300	500	220	697
3B	5	120	-	298	622	310	745
	6	215	273	390	1080	627	1225
	7	113	115	438	620	662	1158
	8	145	247	848	1082	1247	1862
	9	135	98	805	1222	1440	1708

Table 9. Sulfate concentrations detected in the output of each tank throughout the entire trial (mgSO_4/l)

Phase	week	HC	VC	H2	V2	H1R	V1R
1	**1**	138	96	78	116	94	116
2	**2**	122	127	58	152	116	147
3A	**3**	64	78	37	97	60	91
	4	97	127	92	111	122	182
3B	**5**	-	-	-	-	-	-
	6	-	-	-	-	-	-
	7	31.7	93.5	37.4	62.06	56.5	129.4
	8	-	-	-	-	-	-
	9	-	-	-	-	-	-

ANNEX 3

Progression of sunflowers growth during the trial.

Figure 8. Greenhouse view at week 1

Figure 9. Greenhouse view at week 2

Figure 10. Greenhouse view at week 3

Figure 11. Greenhouse view at week 4

Figure 12. Greenhouse view at week 6

Figure 13.Greenhouse view at week 7

Figure 14. Greenhouse view at week 8

Figure 15. Greenhouse view at week 9, left side

Figure 16. Greenhouse view at week 9, right side