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Department of Industrial Engineering, XXXI Cycle

Phytotreatment of contaminated wastewater with energy crops

Coordinator: **Ch.mo Prof. Paolo Colombo**

Supervisor: **Ch.mo Prof. Raffaello Cossu**

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Ciclo XXXI

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Ph.D. thesis, October 2018

Phytotreatment of contaminated wastewater with energy crops

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Abstract

Nowadays, advanced industrial societies still depend on fossil fuels supply. However, in the last decades, industrial and scientific efforts have been made toward renewable sources. The use of energy crops, fast-growing plants, aimed to produce biofuels or generate energy, represents an important alternative to traditional sources of energy, with advantages also with respect to environment and agricultural and economic development. However, the cultivation of energy crops to produce significant amounts of biomass may lead to the dilemma "Agriculture for food or energy production?". Wastewater phytrotreatment using energy crops could be considered an optimal compromise among different needs, especially if applied in derelict areas:

- simple and low-cost wastewater treatment
- renewable energy production
- conservation of fresh water storages
- preservation of land for food production

A series of researches were carried out at the University of Padova to evaluate the performances of some oily crops (sunflower, soybean, rapeseed) and *Pennisetum Purpureum* (elephant grass) in the phytotreatment of source separated municipal wastewater and/or MSW landfill leachate.

The results proved that oleaginous species, cultivated in 20 L pots and irrigated with increasing nitrogen concentrations in the feedstock, can be used for the phytotreatment of grey and yellow waters from source segregation of domestic sewage [Chapter 2], displaying high removal efficiencies of nutrients and organic substances (total N η >80%; total P η >90%; COD nearly 90%). No inhibition was registered in the growth of plants irrigated with different mixtures.

The three oleaginous species were also used to treat MSW landfill leachate. An experimental research was carried out using 20 L volume pots irrigated with old landfill leachate under different percentages in the feeding and subsequent COD, N and P loads [Chapter 3]. Significant removal efficiencies were achieved: COD (η >80%), total N (η >70%) and total P (η >95%). Plants irrigated with leachate, when compared to control units fed only with water and nutrient solution (Hoagland solution), developed a larger plant mass. Sunflower was the best performing species. Leachate irrigation seemed to stimulate the oil production, with a favorable Free Fatty Acids composition in view of the biodiesel production.

As a further step, sunflowers were grown in 130 L rectangular tanks irrigated with increasing dosages of old MSW landfill leachate [Chapter 4]. Two different irrigation systems were tested: vertical and horizontal sub-superficial flows, with or without effluent recirculation. The experiment revealed good removal efficiencies for COD (η >50%) up until flowering, while phosphorous removal invariably exceeded 60%. In general, horizontal flow units showed the best performances in terms of contaminant removal capacity; the effluent recirculation procedure did not improve performance. Significant evapotranspiration was observed, promoting the removal of up to 80% of the irrigation volume.

Based on the previous researches, sunflowers were grown in a waste-derived substrate, a mixture of sand from sweeping of streets and compost containing sewage sludge, and irrigated with increasing dosages of old MSW landfill leachate [Chapter 5]. Plants were grown in 300 L reactors characterized by vertical and horizontal sub-superficial flows. Vertical and horizontal flow units were connected in series to enhance nitrification and denitrification: nitrogen losses in gaseous form were approximately 40-45%. The connection in series proved to be effective in removing the influent total nitrogen (η > 80%) and in reducing the influent volumes due to evapotranspiration (more than 80%). Leachate irrigation did not inhibit the biomass development and resulted in a favorable oil composition for biodiesel production.

Pennisetum Purpureum (elephant grass) was also tested [Chapter 6]. The plants were grown in lysimeters, 1 m deep, to simulate the superficial layer of landfills top covers. Plants were irrigated with MSW landfill leachate produced by a landfill in operation; the pollutants loads were increased over time. The removal efficiencies were in the range 95-99% for all the investigated contaminants. The vertical sub-superficial flow led to an almost total nitrification, as expected, but a partial denitrification was detected too. A simple mathematical model was developed to study the kinetics of nitrogen removal, which confirmed the occurrence of a fast nitrification process. *Pennisetum Purpureum* growth seemed to be stimulated by the leachate irrigation; no significant accumulation of heavy metals was observed in the biological tissues.

Samples of soil used as substrate in a lab-scale leachate phytotreatment test with sunflowers were analysed to provide chemical characterization before, during, and at the end of the experiment [Chapter 7]. The results showed that the phytotreatment activity did not increase initial contaminant concentrations (e.g.: heavy metals). These results were reinforced by those from ecotoxicological bioassays in which *Eisenia fetida* (earthworms), *Lepidium sativum* (cress), *Folsomia candida* (collembola), and *Caenorhabditis elegans* and *Steinernema carpocapsae* (nematodes) were used.

A Multi-Criteria Analysis, based on economic, energetic, and environmental aspects was developed to assess four potential scenarios of energy crops application to the top of closed landfills [Chapter 8]. In this study, the scenarios have been assessed and compared with respect to a reference case defined for northern Italy. The first three scenarios were based on energy maximisation, leachate phytotreatment capacity, and environmental impact, respectively. The fourth scenario was a combination of the characteristics emphasised by the previous scenarios. The combination scenario resulted to be the best. The economic criterion emerged as weak, as all the considered scenarios showed some limits from this point of view. The decrease of leachate production due to the presence of energy crops on the top cover, which enhances evapotranspiration, represented a favourable but problematic aspect in the definition of the results. This analysis provided important indications: the presence of energy crops on the top cover represents a positive but also critical option which must be addressed in the authorization and design phase.

List of publications

Papers published on peer-reviewed journals

Cossu, R., Garbo, F., Girotto, F., Simion, F., Pivato, A. (2017). *Plasmix management: LCA of six possible scenarios.* Waste Manage. 69, 567-576. http://dx.doi.org/10.1016/j.wasman.2017.08.007

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Garbo, F., Cossu, R. (2017). *Landfill cover systems - an overview.* In: Proceedings of the Sardinia 2017 - 16th International Waste Management and Landfill Symposium. ISBN: 9788862650106, Santa Margherita di Pula (CA), Italy, 02-06 October 2017

Garbo, F., Lavagnolo, M.C. (2017). *Decentralized wastewater system: a case study.* In: Proceedings of the WasteSafe2017 - 5th International Conference on Solid Waste Management in South Asian Countries. ISBN: 9789843423061, Khulna, Bangladesh, 25-27 February 2017

Garbo, F., Lavagnolo, M.C., Malagoli, M. (2017). *Wetland lab scale investigations for leachate treatment.* In: Proceedings of the Sardinia 2017 - 16th International Waste Management and Landfill Symposium. ISBN: 9788862650106, Santa Margherita di Pula (CA), Italy, 02-06 October 2017

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Malagoli, M., Garbo, F., Lavagnolo, M.C. (2017). *Sustainable production of renewable energy from leachate phytotreatment.* In: Proceedings of the WasteSafe2017 - 5th International Conference on Solid Waste Management in South Asian Countries. ISBN: 9789843423061, Khulna, Bangladesh, 25-27 February 2017

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Oral presentations given at international conferences

Decentralized wastewater system: a case study. Garbo, F., Lavagnolo, M.C.; WasteSafe2017 - 5th International Conference on Solid Waste Management in South Asian Countries, Khulna, Bangladesh, 25-27 February 2017.

Energy recovery from oily crops in landfills. Garbo, F., Lavagnolo, M.C., Malagoli, M., Cossu, R.; Venice 2016 - 6th International Symposium on Energy from Biomass and Waste, Venice, Italy, 14-17 November 2016.

Landfill cover systems - an overview. Garbo, F., Cossu, R.; Sardinia 2017 - 16th International Waste Management and Landfill Symposium. Santa Margherita di Pula, Cagliari, Italy, 02-06 October 2017.

LCA approach for Plasmix management. Garbo, F., Cerminara, G., Morello, L., Lavagnolo, M.C., Cossu, R.; SUM 2016 - 3rd Symposium on Urban Mining and Circular Economy, Bergamo, Italy, 23-25 May 2016.

Leachate irrigation of different oleaginous plants. Lavagnolo, M.C., Garbo, F., Malagoli, M., Cossu, R.; Sardinia 2015 - 15th International Waste Management and Landfill Symposium, Santa Margherita di Pula, Cagliari, Italy, 5-9 October 2015.

Phytotreatment of leachate using energy crops. Lavagnolo, M.C., Garbo, F., Malagoli, M., Cossu, R.; ICLRS 2016 - The 9th Intercontinental Landfill Research Symposium, Noboribetsu Onsen, Hokkaido, Japan, 13-15 June 2016.

Wastewater management in the urban mining context. Lavagnolo, M.C., Garbo, F., Malagoli, M., Cossu, R.; SUM 2016 - 3rd Symposium on Urban Mining and Circular Economy, Bergamo, Italy, 23-25 May 2016.

Wetland lab scale investigations for leachate treatment. Garbo, F., Lavagnolo, M.C., Malagoli, M.; Sardinia 2017 - 16th International Waste Management and Landfill Symposium. Santa Margherita di Pula, Cagliari, Italy, 02-06 October 2017.

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Lavagnolo, M.C., Garbo, F., Malagoli, M., Cossu, R. (2016). *Phytotreatment of leachate using energy crops*. The 9th Intercontinental Landfill Research Symposium. Noboribetsu Onsen, Hokkaido, Japan, 13-15 June 2016.

Chapter 1: Introduction and outline of the Ph.D. activity

1.1. Municipal wastewater management: traditional and sustainable approaches

Traditionally, wastewater management approach was based on the centralization of the different household streams (e.g.: toilet, shower, bath, and kitchen sink) to a unique treatment unit in each territorial entity (e.g.: municipalities, provinces).

More recently decision makers started to question this commonly accepted approach (Marlow et al., 2013). As an alternative to wastewater management schemes strongly dependent on centralized infrastructures, novel principles and strategies have been proposed especially to serve those realities (e.g.: isolated communities, small villages, touristic centres) where centralization is clearly challenging and probably not sustainable to perform (Lijó et al., 2017; Machado et al., 2017; Massoud et al., 2009). Moreover, the wastewater transport to a centralized wastewater treatment plant involves costs that are not always advantageous. Considering calculations based on typical infrastructure lifetimes of 25 years for wastewater treatment plants (WWTP) and 80 years for sewers, this trade-off is further aggravated as typically more than 80% of the investment costs have to be spent on sewer infrastructures (Maurer et al., 2006; Bakir, 2001; Hong et al., 2005). Decentralized approaches are based on source separation of wastewater streams and on their separate treatment next to source of generation. Wastewater still requires being collected, but the use of large and long pipes is avoided, as well as the related excavation works to create a more or less composite collection system network (Libralato et al., 2011).

A very important aspect of this approach is that, nowadays, wastewater can and should be considered a renewable resource to be reused as non-potable water after treatment or from which energy and also nutrients could be recovered (Skambraks et al., 2017). Urine contributes on average 79%, 47% and 71% to the total N, P and K content of household wastewater respectively, but currently its volumetric fraction of the total wastewater flow is only around 1% (Ledezma et al., 2015). In other words, when nutrients from urine reach the wastewater treatment plant (WWTP), they have already been diluted about 100-fold, which complicates their recovery or removal (Ledezma et al., 2015).

An experimental system for the decentralized collection and separate treatment of different wastewater streams has been arranged at the University of Padova; it is named *Aquanova* (Fig. 1.1). *Aquanova* system looks at the separation at the source of domestic wastewater fluxes, namely brown water (faecal matter), yellow water (urine) and greywater (produced in baths, showers, washbasins, kitchen sinks). It includes a phytotreatment plant to treat mixtures of grey and yellow waters, and a small scale anaerobic biodigestor for the co-treatment of brown water and kitchen waste. To perform the separation between brown and yellow waters, a special toilet (Otterpol et al., 1999) should be installed. In this system, phytotreatment represents an optimal solution for the treatment of grey and yellow waters: grey waters represent the major fraction in terms of volume, up to 80%; yellow water is rich in nutrients, fundamental for the plants development (Langergraber and Muellegger, 2005). The goal is to produce a phytotreatment effluent respecting the quality requirements for wastewater reuse.

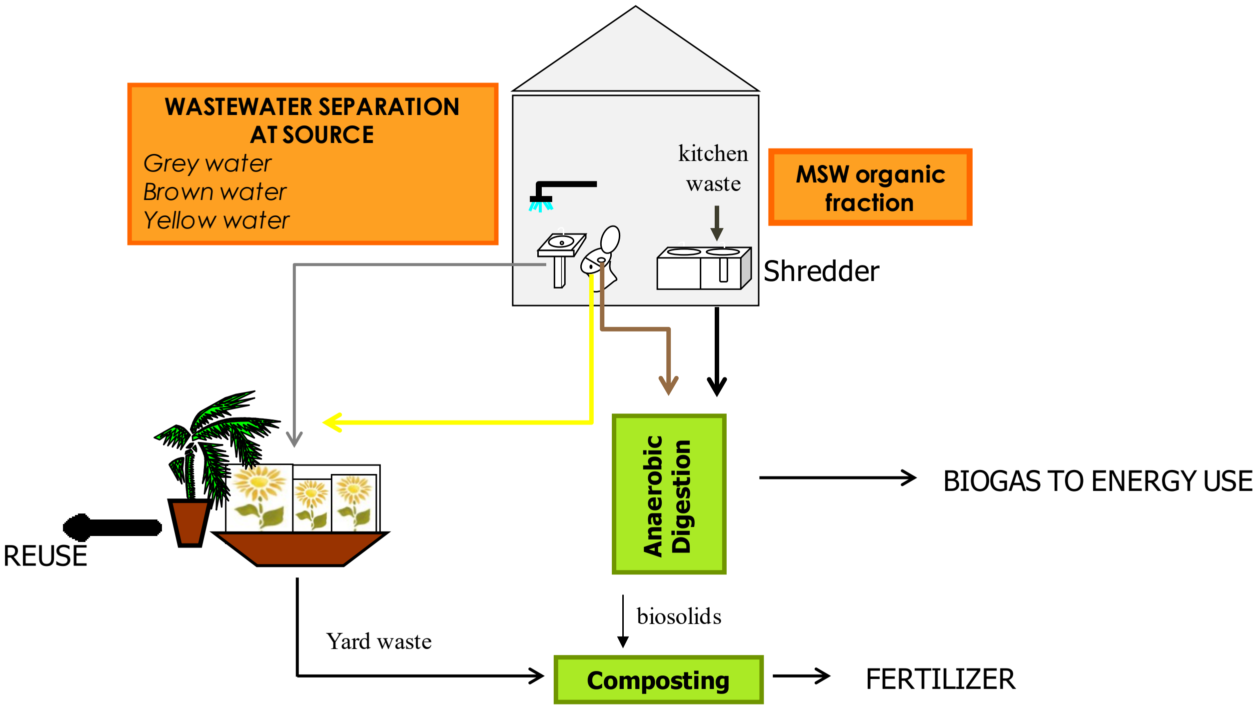

Fig. 1.1. Sketch of the Aquanova system

1.2. Municipal Solid Waste (MSW) landfill leachate management: traditional and sustainable approaches

Currently, most of Municipal Solid Waste (MSW) is still disposed of in landfills due to its economic convenience (Qi et al., 2018; Pubali et al., 2017; Renou et al., 2008). According to the current European regulation (99/31/CE), several toxic and hazardous materials are now banned from MSW landfills and modern landfills (e.g.: sanitary landfills) are designed and managed to collect and treat the leachate, minimizing the environmental risks. However, now researchers have to face the environmental risks posed by leachates produced by closed landfills which were active in the past decades, when the regulations were less stringent and the landfills were not managed in sustainable ways (Sil and Kumar, 2017).

Landfill leachate is defined as the effluent produced by the rainwater percolation through the waste layers, by the biochemical processes occurring in the landfill body and by the intrinsic water content of waste (Renou et al., 2008). Leachate is composed of a wide variety of inorganic, natural and xenobiotic compounds. It contains organic matter (biodegradable and refractory), ammonia nitrogen, heavy metals, chlorinated organics and inorganic salts (Qi et al., 2018; Pubali et al., 2017; Renou et al., 2008) and may also contain immiscible liquids (e.g.: oils), small particulates (e.g.: suspended solids) and a range of organisms (e.g.: bacteria or viruses) (Kjeldsen et al., 2002).

Its quality is highly site specific and depends on the type and age of the landfilled waste, type of landfill (e.g.: anaerobic, semi-aerobic), landfill temperature, etc. (Jones et al., 2006). In particular, the composition strongly depends on the age of the landfill (Renou et al., 2008). Although composition varies during the different phases (aerobic, acetogenic, methanogenic) of landfills, leachates can be classified according to the landfill age (Table 1.1) using the following parameters: COD, BOD_5, BOD_5/COD, pH, suspended solids (SS), ammonia, Total Kjeldahl Nitrogen (TKN) and heavy metals.

Table 1.1. Landfill leachate characterization based on the landfill age (Renou et al., 2008; Kjeldsen et al., 2002)

	Old	Intermediate	Recent
Age (years)	>10	5-10	<5
pH	>7.5	6.5-7.5	<6.5
COD (mg/L)	<5000	5000-10000	>10000
BOD_5/COD	<0.1	0.1-0.3	>0.3
Heavy metals	Low	Low-medium	Low-medium
Biodegradability	Low	Medium	High

Conventional landfill leachate treatments can be classified into four categories:

a. leachate recirculation into the landfill body
b. combined treatment with domestic sewage
c. biodegradation: aerobic and anaerobic processes
d. chemical and physical methods

Advantages and disadvantages of all these methods are summarized in Table 1.2.

Currently, the most commonly adopted method for leachate treatment is the biological process with nitrification and denitrification steps followed by membrane technology (Fernandes et al., 2015). However these traditional approaches are often undesirable because off-site transport is dangerous and expensive, while on-site facilities (e.g.: filtration units) require high investment and maintenance costs and are highly energy-consuming.

The low-cost treatment alternative is represented by phytotreatment. The success of this option depends on the capacity of the soil-vegetation system to tolerate the environmental stresses produced by the landfill leachate (Jones et al., 2006). The use of energy crops, cultivated on the top of closed landfills, may increase the competitiveness of this treatment system. The leachate, collected and re-circulated on the top of the landfill, is treated in phytotreatment basins in which crops are cultivated, acting as a source of nutrients for the plant while plants are actively involved in the removal of contaminants (Fig. 1.2).

This solution, which represents the core idea of the researches reported in this Thesis, implies a series of advantages:

- Contemporary leachate treatment and energy generation
- Saving fresh water for the crops irrigation, re-using the leachate
- No CO_2 emissions for leachate transport to off-site treatment plants
- Re-utilisation of derelict areas
- No competition of land for food and energy production
- Increase of the aesthetic value of the landfill site

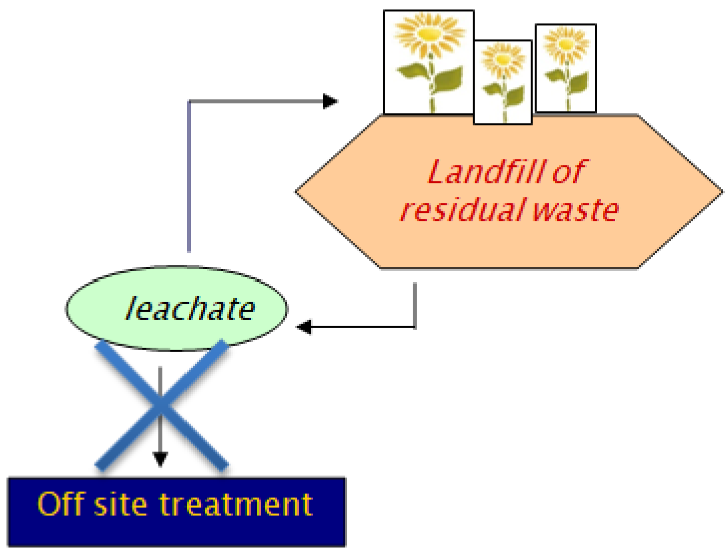

Fig. 1.2. Sketch of the in-situ landfill leachate phytotreatment using energy crops

Table 1.2. Summary of landfill leachate treatment options (Renou et al., 2008; Qi et al., 2018; Pubali et al., 2017)

	Alternatives	Advantages	Disadvantages
Leachate recirculation	-	• Faster waste stabilization • Enhanced natural evaporation	• Inhibition of methanogenesis if the leachate has acid pH • Risk of saturation or ponding of the waste layers
Combined treatment with domestic sewage	-	• Low-operating costs • No need of customized treatment plants	• Presence of recalcitrant compounds and heavy metals that may increase the effluent concentrations
Biological treatments	• Aerobic treatments: Suspended-growth biomass processes (Lagooning, Phytotreatment, Activated sludge); Attached-growth biomass systems (Trickling filters, Moving-Bed Biofilm Reactor - MBBR) • Anaerobic treatments: Suspended-growth biomass processes (Digester, Up-flow anaerobic sludge blanket-UASB); Attached-growth biomass systems (Anaerobic filter)	• Reliability, simplicity and high cost effectiveness • Effective in removing organics and nitrogen from young leachates when the $BOD_5/COD>0.5$	• In old leachates, the presence of refractory com-pounds limits the effectiveness • Inhibition due to excessive ammonia concentrations may occur
Physical/chemical treatments	Flotation, Coagulation-Flocculation, Chemical Precipitation, Adsorption, Chemical Oxidation, Filtration (Microfiltration, Ultrafiltration, Nanofiltration, Reverse Osmosis)	• Removal of recalcitrant compounds, ammonia, suspended solids, colloidal particles, floating material, color and toxic compounds • Reliability, effectiveness on a broad spectrum of contaminants	• Need of highly sophisticated plants • Costs of chemical compounds • Energy-consuming processes (especially filtration)

1.3. Phytotreatment and wetland systems

Phytotreatment can be applied to remediate municipal wastewater or landfill leachates (Pilon-Smits, 2005; Jones et al., 2006). It implies the utilization of the soil-plant system to detoxify, degrade or inactivate pollutants. The remediation capacity is due to the combination of different processes (Jones et al., 2006) (Fig. 1.3):

- Phytodegradation (often called rhizodegradation): it refers to the process of pollutants removal due to the direct degradation of the roots' biological tissues or in association with the microorganism living in symbiosis in the rhizosphere
- Phytostabilization: it refers to the immobilization of contaminants in the root zone. The plants can release in the rhizosphere exuded radicals that enhance the precipitation of some compounds (e.g.: heavy metals)
- Phytoextraction: it refers to the uptake of organics, nutrients or other compounds (e.g.: heavy metals) and their utilization for new biomass development, or sequestration in the roots and/or in the aerial part (accumulation)
- Phytovolatilization: it refers to the pollutants uptake from the soil and their release in the atmosphere as volatile compounds. It is particularly effective on volatile contaminants (e.g.: mercury)

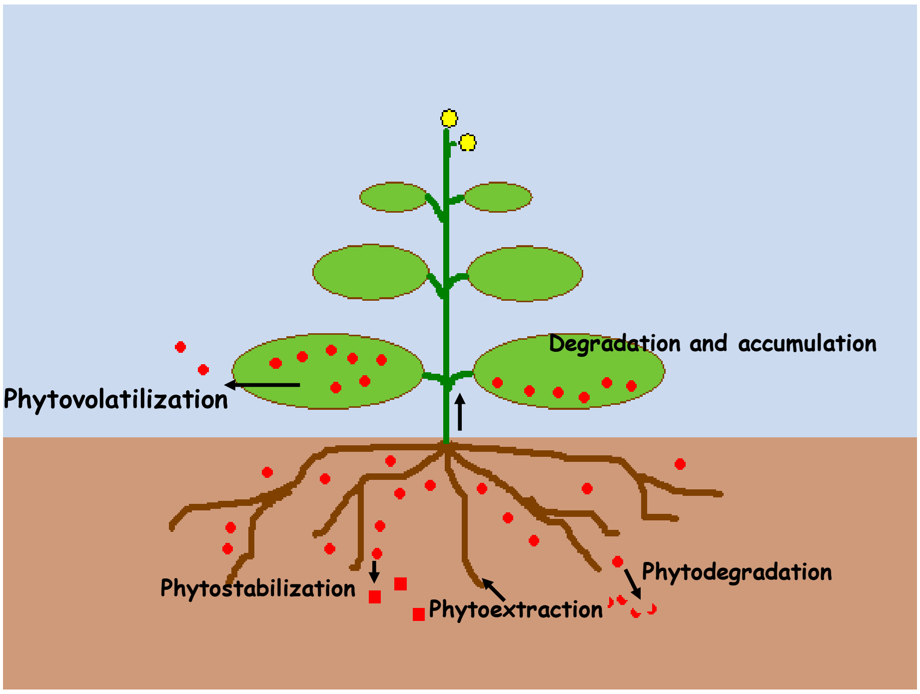

Fig. 1.3. Main processes involved in phytotreatment

Phytotreatment is performed on constructed wetlands: systems that reproduce the conditions existing in natural wetland areas. In these systems the self-depurative capacities are basically determined by the interaction of biological, chemical and physical processes (Fig. 1.4) with a predominant role played by plants and bacterial colonies living in symbiosis with the latter (Kadlec and Wallace, 2008). The main advantages of treatment wetlands include (Massoud et al., 2009; Kadlec and Wallace, 2008):

- High biological activity rate: they can transform most of contaminants (suspended solids, BOD_5, COD, nitrogen, phosphorus, bacteria and virus, heavy metals) found in wastewaters into harmless by-products or essential nutrients that can be used for additional biological productivity
- No need of chemicals addition
- Simplicity and economy of construction and management (low cost of excavation, piping, pumping, etc.)
- Low energy demand (natural environmental energies, minimal fossil-fuel energy); little skilled personnel; use of simple equipment
- Possibility of landscapes restoration: wetlands are among the most productive ecological systems and are rich in biodiversity, well accepted by local population (compared to conventional plants)
- Possibility of reusing the treated water
- Possibility to harvest the essences

The main disadvantages of treatment wetlands include (Massoud et al., 2009; Kadlec and Wallace, 2008):

- Considerable surface demand
- Significant performances decline during the coldest months
- Problem of odours
- Proliferation of mosquitoes
- Sensitive to hydraulic overloading

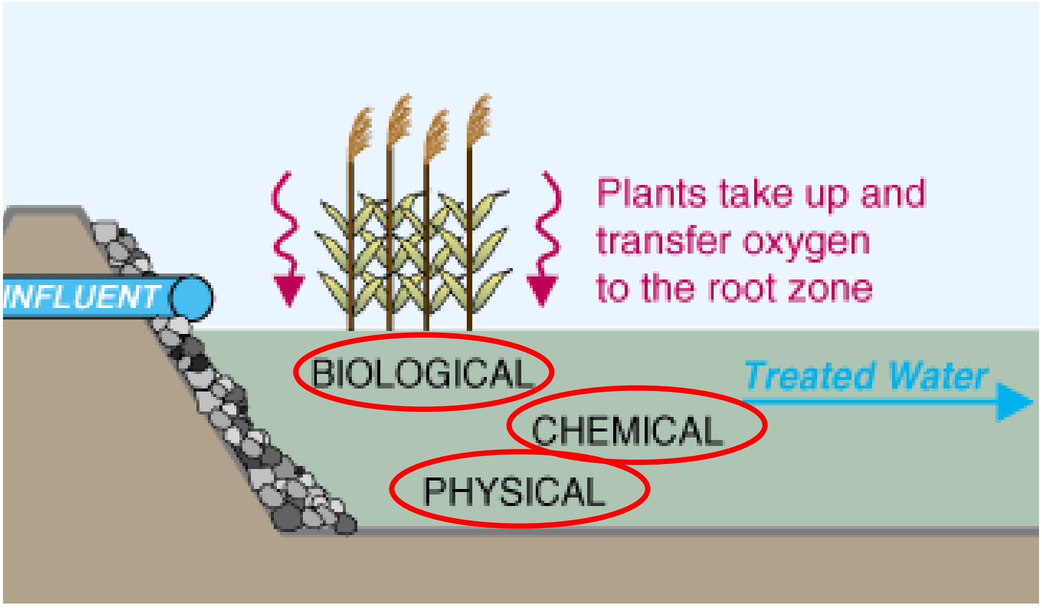

Fig. 1.4. Removal processes occurring in wetlands (Kadlec and Wallace, 2008)

There are two basic types of constructed wetlands, depending on the type of flow: surface flow and subsurface flow systems (Saeed and Sun, 2012). Surface flow wetlands are similar to natural wetlands, with a shallow flow of wastewater over the saturated soil substrate (Fig. 1.5).

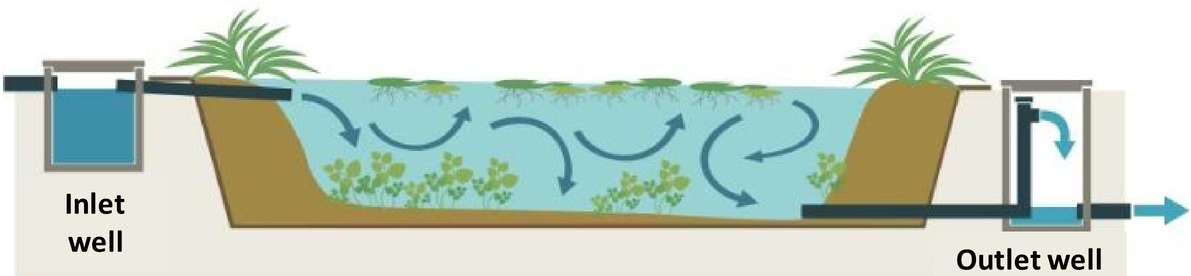

Fig. 1.5. Surface flow constructed wetlands (ISPRA, 2012)

Subsurface flow wetlands are filled with a porous medium; wastewater flows vertically (Fig. 1.6) or horizontally (Fig. 1.7) through the substrate soil where it comes in contact with the microorganisms living on the surfaces of plants roots and substrate, allowing the removal of pollutants. The main advantages and disadvantages of vertical and horizontal subsurface flow wetlands are summarized in Table 1.3. Hybrid wetlands (e.g.: vertical and horizontal subsurface flow wetlands connected in series) are often used to maximize the advantages and minimize the drawbacks of each single wetland type.

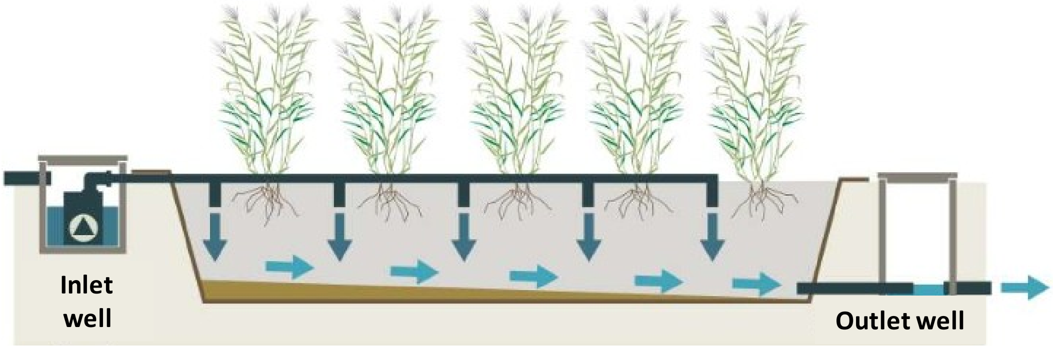

Fig. 1.6. Vertical subsurface flow constructed wetlands (ISPRA, 2012)

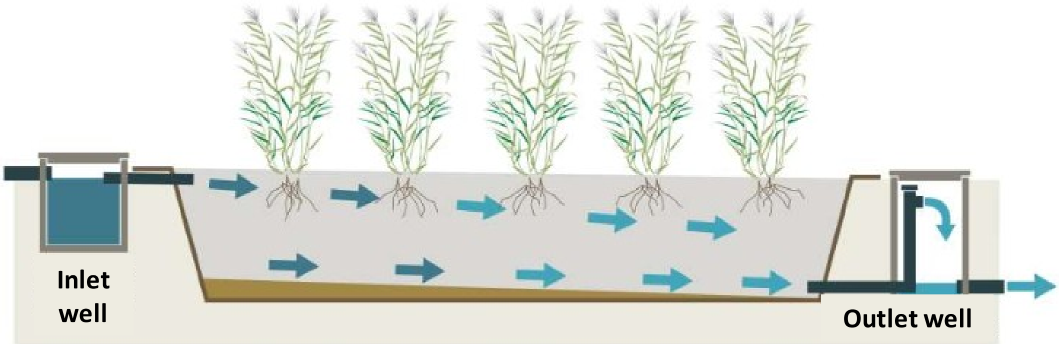

Fig. 1.7. Horizontal subsurface flow constructed wetlands (ISPRA, 2012)

Table 1.3. Advantages and disadvantages of vertical and horizontal subsurface flow wetlands (Saeed and Sun, 2012; Massoud et al., 2009)

Type	Advantages	Disadvantages
Vertical subsurface	• Smaller area demand compared to horizontal flow systems • Water flows from the surface to the bottom, enhancing oxygen intrusion in the substrate soil • Oxygen supply results in a good nitrification capacity • Better organics and solids removal	• Short flow distances • Poor denitrification • Low nitrate removal efficiency • Loss of performance (especially P removal)
Horizontal subsurface	• Longer flow distance • Efficient in removing suspended solids and organics • Effective for nitrate removal (denitrification)	• Higher area demand compared to vertical flow systems • Clogging problems may occur • Careful calculation of hydraulics is required to ensure minimum oxygen supply • Reduced ammonia oxidation capacity • Uniform flow of wastewater throughout the packed media is complicated to be achieved

1.4. Crops for food or energy production?

The global energy consumption is continuously increasing. According to the International Energy Outlook (EIA, 2017), the total world consumption will rise by 28% between 2015 and 2040, mainly due to the increasing demand in developing countries. Currently, most of the global energy demand is supplied by fossil fuels, which contribute to increase the greenhouse gas content in the atmosphere, leading to the climate changes (IEA, 2015; Ellaban et al., 2014). Therefore, the identification of alternative energy sources is fundamental to minimize the impacts of domestic, industrial, transportation and agricultural sectors on the environment.

The interest on energy crops increased over the last 20 years due to their potential use as alternatives to conventional, not-sustainable sources of energy (Pandey et al., 2016). Energy crops, fast-growing plants with high biomass production rates, are cultivated for bioenergy generation in terms of heat, electrical power or biofuels (biodiesel, bioethanol, biomethane). Approximately 2% of global agricultural land is currently used for biomass-for-energy, supplying about 10% of the global energy mix. But a further increase of the surface utilized for biomass-for-energy will inevitably lead to the following dilemma: "Agriculture for food or energy production"? (Paschalidou et al., 2016). Sustainable renewable energy generation implies that energy crops shall not compete with food crops in terms of land, water and nutrients availability. Therefore, energy crops should be preferably cultivated on derelict or contaminated areas (Pandey et al., 2016). In this optic, the use of energy crops for wastewater phytotreatment, performed in derelict areas (e.g.: top of closed landfills), or in contaminated soil remediation programs might be useful to achieve multiple goals: generation of renewable energy, remediation of the contaminated matrix (soil or wastewater), no competition with food crops.

1.5. Biodiesel production and characterization

Biodiesel (defined as methyl esters of fatty acids) production is of primary interest as it may be considered the optimal alternative to oil-derived fuels and can be used without the need to modify the existing engines (Gebremariam and Marchetti, 2018). When compared to conventional diesel, it has no sulphur and produces less carbon monoxide, particulate matters, smoke, hydrocarbons and has more free oxygen than conventional petrol diesel (Hasan and Rahman, 2017) resulting in complete combustion and reduced emissions (Fazal et al., 2011; Nguyen et al., 2010; Baskar and Aiswarya, 2016). Biodiesel can be produced from a variety of feedstock, including vegetable oils (edible or non-edible), waste oils and animal waste fats (Olkiewicz et al., 2016; Mandolesi de Araújo et al., 2013). Among others, transesterification (Fig. 1.8) is the most used production method, generating biodiesel and glycerol from the oil (Bet-Moushoul et al., 2016; Singh and

Singh, 2010). In a transesterification reaction, one mole of triglyceride reacts with three moles of alcohol (molar ratio of methanol to vegetable oil of 3:1) to form one mole of glycerol and three moles of the respective fatty acid alkyl esters. The purity and quality of biodiesel is determined by the amounts of free and bonded glycerine (Li-Hua et al., 2009). Highly purified biodiesel is necessary to achieve the stringent standard specifications set by the European Union (EN 14214) for biodiesel fuel. In particular, according to the EN 14214, the ester content must not be less than 96.5%.

Many researches focused on the conversion of edible oils to biodiesel, including sunflower oil, rapeseed oil and soybean oil, highlighting the risk of imbalance with food use (Bet-Moushoul et al., 2016; Gashaw and Lakachew, 2014; Karmakar et al., 2010). Therefore, attention has been paid on non-edible oils, like castor oil (Diaz et al., 2013), jatropha oil (Anr et al., 2016), neem oil (Gurunathan and Ravi, 2016) or on microalgae (Baskar and Aiswarya, 2016; Singh and Singh, 2010). According to its origin, biodiesel can be divided into three generations: first generation biodiesel, produced from edible oils (e.g.: sunflower oil); second generation biodiesel, produced from non-edible oils (e.g.: jatropha oils); third generation produced from microalgae (Ambat et al., 2018).

Advantages and disadvantages of the three categories of biodiesel are summarized in Table 1.4.

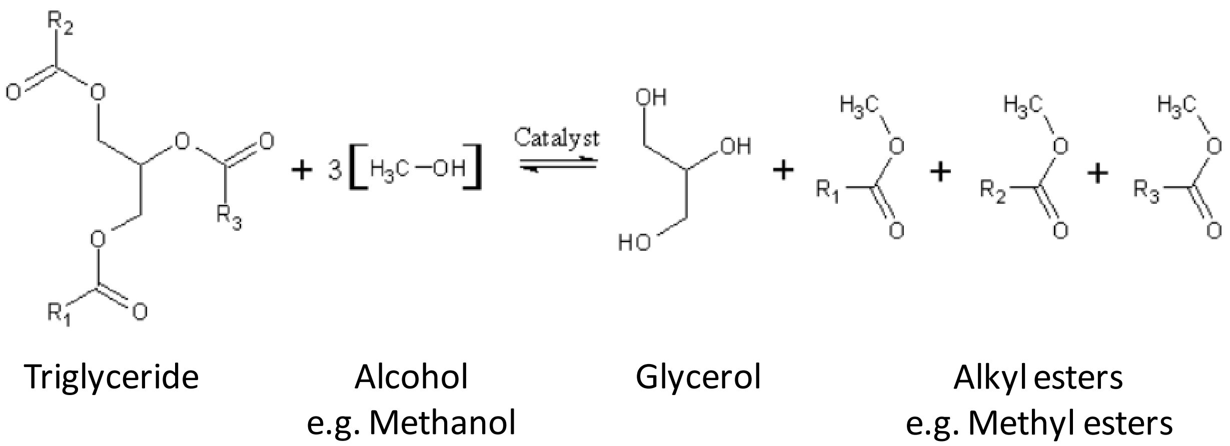

Fig. 1.8. Transesterification process for biodiesel production (modified from Singh and Singh, 2010)

Table 1.4. Advantages and disadvantages of the three biodiesel generations (Ambat et al., 2018)

Biodiesel	Origin	Advantages	Disadvantages
First generation	Edible oils	• Production can match the industrial needs • Performances comparable to conventional diesel	• Imbalance with food use • Competition of land
Second generation	Non-edible oils	• No food imbalance • Less requirement of land • Eco-friendly nature	• High viscosity • Production not up to industrial needs • More alcohol needed
Third generation	Microalgae	• High growth rate and productivity • High percentage of oil content • No competition with food chain / no land requirements	• Difficulties in oil extraction • Requirement of sunlight • High investment costs

1.6. Outline of the Ph.D. activity: the work packages

Based on the above-mentioned ideas, concepts and processes, it has been decided to focus on phytotreatment with energy crops to remediate mixtures of yellow, grey and kitchen waters as well as MSW landfill leachate.

The specific aims of the Ph.D. activities included:

- Getting a deep knowledge of the mechanisms involved in the phytotreatment process
- Assessment of nutrients and other contaminants removal capacity
- Identification of the plant species with the highest performances
- Identification of the most suitable substrate soils
- Assessment of the role of soil as "sink" for nutrients and heavy metals
- Assessment of the crops biomass development to check the occurrence of inhibition phenomena
- Use of the mass balance approach to identify the pathways of nutrients removal
- Study of the kinetics of nitrogen removal
- Characterization of the vegetable oil in view of the biodiesel production

- Evaluation of the heavy metals accumulation in the biological tissues of plants irrigated with landfill leachate
- Assessment of the quality of the substrate soil at the end of the phytotreatment process to check whether accumulation of contaminants (e.g.: heavy metals) occurred
- Evaluation of the economic, energetic, environmental feasibility of the suggested treatment system

The experimental activities reported in this Thesis can be divided into three work packages (Fig. 1.9):

- Work package 1: *Source-segregated municipal wastewater and landfill leachate phytotreatment using soybean, rapeseed, sunflower and elephant grass*. It includes five research projects named Experiment A, B, C, D, and E; reported in Chapters 2, 3, 4, 5, and 6, respectively
- Work package 2: *Assessment of the quality of phytotreatment substrate soil as landfill cover material for in-situ leachate management*. It includes one research project, reported in Chapter 7
- Work package 3: *Energy crops on landfills: functional, environmental, and costs analysis of different landfill configurations*. It includes one research project, reported in Chapter 8

In the first Ph.D. year, much attention was focused on bibliographic research, in order to have a general vision on the topic. In parallel, the elaboration of available data was done. The results have been included in three scientific manuscripts:

- "Use of oleaginous plants in phytotreatment of grey water and yellow water from source separation of sewage" [Chapter 2 - Experiment A] which describes the research in which sunflower, soybean and rapeseed were used to treat different mixtures of grey waters from bathroom sinks, kitchen waters from kitchen sink and yellow waters source-separated with a special toilet (*Aquanova* system). This paper has been published on *Journal of Environmental Sciences.*
- "Lab-scale phytotreatment of old landfill leachate using different energy crops" [Chapter 3 - Experiment B] which describes the research in which sunflower, soybean and rapeseed were grown in two different substrates (sandy or clayey soil) and irrigated with old landfill leachate. The seeds oil was analyzed in view of the biodiesel production. This paper has been published on *Waste Management.*

- "Different leachate phytotreatment systems using sunflowers" [Chapter 4 - Experiment C] which describes the research in which sunflowers, grown on sandy soil, were irrigated with old landfill leachate. Two different irrigation systems were tested: vertical flow and horizontal subsurface flow, with or without effluent recirculation. This paper has been published on *Waste Management.*

Helianthus annuus (sunflower), *Glycine max* (soybean) and *Brassica napus* (rapeseed) plants were used, in view of the biodiesel production from the seeds oil, as these are contingent in Mediterranean and Continental areas. Although these species are commonly used to produce edible oils and their utilization for biodiesel production may be critic in terms of sustainability (Ambat et al., 2018), it has been decided to simulate their cultivation in derelict areas (e.g.: top cover of closed landfills) to combine the advantages of first and second generations biodiesels, listed in Table 1.4.

During the second and third Ph.D. years, new researches were designed and performed. Based on the literature review and on the results of the previous researches, a special focus was paid on the use of sunflowers. Also a new promising crop, *Pennisetum Purpureum* (elephant grass), particularly suitable for biogas or heat production in developing countries, was tested. Plants were irrigated with municipal solid waste landfill leachate (to simulate the phytotreatment on the top of closed landfills) and were harvested after clear senescence was reached.

The results have been included in three scientific manuscripts:

- "Landfill leachate phytotreatment with sunflowers grown in a waste-derived substrate" [Chapter 5 - Experiment D] which describes the research in which sunflowers, irrigated with old landfill leachate, were grown in a waste-derived substrate in reactors characterized by vertical and horizontal subsurface flow, connected in series. The seeds oil was analyzed in view of the biodiesel production. The results were presented at the Venice 2016 - 6th International Symposium on Energy from Biomass and Waste, Venice, Italy, 14-17 November 2016
- "Leachate phytotreatment with *Pennisetum Purpureum* (elephant grass) in view of its cultivation on the top of closed landfills" [Chapter 6 - Experiment E] which describes the research in which elephant grass, irrigated with leachate produced by a landfill in operation, was grown in columnar reactors simulating the superficial layer of landfill top covers. The results were presented at the Sardinia 2017 - 16th International Waste Management and Landfill Symposium, Santa Margherita di Pula (Cagliari), Italy, 02-06 October 2017.
- "Assessment of the ecotoxicity of phytotreatment substrate soil as landfill cover material for in-situ leachate management" [Chapter 7] which describes the research in which the substrate soil was analyzed before, during and after the applied leachate phytotreatment

process with sunflowers. The assessment, based on chemical and ecotoxicological characterizations, was used to check wheter phytotreatment causes a degradation of the substrate's initial quality. This paper has been published on *Journal of Environmental Management.*

In order to check the feasibility and convenience of energy crops application to landfills, a Multi-Criteria Analysis has been also developed. The results have been included in following manuscript:

- "Energy crops on landfills: functional, environmental, and costs analysis of different landfill configurations" [Chapter 8] which describes the development of a Multi-Criteria Analysis, based on economic, energetic, and environmental aspects, to assess four different configuration scenarios in which energy crops were applied to landfills. This paper has been published on *Environmental Science and Pollution Research.*

Although this Thesis has been conceived as a unique work, each Chapter has been organized as a single-standing paper and includes: Abstract, Introduction, Materials and Methods, Results and Discussion, Conclusion, References.

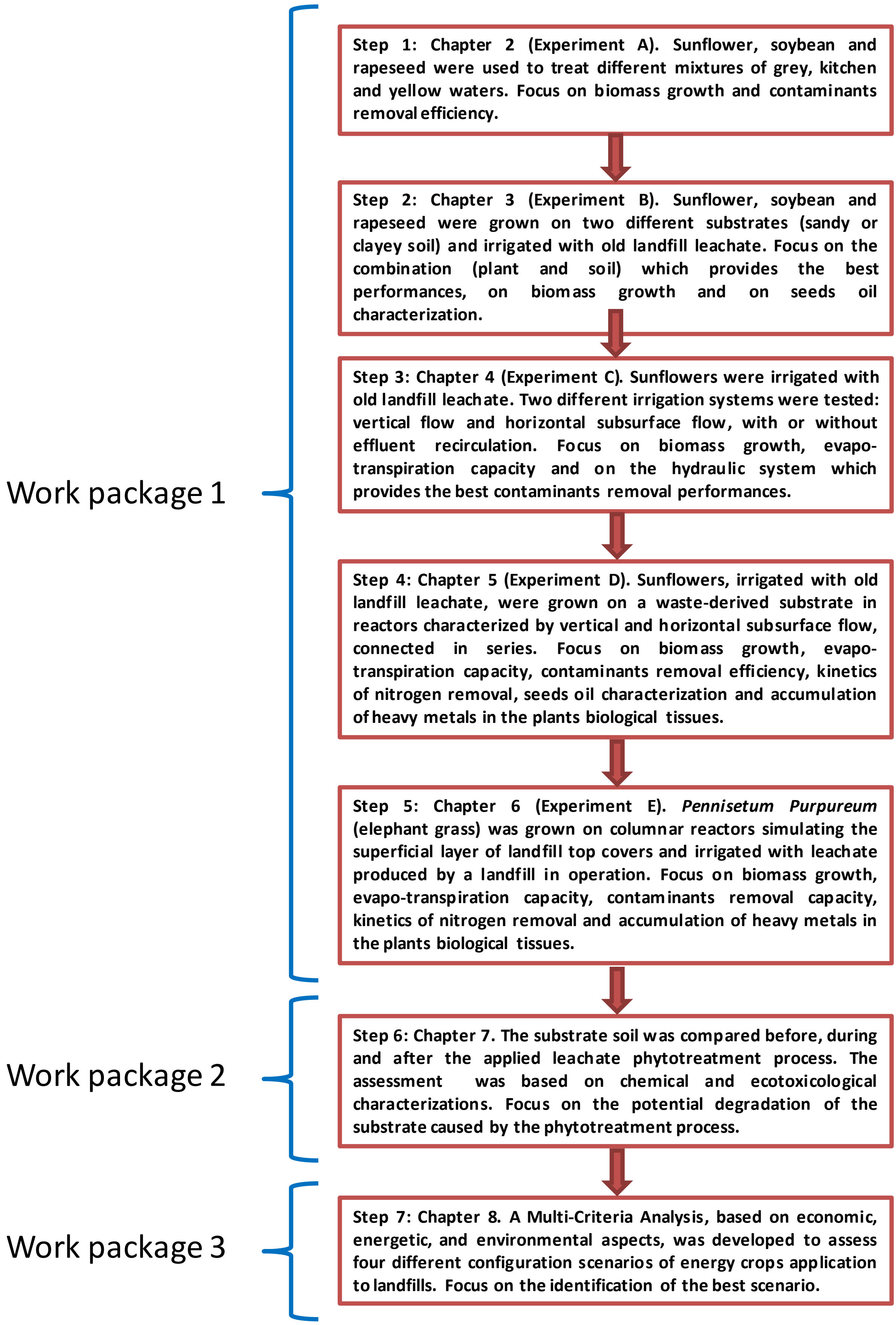

Fig. 1.9. Schematic representation of the work packages

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Chapter 2: Use of oleaginous plants in phytotreatment of grey water and yellow water from source separation of sewage

Lavagnolo, M.C., Malagoli, M., Alibardi, L., **Garbo**, **F.**, Pivato, A., Cossu, R., 2017. Use of oleaginous plants in phytotreatment of grey water and yellow water from source separation of sewage. J. Environ. Sci. 55, 274-282. http://dx.doi.org/10.1016/j.jes.2016.08.013
Readapted from the original publication.

Abstract

Efficient and economic reuse of waste is one of the pillars of modern environmental engineering. In the field of domestic sewage management, source separation of yellow (urine), brown (faecal matter) and grey waters aims to recover the organic substances concentrated in brown water, the nutrients (nitrogen and phosphorous) in the urine and to ensure an easier treatment and recycling of grey waters. With the objective of emphasizing the potential of recovery of resources from sewage management, a lab-scale research study was carried out at the University of Padova in order to evaluate the performances of oleaginous plants (suitable for biodiesel production) in the phytotreatment of source separated yellow and grey waters. The plant species used were *Brassica napus* (rapeseed), *Glycine max* (soybean) and *Helianthus annuus* (sunflower). Phytotreatment tests were carried out using 20 L pots. Different testing runs were performed at an increasing nitrogen concentration in the feedstock. The results proved that oleaginous species can conveniently be used for the phytotreatment of grey and yellow waters from source separation of domestic sewage, displaying high removal efficiencies of nutrients and organic substances (nitrogen>80%; phosphorous >90%; COD nearly 90%). No inhibition was registered in the growth of plants irrigated with different mixtures of yellow and grey waters, where the characteristics of the two streams were reciprocally and beneficially integrated.

Keywords: phytotreatment, sewage, source separation, decentralization, grey water, urine, energy crops

2.1. INTRODUCTION

The traditional concept of using huge quantities of water to transport domestic waste away from households, resulting in the production of diluted wastewater streams and treatment at centralised facilities, has often been reconsidered due to the related costs, high use of resources and significant surface occupancy (Butler and Parkinson, 1997; GTZ, 2003; Gandini, 2004).

More and more attention is being focused on sustainable sanitation systems, aimed at closing nutrient and water cycles, with low material and energy consumption. In these systems, sewage is considered a valuable source of nutrients and water for plant growth. Sustainable sanitation systems are generally based on collection and treatment of different source-separated sewage streams: yellow water (urine); brown water (faeces) and grey waters from kitchen, laundry, dishwasher, shower, etc (Langergraber and Muellegger, 2005; Cossu et al., 2003a, 2003b; Borin et al., 2004). Source separation is carried out to optimise the potential for reuse when compared to "end-of-pipe" technologies (Larsen and Maurer, 2011).

Depending on the purpose of reuse, several studies focusing on the treatment of source-separated sewage streams applied technologies largely similar to those adopted in the conventional treatment of combined wastewater (Jefferson et al., 1999; Maurer et al., 2006; Escher et al., 2006; Kujawa-Roeleveld and Zeeman, 2006; Leal et al., 2010; Larsen and Maurer, 2011; Saeed et al., 2014; Zhang et al., 2015), whilst only a few cases have been studied and used for the phytotreatment of grey waters (Frazer-Williams et al., 2008; Fangyue et al, 2009; Vymazal, 2009).

A sustainable source-separated system, named “Aquanova”, has been developed since the early nineties at the University of Padova. The system is aimed at optimizing the integrated management of various source separated sewage stream and biodegradable fractions of solid waste (Cossu et al., 2003a, 2003b).

The Aquanova system is graphically described in Fig. 2.1. Three different sewage streams are segregated using a source separation toilet and separate piping for grey water outflows. Yellow water and grey waters undergo phytotreatment, while brown waters mixed with shredded kitchen waste undergo anaerobic digestion.

Several aquatic plant species - such as *Acorus Calamus Variegatus, Alisma Plantago Aquatica, Calla Palustris, Canna Indica, Eupatorium Cannabium, Iris Pseudocorus, Lytrum Salicaria, Lobelia Cardinalis, Lysimachia Nummularia, Mentha Aquatica Rubra, Thalia Dealbata, Typha Latifolia, Lemna Minor, Eichornia Crassipes, Phragmites australi, Typha* - and natural mountain flora - such as *Aconitum napellum*, *Senecio cordatum, Senecio rupestre, Epilobium alpestre*, *Achillea millefolium* - have been tested in lab-scale and full scale phytotreatment units under different operative conditions, in previous research programmes performed by the authors of this paper (Cossu et al., 2003a).

The results of these studies confirmed the good performances of a wide species of plants in the phytotreatment of grey and yellow waters (Borin et al., 2004).

Considering the interest developed in recent years in the production of alternative energy from oleaginous crops, and the related concern for competing land use by energy crops (the "table or tank dilemma"), the present research was conceived in order to investigate the phytotreatment of source segregated sewage fractions using oleaginous crops active under temperate climatic conditions such as soybean (*Glycine max*), rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*), already taken into consideration for use in the production of industrial biodiesel (Lavagnolo et al., 2016, Meher et al., 2006; Zegada-Lizarazu and Monti, 2011). In particular, biofuel obtained from sunflower and rapeseed was found to be of excellent quality due to the high content of monounsaturated esters (Ramos et al., 2009).

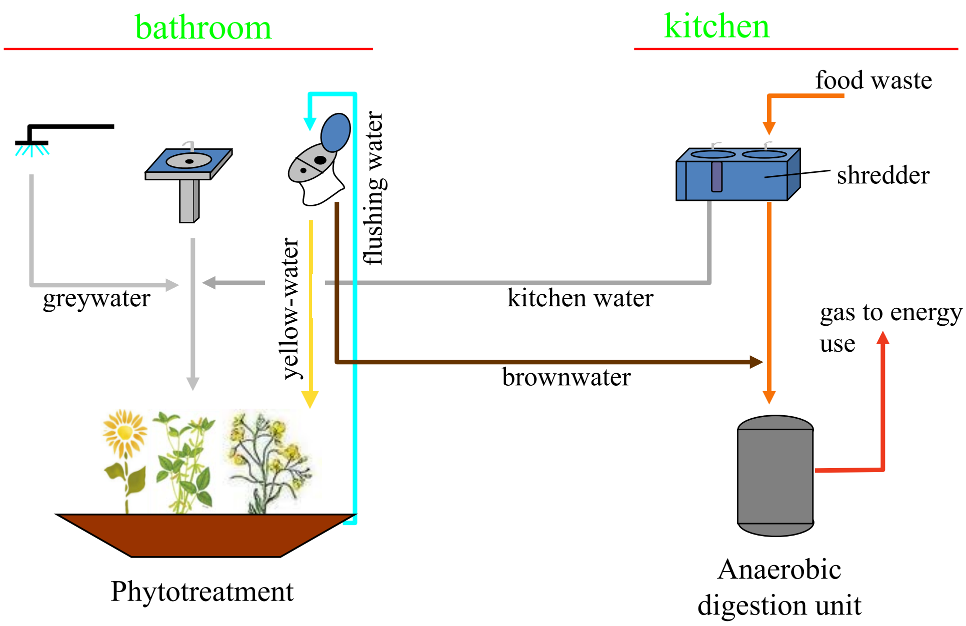

Fig. 2.1. Scheme of the Aquanova system for the integrated management of sewage and kitchen waste (Cossu et al., 2001).

2.2. MATERIALS AND METHODS

2.2.1. Wastewaters

The experiment was carried out at the Environmental Engineering Centre, Department of Industrial Engineering, University of Padova, where the Aquanova system has been implemented.

The following waters were used as feedstock: grey waters from bathroom sinks (GW); kitchen waters from kitchen sink (KW); yellow waters (YW) from a source segregation toilet (Fig. 2.2a).

Table 2.1. Mean values of pH and concentration of different analytical parameters monitored in the grey, kitchen and yellow water samples used in the phytotreatment runs

	Grey water	Kitchen water	Yellow water
pH	7.7 ± 0.2	6.9 ± 1.5	8.0 ± 1.0
Alkalinity (mg $CaCO_3$/L)	258 ± 20	414.7 ± 37	5000 ± 925
BOD_5 (mg O_2/L)	30 ± 5	90 ± 5	842 ± 17
COD (mg O_2/L)	54 ±17	1002 ± 80	2924 ± 76
TKN (mg N/L)	1.5 ± 0.4	0.8 ± 1.2	3320 ± 740
N-NH_4^+ (mg N/L)	1.5 ± 0.4	0.8 ± 1.2	3100 ± 504
P (mg P/L)	3.1 ± 0.1	5.3 ± 1.0	350 ± 130
TS (mg/L)	401 ± 10	987 ± 63	9387 ± 928
VS (mg/L)	133 ± 6	859 ± 130	3647 ± 999
Cl^- (mg Cl/L)	27.6 ± 1.1	29 ± 15	1597 ± 67
SO_4^{2-} (mg S/L)	23.9 ± 3.3	12.6 ± 4.2	187 ± 6.0
MBAS (mg/L)	0.30 ± 0.02	115 ± 52	-
Cu (μg/L)	72.2 ± 15	154 ± 110	117 ± 43
Fe (μg/L)	381 ± 59	239 ± 54	419 ± 77

BOD_5: Biochemical Oxygen Demand, measured in 5 days; COD: Chemical Oxygen Demand; TKN: Total Kjeldahl Nitrogen; TS: Total Solids; VS: Volatile Solids; MBAS: Methylene Blue Active Substances

Wastewaters samples were analysed according to the Italian standard analytical methods (CNR-IRSA, 29/2003) and measured in triplicate. pH, alkalinity, Total Solids, Volatile Solids, Biochemical Oxygen Demand, Chemical Oxygen Demand, Total Kjeldahl Nitrogen, N-NH_4^+ and the other parameters listed in Table 2.1 were taken into account to characterize the feedstock. COD was evaluated by the potassium dichromate oxidation method. BOD_5 was evaluated using a respirometer apparatus (Sapromat E). BOD_5 of kitchen water was performed after pre-filtration at 2 μm in order to detect the soluble BOD compounds. TKN and N-NH_4^+ was evaluated by means of a distillation-titration procedure, while TKN was measured after an acid digestion phase. Dissolved components (nitrate, phosphate and sulphate ions) were determined using a UV-VIS spectrophotometer (UV-1601, Shimadzu, Japan) preceded by filtration with a 0.45 μm pore membrane. The colorimetric method was used to detect total phosphorus after sample digestion. Chloride and sulphide were measured by titration, whereas metal content was measured by

Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES-4200 DV, Perkin Elmer, USA).

Wastewaters were analysed twice a week throughout the entire study period. The analytical results are summarised, as mean values, in Table 2.1. Heavy metals concentrations, with the exception of Cu and Fe, were below detection limits.

2.2.2. Plants and inflow waters

Phytotreatment tests were carried out in 20 L plastic pots with a layer of 30 cm of sandy substrate (sand: 82%, clay: 10%, silt: 8%; density: 1.5 kg/L) and a layer of 10 cm medium-sized gravel as bottom drainage. A drainage tube was fitted at the base of each pot to drain off the outflow. The analytical quality of the sandy substrate used is described in Table 2.2. Three plant species were tested: *Brassica napus* (rapeseed), *Glycine max* (soybean) and *Helianthus annuus* (sunflower). Seeds were provided by the Seed Data Bank of the DAFNAE Department, University of Padova. Eight pots per each plant species were used: four as testing units and four as control units. One plant was grown in each experimental unit. The pots were arranged in a greenhouse (Fig. 2.2b) where an average daily temperature of 24°C, average night temperature of 12°C and a photoperiod of 14 hr were maintained.

Table 2.2. Quality of the sandy substrate used in the experimental pots. Data are refer to dry solid matrix of the substrate.

Parameter	Value	Parameter	Value
TS (% w/w)	98.0±1	Ca (mg/kg)	157761.0±156.0
VS (%TS)	1.2±0.3	Cd (mg/kg)	< 0.7
TOC (%)	< 1.0	Cr (mg/kg)	2.5±0.2
TKN (mg/kg)	77.6±2.2	Cu (mg/kg)	6.5±0.3
NH_4^+-N (mg/kg)	55.1±1.8	Fe (mg/kg)	3955.8±178.8
NO_3-N (mg/kg)	< 10.0	K (mg/kg)	810.2±25.6
P-tot (mg/kg)	173.0±12.2	Mg (mg/kg)	51665.1±223.2
Cl^- (mg/kg)	2278.9±125.1	Mn (mg/kg)	179.3±3.1
Si (mg/kg)	125.1±8.3	S (mg/kg)	108.8±2.7
Na (mg/kg)	357.5±12.5	Pb (mg/kg)	2.7±0.4
Ni (mg/kg)	3.3±0.3	Zn (mg/kg)	20.7±1.9

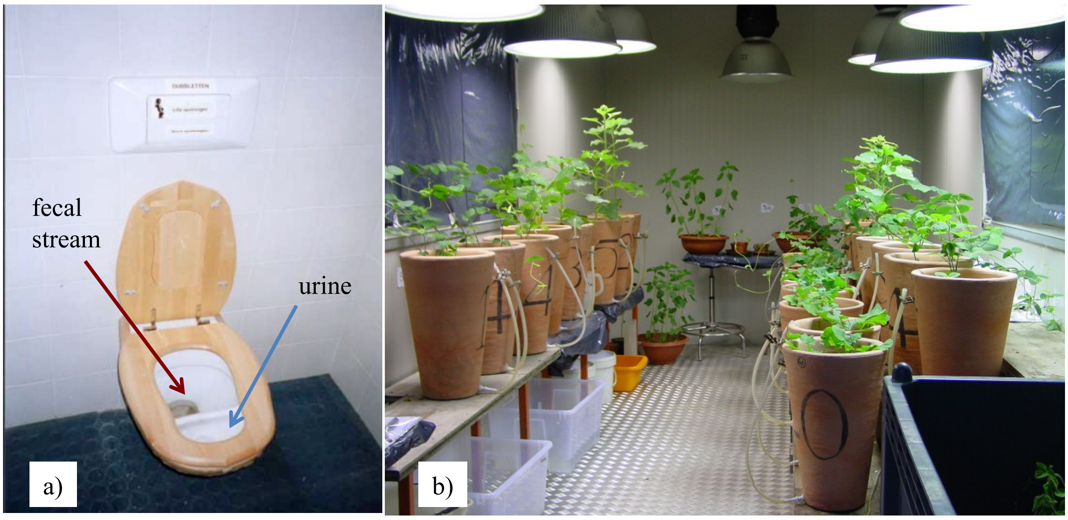

Fig. 2.2. Equipment used in the research: (a) source separate toilet; (b) greenhouse.

At the beginning of the experiment (acclimatization period), all experimental units were irrigated using tap water and Hoagland's nutrient solution (Hoagland and Arnon, 1950) in order to promote initial plant growth. From the acclimatization phase onwards, testing units (four of each species) were watered first with grey water and subsequently with different combinations of grey, kitchen and yellow waters. The research was divided into four phases, each characterised by different dosing, with the aim of gradually increasing the load of nitrogen. The duration and settings of the four phases are described in Table 2.3.

The remaining pots (four of each species) were watered with tap water and Hoagland's solution and used as control units according to a well established procedure (Holmes, 1980; Hocking and Steer, 1983; Salvagiotti et al., 2008).

Hydraulic loading was provided according to the individual plant growth demand and the hydraulic retention time (HRT) was kept equal to 7 days minimum in all experimental units. In a similar experiment, Sawaittayothin and Polprasert (2007) demonstrated that the minimum HRT must be between 5 and 8 days, depending on the contaminants to be removed. The experiment was extended for the entire vegetative period of the three species until plant senescence was reached (end of phase IV).

During the research phases the outflow streams from the different pots were sampled and analysed twice a week, according to the Italian standard analytical methods (CNR-IRSA, 29/2003), with four replicates.

At the end of phase IV the plants were individually uprooted. Shoot and root length and fresh weight were measured. The plant tissues were dried at 65°C for 3 days. Dry weight was measured and the content of total nitrogen (as sum of TKN and $N\text{-}NO_3$), total phosphorous, heavy metals and microelements was determined.

The sandy substrate was analysed for determining total nitrogen and total phosphorous contents, in order to evaluate the role of soil in nutrients removal. Plants and sandy substrate were analysed according to the Italian standard analytical methods for solid samples (CNR-IRSA, 64/1985).

Table 2.3. Description of the feeding mixtures adopted throughout the different research phases.

Phase	Duration (days)	Composition of the inflow water (%, *V/V*)
Acclimatization	19	tap water + nutritive solution (Hoagland solution)
	10	50% tap water + 50% GW
Phase I	18	100% GW
Phase II	10	49.95% GW + 49.95% KW + 0.1% YW
Phase III	10	49.9% GW + 49.9% KW + 0.2% YW
Phase IV	10	49.75% GW + 49.75% KW + 0.5% YW

GW= Grey water, KW = Kitchen water, YW = Yellow water.

2.3. RESULTS AND DISCUSSION

2.3.1. Wastewaters and feeding mixture quality

The quality of the wastewaters used in the research (Table 2.1) of course reflects the water use and residential peculiarities of the community where they were produced, the University (e.g.: no shower was utilised). For all parameters the observed values were, as expected, higher for yellow water rather than for grey water, but nitrogen content found as mean value in the last was lower than values found in other studies (Cossu et al, 2003b; Eriksson et al., 2009; Fangyue et al., 2009; Kattel et al., 2011).

Table 2.4 shows the quality of wastewater feedings measured during the different research phases. The concentration of nutrients (N and P) increased progressively, as purposely planned, from phase I to IV.

Nitrogen load was mainly associated to yellow water. This is clearly evident from the first phase feedings when only grey water was present, and consequently the nitrogen load in the inflow was particularly low. The yellow water acted as fertilizer for the plants, without overloading the hydraulic volume of the system.

COD and solids concentrations in the feeding were mainly linked to the presence of kitchen water and yellow water, while MBAS (Methylene Blue Active Substances) were mainly associated with kitchen waters.

Table 2.4. Value range of the concentrations of the main analytical parameters describing the quality of water feedings during the different phases of the research.

Phases	COD (mgO_2/L)	TKN (mgN/L)	$N\text{-}NO_3$ ($mgNO_3\text{-}N/L$)	Ptot (mgP/L)	Cl^- (mgCl/L)	SO_4^{2-} (mgS/L)	TS (mg/L)	VS (mg/L)	MBAS (mg/L)
Acclimatization	5±1.2	0.3±0.1	41.0±8.8	3.1±0.2	3.7±0.2	48.0±14	440±11	150±2	-
	23±9	0.9±0.2	0.07±0.03	3.3±0.1	13.4±2.4	12.0±1.6	278±18	147±3	0.13±0.02
Phase I	48±17	1.5±0.4	0.01±0.10	3.1±0.1	27.6±1.1	26.0±3.3	401±10	133±6	0.30±0.02
Phase II	528±49	4.5±1.6	0.60±0.10	4.5±0.7	29.8±8.1	19.5±3.7	703±38	499±69	57.59±25.98
Phase III	530±48	7.8±2.3	0.60±0.10	4.9±0,8	31.4±8.2	19.6±3.7	711±40	502±70	57.53±25.95
Phase IV	540±47	17.7±4.5	0.60±0.10	5.9±1.9	36.1±8.3	20.3±3.8	737±41	512±73	57.36±26.00

Water demand naturally increased during the experimental period (Fig. 2.3), due to plant growth. Water demand reached a peak in phase III, whereas the reduction in water consumption during phase IV clearly underlined the onset of plant senescence.

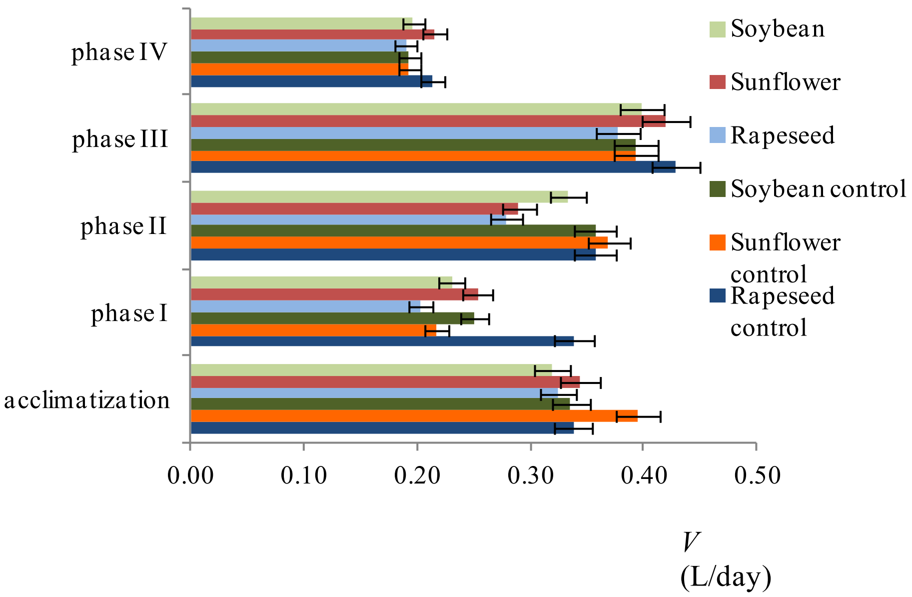

Fig. 2.3. Influent volume of water provided daily in the four experimental phases for the three different tested plant species.

2.3.2. Plants growth

Plants growth parameters are reported in Table 2.5, for both the experimental and the control pots. Biomass development is a good indicator of the plant health which reflects a balanced availability of nutrients and the absence of inhibitory effects by toxic substances. Roots development, either in terms of mass or length allows the evaluation of the soil and of the phytotreatment performance capacity of each individual plant.

A significantly reduced roots development was observed in rapeseed compared to the corresponding control plants, both in terms of mass and length. Total biomass was approximately 21% of the biomass developed by the control plants, while the root biomass was even lower (13%). This is a clear indication that rapeseed is not a suitable species for the phytotreatment of the kinds of wastewater tested.

Sunflower produced less biomass than the control, while roots (in terms of length and weight) grew 10% more. Total biomass and the roots weight of soybean corresponded to approximately 60% that observed in control plants, indicating an equally distributed growth between root and shoot. However, root length was approximately 20% higher than in controls.

Table 2.5. Total biomass, biomass and length of roots measured in the individual plants at the end of the research period, and variation (ɳ) between treated plants and respective controls

Plant species		Rapeseed	Sunflower	Soybean
Total biomass	Test (g/pot)	7.56±0.86	4.41±0.78	7.79±0.23
	Control (g/pot)	36.10±2.69	6.51±1.47	11.99±2.02
	Test/Control (%)	21	68	65
	ɳ (%)	-79	-32	-35
Root mass	Test (g/pot)	3.13±0.23	0.49±0.08	0.99±0.03
	Control (g/pot)	24.40±1.55	0.45±0.12	1.71±0.17
	T/C (%)	13	110	58
	ɳ (%)	-87	10	-42
Main root length	Test (g/pot)	7.24±0.88	7.74±1.25	8.38±1.55
	Control (g/pot)	11.20±1.20	7.00±0.93	6.75±1.13
	Test/Control (%)	65	111	124
	ɳ (%)	-35	11	24

Generally, even when the total amount of nutrients added through wastewater irrigation was comparable to the amount recommended for optimal plant growth (Güsewell, 2004; Hoagland and Arnon, 1950), plants irrigated with wastewaters developed a lower biomass than the corresponding controls.

These results are partially in contrast with those obtained in similar experiments both for sunflowers (Khan et al., 2009) and other plant species (Gandini, 2004), where vegetative growth was enhanced by irrigation with grey, yellow and kitchen waters. These divergent results could be linked to the low content of nitrogen in the greywaters, as highlighted earlier. This fact might have resulted in a shortage of nutrients at the beginning of our experiment (phase I), when only greywaters were fed, which inhibited plants growth, as observed in previous studies (Güsewell, 2004; Jones et al., 2011). This early impairment in plant growth was not recovered in the following phases, despite an increase in nutrient loading with addition of yellow and kitchen waters.

2.3.3 Removal efficiency

Fig. 2.4 describes graphically the variation in time of the loading of nutrients and COD in the feeding, concentrations of the same parameters in the outflow streams and removal yields, as observed throughout the different phases. Loadings are expressed in terms of surface load (mg/m^2/day) in order to allow comparison with literature data. Removal yields (η, %) were obtained by computing the input and output loads:

$$\eta = (V_{in} \cdot C_{in} - V_{out} \cdot C_{out})/(V_{in} \cdot C_{in}) \cdot 100\% \qquad (2.1)$$

where: V_{in} = influent volume (L/week); V_{out} = effluent volume (L/week); C_{in} = influent concentration (mg/L); C_{out} = effluent concentration (mg/L).

High nitrogen removal efficiency (>80%) was observed throughout the experimental period. In phase I nitrogen load was found to be quite low, (3-20 mg N/m^2/day). While this produced a negative effect, as observed earlier, on the development of plant biomass, high removal efficiencies were achieved. In phases II, III and IV the N load was gradually increased from 20 to 198 mg N/m^2/day for sunflower and soybean, and from 10 to 140 mg N/m^2/day for rapeseed, due to the contribution of yellow water (Table 2.4).

The different values of the nitrogen load observed for the three species were the result of the different water demand of the individual plants (Fig. 2.3).

Throughout the last three phases, with the exception of a slight drop at the end of phase II, removal efficiencies remained stable, higher than 90%, indicating a positive and rapid response of the system to the increase of nitrogen loading. Nitrogen concentration in the outflow was constantly below 10 mg/L.

Throughout the entire experiment, mean phosphorous load values ranged between 30 mg P/m^2/day (sunflower) and 35 mg P/m^2/day (rapeseed and soybean) being within the phosphorous plant demand (Holmes, 1980). Removal efficiencies were very high for all plant species, with a concentration in the outflow below 1 mg P/L. This clearly indicates that this nutrient was almost completely removed by the system.

During phase I, due to the low inflow COD concentrations (Table 2.4), the removal efficiencies were very high, with a COD output constantly below 10 mg/L. From phase II the COD concentration was increased by the addition of yellow and kitchen wastewaters. In phases III and IV COD load in the inflow fluctuated as a result of the high variability of the quality of kitchen waters. Nevertheless, COD in the outflow remained below 100 mg/L.

The COD load in the different phases ranged between 200 mg O_2/m^2/day (Acclimatization and Phase I) and 2000-4000 mg O_2/m^2/day (Phases II, III and IV).

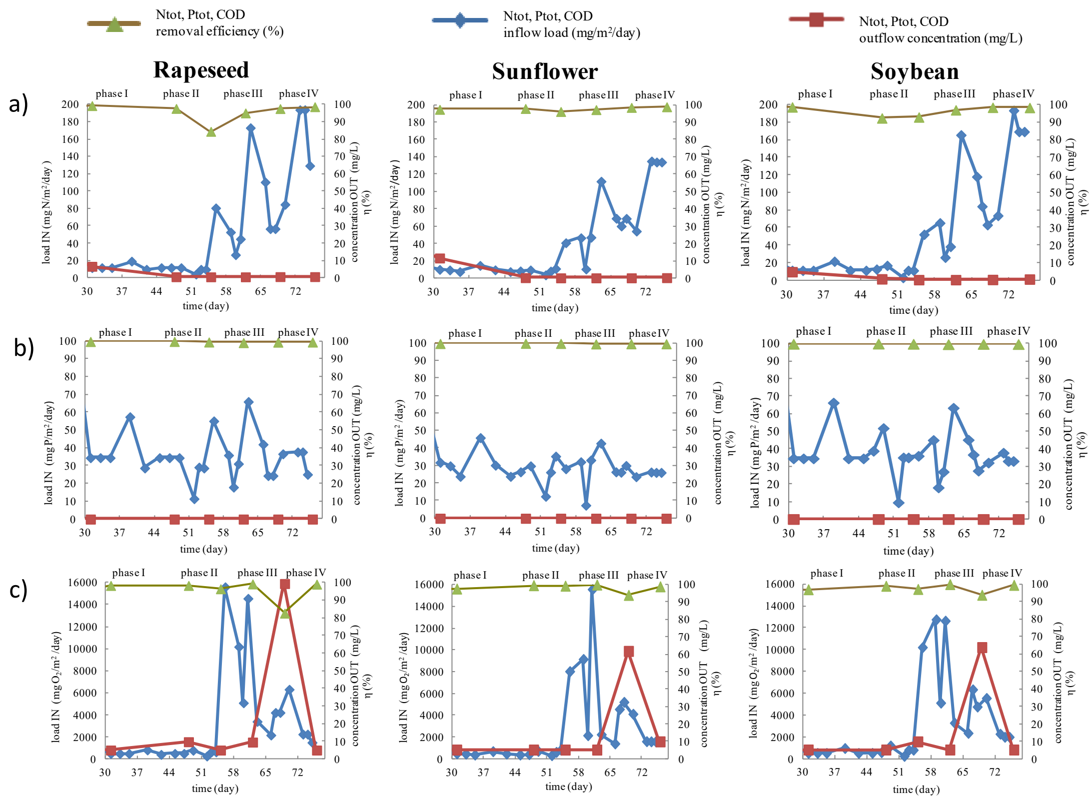

Fig. 2.4. Variation in time of the feeding load, the outflow concentrations and of the removal yields of total nitrogen (N_{tot}) (a), total phosphorous (P_{tot}) (b) and COD (c) along the experiment for the three plant species.

The good COD removal efficiency is related to the synergic effects of the chemical, physical and biological processes occurring in the plant-substrate system (sedimentation, filtration, adsorption in the substrate, biodegradation of the organic matter and uptake by plant roots), as reported by Duggan (2005).

Generally, COD, N and P removal rates are higher than those reported in previous studies (Keffala and Ghrabi, 2005; Khan et al., 2009). Input and output concentrations and related removal efficiencies with regards to parameters other than nutrients and COD are reported in Table 2.6.

The efficiency of MBAS removal was very high (more than 95%) for all plant species, even in the presence of high input concentrations (up to 111 mg/L in phase II) (Table 2.6). Similar findings were recently reported by Ramprasad and Philip (2016).

Table 2.6. Input and output concentrations of several parameters and removal efficiency rates observed for the different plant species

Parameter	Species	IN		OUT		η (%)
		Min - Max	Average	Min - Max	Average	
Cl^- (mgCl/L)	Rapeseed	27.6 - 44.4	30.8	23.6 - 28.5	24.4	21
	Sunflower			22.9 - 27.7	23.6	23
	Soybean			26.1 - 31.5	26.9	13
SO_4^{2-} (mgS/L)	Rapeseed	19.5 - 29.3	21.8	15.1 - 16.9	15.8	23
	Sunflower			13.7 - 15.4	14.4	30
	Soybean			13.2 - 14.8	13.9	32
TS (mg/L)	Rapeseed	401 - 778	732	384 - 542	485	61
	Sunflower			330 - 440	399	79
	Soybean			520 - 684	587	68
VS (mg/L)	Rapeseed	133 - 585	505	118 - 280	223	77
	Sunflower			98 - 160	124	90
	Soybean			136 - 402	281	79
Cu (µg/L)	Rapeseed	27.0 - 82.9	112.0	10.0 - 12.0	11.0	90
	Sunflower			< 10.0	10.0	93
	Soybean			<.10.0	10.0	93
Fe (µg/L)	Rapeseed	31.0-1150.0	647.0	10.0 - 404.0	160.0	86
	Sunflower			10.0 - 510.0	25.0	100
	Soybean			10.0 - 488.0	170.0	99
MBAS (mg/L)	Rapeseed	0.30 - 83.57	43.5	0.1 - 1.3	0.8	98
	Sunflower			0.1 - 1.5	0.9	98
	Soybean			0.1 - 1.3	0.8	98

IN: input concentration; OUT: output concentration; η: removal efficiency

Copper and iron are reported as they are deemed of interest due to the detection of concentrations present in input wastewaters (see Table 2.1). For both heavy metals, which were detected in significant concentrations, removal rates were very high particularly for sunflower. Outflow concentrations lower than 0.01 mg/L for Cu and 0.5 mg/L for Fe were always achieved.

Although the concentrations of chloride and sulphate increased in the feeding due to the increasing percentage of yellow water during the different study phases (Tables 2.1 and 2.4), no effects on plant growth were detected. As expected (Ouyang, 2013), removal rate of chloride and sulphate was limited; in particular, soybean plants were found to be the less efficient to increasing chloride load but displayed the best performance in sulphate removal. Removal efficiencies for TS and VS throughout the different research phases exceeded 60%-70%.

2.3.4. Nitrogen balance

At the end of the entire experiment a total nitrogen balance for each pot was calculated on the basis of the following equation:

$$N_{in} = N_{out} + Np + Ns + Nb \tag{2.2}$$

where, N_{in} = total mass of nitrogen entering the pot plant-soil system, nitrogen input as sum of TKN and N-NO_3 loads (N-NO_2 was negligible) provided throughout the entire experiment (mg); N_{out} = total mass of nitrogen in the outflow (mg); Np = amount of nitrogen accumulated in the plant tissue (mg); Ns = nitrogen accumulated in the substrate (mg); Nb = balancing term for closing the equation (mg). This term takes into account the nitrogen gaseous loss.

The mean values (averages of four replicates) of the nitrogen balance terms are reported in Table 2.7.

Table 2.7. Mean values of the nitrogen balance terms measured at the end of the entire experiment for each individual plant-soil system (pot). Data are the averages of four replicates and are expressed both as mg/pot and percentage of Total N input. Surface pot is 0.045 m^2

Species	N_{in}	N_{out}		Np		Ns		Nb	
	mg	mg	%	mg	%	mg	%	mg	%
Rapeseed	860±54	1.5±1.1	0.2	80±14	9	780±88	91	-1.5±0.3	-0.2
Sunflower	630±38	0.7±1.2	0.1	60±21	10	570±75	91	-0.7±0.9	-0.1
Soybean	850±77	1.3±0.6	0.2	170±36	20	670±53	79	8.7±1.8	1.0
Rapeseed control	780±10	1.0±0.4	0.1	140±23	18	680±43	87	-41.0±7.9	-5.3
Sunflower control	730±12	2.1±1.0	0.3	70±31	10	590±48	81	67.9±13.7	9.3
Soybean control	710±18	1.1±0.3	0.2	260±45	37	460±37	65	-11.1±2.3	-1.6

The input values indicate that the total amount supplied by wastewater irrigation is close to the common nitrogen demand of each plant species (Holmes, 1980) being slightly higher (rapeseed and soybean) or lower (sunflower) with respect to the amount supplied to the corresponding controls. Despite this evidence, N plant uptake for each individual plant was lower than in the controls. This is particularly evident for rapeseed as a consequence of the larger biomass development observed in the control (Table 2.5).

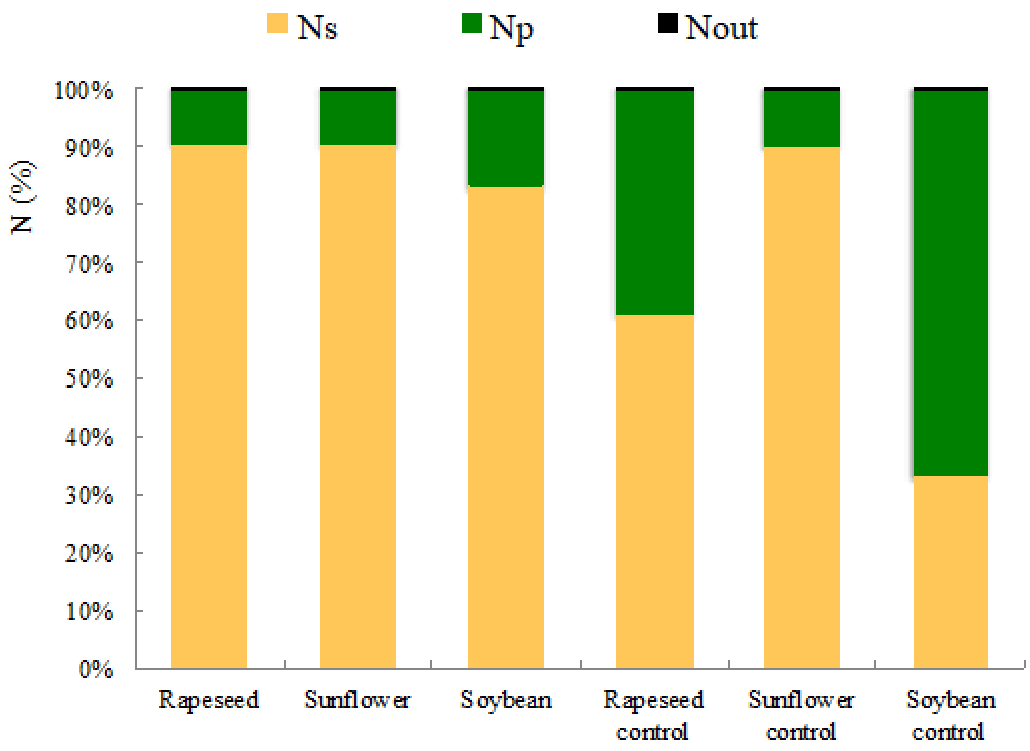

Fig. 2.5. Balance of whereabouts of the total nitrogen mass (N_{in}) entering the soil-plant system. Ns = nitrogen in soil, Np = nitrogen uptake by plants, N_{out} = residual nitrogen in the outflow. All terms are expressed as % of $N_{in.}$

A graphical description of the relevance of the different whereabouts of the nitrogen mass provided with the inflow is given in Fig. 2.5. It clearly shows how the soil plays the most important role in phytotreatment removal, as observed in several other studies, connected in particular to the bacterial metabolism around the root zone (Griffiths and Robinson, 1992).

Similar to nitrogen balance, a mass balance for phosphorous has been drawn and mean values are reported in Table 2.8:

$$P_{in} = P_{out} + Pp + Ps + Pb \tag{2.3}$$

where, P_{in} = total mass of phosphorous entering the pot plant-soil system (mg); P_{out} = total mass of phosphorous in the outflow (mg); Pp = phosphorous plant uptake measured as total mass of phosphorous accumulated in the plant tissue (mg); Ps = phosphorous accumulated in the substrate (mg); Pb = balancing term for closing the equation (mg).

Contrary to nitrogen, the total phosphorous amount supplied by wastewater irrigation is slightly lower than amount provided to the corresponding control pots. Treated plants accumulated less P than their respective controls (Table 2.8). The graphical representation of the whereabouts of phosphorous in the inflow once again highlights the fundamental role of the soil, which appears however less important than for nitrogen (Fig. 2.6).

Table 2.8. Mean values of the phosphorous balance terms measured at the end of the entire experimental period for each individual plant-soil system (pot). Data are the averages of four replicates and are expressed both as mg/pot and percentage of Total P input

Species	P_{in}	P_{out}		Pp		Ps		Pb	
	mg	mg	%	mg	%	mg	%	mg	%
Rapeseed	69±15	0.1±0.1	0.1	12±3	17	59±8	86	-2.0±0.6	-3.0
Sunflower	53±18	0.1±0.1	0.2	10±3	19	40±3	76	2.9±2.1	5.5
Soybean	71±14	0.1±0.3	0.1	18±2	25	50±7	70	2.9±0.8	4.1
Rapeseed control	82±3	0.1±0.2	0.1	31±4	38	54±4	66	-3.1±1.1	-3.8
Sunflower control	78±3	0.2±0.1	0.3	18±2	23	58±5	74	1.8±0.9	2.3
Soybean control	76±2	0.1±0.2	0.1	39±4	51	40±9	53	-3.1±1.7	-4.1

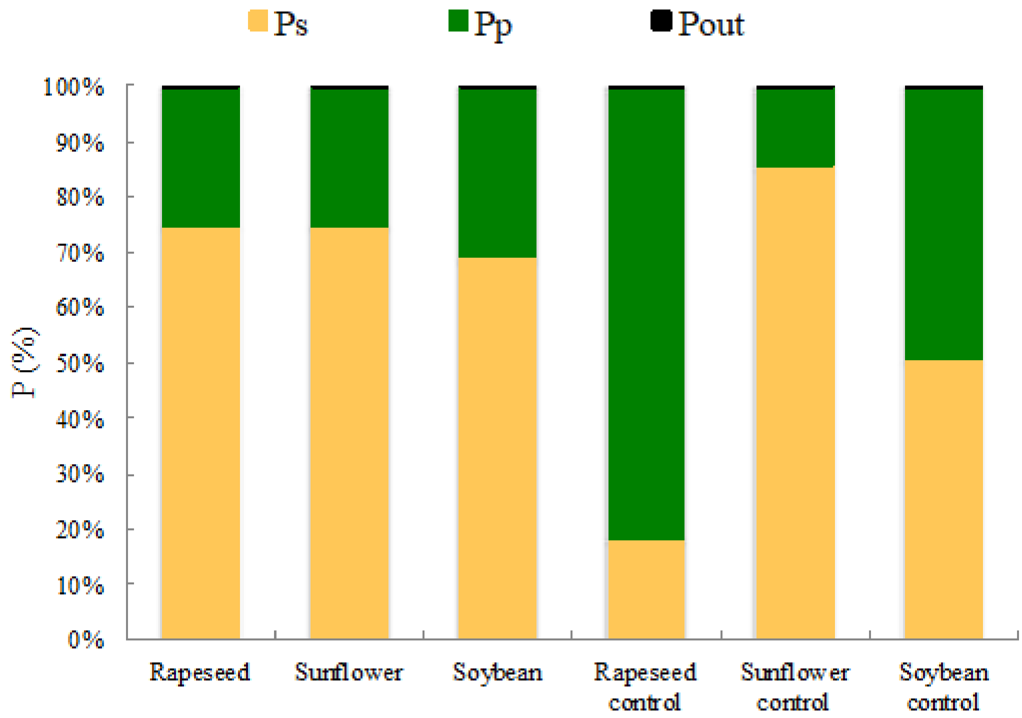

Fig. 2.6. Balance of whereabouts of the total phosphorous mass (P_{in}) entering the soil-plant system. P_s = phosphorous in soil, P_p = phosphorous uptake by plants, P_{out} = residual phosphorous in the outflow. All terms are expressed as % of Pin.

2.4. CONCLUSIONS

The basis underlying the investigation was a pot trial comprising three species of oleaginous plants (rapeseed, soybean and sunflower), aimed at assessing their ability to treat grey, yellow and kitchen wastewaters and at calculating, with respect to the plant-soil system, a balance of the whereabouts of the nutrients (N and P) loads supplied with the wastewaters. The investigation was divided into four distinct phases using different mixtures of the three wastewaters with the aim of progressively increasing nutrients and organic contents in the irrigation water.

The following conclusions could be drawn:

(1) Rapeseed, soybean and sunflower plants treated with wastewaters presented a biomass development lower than the controls. The reduced vegetative growth was mainly due to a general scarcity of available nutrients for plants at the beginning of the growth stage (phase I of the study), when plants were fed with grey waters only.

(2) The addition of yellow waters increased the nitrogen concentration in feed, determining a positive response of plants both in terms of growth and removal efficiency.

(3) The removal efficiencies for N, P and COD remained higher than 80% for all plant species throughout the period. Sunflower plants induced the highest removal rates, whilst rapeseed plants featured the lowest removal rates and the highest biomass reduction.
(4) The most crucial finding is related to the identification of an optimal combination of source-separated wastewaters and nutrient-loaded waters (kitchen water and yellow water), with the aim of achieving a satisfactory degree of plant growth and phytotreatment performance.
(5) The removal mechanisms involve complex interactions between chemical, physical and biological processes.

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Chapter 3: Lab-scale phytotreatment of old landfill leachate using different energy crops

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Abstract

Old landfill leachate was treated in lab-scale phytotreatment units using three oleaginous species: sunflower (H), soybean (S) and rapeseed (R). The specific objectives of this study were to identify the effects of plant species combinations with two different soil textures on the reduction of COD, total N (nitrogen) and total P (phosphorous); to identify the correlation between biomass growth and removal efficiency; to assess the potential of oily seeds for the production of biodiesel The experimental test was carried out using 20 L volume pots installed in a greenhouse under different leachate percentages in the feeding and subsequent COD, N and P loads. Significant removal efficiencies were achieved: COD (η >80%), total N (η >70%) and total P (η >95%). Better performances were displayed by the clayey soil. Plants irrigated with leachate, when compared to control units fed only with water and nutrient solution (Hoagland solution), developed a larger plant mass. Sunflower was the best performing species.

Keywords: phytotreatment, leachate, oleaginous crops, renewable energy

3.1. INTRODUCTION

Leachate treatment represents a massive cost in the management of solid waste. Due to the high concentration of contaminants, advanced technological treatments are required to reach the prescribed emission standards (Liu et al., 2015). A major concern is related to the qualitative changes which leachate undergoes over time during the landfill management phases (Kulikowska and Klimiuk, 2008; Lee et al., 2010). Young leachates with high BOD/COD ratio, high ammonium content and low pH can be successfully treated by means of biological treatment, whilst alternative treatments are used for the old leachates characterized by a significant fraction of recalcitrant compounds (humic acid, fulvic acid) and a high ionic strength (Stegmann and Ehrig, 1981; Cossu et al., 1992; Renou et al., 2008). In the majority of cases, leachate treatment is a combination of different processes, and both the production of secondary wastes (sludge, concentrate, brine) and the consumption of energy by each specific treatment step (Fane, 2007; Ehrig and Robinson, 2010) should be carefully considered at the time of selection of the most appropriate technologies. Phytotreatment has been widely investigated and appears to be a valid alternative to energy-demanding processes (Jones et al., 2006). It is a sustainable process, featuring very low operational and maintenance costs, and is suited for use in treating weak leachates from old landfills, or for polishing leachates that have been pretreated by other biological processes (Ehrig and Robinson, 2010). Several plant species have been tested at lab and full scale by a series of authors and, in general, phytotreatment has displayed efficiency in the removal of recalcitrant contaminants, mainly due to soil and plant synergic effects (Fraser et al., 2004; Hasselgreen, 1992, Akinbile et al., 2012). Plants treated with leachate grew better than those irrigated with water or did not differ significantly from plants treated with fertilizers (Cheng and Chu, 2011; Sang et al., 2010; Duggan, 2005; Jones et al., 2006; Marchiol et al., 2007; Tyrrel et al., 2001; Zalesny et al., 2007; Zupančič Justin et al., 2010). The use of plants for leachate treatment greatly increases evapo-transpiration compared to unvegetated sites , in which transpiration does not take place (Ettala, 1989). Increased evapo-transpiration is a desirable effect as it causes a reduction in the volume of leachate to be treated (Duggan, 2005; Ogata et al., 2015). Phytoremediation of wastewater could be combined with the production of renewable energy, such as wood from short rotation coppice, bioethanol from lignocellulosic biomass or biodiesel from oleaginous crops (Pandey et al., 2016). The growth of energy crops, combined with wastewater treatment, increases the economic competitiveness of the system, reducing the costs associated with irrigation and fertilization (Duggan, 2005; Rockwood et al., 2004; Hasselgren, 1989). In recent years, the phytotreatment of leachate using plants for biomass and/or bioethanol production, has been partially explored by the scientific community, yielding interesting results on pollutant removal performances (Dimitriou and Aronsson, 2010; Duggan, 2005; Jones et al., 2006, Zalesny et al., 2007; Zupančič J. and Zupančič, 2009).

This paper describes the results of an original research program in which three oleaginous plant species, *Helianthus annuus* (sunflower), *Glycine max* (soybean) and *Brassica napus* (rapeseed) were used to treat old landfill leachate. The three plant species have proved to be resistant to a series of organic and inorganic contaminants in different phytoremediation tests (Brunetti et al., 2011; January et al., 2008; Marchiol et al., 2007; Agostini et al., 2003; Schnoor et al., 1995). Moreover, oil for biodiesel production can be extracted from their seeds (Singh and Singh, 2010). Biodiesel yield and quality are influenced by oil composition; in particular the amount of Free Fatty

Acids (FFA) should be < 1% w/w. Feedstock with high FFA content decreases biodiesel yield and increases production costs. Different vegetable oils with varying fatty acid compositions can be used, although soybean, sunflower and rapeseed are the most widely employed. More than 95% of the world biodiesel is produced from edible oils such as rapeseed (84%),sunflower oil (13%), palm oil (1%), soybean oil and others (2%) (Atabani, 2012).

In this study, plants were grown both on sandy and clayey soil to test the effectiveness of leachate treatment in two different growing textures. The quality of the treated effluent depends on soil-water physical and chemical interaction, although leachate percolating through clayey soil should contain lower amounts of ammonia (Pivato and Raga, 2006) and COD than leachate percolating through sandy soil (Duggan, 2005). The outcomes produced on plant growth and phytotreatment efficiencies have been discussed, together with the support of nitrogen and phosphorous mass balance, to better evaluate interactions between the different plants-soil-leachate components (Duggan, 2005).

3.2. MATERIALS AND METHODS

3.2.1. Equipment

A total of 24 pots (20 L volume each), 50 cm high, were equipped with a flexible tube at the bottom to control the water level inside the pot and drain off outflow. 10 cm of coarse gravels (8-16 mm in size) were arranged at the bottom as drainage layer. Twelve pots were filled with 30 cm of pure sand and twelve pots with 30 cm of clayey soil (Fig. 3.1). Pots were placed inside a greenhouse, with controlled temperature (21°C - 24°C) and a 14-hours photoperiod with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity.

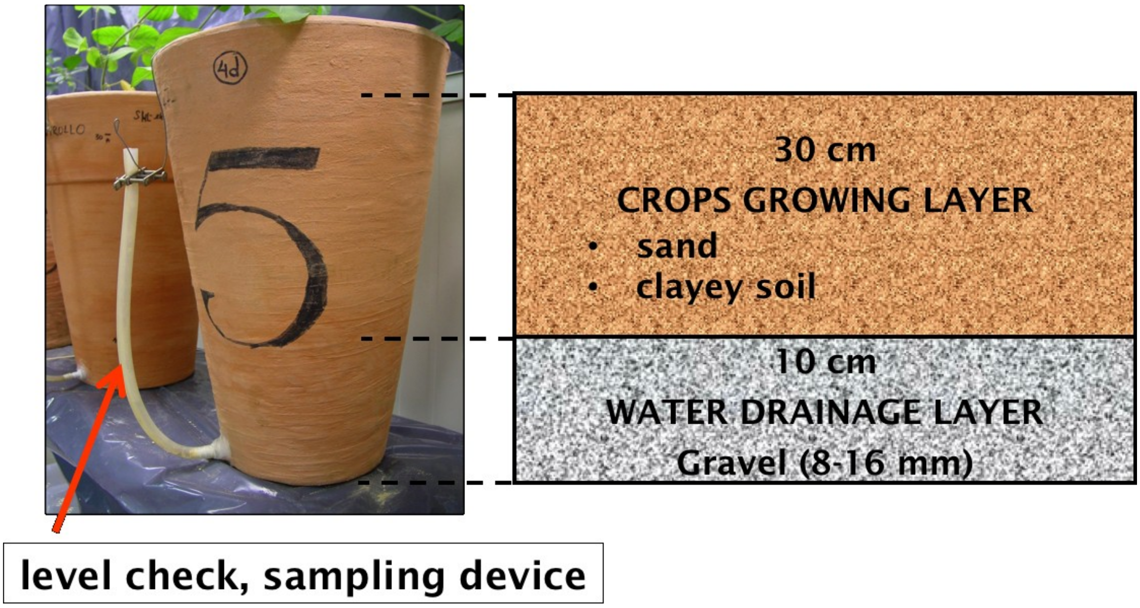


Fig. 3.1. Pots used during the experiment

3.2.2. Landfill leachate

Leachate was collected from a confined capped landfill receiving unsorted municipal solid waste from 1987 to 1999, located in the North East of Italy. This leachate can be classified as old landfill leachate (Table 3.1), as the pH value is high and BOD_5/COD below 0.1 (Kjeldsen et al., 2002; Andreottola and Cannas, 1992; Stegmann et al., 2005).

Table 3.1. Characteristics of the leachate during the research period (mean values ± SD)

Parameter	Values	Parameter	Values
pH	8.02±0.05	S^- (mg/L)	<4
TS (mg/L)	6315±636	Cl^- (mg/L)	239±12
VS (mg/L)	1548±607	Ca (mg/L)	245±13
COD (mg/L)	2255±698	K (mg/L)	1075±57
BOD_5 (mg/L)	75±19	Mg (mg/L)	97.50±5.20
TOC (mg/L)	1953±259	Na (mg/L)	2705±144
IC (mg/L)	140±10	Cr (µg/L)	431±23
TKN (mg/L)	1204±30	Cu (µg/L)	54±3
$N\text{-}NH_4^+$ (mg/L)	1117±3	Fe (µg/L)	6690±356
$N\text{-}NO_3^-$ (mg/L)	0.57±0.13	Mn (µg/L)	171±9
P (mg/L)	22±3	Ni (µg/L)	144±8
$P\text{-}PO_4^{3-}$ (mg/L)	20±1	Pb (µg/L)	49±3
SO_4^{2-} (mg/L)	<10	Zn (µg/L)	171±9

3.2.3. Chemical and physical properties of soils

Soil textures, determined using the Bouyoucos methods (Bouyoucos, 1962), are reported in Fig. 3.2. As expected, sandy soil belongs to the sand category, since pots were filled with pure sand.

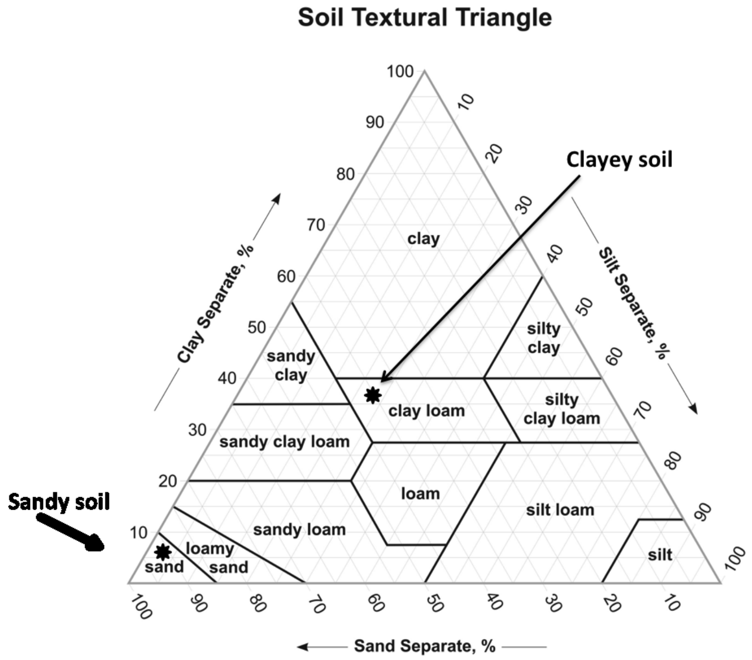


Fig. 3.2. Soils textures, classified according to USDA standards (USDA-NRCS, 1999)

The main characteristics of both clayey and sandy soils are reported in Table 3.2. As expected, the clayey soil featured a higher concentration of micro-nutrients (iron, copper, manganese) and macro-nutrients (nitrogen, phosphorous, potassium, calcium, magnesium, sulphur) than sandy soil.

Table 3.2. Quality of sand and clayey substrate used in the experiment (DM = dry matter)

Parameter	Sandy soil	Clayey soil	Parameter	Sandy soil	Clayey soil
Field capacity (% DM)	20.40	35.50	Ca (mg/kg$_{DM}$)	157761	89826
VS (% DM)	1.20	2.08	Cr (mg/kg$_{DM}$)	2.50	11.20
TOC (% DM)	< 1	< 1	Cu (mg/kg$_{DM}$)	6.50	16.90
TKN (mg/kg$_{DM}$)	77.60	392	Fe (mg/kg$_{DM}$)	3956	15567
NH_4^+-N (mg/kg$_{DM}$)	55.10	79	K (mg/kg$_{DM}$)	810	2204
NO_3-N (mg/kg$_{DM}$)	< 10	64	Mg (mg/kg$_{DM}$)	51665	35182
P-tot (mg/kg$_{DM}$)	111	313	Mn (mg/kg$_{DM}$)	179	325
Cl^- (mg/kg$_{DM}$)	2279	2382	Pb (mg/kg$_{DM}$))	2.70	8.40
Na (mg/kg$_{DM}$)	358	356	Zn (mg/kg$_{DM}$)	20.70	38
Ni (mg/kg$_{DM}$)	3.30	11.10	S (mg/kg$_{DM}$)	0.90	36.10

3.2.4. Plants seeding and irrigation program

Seeds of the three plant species (sunflower, soybean and rapeseed) were germinated in peat soil; seedlings were irrigated with tap water before transplantation into the experimental pots. Two plants, 0.5 g dry weight each, were transplanted in each pot. In the first week following transplantation, seedlings were irrigated with Hoagland nutrient solution (Hoagland and Arnon, 1950). Subsequently, 12 plants were irrigated with increasing leachate dosages (L); 6 control plants, growing on clayey soil (C_c) were irrigated with tap water; the remaining 6 control plants growing on sandy soil (C_s) were irrigated with Hoagland nutrient solution in order to balance poor soil nutrient content (Table 3.3). A scheme reporting details of the tested combinations is represented in Fig. 3.3. Daily irrigation volumes were gradually increased during the experiment, proportionally to biomass development, from 0.2 L/pot to 0.4 L/pot. Nitrogen load was the reference parameter for the calibration of feeding volume provided to the leachate irrigated essences during the three months of irrigation.

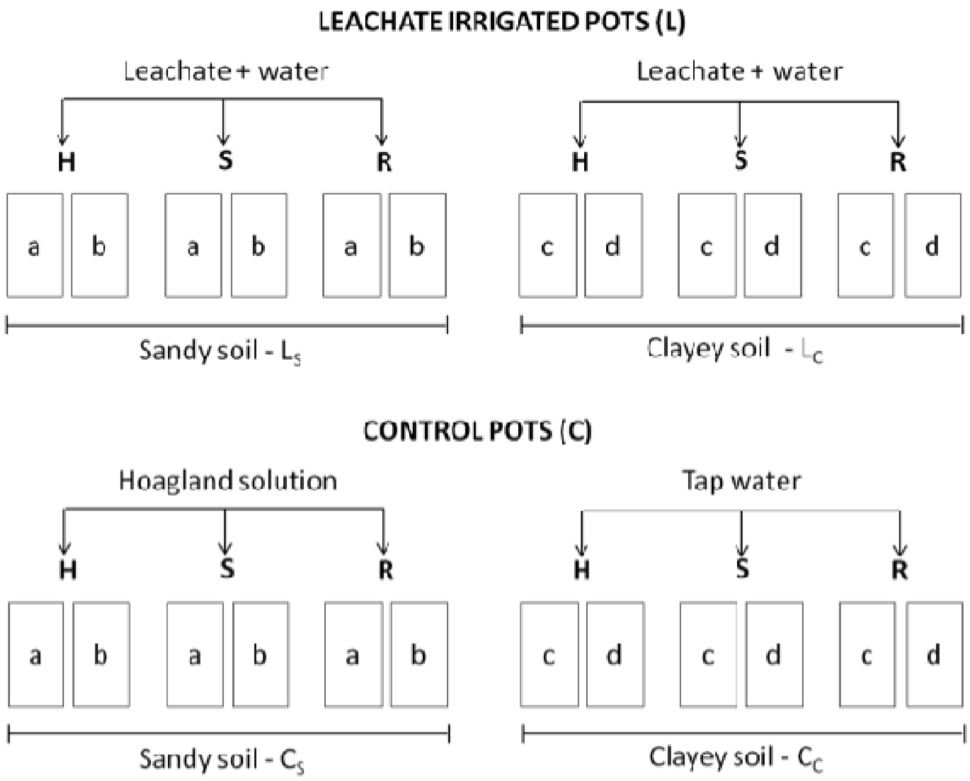


Fig. 3.3. Combination of plant species, soil textures and feeding water. (H=sunflower pots, S=soybean pots, R=rapeseed pots; a,b= replicates in sandy soil; c,d=replicates in clayey soil)

Table 3.3. Main characteristics of the irrigation water over the whole experimental period

	Irrigation water composition			Main pollutant concentration		
Weeks	Duration in days	Tap water	Leachate	TKN	COD	P_{tot}
		% v/v	% v/v		mg/L	
1	7	98%	2%	26.80	54.50	0.54
2-3	14	96%	4%	50.80	99.40	0.99
4-5	14	92%	8%	98.90	189	1.87
6	7	86%	14%	171	316	3.20
7	7	83%	17%	207	383	3.86
8	7	80%	20%	243	451	4.53
9	7	75%	25%	303	564	5.64
10-11	14	73%	27%	327	609	6.08
12	7	70%	30%	363	676	6.74

3.2.5. Analytical methods

The outflow of each pot was drained once a week: COD, N and P concentrations were detected. Leachate and all liquid samples were analyzed according to Italian CNR-IRSA analytical methods (VV.AA, 1985; VV.AA, 2003). BOD_5 was evaluated using a respirometer (Sapromat E); TOC, IC and TC were analyzed using the Shimadzu TOC-VCSN analyzer; N-NH_4^+ was evaluated by means of a distillation-titration procedure, while TKN was measured after an acid digestion phase; dissolved components (nitrate, phosphate and sulphate ions) were determined using a UV-VIS spectrophotometer (Shimatzu UV-1601) preceded by filtration with a 0.45 µm pore membrane. The colorimetric method was used to detect total phosphorus after sample digestion. Chloride and sulphide were measured by titration, whereas metal content was measured by ICP-OES (Perkin Elmer ICP-OES-4200 DV). At the end of the experimental period, plants were harvested and weighed. TKN, nitrate and phosphorus were analyzed after splitting plants into leaves, stem, roots and seeds. Soil specimens were sampled from each pot at the end of the trial: they were analyzed and the concentrations of TS, VS, TOC, N, P, Chloride and metals were measured. Plants and soil samples were analyzed according to Italian CNR-IRSA analytical guidelines for solid specimens (CNR-IRSA, 64/1986). Oil seeds were analyzed in oil content and Free Fatty Acids (FFA) quality according to European standards (Reg. CEE 2568/2011, G.U. CEE L248/91 All. II, Reg. CE 702/2007, G.U. CE L161/2007).

3.3. RESULTS AND DISCUSSION

3.3.1. Biomass growth

At the end of the trial, dry weight of plants was measured: in general, leachate irrigation influenced plant growth, as crops receiving leachate (L) developed larger biomasses than corresponding controls (C), with the exception of sunflower and soybean growing on sandy seedbed. Sunflower and soybean grew better in clayey soil, while rapeseed produced more biomass in sandy media.
Optimal growth was detected for sunflower growing on clayey substrate irrigated with diluted leachate (Fig. 3.4).

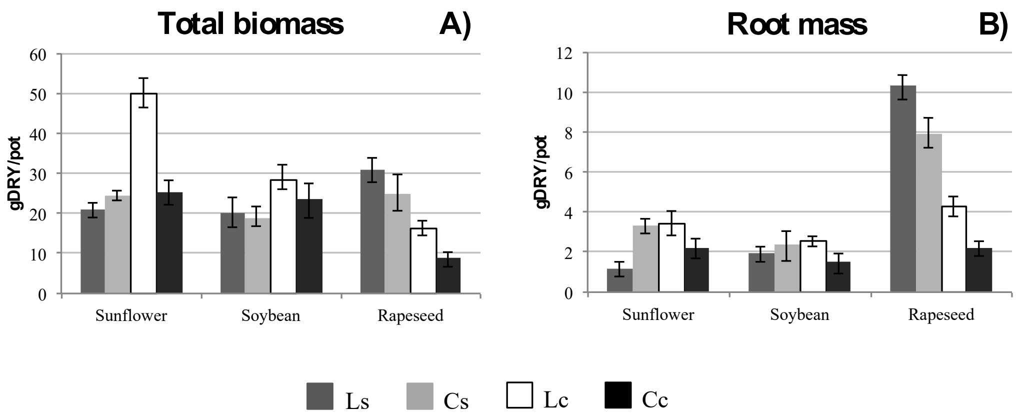


Fig. 3.4. Total biomass A) and root mass B) of the different crops at the end of the trial. Mean value (n=4) and standard deviation (L = leachate irrigated pots; C = control pots; s = sandy soil; c = clayey soil).

The difference in plant development could be ascribed to the interaction between plants and leachate-soil chemical compositions (Chinnusamy et al., 2005), with analogous behaviours being registered in a previous study on leachate treatment with willows and poplars growing respectively on sandy and clayey soil (Dimitriou and Aronsson, 2010). Leachate drainage through a more permeable substrate (sand) would limit the availability of nutrients to plants, especially for sunflower and soybean, the roots of which were not as well developed as rapeseed. Sunflower and soybean blooming occurred approximately two months after transplantation, and in particular both control and leachate irrigated sunflowers in sandy soil produced multiple flowering. Rapeseed failed to produce flowers, possibly due to the period of captivity in the greenhouse. Nevertheless, rapeseed reached senescence as leaves became yellow and started to fall, without producing any seeds. At the end of the experiment, sunflower and soybean seeds were analyzed to verify possible influences of leachate irrigation on composition, and therefore on biodiesel potential production. In particular, oil content and FFA composition were analyzed and compared with values detected in sunflowers and soybeans grown in traditional ways (Table 3.4). Oil content in soybean seeds was found in the range suggested by Karmakar et al. (2010), while the amount detected in sunflower seeds was two-fold the amount reported by Karmakar et al. (2010): leachate seems to stimulate the accumulation of oil in the seeds.

In the biodiesel production process, transesterification does not alter the fatty acid composition of oil that affects many of the critical parameters of biodiesel, such as Cetane Number (CN), for which a high value indicates the ability of fuel to auto-ignite rapidly after being injected. High CNs have been associated with less highly unsaturated components in seed oil, such as C18:2 and C18:3, and more highly saturated fatty acids such as C18:1 (Karmakar et al., 2010; Ramos et al., 2009). Sunflower seeds registered a particularly favourable composition in both growing seedbeds, confirming the positive effects of leachate irrigation.

Table 3.4. Oil content in seeds and Free Fatty Acids concentration in seeds of Sunflower and Soybean, compared with literature data (Karmakar et al., 2010). Mean values of three independent analyses are reported; standard deviation was in the range of 1-5% for each value.

Plant species	*Sunflower*	*Sunflower*	*Literature data (Karmakar et al., 2010)*	*Soybean*	*Soybean*	*Literature data (Karmakar et al., 2010)*
Feeding	Leachate	Leachate		Leachate	Leachate	
Soil	Sandy soil	Clayey soil		Sandy soil	Clayey soil	
Oil content in seeds (%)	43.03	45.13	25-35	17.03	16.26	15-20
Individual free fatty acid concentration (% v/v)						
Lauric (c12:0)	0.01	0.011	-	0.01	0.01	-
Myristic (c14:0)	0.06	0.06	< 1.00	0.04	0.04	< 0.50
Palmitic (c16:0)	1.82	1.87	3.00-6.00	2.49	2.41	7.00-11.00
Palmitoleic (c16:1)	0.07	0.08	-	0.04	0.04	-
Stearic (c18:0)	0.77	0.67	1.00-3.00	0.46	0.46	2.00-6.00
Oleic (c18:1)	36.28	37.90	14.00-35.00	2.91	2.90	19.00-34.00
Linoleic (c18:2)	1.59	2.02	44.00-75.00	8.60	7.76	43.00-56.00
Linolenic (c18:3)	0.11	0.08	< 1.50	1.59	1.78	5.00-11.00
Arachidic (c20:0)	0.08	0.09	0.60-4.00	0.04	0.04	<1.00
Gadoleic (c20:1)	0.09	0.11	-	0.02	0.02	-
Behenic (c22:0)	0.00	0.00	0.80	0.01	0.01	-
Erucic (c22:1)	0.00	0.00	-	0.00	0.00	-
Lignoceric (c24:0)	0.22	0.24	-	0.06	0.07	-
Nervotic (c12:0)	0.02	0.00	-	0.00	0.00	-

3.3.2. Contaminant removal

The discussion on removal rates has been based on values obtained as mean of the two single research units. Removal rates have been calculated on load basis (mg/week in each pot) to evaluate the mass removal efficiency:

$$\text{removal rate} = \frac{C_{in} \cdot V_{in} - C_{out} \cdot V_{out}}{C_{in} \cdot V_{in}} \tag{3.1}$$

where:
C_{in}, C_{out} = concentration of the pollutant in the inflow and outflow (mg/L)
V_{in}, V_{out} = volume of the inflow and outflow (L/week in each pot)

3.3.2.1. COD removal

Plants irrigated with leachate were submitted to increasing contaminant loads: COD load varied approximately from 50 mg/pot/week (188 mg/m^2/day) to 1082 mg/pot/week (4068 mg/m^2/day), with a removal efficiency of more than 80% throughout the whole irrigation period, irrespective of the specific plant-substrate combination (Fig. 3.5). The results obtained are similar to those yielded by experiments involving leachate irrigation of perennial plants with an average removal rate of 85% (Jones et al., 2006). Other results of leachate treatment with wetland hydrophytes can be compared, although their cultivation differs substantially from that applied in the present research: a 45% COD removal rate was obtained using a constructed wetland with *Phragmites australis* (Zupančič Justin and Zupančič, 2009); between 39% and 91% with *Cyperus haspan* (Akinbile et al., 2012), thus confirming the good performance of the plant species tested in our study.

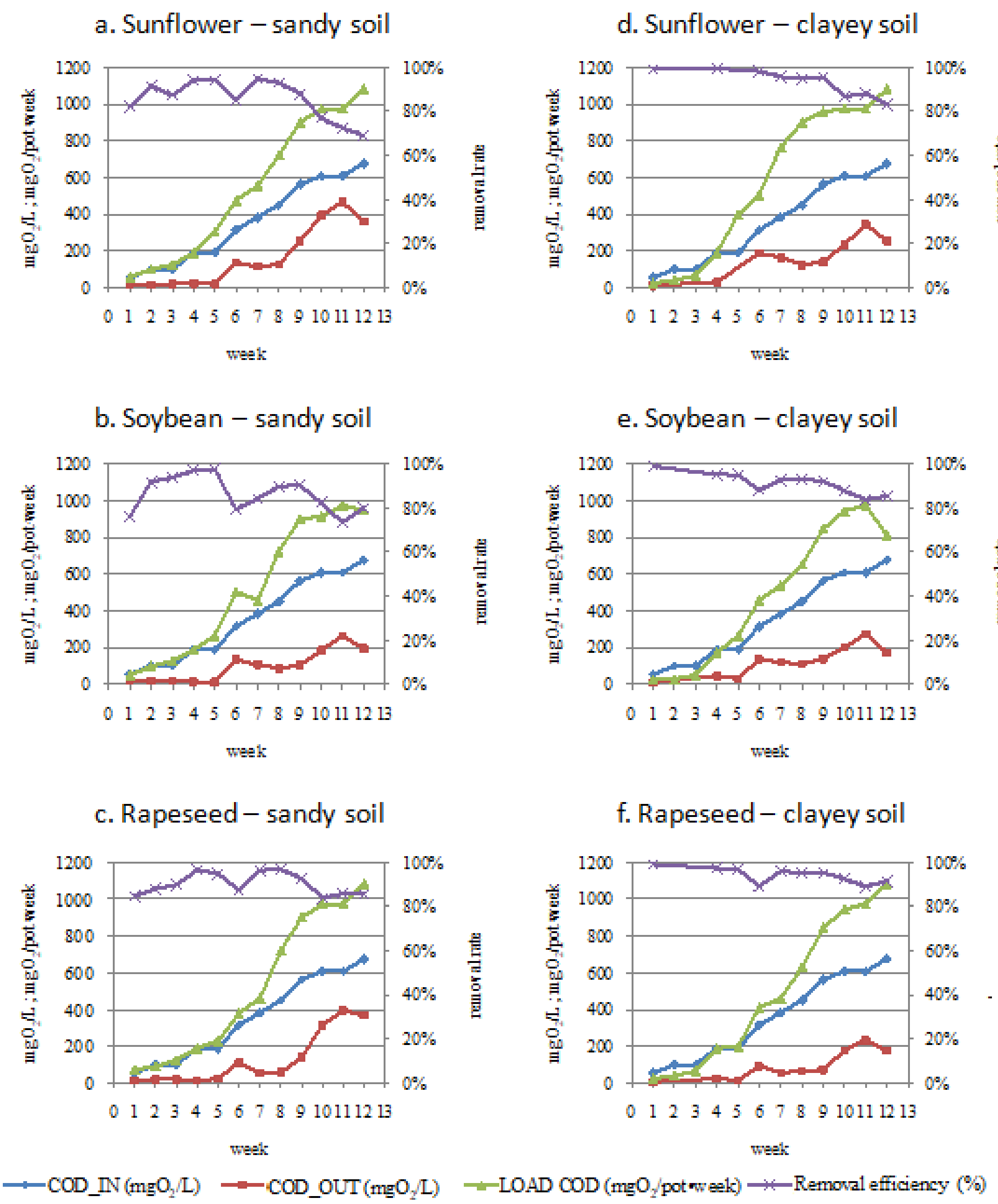


Fig. 3.5. COD input and output concentration (mgO_2/L), COD input load (mg/pot/week) and COD removal efficiency (%) throughout the whole experimental period. Standard deviation values were calculated in the range 4-10%.

During the first 8 weeks of the experiment, COD concentrations in the outflow met the Italian discharge limit of 120 mg/L (Legislative Decree 152/2006); subsequently, with a COD input close to 720 mg/pot/week (2700 $mg/m^2/day$), COD values increased in the effluent of each plant-soil combination. It should be taken into consideration that, 8 - 9 weeks after planting, plants reached maximum development and the decrease in COD removal rate could be due in part to plant senescence. Generally, plants are known to favour growth of microorganisms, which play a dominant role in the degradation of organics and nitrification, by conveying oxygen to the rhizosphere (Akinbile et al., 2012). As senescence occurs, oxygen translocation to the soil by plants is reduced, thus decreasing the possibility of aerobic removal of organics. In clayey soil filled pots higher COD removal efficiencies were observed compared to sandy soil filled pots (Fig. 3.5) likely due to the pollutants adsorption on the clay component, confirming previous experimental results on leachate treatment in various soils (Duggan, 2005; Wong et al., 1990).

3.3.2.2. Nitrogen and phosphorous removal

At the beginning of the experiment, nitrogen input was set at 15 mgN/pot/week (56 mgN/m^2/day). During the experiment the nitrogen load increased up to 600 mgN/pot/week (2256 mgN/m^2/day) in sunflower and rapeseed growing pots. Load applied was lower in soybean pots (about 500 mgN/pot/week), due to the lower water demand (Fig. 3.6). Nitrogen removal efficiency was generally above 70% during the first eight weeks. Afterwards, as registered with COD, nitrogen removal efficiency decreased and the loss of efficiency was higher in sandy soil units. In the same period, outflow nitrogen concentrations were below 100 mgN/L. In a similar experience, Cheng and Chu (2011) registered an effluent nitrogen concentration close to 90 mgN/L.

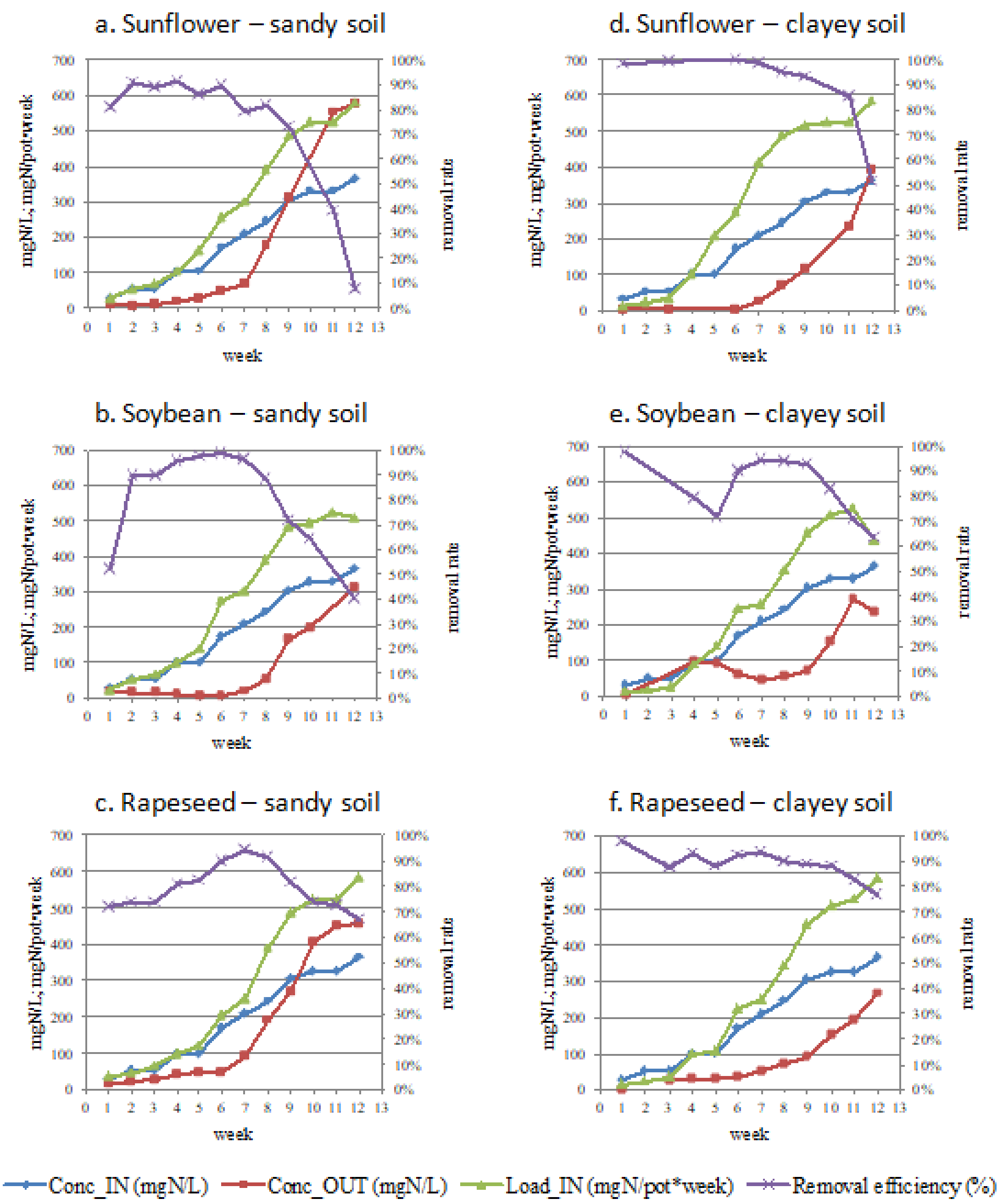
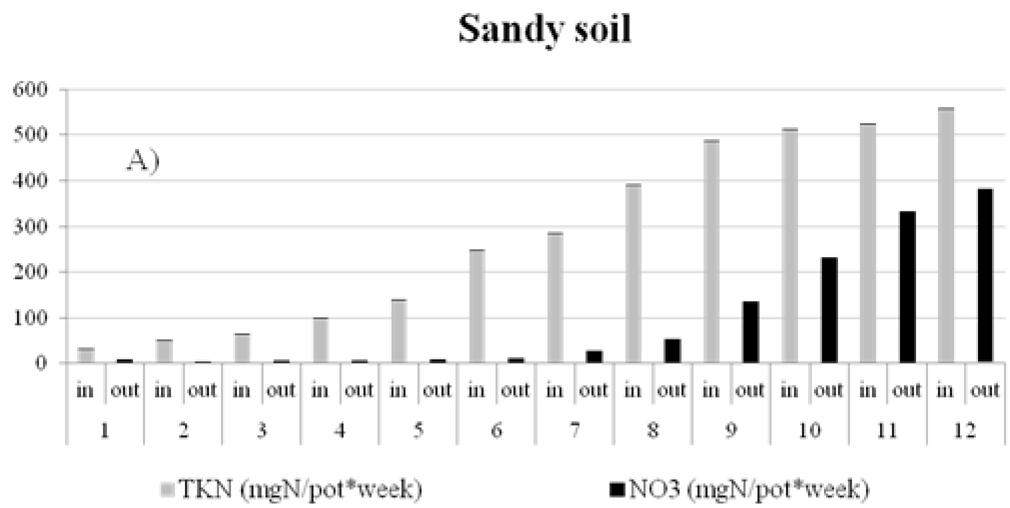


Fig. 3.6. Nitrogen input and output concentration (mgN/L), nitrogen input load (mgN/pot/week) and nitrogen removal efficiency (%) throughout the whole experimental period. Standard deviation values were calculated in the range 4-10%

The prevalent form of nitrogen in leachate was ammonium, which was mostly transformed into nitrate in the effluents (Fig. 3.7). Fig. 3.7 was elaborated taking into account average values of the different forms of nitrogen as input and output loads. Residual ammonia has not been represented as it was negligible with respect to nitrates, with values ranging between 0.00 mgN/pot/week and 5.44 mgN/pot/week in sandy units and between 0.25 mgN/pot/week and 6.52 mgN/pot/week in clayey units. During the research period, nitrates in the outflow increased on a par with TKN increase in the influent. Nitrification occurred at higher rate in sandy than in clayey soil as oxygen dispersed better in the more permeable medium. Nitrate is the form of nitrogen preferentially taken up by plants, but may also act as a primary factor for the eutrophication of water bodies. Thus, horizontal phytotreatment systems are often implemented for final nitrogen removal by denitrification (Cheng and Chu, 2011; Tyrrel et al., 2001). Although in clayey soil nitrification was lower compared to sandy soil, total nitrogen concentration in the effluent was much lower. This issue will be better discussed in the mass balance section.



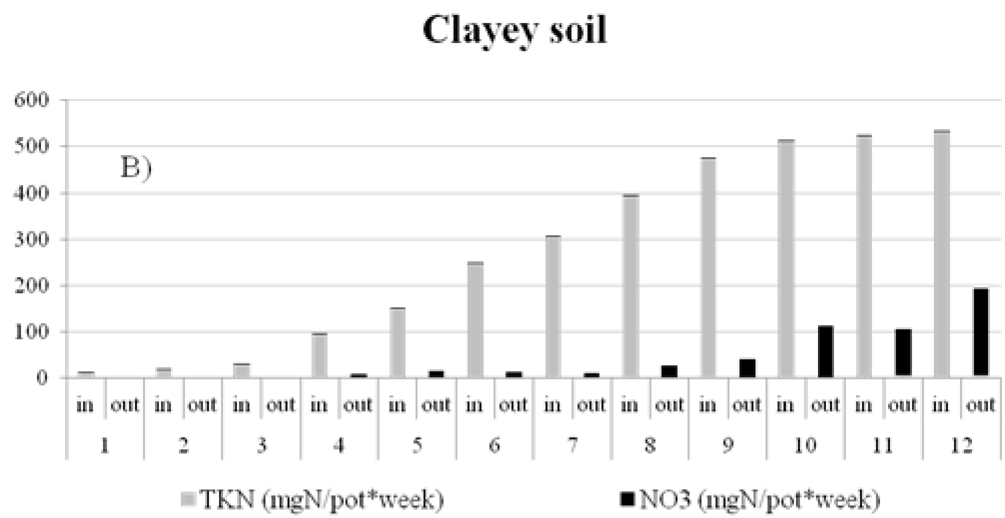


Fig. 3.7. Input and output nitrogen compositions in the effluents throughout the whole experimental period. Mean values from all the sandy A) and clayey units B) and from the three plant species . Standard deviation values were calculated in the range 4-10%.

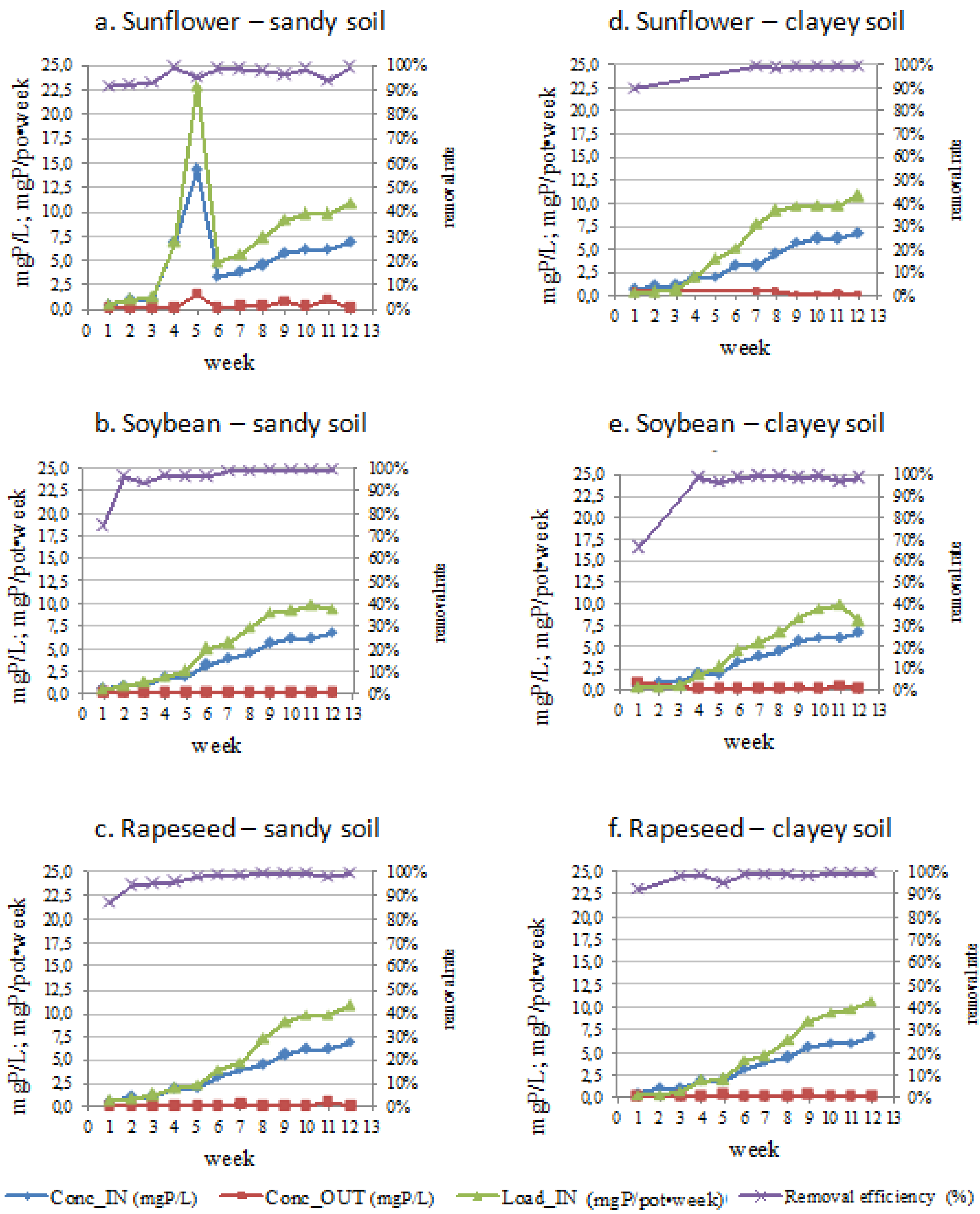


Fig. 3.8. Phosphorous input and output concentration (mgP/L), phosphorous input load (mgP/pot/week) and phosphorous removal efficiency (%) throughout the whole experimental period. Standard deviation values were calculated in the range 2-5%

Phosphorous concentration in leachate is low when compared to other contaminants and is of minor concern. Phosphorous load ranged from 0.57 mgP/pot/week (2.14 mgP/m^2/day) to 10.8 mgP/pot/week (40.27 mgP/m^2/day) (Fig. 3.8). The peak evident in Fig. 3.8.a at the fifth week was due to an addition of 0.04 mmol/L of KH_2PO_4 to the water fed to sunflower plants grown in sandy soil; indeed, in that specific period plants seemed to be affected by a lack of phosphorous, as in both leachate- and water- irrigated units, plants displayed a limited leaf production and progressive foliage dryness. Excellent phosphorus removal rates were observed throughout the whole experimental period in each combination soil-plant-feeding: phosphorous outflow concentration remained below 1 mg/L. In phytotreatment systems, phosphorous is mainly sorbed by or precipitated in the filter medium and removal efficiency is largely associated with the physical, chemical and hydrological properties of filter material. The high phosphorus removal rates obtained in our study may have been due to the combination of the favorable leachate pH (>6), and the mineral composition of both soil textures, as the presence of Fe and Ca has been demonstrated to enhance P removal (Vohla et al., 2011).

3.3.3. Nitrogen and phosphorus mass balance

Nutrient mass balance was performed on leachate-irrigated pots. Nitrogen and phosphorus distributions for the main system components (waters, soils and plants) are reported in Table 3.5 and Fig. 3.9 and 3.10. Dry mass of the substrate in each single pot was 15 Kg on average.
At the beginning of the experiment, nitrogen was present mainly in organic form and as ammonium in both seedbeds (Table 3.2), and was added to the system largely by irrigation as ammonium (Table 3.1). During the experimental period a portion of nitrogen was adsorbed by the substrate (Table 3.5), part was oxidized to nitrate and discharged with the effluent (Fig. 3.6), and part was taken up by plants.

Table 3.5. Nitrogen distribution between the different system components in the leachate tested pots (mgN/pot). Standard deviation values were calculated in the range 4-10% for water components, 3-10% for substrates and plants

	Sunflower				Soybean				Rapeseed			
	Sandy soil		Clayey soil		Sandy soil		Clayey soil		Sandy soil		Clayey soil	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT
Water*	3513	1545	3711	635	3401	1060	3076	578	3372	720	3166	432
	START	END	START	END	START	END	START	END	START	END	START	END
Substrate**	1180	1938	7070	7354	1249	2824	5476	5957	1180	2195	5005	5757
Plant	-	440	-	1017	-	702	-	1032	-	1053	-	633
Total	4692	3922	10781	9006	4650	4686	8552	7567	4552	3968	8171	6822

*IN = total amount provided during the whole experiment, OUT = total content in the outflows
**START = content in the soil at the beginning of the trial, END = final total content at the end of the experiment

The importance of the medium in the performance of proposed system process is confirmed by mass balance, although sandy and clayey soils contributed differently to the nitrification and denitrification processes. Nitrogen losses (ΔN in Fig. 3.9) have been calculated as difference between the measured total N input and total N output from the system. Nitrogen was likely lost in gaseous form, as reported by previous studies on nitrogen removal in phytotreatment processes (Cheng and Chu, 2011; Tyrrel et al., 2001). On average, approximately 40% of nitrogen was lost from the pots with clayey soil and about 14% from sandy soil pots (Fig. 3.9). The formation of gaseous N was the result of denitrification processes, which were likely favored in clayey soil pots due to the fine soil texture. On the contrary, sandy soil allowed a better oxygen permeation and enhanced nitrification, leading to a lower N-gas production.

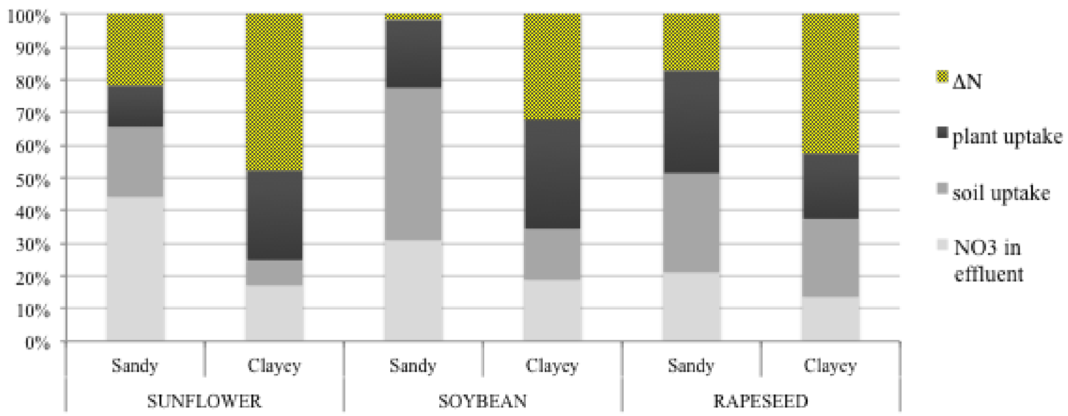


Fig. 3.9. Fate of nitrogen in system components at the end of the experimental period. ΔN is the calculated loss of nitrogen in gaseous forms.

Fig. 3.10 shows phosphorus distribution between plant tissues, substrate and drainage water at the end of the experiment. Soils played a key role in the accumulation of phosphorus, with no particular differences being observed between the different plant-soil combinations, as found in another phytotreatment study (Fraser et al., 2004). Phosphorous concentration in the effluent was almost completely negligible. Both sandy and clayey soils retained N and P during the irrigation period, confirming that phytoremediation is a unique process, consisting in a combination of different phenomena, rather than the isolated action of plants (Jones et al., 2006). Indeed plants play an important role in cooperation with the substrate media. The combination of two plants with two substrates revealed different synergic effects: in the reduction of volume by evapotraspiration, caused by the different plants growth and soil characteristics; in N and P removal by soil adsorption and plant uptake.

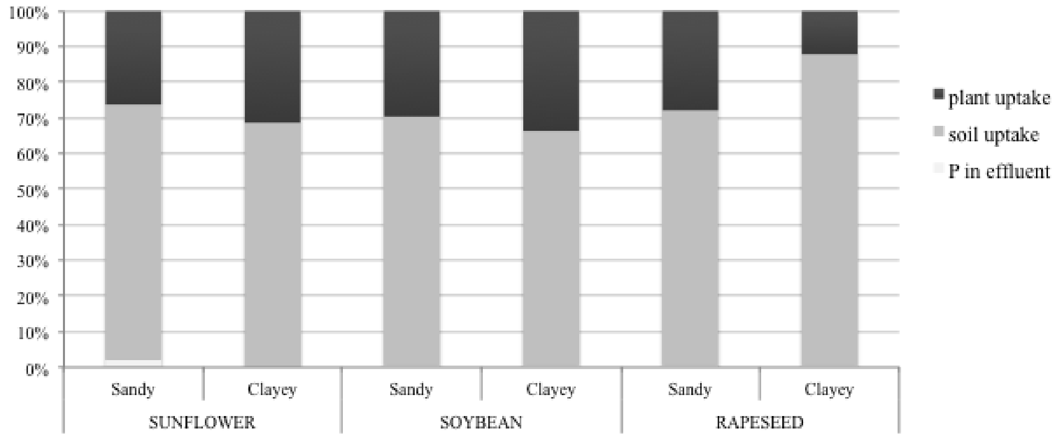


Fig. 3.10. Phosphorus distribution in the different components of the system in the tested units at the end of the research period

Leachate irrigated plants accumulated more N in their tissues than corresponding controls (Table 3.6), due to the higher load of nitrogen provided during the experiment (Gϋsewell, 2004). On the contrary, phosphorus content in plant tissues was not influenced by the different supply. Plant tissue N/P ratio in control plants (Table 3.6) is quite similar to that recorded for various plants (Gϋsewell, 2004). On the contrary, N/P ratio of leachate treated plants is much greater than literature values, proving that the tested plant species may grow under conditions of unbalanced nutrient availability, a common problem in many leachate phytotreatment applications (Vymazal, 2009).

Table 3.6. Nitrogen and phosphorus in plant tissues (mean values with ± SD). Percent of nitrogen (N/DM) and phosphorus (P/DM) on plant dry biomass. Ratio between nitrogen and phosphorus (N/P). L = leachate fed pots; C = control pots; s = sandy soil; c = clayey soil. (DM = dry matter)

		Nitrogen		Phosphorous		N/P
		mgN/pot	N/DM (%)	mgP/pot	P/DM (%)	
Sunflower	L_S	440±21	2.08	45.30±2.2	0.21	9.70
	C_S	324±17	1.10	32.50±1.3	0.11	9.96
	L_C	1017±65	2.04	77.40±5.0	0.16	13.10
	C_C	316±19	1.24	48.50±2.1	0.19	6.51
Soybean	L_S	702±48	3.45	43.00±1.7	0.21	16.30
	C_S	375±16	2.01	39.00±1.0	0.21	9.60
	L_C	1032±54	3.63	58.50±2.8	0.21	17.60
	C_C	341±29	1.46	54.90±3.4	0.23	6.22
Rapeseed	L_S	1053±60	3.39	39.00±2.8	0.13	27.00
	C_S	362±30	1.46	28.40±1.0	0.11	12.70
	L_C	633±29	3.85	20.40±1.1	0.12	31.00
	C_C	137±11	1.54	12.60±0.8	0.14	10.90

3.4. CONCLUSION

Phytotreatment of old landfill leachate using oily crops proved to be feasible under lab-scale conditions, yielding a series of favorable results both in terms of biomass growth and pollutant removal rates. To our knowledge, this is the first time that energy crops, such as sunflower, rapeseed and soybean have been exposed to landfill leachate treatment. High pollutant removal rates have been obtained in each plant-soil texture combination, although efficiencies started to decrease from week eight, corresponding to 2900 mgCOD/m^2 and 1493 mgN/m^2 irrigation per day, when plants reached their maximum development, and began their senescence period. Further investigations should be carried out to test the leachate dilution on plant senescence time with the aim of increasing removal efficiencies. The oil composition of sunflower seeds seemed to be particularly favorable to biodiesel production as the content of unsaturated long chain free fatty acids was much lower than values commonly detected. Clayey soil proved to be more suitable for COD removal, while nitrification was better in sandy soil. Sandy soil revealed to be less suitable for sunflowers growth as lacking in phosphorous as leachate. This experiment was fundamental in evaluating pollutant removal efficiency of the system and the capacity of plants to tolerate leachate supply. Sunflower proved to be the best performing species in the removal of pollutants. A scale-up of the current experience should subsequently be implemented to analyze system dynamics in a close-to-reality context.

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Chapter 4: Different leachate phytotreatment systems using sunflowers

Garbo, **F**., Lavagnolo, M.C., Malagoli, M., Schiavon, M., Cossu, R., 2017. Different leachate phytotreatment systems using sunflowers. Waste Manage. 59, 267-275. http://dx.doi.org/10.1016/j.wasman.2016.10.035
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Abstract

The use of energy crops in the treatment of wastewaters is of increasing interest, particularly in view of the widespread scarcity of water in many countries and the possibility of obtaining renewable fuels of vegetable origin. The aim of this study was to evaluate the feasibility of landfill leachate phytotreatment using sunflowers, particularly as seeds from this crop are suitable for use in biodiesel production. Two different irrigation systems were tested: vertical flow and horizontal subsurface flow, with or without effluent recirculation. Plants were grown in 130 L rectangular tanks placed in a special climatic chamber. Leachate irrigated units were submitted to increasing nitrogen concentrations up to 372 mgN/L. Leachate was successfully tested as an alternative fertilizer for plants and was not found to inhibit biomass development. The experiment revealed good removal efficiencies for COD (η >50%) up until flowering, while phosphorous removal invariably exceeded 60%. Nitrogen removal rates decreased over time in all experimental units, particularly in vertical flow tanks. In general, horizontal flow units showed the best performances in terms of contaminant removal capacity; the effluent recirculation procedure did not improve performance. Significant evapo-transpiration was observed, particularly in vertical flow units, promoting removal of up to 80% of the inlet irrigation volume.

Keywords: sunflowers, landfill leachate, phytotreatment, vertical flow, horizontal sub-superficial flow

4.1. INTRODUCTION

The use of energy crops in the decontamination of wastewaters is of increasing interest, particularly in view of the widespread scarcity of water in many countries worldwide and of the possibility of obtaining renewable sources of energy (Tsoutsos et al., 2013, Zema et al., 2012).

Energy crops are defined as low-cost and fast-growing plants used to produce bioenergy and biofuels (such as bioethanol or biodiesel) or which can be burned to generate electricity or heat (Lal R., 2008; Nges et al., 2012; Rowe et al, 2009).

Recent developments in the cultivation of energy crops have been driven by the need of advanced industrial societies to reduce both their dependence on fossil fuels as a source of energy, and the emission of greenhouse gases (Fernando et al., 2014, Lal R., 2008).

The European Union strongly encourages and actively solicits the identification of means of improving the production of renewable energy (EC, 2009). Directive 2009/28/EC sets targets for each Member State, with the aim of reaching the 20-20-20 objective by 2020: reduction of 20% of greenhouse gas emissions compared to emissions in 1990; reduction of 20% of energy consumption due to the improvement of energy efficiency; 20% of energy consumption from renewable sources. The Directive, moreover, specifies a 10 % mandatory target for biofuel utilization (Manãs et al., 2014; Spugnoli et al., 2012).

However, the specific cultivation of energy crops to fulfill the EU mandate may involve the use of high irrigation rates in order to produce relevant amounts of biomass. As a consequence, shortages in the supply of fresh water may ensue (Tsoutsos et al., 2013, Zema et al., 2012).

Nowadays, almost 70% of water consumption is linked to agricultural cultivations (FAO, 2014; United Nations, 2015), estimating an increase of 19% by 2050 (United Nations, 2015).

The use of unconventional water resources (raw or treated urban or industrial wastewater, landfill leachate) may represent an optimal compromise between the need to produce renewable energy and conservation of water supply (Zema et al., 2012).

Raw municipal wastewater has been tested on cultivations of Typha latifolia, Arundo donax and Phragmites australis, resulting in an up to 54% increase in average biomass yields compared to plants irrigated with conventional water (Zema et al., 2012).

Sewage sludge proved to be even more effective than commercial inorganic fertilizers in promoting biomass development of Cynara cardunculus L (Manãs et al., 2014)

Helianthus annuus (sunflower) and Ricinus communis (castor) fed with the final effluent from a municipal wastewater treatment plant was characterized by a lower acidity value of extracted oil and a slightly lower viscosity compared to freshwater irrigated controls: wastewater irrigation seems to have a positive effect on biodiesel production as it simplifies the production process, as reported by Tsoutsos et al. (2013).

Poplar irrigated with diluted leachate had a greater height, diameter, and number of leaves, respectively, than control trees (Zalesny et al., 2009). In another study, diluted landfill leachate supplied to poplar and willow trees increased willow biomass production compared to corresponding controls, but not that of poplar (Dimitriou and Aronsson, 2010). In general, irrigation with wastewater or diluted landfill leachate is capable of effectively promoting the growth of energy plants, with plants then contributing to the removal of contaminants.

Helianthus annuus (sunflower), Glycine max (soybean) and Brassica napus (rapeseed) plants are considered optimal energy crops for use in Mediterranean and Continental areas. Lab-scale tests conducted using these three plant species irrigated with diluted landfill leachate and source separated sewage demonstrated how significant removal efficiencies can be achieved: COD (η >80%), total N (η >70%) and total P (η >95%) (Lavagnolo et al., 2017, 2016, 2011).

Sunflowers grow well in fertile and not-so-fertile areas, as well as in the presence of limited water availability (Skolou et al., 2011). Sunflower plants grown in contaminated areas, or irrigated with landfill leachate or municipal/industrial wastewater, may represent a convenient option for use in producing alternative biofuel such as biodiesel.

The present study investigates the effects of diluted leachate irrigation on sunflower plants grown in tanks filled with soil under two different hydraulic regimes: vertical and horizontal sub-superficial flow. Nitrogen, phosphorous and organic (COD) contents were also monitored to control the ongoing process and to verify the efficiency of the treatment.

4.2. MATERIALS AND METHODS

4.2.1. Research program

The experiment was performed at the Laboratory of Environmental Engineering of the University of Padua (Italy).

Twelve 130 L polyethylene tanks were used: six set up as horizontal subsurface flow (HSSF) systems and six as vertical flow (VF) systems. Four HSSF tanks and four VF tanks were irrigated with diluted leachate; two HSSF and two VF were fed exclusively with tap water and used as controls ($HSSF_C$ and VF_C). All tanks were placed in a controlled climatic chamber. The experiment lasted 9 weeks and was divided into 4 phases, characterized by changes in the irrigation scheme (Fig. 4.1). The leachate dose was increased gradually to adapt the plants to the increasing concentration of contaminants and avoid a sudden failure caused by phyto-toxicity phenomena (Cheng and Chu, 2011).

Nitrogen concentration in the feed was used as a reference parameter in setting the irrigation timetable, as previous studies had revealed that nitrogen concentrations exceeding 400 mgN/L (Lavagnolo et al., 2016b; Leigue Fernàndez, 2014) may produce a negative effect on plants.

In the first week (first phase) plants were irrigated with 10% leachate and 90% tap water with 124 mgN/L, expressed as Total Kjeldahl Nitrogen (TKN). During week 2 (second phase) the amount of leachate was increased to 20% (248 mgN/L of TKN); from week 3 the third phase of the experiment started and the leachate dose was increased to 30% (372 mgN/L of TKN). Starting from Phase 3B (from week 5) effluent recirculation was applied to four leachate-irrigated units, two HSSF and two VF units, which were renamed $HSSF_R$ and $VF_{R.}$ The re-circulated fraction represented 50% by volume of the feed: 30% diluted leachate was used for the remaining 50% of the volume.

Effluent recirculation was applied to evaluate whether this practice could enhance the nitrification and denitrification processes.

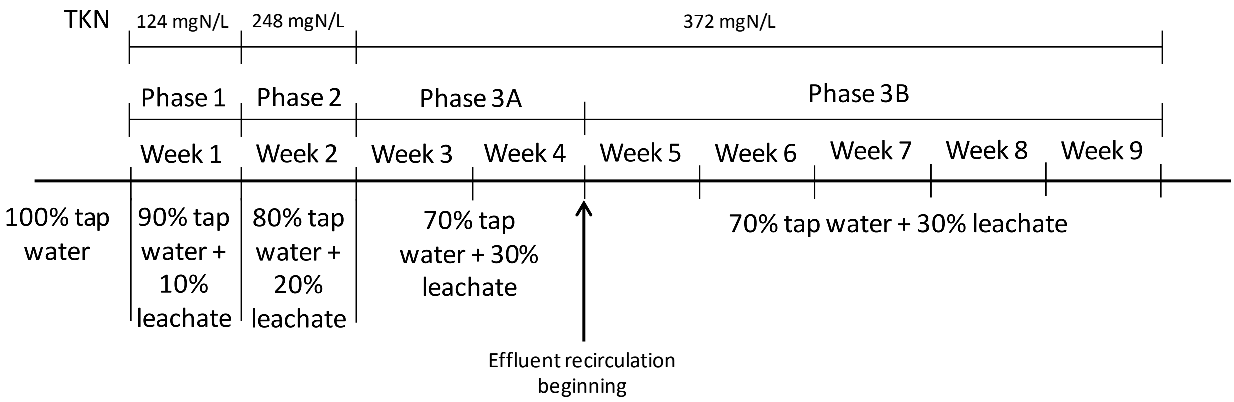

Fig. 4.1. Schematic research program of the entire experiment

4.2.2. Equipment

VF tanks were irrigated daily over the entire surface to simulate the vertical flux. The growing medium was made up of 200 mm soil above 100 mm gravel (Fig. 4.2a). The substrate surface was 0.375 m^2. Six sunflower plants were grown in each reactor. Irrigation was provided intermittently, every 12 hours, to replenish the level of water inside the tanks and promote air intrusion into the soil for stimulating the nitrification (Pellissari et al., 2017).

In HSSF tanks the influent was irrigated daily (addition every 3 hours, from Monday to Friday) and distributed homogeneously in the inlet zone, which was filled with coarse gravel (40-60 mm), till its saturation to support the horizontal movement of water. The outlet zone was made up of finer gravels (10-20 mm). The 550 mm thick soil as growing substrate was placed between the two gravel zones (Fig. 4.2b). The substrate surface was 0.275 m^2. Four plants were grown in each reactor.

All units were drained once a week; the minimum Hydraulic Retention Time (HRT) was kept equal to 7 days: in a similar experimental trial, Sawaittayothin and Polprasert (2007) demonstrated that the minimum HRT must be between 5 and 8 days.The tanks were placed into a climatic chamber in which a 14-hour photoperiod with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity was imposed. Mean air temperature was maintained at 24 °C (MIN=17 °C, MAX=35 °C).

At the end of the experiment, plants were harvested, oven dried at 60 °C, and weighed. Nitrogen contents in leaves, roots and stems were determined. Soil samples were collected from each tank at the end of the experiment: final nitrogen concentration was measured to complete the nitrogen mass balance.

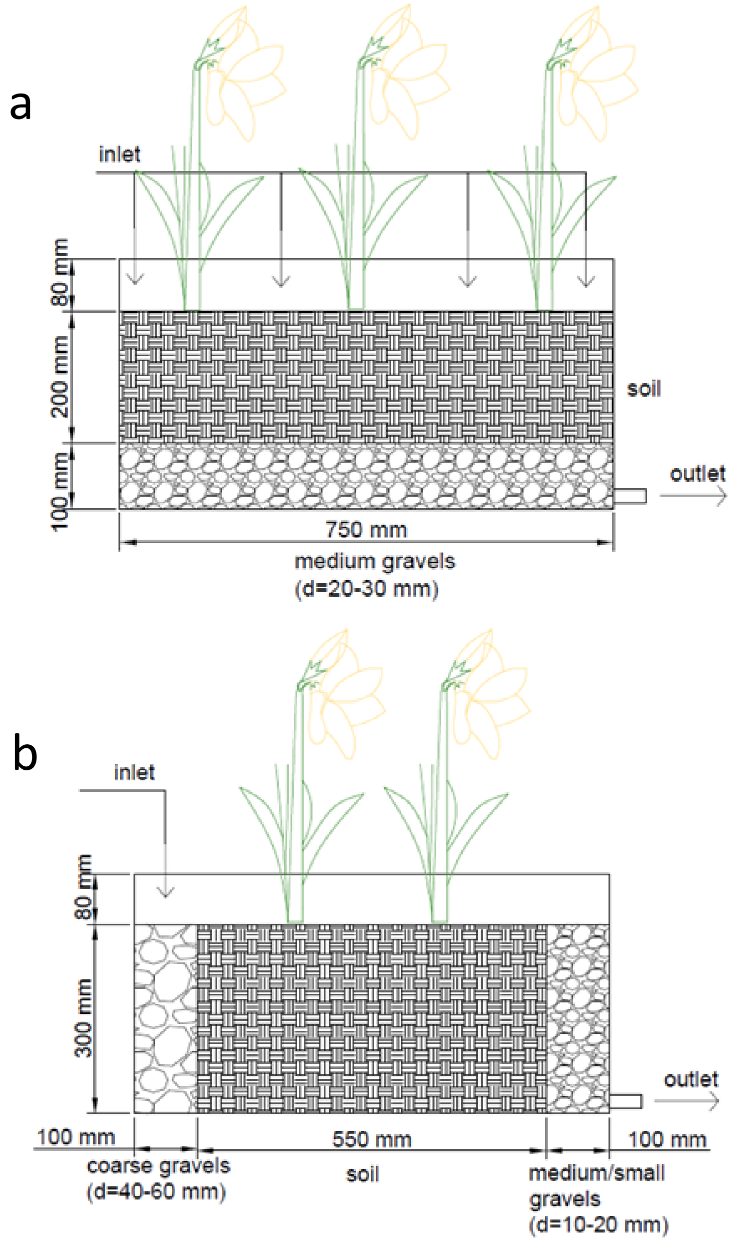

Fig. 4.2. Cross section of (a) vertical flow tank (VF, VF_R ,VF_C) and (b) horizontal subsurface flow tank (HSSF, $HSSF_R$, $HSSF_C$)

4.2.3. Substrate characteristics

A suitable substrate should ensure an acceptable compromise between the needs for macro-porosity, air circulation and root development, guaranteeing at the same time satisfactorily removal efficiencies (Jones et al., 2006; Leigue Fernàndez, 2014).

Long-term studies conducted on both innovative and traditional constructed wetlands indicate that mixtures of soil and sand represent the optimal combination (Lavagnolo et al., 2016b; Stottmeister et al., 2003; Weerakoon et al., 2013).

Two different growing substrates were used for horizontal and vertical flow, respectively. Soil textures, determined with the Bouyoucos Methods (Bouyoucos, 1962), are reported in Table 4.1. According to the soil taxonomy proposed by USDA (USDA-NRCS, 1999), they were both classified as sandy loam.

The main characteristics of the substrates, determined according to standard international methods, are reported in Table 4.2.

Table 4.1. Texture of substrates 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 4.2. Substrate parameters

Parameter	Unit	Horizontal flow	Vertical flow
VS	mg/kg_{DM}	1.53	2.03
Total Carbon	mg/kg_{DM}	31080.00	27500.00
Total Organic Carbon (TOC)	mg/kg_{DM}	4530.00	2770.00
Total Nitrogen	mg/kg_{DM}	420.00	320.00
Total Phosphorous	mg/kg_{DM}	255.46	194.63

4.2.4. Landfill leachate

Leachate was collected in a closed anaerobic municipal solid waste landfill located in the North of Italy, in which untreated municipal solid wastes were disposed of between 1983 and 1990.

The results of the chemical characterization of raw leachate samples, are reported in Table 4.3. Nitrites and nitrates were absent, therefore TKN is representative of total influent nitrogen. Values of TKN, ammonium nitrogen and BOD to COD ratio (BOD/COD equal to 0.04) are typical of a leachate produced during the stable methanogenic phase (Jones et al., 2006; Stegmann et al., 2005).

The TKN/COD ratio is approximately 1: conventional biological processes might be limited by the excessive amount of nitrogen, inhibitory to microorganisms (Renou et al., 2008).

Table 4.3. Chemical characterization of the raw leachate used. (Units: mg/L).

Chemical analysis	
Parameter	Value
pH	8±0.2
TKN	1240±35
NH_4^+	1221±28
P_{TOT}	12±2
PO_4^{3-}	11±1
TS	4277±365
VS	1102±259
COD	1325±27
BOD_5	50±8
Cl^-	1138±26
NO_2^-	0±0.1
NO_3^-	0±0.3
SO_4^{2-}	0±0.7

4.2.5. Analytical methods

The effluents of all experimental units were drained once a week and their volumes recorded. Nitrogen, phosphorous and organic content (expressed as COD) of the effluents were evaluated to control the ongoing process.

Leachate and all liquid samples were analyzed according to the CNR-IRSA standard Italian analytical methods (CNR-IRSA, 29/2003). BOD_5 was measured with a respirometer apparatus (Sapromat E); ammonia was evaluated by means of a distillation-titration procedure; TKN was measured through a distillation-titration procedure after an acid digestion phase; dissolved components (nitrate, phosphate and sulfate ions) were determined using a UV-VIS spectrophotometer (Shimadzu UV-1601). The same spectrophotometer was used to detect total phosphorus after sample digestion; chloride was measured by titration. Nitrogen content in soil and plants at the end of the trial was analyzed according to CNR-IRSA standard Italian analytical guidelines for solid specimens (CNR-IRSA, 64/1986).

4.2.6. Nitrogen balance

At the end of the entire experiment a total nitrogen balance for each experimental unit was calculated, based on the following equation:

$$N_{in} = N_{out} + \Delta N_P + \Delta N_S + \Delta N_L \qquad (4.1)$$

where:

N_{in} = Total mass of nitrogen entering each unit

N_{out} = Total mass of nitrogen in the outflow

ΔN_P = Amount of nitrogen accumulated in the plant tissue

ΔN_S = Nitrogen accumulated in the substrate

ΔN_L = Nitrogen gaseous losses.

4.3. RESULTS AND DISCUSSION

4.3.1. Sunflower growth

Plants grew vigorously and uniformly throughout all experimental units. Blooms occurred in the middle of Phase 3B (week 7), followed by a sudden senescence. Sunflowers showed no symptoms of toxicity and were not detrimentally affected by the presence of leachate in the irrigation water. Final dry weight of plants was on average 16.8 g/plant; no significant differences were detected among the plants growing on the different experimental units: standard deviation values were calculated in the range 2-5%. Fig. 4.3 reports the dry weight distribution among the plant components: no significant differences were detected between leachate irrigated essences and corresponding controls. Stems always represented the heavier fraction, followed by leaves and seeds.

Plant growth is affected by the bioavailability of nitrogen and phosphorous. In general, optimal N:P ratio in plant tissues ranges from 10 to 20 (Gusewell, 2004). The plants grown in horizontal flow tanks were characterized by N:P ratios above 20, likely indicating excess uptake of nitrogen (Table

4.4). Nevertheless, the absence of significant differences among the final dry weight of plants proves that the excessive uptake of nitrogen did not produce negative effects on plant growth.

Table 4.4. N:P ratios detected in sunflowers

Flow system	N : P ratios (mgN/mgP)
HSSF	28.31
$HSSF_R$	27.50
$HSSF_C$	21.75
VF	16.17
VF_R	19.81
VF_C	19.67

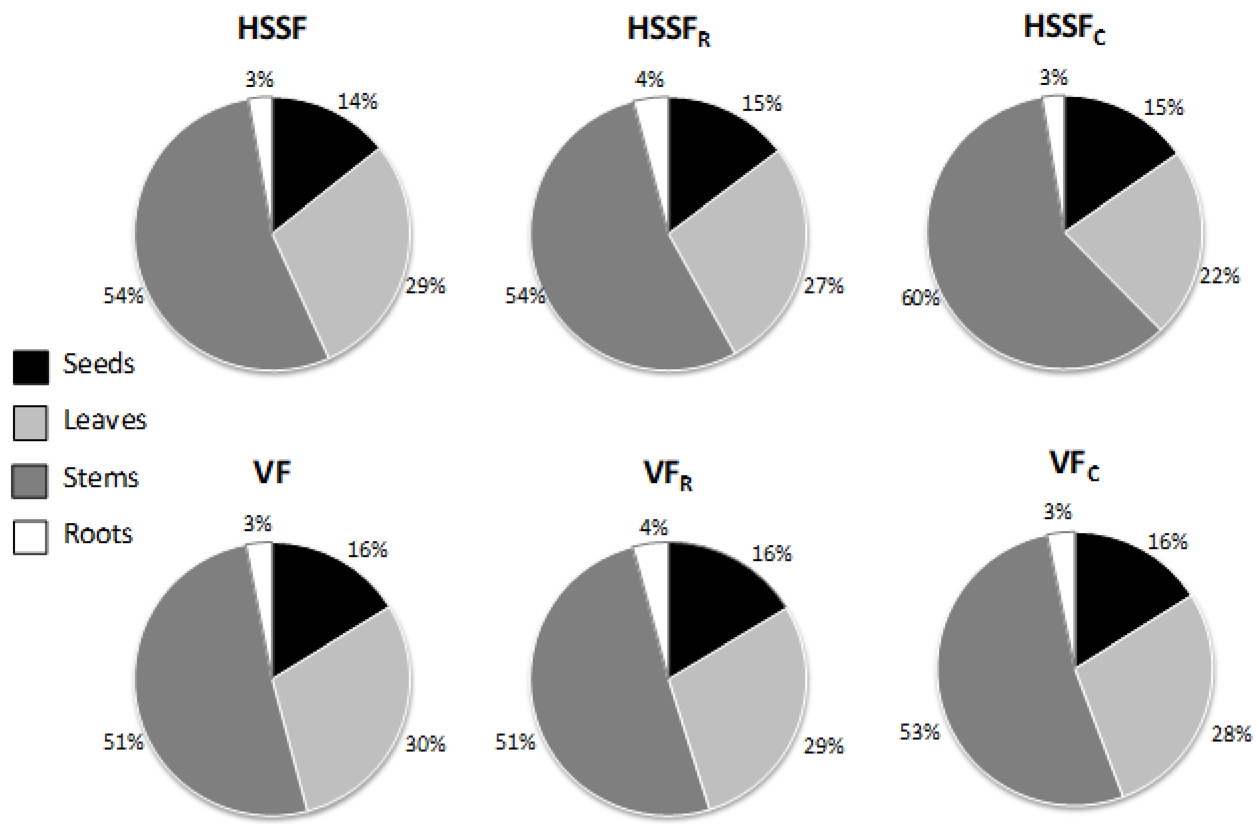

Fig. 4.3. Distribution of the dry weight among the different plants units in percentage. Average results. Standard deviation values were below 5%. (HSSF=Horizontal subsurface flow, VF=vertical flow, C=control, R=effluent recirculation)

4.3.2. Influent and effluent volumes

From week 1 (Phase 1) to week 4 (Phase 3A) watering was gradually reduced in all tanks from about 20 L/week to 10 L/week to balance the increased amount of leachate added (Fig. 4.4). During this initial period evapo-transpiration was comparable in all experimental units and ranged between 40% and 60% of the corresponding influent volume.

Starting from Phase 3B, the inlet volume was increased in all tanks proportionally to the increasing requirements of each unit. In general, horizontal flow units were able to receive and treat slightly higher volumes than vertical flow units. The HSSF tanks showed evapo-transpiration rates comparable to the previous phases while VF units showed peaks of evapo-transpiration up to 80% of the corresponding inlet.

Applied Hydraulic Loading Rates (HLR) are reported in Fig. 4.4. Horizontal subsurface flow units were characterized by higher HLRs compared to vertical flow systems. Similar HLRs have been applied by Ogata et al. (2015) to HSSF constructed wetlands treating diluted landfill leachate in which traditional hydrophytes were grown.

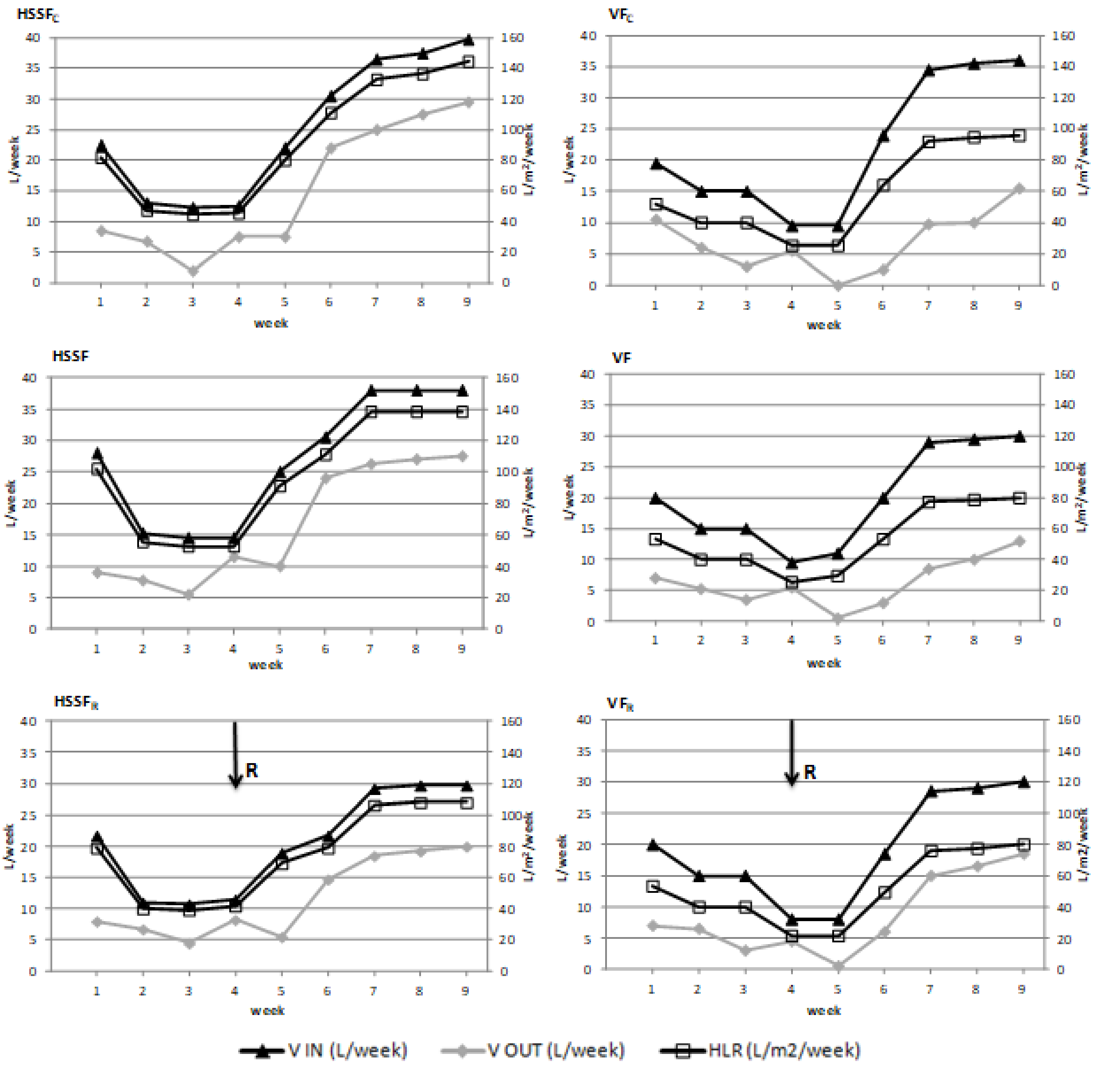

Fig. 4.4. Volumes (L/week) added, drained and Hydraulic Loading Rate (HLR) over the whole experimental period. (Phase 1 = week 1; Phase 2 = week 2; Phase 3A = weeks 3-4; Phase 3B = weeks 5-9). Average results. Standard deviation values were below 5%. (HSSF=Horizontal subsurface flow, VF=vertical flow, C=control, R=effluent recirculation)

4.3.3. COD removal

COD removal efficiencies, based on influent and effluent concentrations, were above 50% in all tanks until flowering (Fig. 4.5). During this period, effluent COD concentration remained constantly below 200 mgO_2/L. Comparable COD removal (40%) was observed in a wetland with Phragmites australis (Zupančič Justin and Zupančič, 2009) and ranges between 39% and 91% were assessed in a wetland with Cyperus haspan (Akinbile et al., 2012). After flowering, plants progress into senescence, reducing several physiological processes. Plants promote microbial aerobic degradation in the medium by transferring oxygen from the leaves to the root zone (Akinbile et al., 2012). When plant senescence begins, the oxygen transfer capacity is reduced, thus limiting aerobic microbial removal. This may explain the decrease in COD removal efficiency noted since week 8. When effluent recirculation was applied, lower COD concentrations were measured in the outlet of $HSSF_R$ and VF_R compared to both HSSF and VF. However, this was due to reduced COD concentrations in the feeding water rather than to an increase in removal capacity.

Summarizing, HSSF and $HSSF_R$ systems, although subjected to higher hydraulic loading rates, were more effective in removing organic compounds than VF and VF_R, respectively, as shown by the average areal load removal capacity: 2.74 gCOD/m^2/d for HSSF; 1.47 gCOD/m^2/d for $HSSF_R$; 1.80 gCOD/m^2/d for VF; 1.27 gCOD/m^2/d for VF_R.

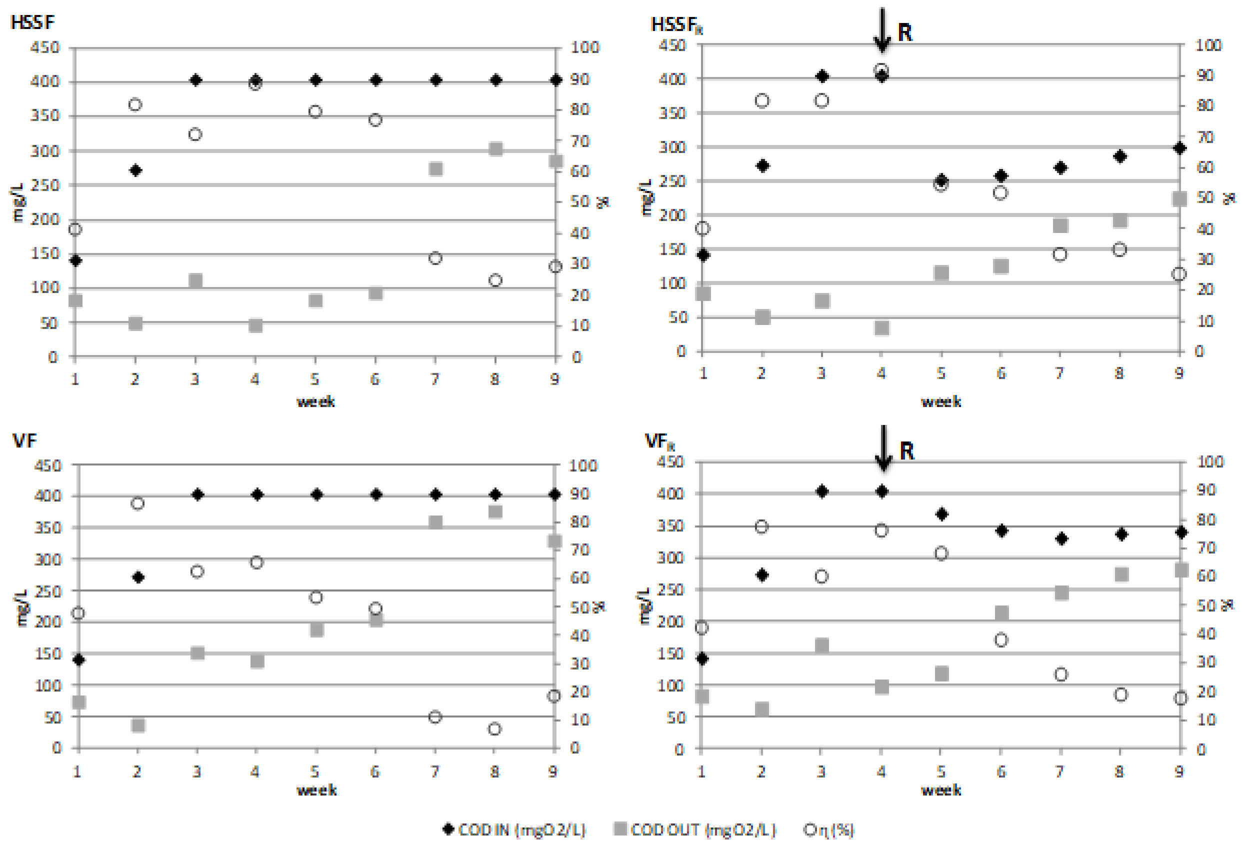

Fig. 4.5. COD influent concentration (mgO_2/L), COD effluent concentration (mgO_2/L) and removal efficiency (%). (Phase 1 = week 1; Phase 2 = week 2; Phase 3A = weeks 3-4; Phase 3B = weeks 5-9). Average results. Standard deviation values were below 5%. (HSSF=Horizontal subsurface flow, VF=vertical flow, R=effluent recirculation)

4.3.4. Nitrogen removal

At variance with COD removal capacity, nitrogen removal efficiencies (based on influent and effluent concentrations) decreased over time in all tanks during the experiment. HSSF and $HSSF_R$ displayed excellent performances: nitrogen removal was close to 100% till week 4, subsequently decreasing over time although remaining above 55% (Fig. 4.6). The process of nitrification occurred, as expected, in tanks VF, VF_R, and, to a lower extent, $HSSF_R$: the majority of the nitrogen exited the system as nitrate. Likely, recirculation promoted system oxygenation and enhanced the activity of nitrate-producing bacteria. However, vertical flow units were less effective in removing nitrogen, with total nitrogen concentrations in the effluent exceeding 100 mgN/L after week 5, while in the effluent of HSSF tanks the level of 100 mgN/L was exceeded exclusively during the senescence phase.

The average areal load removal capacity proved that the outflow recirculation was not effective in the overall performances: 3.40 $gN/m^2/d$ for HSSF; 1.34 $gN/m^2/d$ for $HSSF_R$; 1.67 $gN/m^2/d$ for VF; 0.94 $gN/m^2/d$ for $VF_{R.}$

Effluent recirculation procedure enhanced the accumulation of nitrates in the outlet of VF_R units, compared to $HSSF_R$.. A denitrification process likely occurred in horizontal flow tanks, as reported by Cheng and Chu, 2011 and Tyrrel et al., 2001. This suggests that the effluent of vertical flow tanks might be conveyed to horizontal flow units in order to achieve complete nitrogen removal by nitrification/denitrification (Pellissari et al., 2017; Vymazal, 2013) .

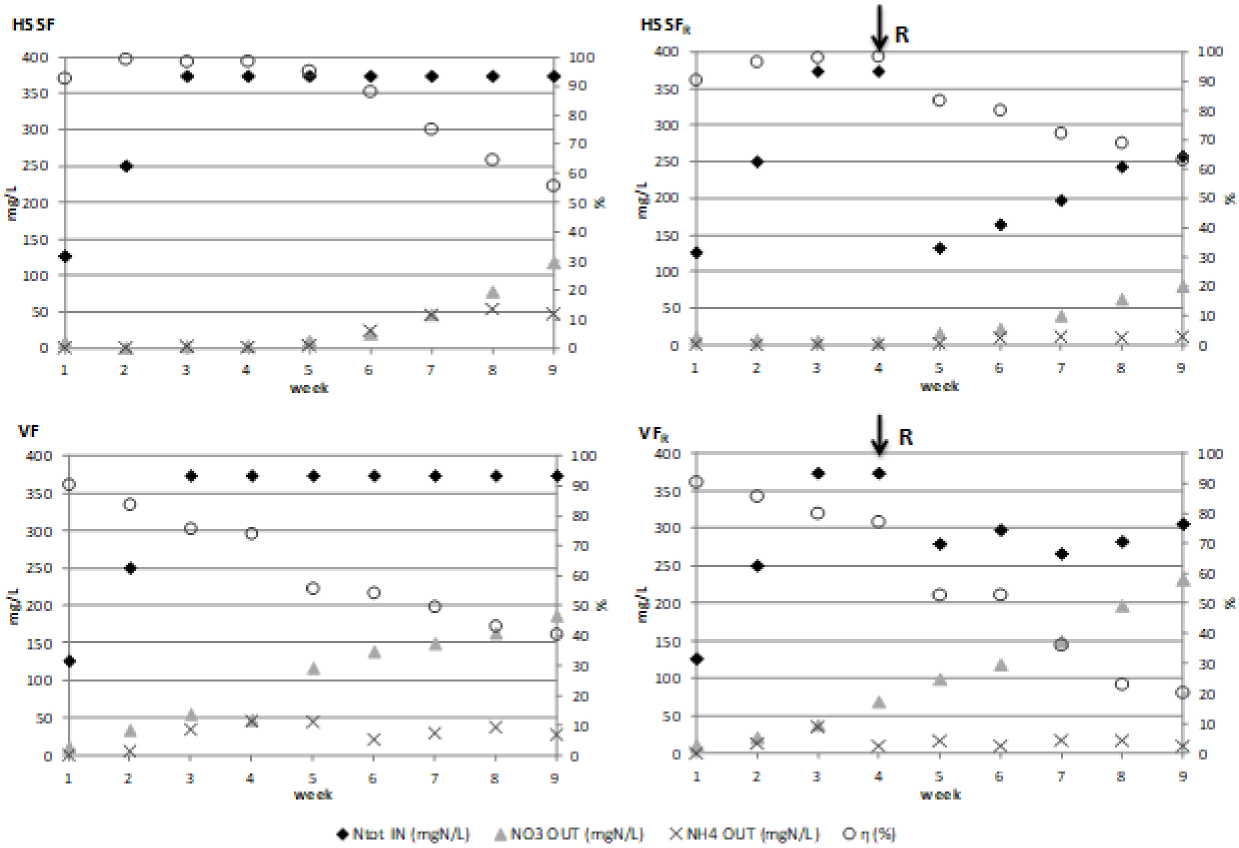

Fig. 4.6. Total nitrogen influent concentration (mgN/L), nitrate effluent concentration ($mgNO_3$-N/L), ammonium nitrogen effluent concentration ($mgNH_4$-N/L) and total nitrogen removal efficiency (%). (Phase 1 = week 1; Phase 2 = week 2; Phase 3A = weeks 3-4; Phase 3B = weeks 5-9). Average results. Standard deviation values were below 5%. (HSSF=Horizontal subsurface flow, VF=vertical flow, R=effluent recirculation)

4.3.5. Phosphorous removal

Excellent phosphorus removal efficiencies (based on influent and effluent concentrations) were measured during the entire period in all experimental units, resulting in phosphorous effluent concentrations constantly below 1 mgP/L (Fig. 4.7).

The average areal load removal capacity evidenced that the performances were satisfactory in all reactors, although horizontal flow tanks were more efficient than the corresponding vertical flow units: 0.03 $gP/m^2/d$ for HSSF; 0.01 $gP/m^2/d$ for $HSSF_R$; 0.02 $gP/m^2/d$ for VF; 0.01 $gP/m^2/d$ for VF_R.

In phytotreatment units, phosphorous is mainly sorbed or precipitated in the filter medium, and removal capacity is mainly associated with the physical, chemical and hydrological properties of the medium. The crucial role played by plants was however demonstrated by our study, particularly in vertical flow units, in which performances dropped following flowering.

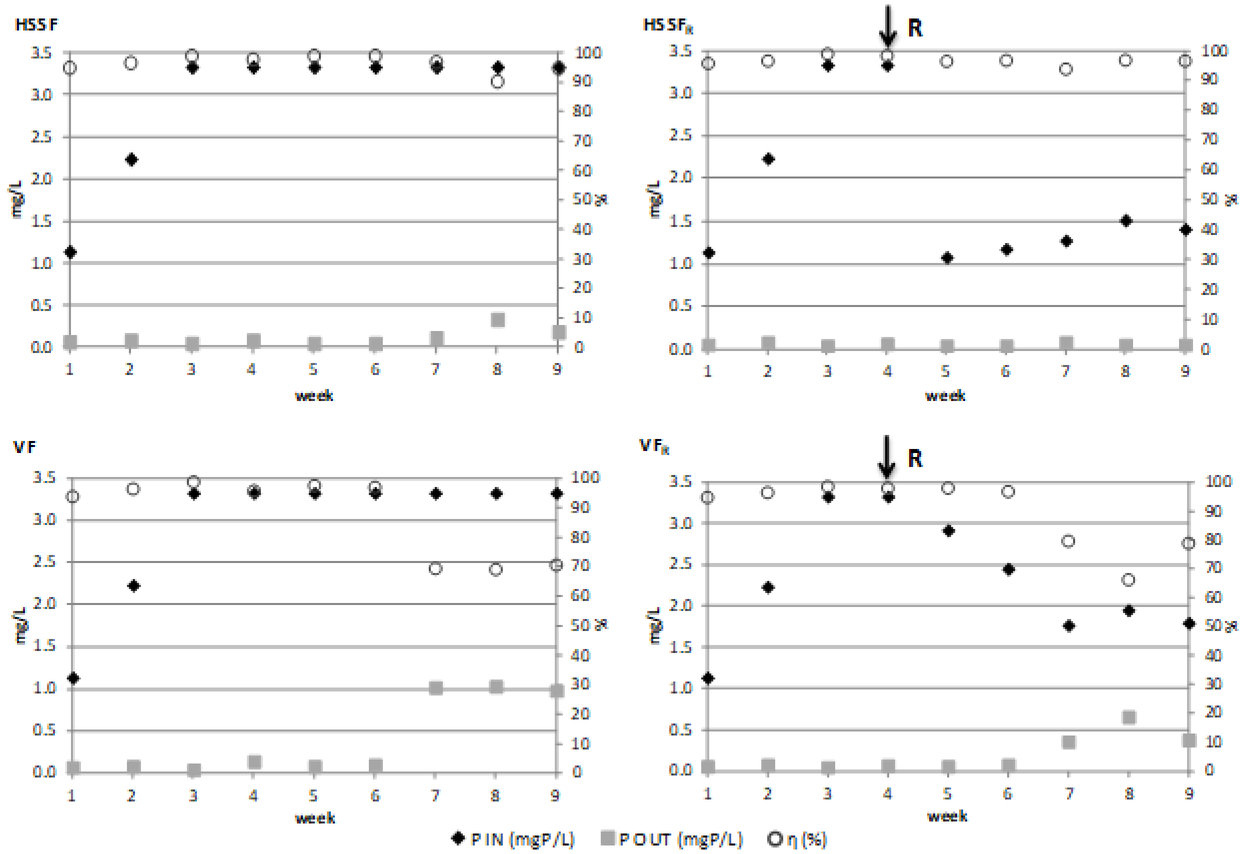

Fig. 4.7. Total phosphorous influent concentration (mgP/L), total phosphorous effluent concentration (mgP/L) and total phosphorous removal efficiency (%). (Phase 1 = week 1; Phase 2 = week 2; Phase 3A = weeks 3-4; Phase 3B = weeks 5-9). Average results. Standard deviation values were below 5%. (HSSF=Horizontal subsurface flow, VF=vertical flow, R=effluent recirculation

4.3.6. Nitrogen mass balance

Nitrogen was the reference parameter used to set the entire research program. Thus, nitrogen mass balance was calculated on all units to evaluate distribution among the main system components: water, soil and plants (Table 4.5).

The majority of nitrogen entered the system in form of ammonium ion (Table 4.3): a fraction was detected in the effluent, partially converted to nitrates (as shown in Fig. 4.6), a portion was adsorbed by the soil matrix and a small part was taken up by plants (Table 4.5). A significant nitrogen loss from the system (ΔN_L) occurred in all units, particularly in horizontal flow tanks, suggesting the formation of gaseous nitrogen compounds, as previously observed by Cheng and Chu (2011). The operating conditions inside the climatic chamber and the pH of water (almost 8), may indeed have promoted ammonia volatilization, as reported by Freney and Simpson (1983).

HSSF and VF systems showed a larger nitrogen loss than $HSSF_R$ and VF_R, respectively, suggesting that effluent recirculation, although enhanced the nitrification process, did not improve the overall nitrogen removal capacity. Effluent recirculation increased the nitrogen accumulation in the substrate, without affecting biomass development.

Based on nitrogen mass balance, sunflower plants appeared to play a limited role in the removal of nitrogen, confirming therefore that efficiency in phytoremediation systems is the result of a synergic action between the different components (Duggan, 2005).

Table 4.5. Nitrogen distribution between system components (mg/tank). Average results. Standard deviation values were below 5 %. (HSSF=Horizontal sub-superficial flow, VF=vertical flow, R=effluent recirculation)

	N_{tot} in influent - N_{in}	N_{tot} in effluent - N_{out}	Plant uptake - ΔN_P	Substrate accumulation - ΔN_S	ΔN_L
HSSF	78199	12531	1597	3653	60418
% (on N_{tot} in influent)	-	*16*	*2*	*5*	*77*
$HSSF_R$	31659	5682	1057	6784	18136
% (on N_{tot} in influent)	-	*18*	*3*	*21*	*58*
VF	59781	15570	2008	16353	25851
% (on N_{tot} in influent)	-	*26*	*3*	*27*	*44*
VF_R	34852	10134	1507	13352	9860
% (on N_{tot} in influent)	-	*29*	*4*	*38*	*29*

4.4. CONCLUSIONS

Leachate decontamination achieved by means of sunflower phytotreatment proved to be feasible under lab-scale conditions, as no inhibition of sunflowers was detected.

Wastewater volume reduction was significant: up to 80% of inlet wastewater was removed by evapo-transpiration. Moreover, the limited volume of effluent was characterized by low concentrations of contaminants, demonstrating the efficiency of the system in abating both volumes and pollutant concentrations.

This study suggests that plants are actively involved in the removal of contaminants, particularly phosphorous and organics, up until flowering; subsequently, pollutant removal capacity tends to decrease rapidly. This could be ascribed to the reduction of oxygen transfer from the leaves to the root zone, which occurs during senescence and limits activity of the bacterial population living in symbiosis in the root zone. The same consideration cannot be extended to the nitrogen removal process, which seems to result from a synergy between the different system components.

Further tests will be performed to investigate the set of processes involved in the removal mechanisms. Moreover, additional experiments will assess whether a combination of vertical and horizontal flow tanks might be capable of further increasing volume reduction and/or the contaminant concentrations in the effluent.

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Chapter 5: Landfill leachate phytotreatment with sunflowers grown in a waste-derived substrate

Based on:

Garbo, **F.**, Lavagnolo, M.C., Malagoli, M., Cossu, R. (2016). Energy recovery from oily crops in landfills. In: Proceedings of the Venice 2016 - 6th International Symposium on Energy from Biomass and Waste. ISBN: 9788862650090, Venice, Italy, 14-17 November 2016.

Abstract

Sunflowers, irrigated with old landfill leachate, were cultivated in a waste-derived substrate: a mixture of sand from sweeping of streets and compost containing sewage sludge. Plants were grown in 300 L reactors characterized by vertical and horizontal subsurface flows, placed in a controlled climatic chamber. Vertical and horizontal flow units were connected in series to enhance nitrification and denitrification: nitrogen losses in gaseous form were approximately 40-45%. Significant removal efficiencies were achieved for total N (η >80%) and total P (η >60%). The influent volumes were strongly reduced by evapotranspiration (more than 80%). Effluent concentration of organic compounds (expressed as COD) was influenced by the leaching from the growing medium. Leachate irrigation did not inhibit the biomass development and seemed to stimulate the oil production, with a favorable Free Fatty Acids composition in view of the biodiesel production. A simple model was developed to study the kinetics of nitrogen removal in vertical flow units, which revealed the occurrence of a fast nitrification process.

Keywords: landfill leachate phytotreatment; sunflowers, waste-derived substrate; seeds oil characterization; kinetics of nitrogen removal

5.1. INTRODUCTION

Phytotreatment is a well-known alternative in the field of wastewater treatment; although considered to be simple and low-cost, it shows high pollutants removal efficiencies (Salt et al., 1998). It can be even more interesting when combined with energy crops cultivation. This solution is particularly suitable in nowadays societies, in which water pollution and freshwater depletion are severe problems, as it combines the possibility to obtain renewable energy while remediating contaminated wastewater (Duggan, 2005). The use of non-conventional water resources, such as landfill leachate, seems to be, in this terms, an optimal compromise between the need to produce biofuels (e.g.: biodiesel or bioethanol) and preserve water storages. Furthermore, energy crops cultivation represents an alternative to reduce greenhouse gases emissions that are typical of energy production systems which rely on fossil fuels (Zema et al., 2012).

Landfill leachate is a source of nitrogen (N) and its land application is an opportunity to return the bio-available N to the ecosystem, closing in this way the nitrogen cycle; moreover the soil-plant system works as a sink where nitrogen is taken up and stored (Cheng and Chu, 2011). Landfill leachate was successfully used to irrigate poplars and willows, paying attention to the irrigation rates that must be properly adjusted to minimize groundwater disturbances (Dimitriou and Aronsson, 2010). Irrigation rate, in fact, is one of the most important parameters to be monitored because it influences the efficiency of the system and the plants response (Zalesny et al., 2009).

Landfill leachate phytotreatment with oily crops for biodiesel production is a promising option which can be applied on the top of landfills in post-closure. It is a chance to exploit derelict areas, avoiding at the same time the competition of land for food and energy production (Lavagnolo et al., 2016).

The most suitable oily crop should be chosen on the basis of different requirements: contaminants removal capacity, quality of the biodiesel produced, possibility to be cultivated in different countries. Crops with these characteristics include *Helianthus annuus* (sunflower), *Glycine max* (soybean) and *Brassica napus* (rapeseed) (Singh and Singh, 2010; Marchiol et al., 2007; Lavagnolo et al., 2016).

This paper describes an experimental trial in which sunflowers, irrigated with diluted landfill leachate, were grown in a waste-derived substrate, a mixture of sand from sweeping of streets and compost containing sewage sludge.

Sand from sweeping of streets can be technically used as construction material (e.g.: filler for the construction of new roads), but is often transported to landfills and used to build the daily/temporary/final top cover. Composted food waste or the composted solid fraction of anaerobic digestate are conventionally used in agriculture as soil improvers. Compost containing sewage sludge, on the contrary, although usable in agriculture, is often refused by Italian farmers because of its origin, which is considered "hazardous" and "dirty". Even the introduction of strict national and regional regulations (Italian Legislative Decree 75/2010; DGR 568/2005), which define in details the characteristics of this kind of compost (in terms of residual contaminants, e.g.: heavy metals) was not successful. As a result, compost containing sewage sludge is often disposed of in landfills (mainly used as temporary cover).

The idea at the basis of the experimental project described in this paper is to try to take advantage of the above-mentioned issues: compost containing sewage sludge and sand from sweeping of streets should be mixed and used to build the superficial layer of the top cover of closed landfills, on which energy crops (e.g.: sunflowers), irrigated with the leachate produced by the same landfill, are cultivated for energy production (e.g.: biodiesel). In this way, these materials that close the cycle of waste management can be valorized as new resources for the production of renewable energy, in the framework of the circular economy concept (Cossu, 2015). Moreover, there will be no more the need to use virgin soil to construct the superficial layer of landfills top covers.

In this research, sunflowers were cultivated in tanks characterized by different hydraulic systems: vertical and horizontal sub-superficial flows, operated in series to simulate nitrification and denitrification. The aims include: assessment of the removal efficiencies of the pollutants provided by the leachate, evaluation of the bio-concentration of heavy metals in the plants biological tissues, study of the kinetics of nitrogen removal in vertical flow reactors and analysis of the quality of seeds oil in view of the biodiesel production.

5.2. MATERIAL AND METHODS

5.2.1. Research program

The research was carried out at the Laboratory of Environmental Engineering of the University of Padova (Italy). Six reactors, filled with the growing substrate, were placed in a controlled climatic chamber. Three reactors were operated as Vertical (V) flow systems; three as Horizontal (H) sub-superficial flow systems. Vertical flow units were named V1, V2, and VC; horizontal flow units were named H1, H2, and HC. V and H units were connected in series to promote the occurrence of nitrification and denitrification (Wang et al., 2017; Vymazal, 2013). V1 and V2 were irrigated with diluted leachate and the effluents were used to feed H1 and H2, respectively; VC was fed with tap water throughout the entire duration of the experiment and the effluent was used to feed HC: they were used as plants and substrate control units (Table 5.1). The vertical flow units were fed twice per day and drained once per week; the horizontal flow units were fed and drained once per week (irrigation provided after the total weekly drainage).

After an initial acclimation period, lasting 7 days, in which all the plants were irrigated with tap water, V1 and V2 were irrigated with increasing leachate dosages (up to a maximum nitrogen influent concentration of 370 mgN/L) (Table 5.2). Dilution rates were set taking into account that the maximum tolerable nitrogen concentration (N was used as reference parameter) for sunflowers is 400 mgN/L (Garbo et al., 2017; Lavagnolo et al., 2016).

In H units, the initial acclimation period with tap water lasted for 14 days as during week 1 the effluents of vertical flow units were not available.

After drainage, effluent samples were stored at -20°C and subsequently analyzed for the following parameters: Total Kjeldhal Nitrogen (TKN), ammonia, nitrate, nitrite, phosphorous and COD. Once clear senescence was reached, plants were harvested, oven dried at 60 °C, and weighed. Nitrogen and heavy metals content in leaves, roots, stems and seeds was determined. At the end of the experiment, the substrate was sampled from each experimental unit: samples were air-dried and both TKN and nitrate contents were determined to complete the final nitrogen mass balance.

Table 5.1. Set up of experimental reactors

Experimental unit	Irrigation	Column description	Irrigation frequency	Drainage frequency
V1	Diluted leachate	Experimental unit	Daily	Once per week
V2	Diluted leachate	Experimental unit	Daily	Once per week
VC	Tap water	Plants and substrate control unit	Daily	Once per week
H1	V1 effluent	Experimental unit	Once per week	Once per week
H2	V2 effluent	Experimental unit	Once per week	Once per week
HC	VC effluent	Plants and substrate control unit	Once per week	Once per week

Table 5.2. Leachate dosages and contaminants concentrations in the feeding of V1 and V2

Week	Feeding quality (V1 and V2)	TKN influent concentration (mgN/L)	COD influent concentration (mgO_2/L)	P influent concentration (mgP/L)
1	90% tap water + 10% leachate	185	143	2.5
2-12	80% tap water + 20% leachate	370	286	5

5.2.2. Equipment

300 L HDPE tanks, sized 95 x 50 x 65 cm, were used for the experiment.

In V units, the growing medium was made up of a 35 cm substrate layer, laying above a gravel drainage layer, 10 cm thick, composed by medium-size gravels (d=20-30mm) (Fig. 5.1a). Six plants, organized in two lines of three sunflowers each, were grown in each reactor (Fig.5.1b). A plant density of 16 plants/m^2 was applied to match the optimal values for Mediterranean areas (Barros et al., 2003). Irrigation was provided over the entire surface to simulate the vertical flux from the top the bottom. A step-feeding strategy (irrigation every 12 hours) was applied to allow the oxygen intrusion, essential for the nitrification process (Pellissari et al., 2016).

In H units, two vertical drainage layers were placed upstream and downstream the substrate, composed by coarse (40-60 mm) and medium (20-30 mm) gravels, respectively (Fig. 5.2a). Four plants were grown in each reactor to maintain the same plant density of vertical flow reactors (Fig. 5.2b) The effluent of V units was distributed homogeneously in the upstream zone of H reactors (Fig. 5.3) to allow a plug-flow movement of water, driven by the hydraulic gradient, and favour the occurrence of denitrification (Pellissari et al., 2016).

The V-H connection in series allowed to maintain an Hydraulic Retention Time (HRT) of 11 days, significantly longer than the suggested minimum of 7 days (Garbo et al., 2017; Sawaittayothin and Polprasert 2007).

The units were placed into a climatic chamber in which a 14-hour photoperiod with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity was imposed. Average air temperature was maintained at 24 °C (MIN=17 °C, MAX=35 °C).

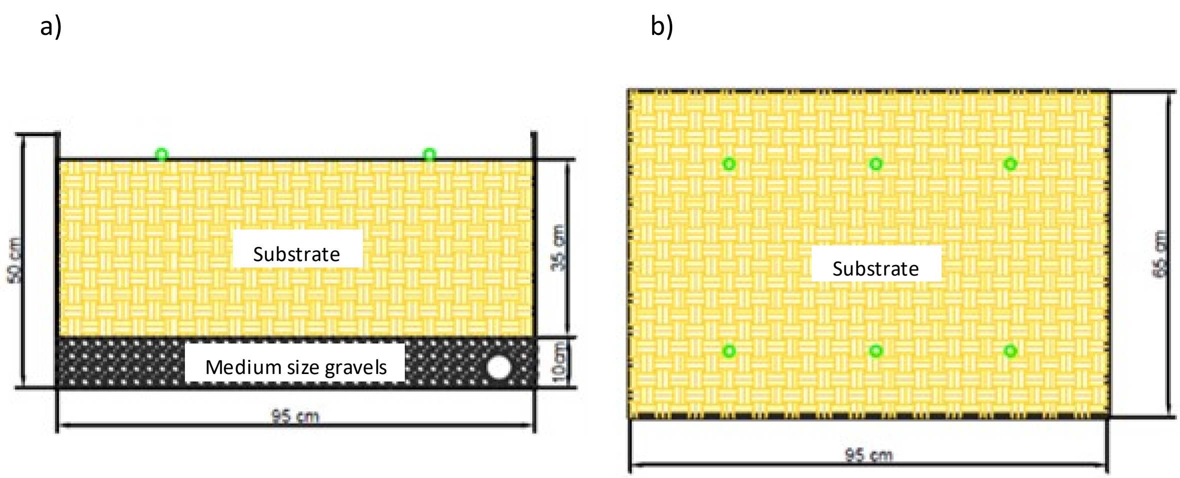


Fig. 5.1. Cross section (a) and plan view (b) of vertical flow units (green dots represent the position of sunflowers)

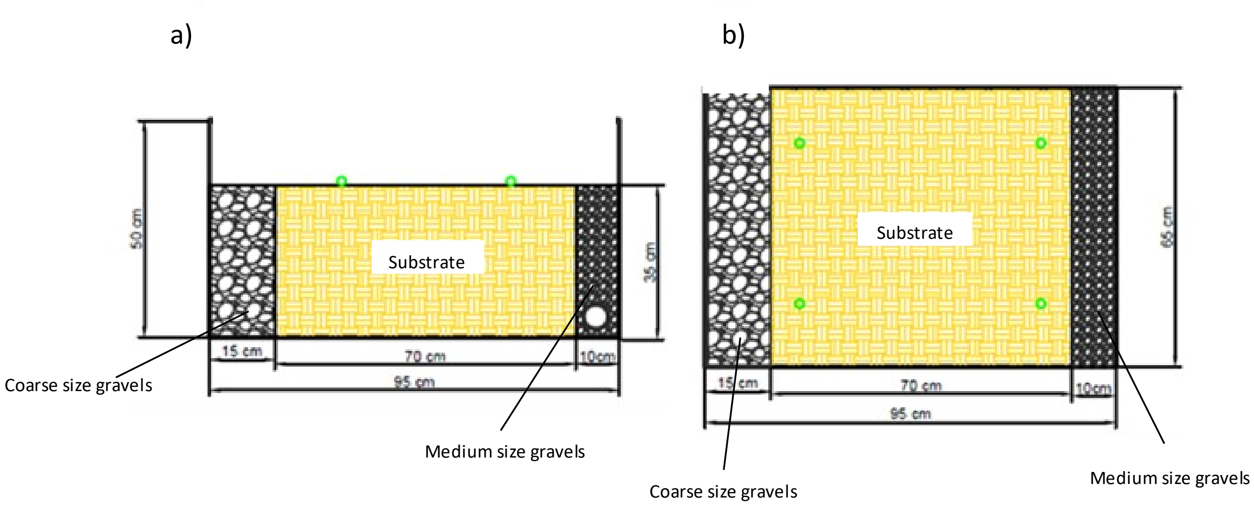


Fig. 5.2. Cross section (a) and plan view (b) of horizontal sub-superficial flow units (green dots represent the position of sunflowers)

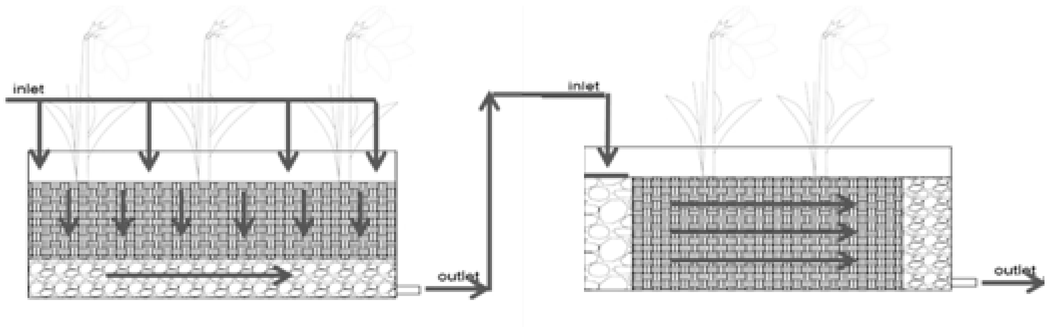


Fig. 5.3. Sketch of the reactors connected in series

5.2.3. Substrate

A mixture of compost containing sewage sludge (25% on volume basis) and sand from sweeping of streets (75% on volume basis) was used as medium to fill the experimental units. The use of a nutrients rich material (compost) and a material with high porosity (sand) provides the optimal living conditions for plants and microorganisms involved in the phytotreatment process (Stottmeister et al., 2003; Weerakoon et al., 2013; Lavagnolo et al., 2016; Garbo et al., 2017).

Sand from sweeping of streets was collected from a plant in which it is mechanically separated from other wastes and then washed to remove residual hydrocarbons and heavy metals. Leaching tests were performed according to the standard UNI EN 12457-2 (L/S was brought to 10 L/kgTS, mixed for 24 h and filtered at 45 µm) to check wheter such contaminants were still present, but the concentrations in the eluate were always below the detection limits.

Compost containing sewage sludge was collected from a composting plant located in the Veneto Region (Italy) and complied with the regional requirements for high quality compost, defined in DGR 568/2005. It was produced from dried sewage slude (1/8 by volume) and shredded green waste (7/8 by volume).

The texture of the mixture (Fig. 5.4) was determined with the Bouyoucos method (Bouyoucos, 1962) and, according to the soil taxonomy proposed by the USDA (USDA-NRCS, 1999), was classified as sand (94% sand, 2% silt, 4% clay). The substrate soil chemical characterization is reported in Table 5.3. Heavy metals concentration in the substrate was similar to that of the experiment reported by Lavagnolo et al. (2016), in which sandy and clayey soils were used.

Table 5.3. Chemical characterization of the mixture (DM = Dry Matter)

Parameter	Unit	Value
VS	% on DM	15
TOC	% on DM	10.3
TKN	mgN/kg_{DM}	1439
NO_3^-	$mgNO_3\text{-}N/kg_{DM}$	24
Cr	mg/kg_{DM}	41.3
Cu	mg/kg_{DM}	33
Ni	mg/kg_{DM}	13
Zn	mg/kg_{DM}	82
Fe	mg/kg_{DM}	8144
Mn	mg/kg_{DM}	307

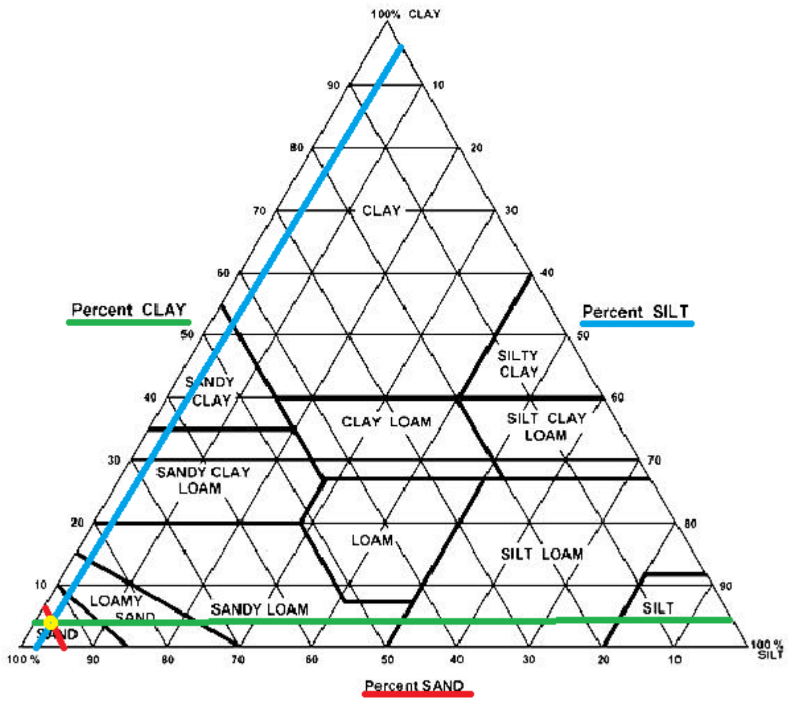


Fig. 5.4. Substrate texture, classified according to USDA standards (USDA-NRCS, 1999).

5.2.4. Landfill leachate

The leachate used for the experiment was collected in a closed landfill located in the North of Italy, in which the residual waste fraction of Municipal Solid Waste (MSW) was disposed. The chemical characteristics of leachate are reported in Table 5.4. NO_2^- was absent, while NO_3^- was 5 mgNO_3-N/L: TKN was representative of almost all the total influent nitrogen. Values of TKN, ammonia and the BOD to COD ratio (BOD/COD equal to 0.35) are typical of a leachate produced during the methanogenic phase (Jones et al., 2006; Stegmann et al., 2005).

Table 5.4. Landfill leachate characteristics

Parameter	Unit	Value
TS	mg/L	7771
VS	mg/L	2525
COD	mgO_2/L	1430
TOC	mgC/L	1145
BOD_5	mgO_2/L	495
TKN	mgN/L	1849
NH_4^+-N	mgNH_4-N/L	1714
NO_2^-	mgNO_2-N/L	0
NO_3^-	mgNO_3-N/L	5
P_{TOT}	mgP/L	25.74
pH	-	8.5
Alkalinity	mg$CaCO_3$/L	14610
Conductivity	mS/cm	15.5
Cd	µg/L	<10
Cr	µg/L	751
Cu	µg/L	52
Fe	µg/L	3850
Mn	µg/L	176
Ni	µg/L	152
Pb	µg/L	<10
Zn	µg/L	115

5.2.5. Analytical methods

Leachate and all the liquid samples were analyzed according to the CNR-IRSA standard Italian analytical methods (CNR-IRSA, 29/2003). BOD_5 was measured with a respirometer apparatus (Sapromat E); ammonia was evaluated by means of a distillation-titration procedure; TKN was measured through a distillation-titration procedure after an acid digestion phase; dissolved components (nitrite and nitrate) were determined using a UV-VIS spectrophotometer (Shimadzu UV-1601). The same spectrophotometer was used to detect total phosphorus after sample digestion. Nitrogen content in soil and plants at the end of the trial was analyzed according to CNR-IRSA standard Italian analytical guidelines for solid specimens (CNR-IRSA, 64/1986). Oil seeds were analyzed in oil content and Free Fatty Acids (FFA) quality according to the European standards (Reg. CEE 2568/2011, G.U. CEE L248/91 All. II, Reg. CE 702/2007, G.U. CE L161/2007).

5.2.6. Nitrogen balance

At the end of the entire experiment, the total nitrogen balance for the leachate irrigated systems V1-H1 and V2-H2 was calculated, based on the following equation:

$$N_{in} = N_{out} + \Delta N_P + \Delta N_S + \Delta N_L \quad (5.1)$$

where:

N_{in} = Total mass of nitrogen entering the systems

N_{out} = Total mass of nitrogen in the outflow of H units

ΔN_P = Amount of nitrogen accumulated in the plant tissues

ΔN_S = Nitrogen accumulated in the substrate

ΔN_L = Nitrogen gaseous losses

5.2.7. Kinetics of nitrogen removal

The Matlab software was used to calibrate the kinetic parameters of nitrogen removal in vertical flow leachate irrigated reactors (V1 and V2), which were characterized by a fixed irrigation procedure in terms of volumes and concentrations (Table 5.2). According to Kadlec and Wallace, (2009), treatment wetland hydraulics is best represented by the Tank-in-Series (TIS) model,which is an intermediate case between the ideal plug-flow and the continuous flow stirred tank reactor (CSTR). The representation of one wetland by a series of CSTR units is just for mathematical convenience and the exact number of *N* tanks to be used in the representation should be estimated by tracer tests. However, according to Boog et al. (2014), for vertical flow reactors the number of

TIS is approximately 1. Therefore, in this study, the units were assumed to be operated as single CSTR to keep the complexity of the system at minimum. The general mass balance equation of a CSTR is:

$$V\frac{dC}{dt} = Q_{in}\,C_{in} - Q_{out}\,C \pm rV \tag{5.2}$$

where:

V: liquid volume (L)

C: concentration inside the reactor and in the effluent (mg/L)

C_{in}: concentration in the influent (mg/L)

Q_{in}: influent flow rate (L/d)

Q_{out}: effluent flow rate (L/d)

r: reaction rate (mg L^{-1} d^{-1})

The nitrogen removal processes were described using a first order kinetic (Saeed and Sun, 2011; Jorgensen and Bendoricchio, 2001):

$$r = \mathrm{k}\,C \tag{5.3}$$

where:

C = concentration in the reactor (mg/L)

k = reaction rate constant (d^{-1})

The reaction rate constant (k) varies with the temperature according to the Arrhenius equation:

$$k_T = k_{20}\,\theta^{(T-20)} \tag{5.4}$$

where:

k_T: reaction rate constant (d^{-1}) at the operating temperature T (°C);

k_{20}: reaction rate constant at 20° C (d^{-1});

Θ: dimensionless parameter (>1)

5.2.7.1. Mathematical model conceptualization

A mathematical model was developed to describe the nitrogen transformations occurring in the experimental reactors V1 and V2 and to calibrate the values of the reaction rate constants. The modelled processes were:

- Mineralization of organic nitrogen to ammonia
- Oxidation of ammonia to nitrate (nitrification)
- Reduction of nitrate to atmospheric nitrogen (denitrification)
- Plants uptake of ammonia and nitrate (assuming that the uptake of ammonia and nitrate are similar and could be jointly modelled)
- Settling/adsorption/biological degradation of organic nitrogen and ammonia in the medium

The forcing functions which were modeled were:

- Influent volumes
- Effluent volumes
- Influent concentrations
- Effluent concentrations
- Temperature of the liquid volumes

The modelled state variables were:

- Organic nitrogen concentration (Norg)
- Ammonia concentration (NH_4^+)
- Nitrate concentration (NO_3^-)

5.2.7.2. Mathematical formulation

Eq. 5.2 and Eq. 5.3 were combined to link the inlet and outlet concentrations of the state variables across the V units (Saeed and Sun, 2011). The resulting mass balances of the state variables were expresses as Ordinary Differential Equations (ODE):

$$\frac{dNorg}{dt} = \frac{Q_{in}\, Norg_{in}}{V} - \frac{Q_{out}\, Norg_{out}}{V} - k_1\, Norg_{out} - k_5\, Norg_{out} \qquad (5.5)$$

$$\frac{dNH4}{dt} = \frac{Q_{in}NH4_{in}}{V} - \frac{Q_{out}\,NH4_{out}}{V} - k_2\,NH4_{out} - k_4\,NH4_{out} + k_1\,Norg_{out} - k_5\,NH4_{out} \tag{5.6}$$

$$\frac{dNO3}{dt} = -\frac{Q_{out}\,NO3_{out}}{V} - k_3\,NO3_{out} - k_4\,NO3_{out} + k_2\,NH4_{out} \tag{5.7}$$

The reaction rate constants (k_1, k_2, k_3, k_4, k_5) refer to the transformations reported in Table 5.5.

Table 5.5. Indexes and description of the reaction rate constants calibrated in the model

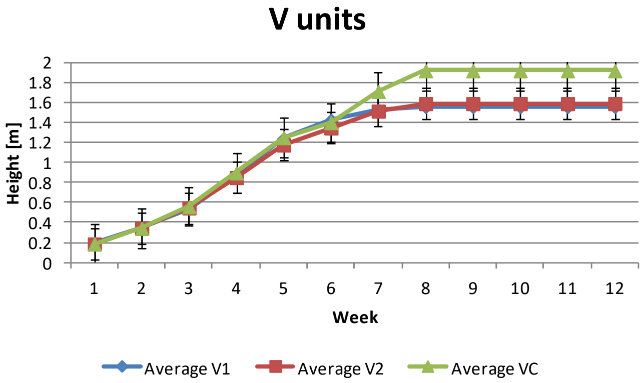
Process	Reaction rate constants
Mineralization of organic nitrogen	k_1
Nitrification	k_2
Denitrification	k_3
Ammonia and nitrate uptake by plants	k_4
Organic nitrogen and ammonia removal due other processes (settling, adsorption, etc)	k_5

5.3. RESULTS AND DISCUSSION

5.3.1. Sunflowers growth

Plants grew vigorously and uniformly throughout all experimental units. Blooming occurred during week 7, followed by a sudden senescence. Sunflowers grown in leachate irrigated reactors showed no symptoms of toxicity and were not affected by the presence of leachate in the irrigation water.
Maximum average height of plants ranged between 1.4 and 1.6 m in both vertical and horizontal flow units, with the only exception of plants grown in VC which reached heights close to 2.00 m (Fig. 5.5). These values match typical height of sunflowers cultivated in conventional ways (Barros et al., 2003). Results reported in Table 5.6 indicate that sunflowers grown in vertical flow reactors developed larger biomasses compared to sunflower cultivated in horizontal flow reactors, as already observed by Garbo et al. (2017), with a peak of 123.02 g_{TS}/plant observed in VC.
Final distribution among the plants components (Fig. 5.6), however, did not reveal any difference among plants grown in V and H units, as well as between leachate irrigated reactors and controls.

a)



b)

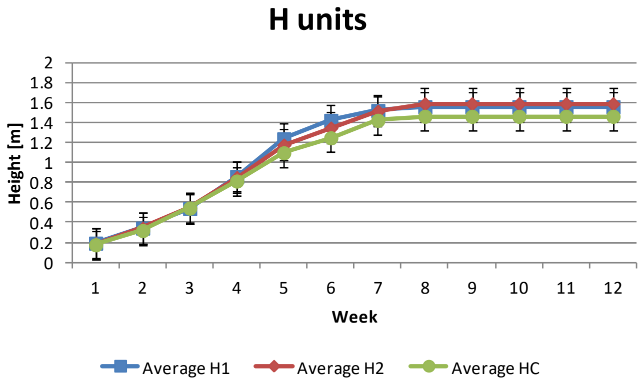


Fig. 5.5. Average height of sunflower grown vertical (a) and horizontal (b) flow units

Table 5.6. Final dry weights among the different plants components. Results expressed as g_{DM}/plant (DM = Dry Matter)

	V1		V2		VC	
	Average	Range	Average	Range	Average	Range
Stems	41.22	19.04-69.74	38.90	15.31-63.19	59.48	47.08-99.71
Leaves	37.37	18.69-57.67	28.61	16.69-40.52	40.73	24.11-73.69
Roots	5.28	1.93-13.56	6.28	1.75-9.65	17.91	9.67-30.66
Seeds	7.55	4.25-13.16	8.10	4.43-14.05	4.90	2.53-8.09
Total biomass	91.42		81.89		123.02	
	H1		H2		HC	
	Average	Range	Average	Range	Average	Range
Stems	32.00	9.48-58.90	34.70	27.47-47.33	33.41	18.86-58.55
Leaves	19.56	5.23-34.97	20.78	18.24-27.65	16.78	9.60-29.12
Roots	2.25	1.22-3.43	3.36	2.21-3.92	3.62	1.22-3.95
Seeds	7.47	2.57-13.16	10.32	5.17-16.66	8.18	2.34-15.92
Total biomass	61.28		69.19		61.99	

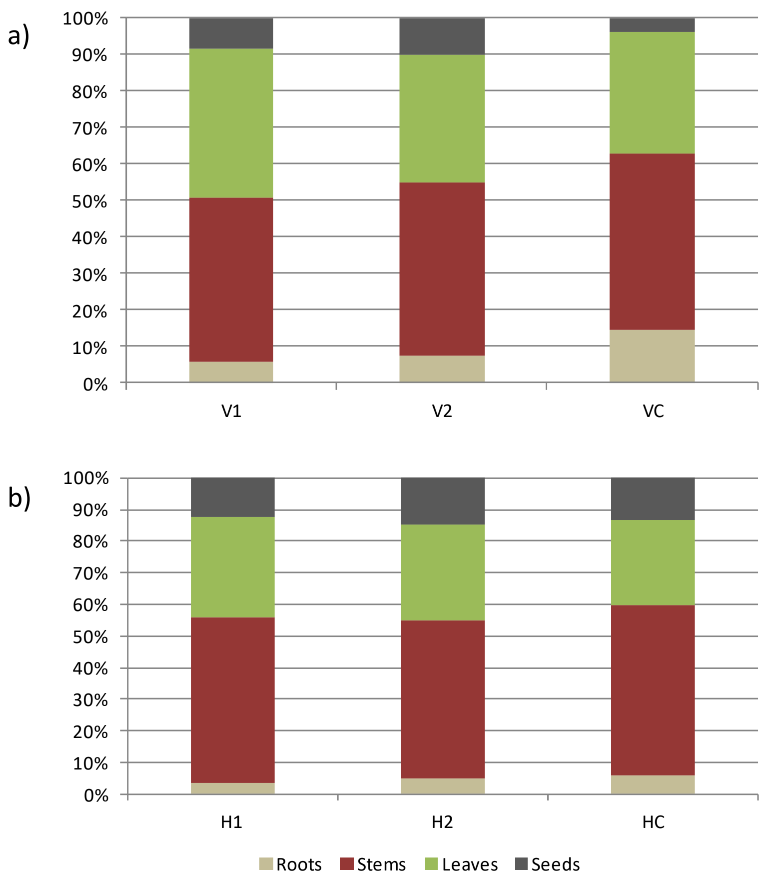


Fig. 5.6. Final dry weight distribution among the different plant components in vertical (a) and horizontal (b) flow units

5.3.2. Influent and effluent volumes

Analysis of influent and outlet volumes was used to estimate the weekly evapo-transpiration (ET), which resulted from the combined effect of plants requirements, evaporation from soil and transpiration from leaves:

$$ET = \frac{V_{IN} - V_{OUT}}{V_{IN}} * 100 \tag{5.8}$$

where:

ET = evapo-transpiration (%)

V_{IN} = inlet volume (L/week)

V_{OUT} = outlet volume (L/week)

The feeding volumes and the volumes exiting each experimental reactor are reported in Fig. 5.7.

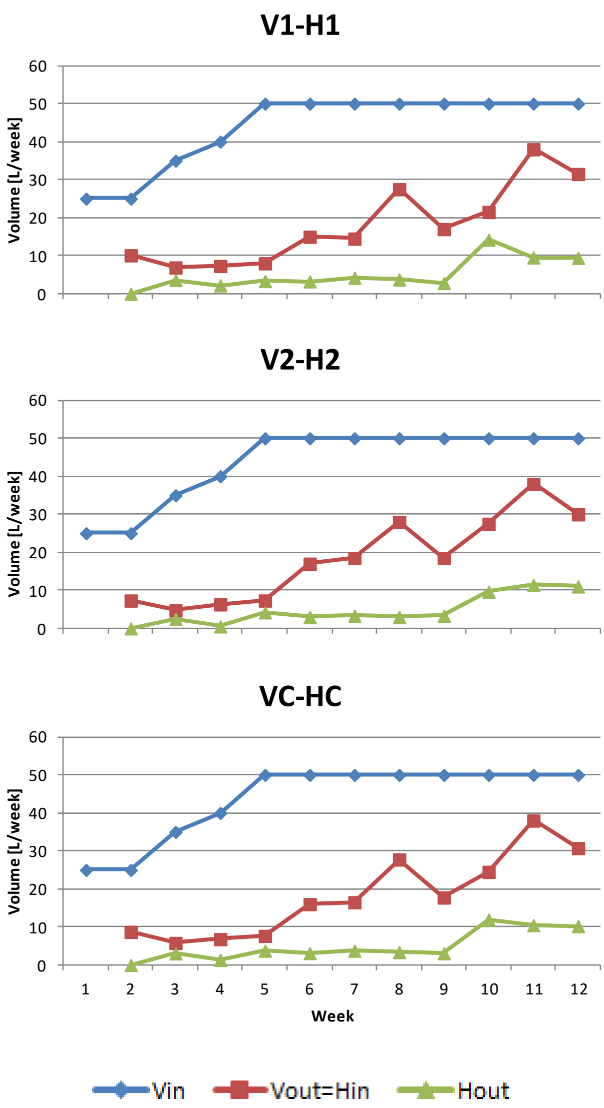


Fig. 5.7. Influent and effluent volumes in systems V1-H1, V2-H2, and VC-HC

The inlet volumes were increased gradually to adapt the plants to the presence of contaminants, starting from 25 L/week (approximately 8.3 mm/d) during the first and second weeks, up to 50 L/week (16.7 mm/d) from week 5: the maximum value was maintained till the end of the experiment. Similar hydraulic loading rates have been applied by Garbo et al. (2017) and Ogata et al. (2015). The effluent volumes were extremely low (0-20 L in V units, 0-5 L in H units) until week 7, when plants blooming occurred. Similar evapo-transpiration rates were obtained by Bialoweic et al. (2014, 2007), but they used reed which are known to have a marked transpiration ability. After blooming, the outlet volumes increased especially in V reactors (effluent volume up to 40 L/week), as a result of a reduced plants water requirement during the senescence phase, as already reported by Lavagnolo et al. (2016). Anyhow, considering the cumulative performances of the V-H systems, evapo-transpiration was always above 80%: similar water losses were observed by Albuquerque et al. (2009) and Bialowiec et al. (2006) in experiments in which vertical and horizontal flow units with hydrophytes were connected in series. No differences were detected between leachate irrigated units and controls, indicating that leachate addition did not influence evaporation from the soil and transpiration from plants.

5.3.3. Contaminants removal

Due to a strong evapo-transpiration effect, evaluation of contaminants removal should take into account the reduction of the liquid volumes, therefore Removal Efficiencies (RE) of the whole systems (V+H) were based on weekly loads:

$$RE = \frac{V_{IN}C_{IN} - V_{OUT}C_{OUT}}{V_{IN}C_{IN}} \quad (5.9)$$

where

V_{IN} = influent volume in vertical flow units (L/week)

V_{OUT} = effluent volume in horizontal flow units (L/week)

C_{IN} = influent concentration in the irrigation of vertical flow units (mg/L)

C_{OUT} = effluent concentration in the horizontal flow units (mg/L)

5.3.3.1. Nitrogen removal

The influent and effluent concentrations of the monitored nitrogen compounds are reported in Fig. 5.8. NO_2^- is not shown because always below the detection limits.

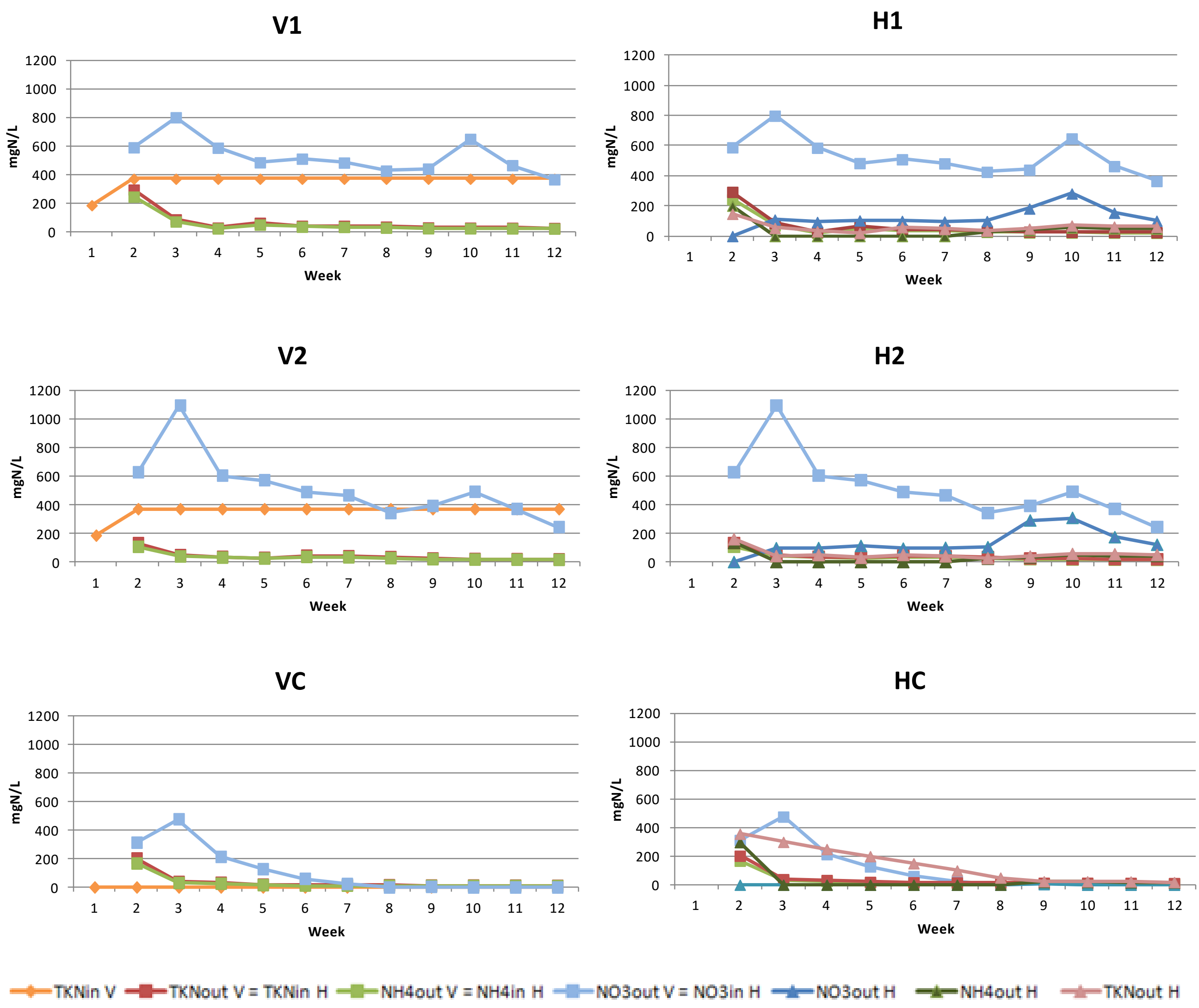


Fig. 5.8. Influent and effluent concentrations of nitrogen compounds in the experimental reactors

In V1 and V2, TKN and ammonia effluent concentrations decreased rapidly from week 1 to week 3 and then remained both below 50 mgN/L, even when the maximum leachate dose was applied (370 mgN/L in the influent). An anomalous increase of NO_3^- concentrations (up to approximately 1000 mgNO_3-N/L) was observed from week 1 to week 3 in the effluents of these units: this phenomenon occurred also in VC and could be related to the leaching of nitrates from the substrate, due to the frequent irrigation procedure. After the peak detected in week 3, nitrate concentrations decreased rapidly reaching values close to 0 mgN/L in VC, while in V1 and V2 the concentrations were almost equal or slightly exceeded the influent nitrogen concentration, suggesting that leachate irrigation likely stimulated the growth of nitrifying bacteria which oxidized not only the influent nitrogen but also nitrogen compounds which were already contained in the substrate (Table 5.3). Focusing on horizontal flow units, the influent was rich in nitrates produced by the vertical flow reactors. Denitrification was observed, as NO_3^- was always below the corresponding influent concentrations. Effluent nitrate concentrations remained below 100 mgN/L in both H1 and H2 till the flowering point (which occurred during week 7), then increased up to 300 $mgNO_3$-N/L but still below the influent concentrations, probably due to a reduced nitrate uptake capacity of plants during the senescence phase. Nevertheless, the reactors proved to be extremely efficient in removing the total nitrogen (TN) supplied with the landfill leachate, even after the flowering point, as shown by Fig. 5.9 in which the performances of the whole leachate irrigated systems (V+H), calculated according to Eq. (5.9), are reported. Removal efficiencies based on weekly loads were always above 80%, aligned with the best performances reported in literature (Cheng and Chu, 2011, Garbo et al., 2017; Lavagnolo et al., 2016).

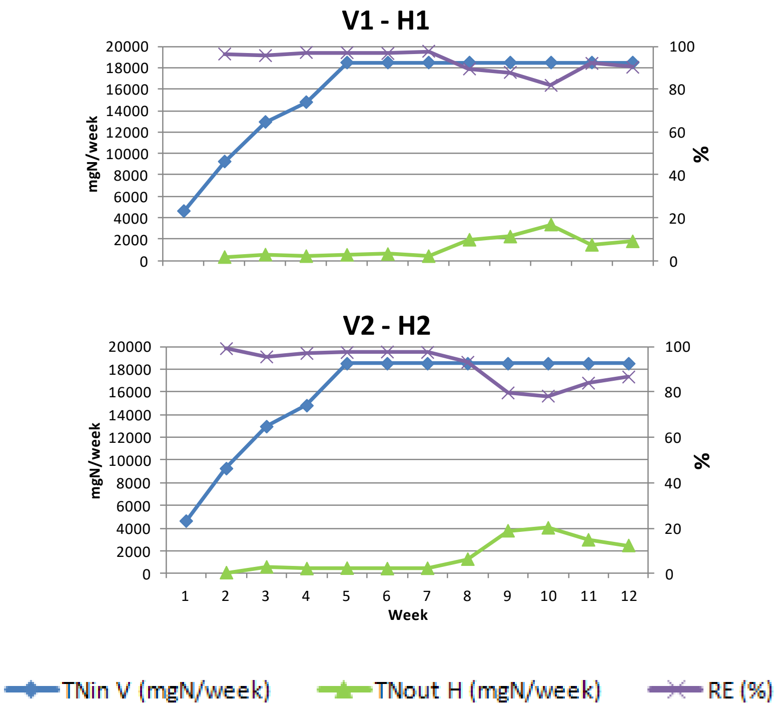


Fig. 5.9. Influent and effluent total nitrogen weekly loads and removal efficiency (RE) in the leachate irrigated systems V1-H1 and V2-H2

5.3.3.2. COD removal

Contrary to nitrogen, COD outlet concentrations were always above the corresponding influent concentrations, in both vertical and horizontal flow reactors, as shown in Fig. 5.10. This phenomenon was observed even in the control reactors (VC and HC), and was due to the release of organic substances from the substrate (in particular from compost).

In vertical flow reactors, effluent concentrations decreased over time to values below 500 mgO_2/L, probably due to the continuous flushing produced by the irrigation modality. Effluent concentrations in H1 and H2 exceeded the influent ones as a result of different aspects:

- H1 and H2 were fed with much higher COD concentrations, compared to V1 and V2
- H units were characterised by lower effluent volumes (Fig. 5.7) which resulted in effluents in which the organics were more concentrated

Anyhow, the removal efficiencies of the systems V1-H1 and V2-H2, based on weekly loads (Eq. 5.9), reported in Fig. 5.11, were satisfactorily till the flowering point (up to 80% in V2-H2), then dropped and remained below 20%.

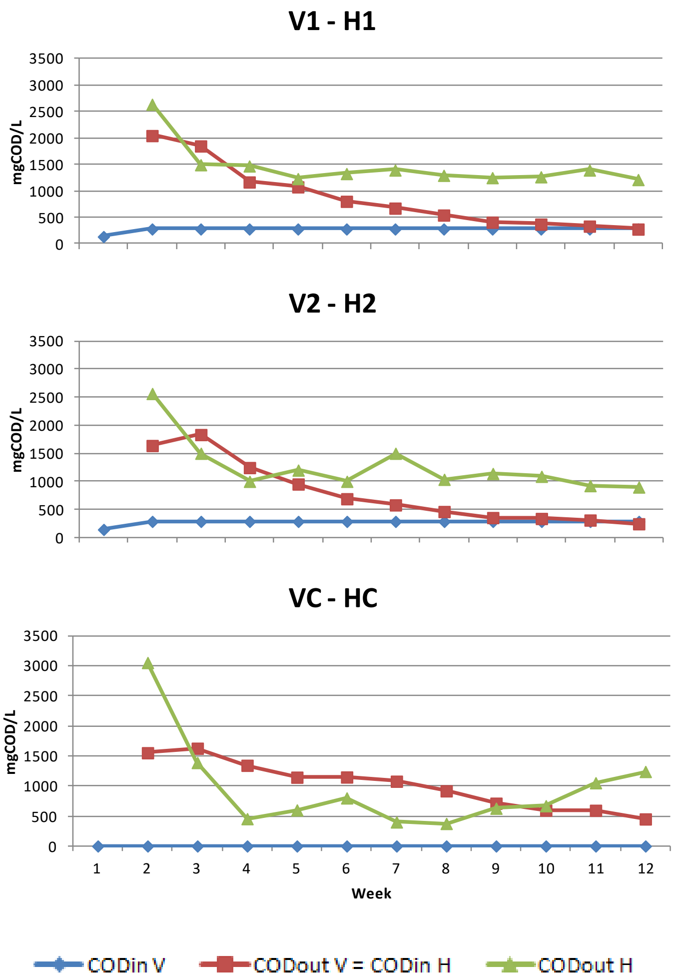


Fig. 5.10. Influent and effluent COD concentrations in the experimental reactors

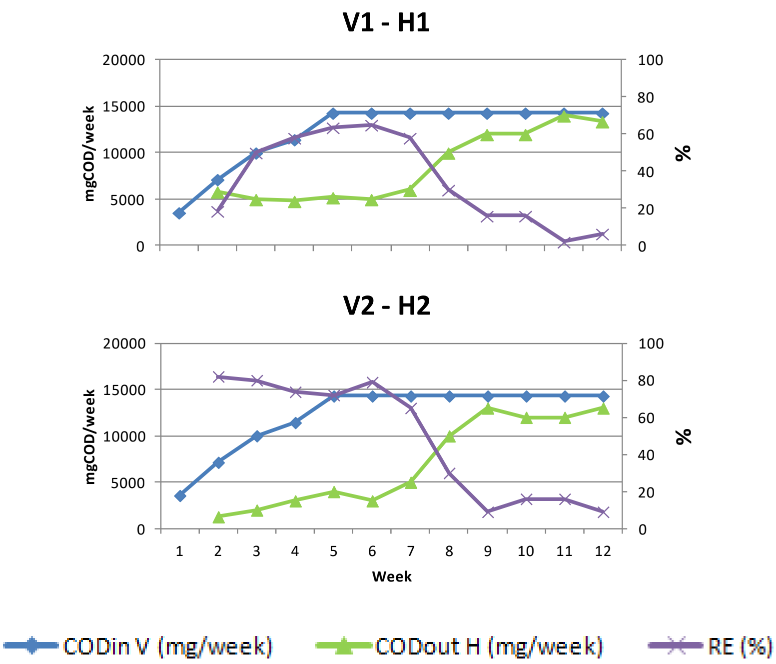


Fig. 5.11. Influent and effluent COD weekly loads and removal efficiency (RE) in the leachate irrigated systems V1-H1 and V2-H2

5.3.3.3. Phosphorous removal

Phosphorous effluent concentrations in systems V1-H1 and V2-H2 were characterised by several fluctuations but remained close to the input values throughout the entire duration of the experiment, with values ranging between 6 and 8 mgP/L, as shown in Fig. 5.12. Effluent concentrations in the controls were comparable (in some cases even higher) than leachate irrigated reactors: leachate irrigation procedure did not lead to an increase of P in the outlet. P removal efficiencies based on weekly loads (Eq. 5.9) were close to 100% till the flowering point (Fig. 5.13); after that they decreased but remained still above 60%. In phytotreatment systems, phosphorous is mainly sorbed by or precipitated in the substrate and the removal efficiency is mainly associated with chemico-physical properties of the material. The high phosphorus removal rates obtained in this experiment could be associated to the mineral composition of the substrate, as the presence of Fe was proved to enhance P removal (Vohla et al., 2011).

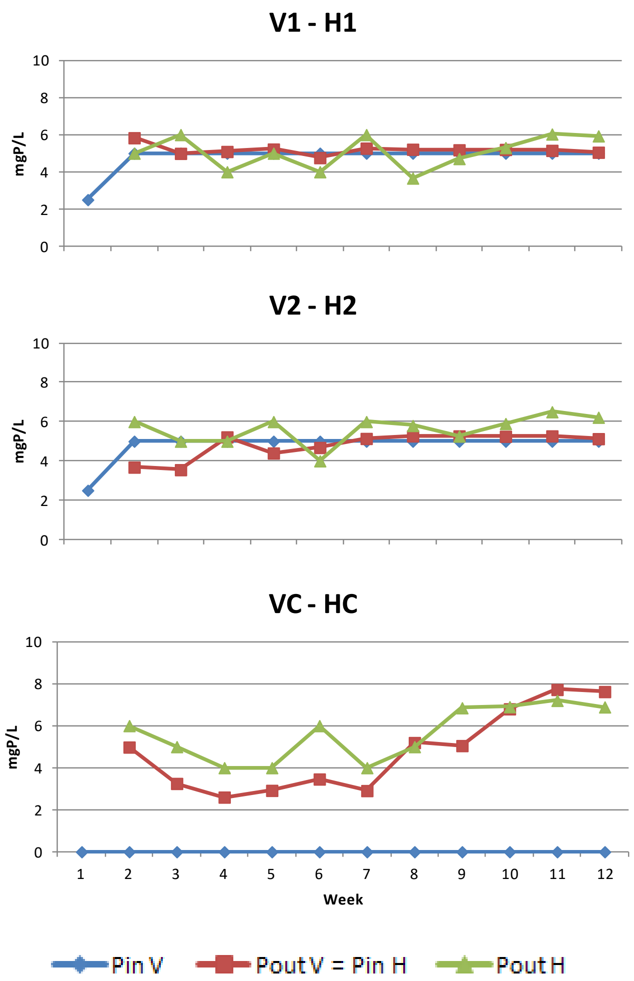


Fig. 5.12. Influent and effluent concentrations of P in the experimental reactors

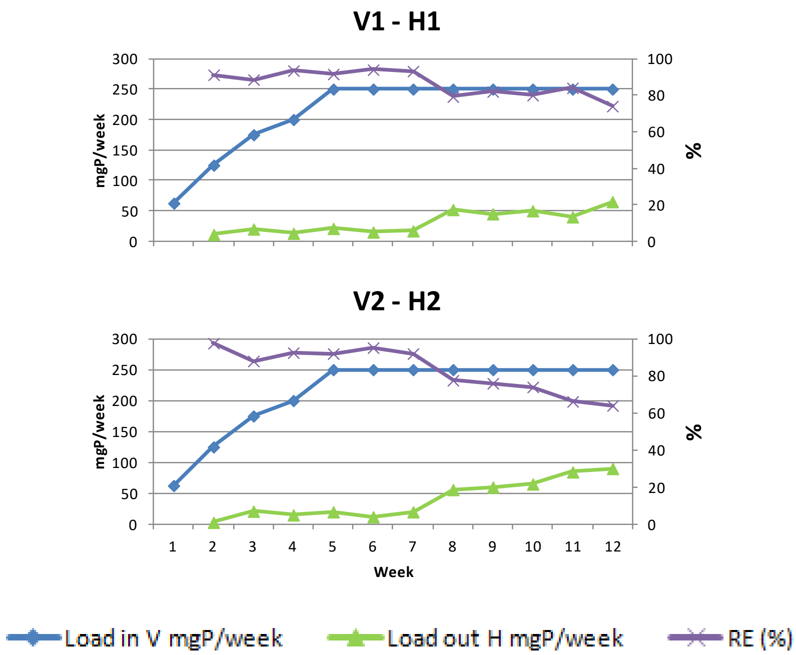


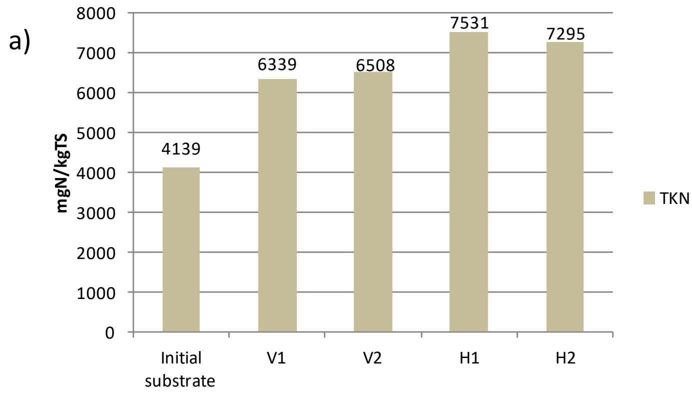
Fig. 5.13. Influent and effluent P weekly loads and removal efficiency (RE) in the leachate irrigated systems V1-H1 and V2-H2

5.3.4. Nitrogen mass balance

Final mass balance of N, the reference parameter, was performed on leachate irrigated systems, V1-H1 and V2-H2. Nitrogen distribution among the main system components (waters, substrates and plants) is reported in Table 5.7. At the beginning of the experiment, nitrogen was present mainly in organic form in the substrate (Table 5.3) and was added to the system by irrigation mostly as ammonium (Table 5.4). The amount of nitrogen found in the effluent of H units (N_{out}) was always below 10% of the nitrogen entering the system (N_{in}), confirming the excellent removal performances reported in Fig. 5.9. As already observed by Lavagnolo et al. (2016), the contribution of the plants (ΔN_P) was negligible: plants taken up only 0.6% of the influent nitrogen in both V1-H1 and V2-H2 systems. The substrate played a primary role in removing nitrogen (ΔN_S): approximately 50% of the N_{in} was accumulated by the substrate, which acted as a filter. Nitrogen losses have been calculated as difference between the observed total input and total output from the systems. The nitrogen losses were likely in gaseous forms, confirming the occurrence of nitrification and denitrification, as reported by Cheng and Chu (2011) and Tyrrel et al. (2001) and were 40.2% and 45.1% in V1-H1 and V2-H2, respectively; aligned with the results reported by Garbo et al. (2017) in a similar experiment. Fig. 5.14 confirms the role of the substrate as filter: final concentrations of TKN (Fig. 5.14a) and NO_3^- (Fig. 5.14b) always exceeded the initial values in all the reactors. Final concentrations of TKN were approximately 1.5 times higher than the initial value in vertical flow tanks (1.8 times in horizontal flow reactors). Accumulation of nitrate was relevant, especially in horizontal flow reactors, which were fed with the effluents of vertical flow reactors, rich in NO_3^- (Fig. 5.8).

Table 5.7. Nitrogen mass balance in the leachate irrigated systems V1-H1 and V2-H2 (gN)

	N entering the system - N_{in}	N in the effluent - N_{out}	N accumulated by the substrate - ΔN_S	N uptake by plants- ΔN_P	ΔN_L
V1-H1 system	1565.3	120.1	805.8 (314.1 by V1, 491.7 by H1)	9.8 (7.5 by V1, 2.3 by H1)	629.6
% on N_{in}	-	7.7	51.4	0.6	40.2
V2-H2 system	1557.4	111.3	734.0 (363.7 by V2, 370.3 by H2)	8.9 (6.7 by V2, 2.2 by H2)	703.2
% on N_{in}	-	7.1	47.1	0.6	45.1



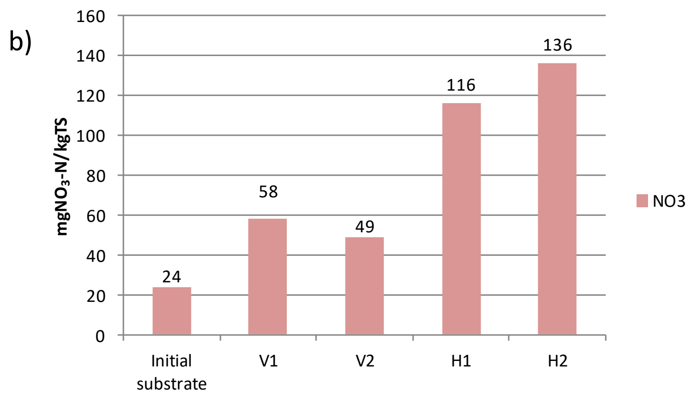


Fig. 5.14. Concentration of TKN (a) and NO_3^- (b) in the initial substrate soil and in each unit at the end of the experiment

5.3.5. Kinetics of nitrogen removal

The calibrated values of the reaction rate constants of nitrogen removal are summarized in Table 5.8. Values detected in reactors V1 and V2 were comparable. Nitrification (k_2) was the fastest process, while nitrogen removal due to other processes (e.g. settling, adsorption, etc), mainly related to the role of the substrate (k_5), seemed to be the slowest one. As expected, k_2 was much greater than k_3 ($k_2/k_3 \approx 100$): vertical flow reactors are optimized for the occurrence of nitrification rather than for the denitrification. The calibrated values are out of the ranges reported in literature for k_1, k_2 and k_3, but a direct comparison is not feasible as literature value are referred to free flow wetlands (Kadlec and Knight, 1996; Jorgensen and Bendoricchio, 2001; Kadlec and Wallace, 2009; Saeed and Sun, 2011). Anyhow, the ratios between the parameters are consistent with the data available, with $k_2 > k_1 \approx k_3$.

The values of k_4 confirm the minor role of plants in terms of nitrogen removal, as already pointed out in Table 5.7. The value of k_5, 10^{-33} d^{-1} in both V1 and V2, is contradictory: according to the modeling procedure the role of the substrate seems to be negligible; while the nitrogen mass balance revealed that the medium played a primary role. Probably the model was an oversimplification of the reality, and was not able to fully describe all the undergoing processes. Indeed, some assumptions (e.g. CSTR, 1° order reactions) might be too strong and could limit the capacity of the model to mimic the behavior of the investigated experimental units.

Table 5.8. Calibrated values of the reaction rates constants and comparison with literature data

	Process	V1	V2	Literature data*
k_1 (d^{-1})	Mineralization of N_{org} to ammonia	0.063	0.035	0.01 – 0.03
k_2 (d^{-1})	Nitrification	0.88	0.94	0.1 – 0.2
k_3 (d^{-1})	Denitrification	0.008	0.0075	0.01 – 0.1
k_4 (d^{-1})	Ammonia and nitrate uptake by plants	0.005	0.005	-
k_5 (d^{-1})	Organic nitrogen and ammonia removal due other processes occurring in the medium	10^{-33}	10^{-33}	-

* Kadlec and Knight, 1996; Jorgensen and Bendoricchio, 2001; Kadlec and Wallace, 2009; Saeed and Sun, 2011

5.3.6. Heavy metals accumulation in plants irrigated with landfill leachate

Heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) concentration in the biological tissues (leaves, stems, roots, seeds) of plants grown in leachate irrigated units was analyzed and compared with the values detected in the control plants, to investigate whether leachate irrigation produced an abnormal accumulation. Average results for Cu, Fe, Mn and Ni are reported in Fig. 5.15; concentrations of Cd, Cr, Pb and Zn were not included because they were always below the detection limits. Cu and Mn were always mainly concentrated in the leaves (concentrations up to 10 mg/kg_{TS} and 200 $mg/kg_{TS,}$ respectively$_)$; Fe was mostly concentrated in the roots (concentrations up to 200 mg/kg_{TS}); Ni was mostly present in stems and roots (values up to up to approximately 3 mg/kg_{TS}). Similar results were reported by Angelova et al. (2005) in a field test in which sunflowers were cultivated in a soil contaminated soil by heavy metals.

No significant differences were detected among leachate irrigated plants and controls, and among plants grown on units characterized by different flows: the applied phytotreatment process did not lead to an accumulation of the investigated heavy metals in the biological tissues.

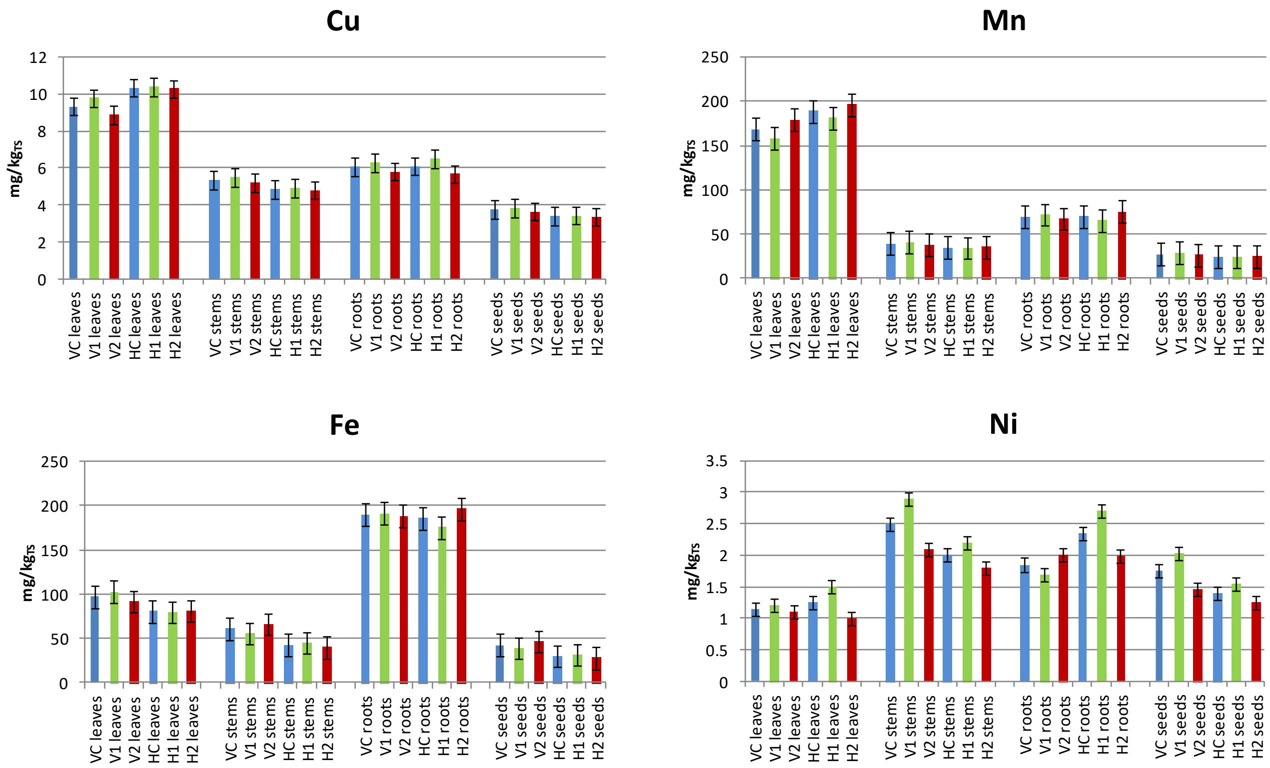


Fig. 5.15. Average concentrations of heavy metals in leachate irrigated sunflower plants and controls at the end of the experimental trial

5.3.7. Sunflower seeds characterization

At the end of the experimental trial, sunflowers seeds were analyzed to verify the effect of leachate irrigation on their composition, thus on biodiesel potential production. In particular, oil content and Free Fatty Acid (FFA) composition have been analyzed and compared with values detected in sunflowers grown in the controls and with values found in plants cultivated in traditional ways (results summarized in Table 5.9, the full characterization is reported in Annex 5.1).

Plants grown in controls VC and HC showed the highest oil content, but the values of the plants grown in the leachate irrigated reactors were found in the upper part of the range suggested by Karmakar et al. (2010): leachate irrigation and the presence of a waste-derived substrate did not inhibit the oil production.

Biodiesel is produced through a transesterefication process that does not alter the oil fatty acids composition, which affects many critical parameters of the biodiesel. The most important parameter is the Cetane number. High Cetane numbers have been associated to less highly polyunsaturated components *(Cx:2,3)* and more saturated fatty acids (*Cx:0; Cx:1)* in the oil (Karmakar et al., 2010; Ramos et al., 2009). Results have been plotted in Fig. 5.16: according to Ramos et al. (2009), vegetable oils falling in the green area satisfy the technical requirements of standard EN 14214:2008+A1:2009 for biodiesel production and utilization in engines. Oil extracted from seeds of sunflowers grown in conventional ways are far from the optimal area, while the oil extracted from the seeds obtained in this study (red area) is closer to the green part of the graph: the cultivation in a waste-derived substrate, even if combined with landfill leachate irrigation, registered a particular positive composition in view of renewable energy production. In the experiment reported by Lavagnolo et al. (2016), in which sunflowers, grown in sandy and clayey soils, were irrigated with old landfill leachate, FFA analysis revealed a higher amount of monounsaturated acids: this is probably due to the use of different cultivars, which may result in significant differences in the oil composition.

Table 5.9**.** Oil content in seeds and Free Fatty Acids content in the oil

	V1	V2	VC	H1	H2	HC	Literature data (Karmakar et al., 2010)
Oil content in seeds (%)	33.7	30.8	35.47	31.9	34.9	40.0	25-35
Free fatty acids composition (% on oil)							
Saturated (Cx:0)	11.29	11.68	10.819	11.66	11.47	11.07	9.00-17.00
Monounsaturated (Cx:1)	36.47	35.75	39.080	46.96	39.67	42.92	19.00-34.00
Polyunsaturated (Cx:2,3)	52.23	52.56	50.102	41.37	48.85	46.00	48.00-67.00

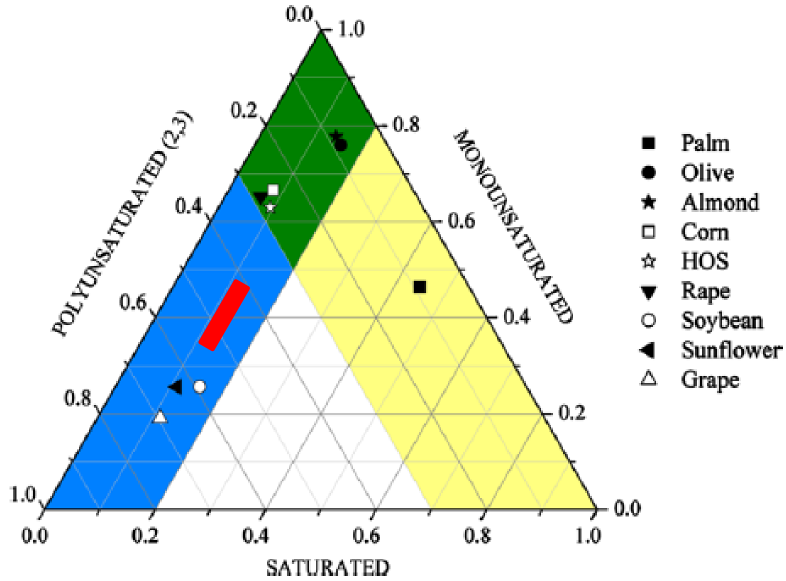


Fig. 5.16. Oil (or biodiesel) characterization by monounsaturated, polyunsaturated and saturated fatty acids. Green area: biodiesel that satisfied EN 14214:2008+A1:2009. Red area: oil from seeds obtained in this experiment (adapted from Barros et al., 2009).

5.4. CONCLUSION

Landfill leachate phytotreatment using sunflowers grown in a waste-derived substrate proved to be feasible under lab-scale conditions, as no inhibition of sunflowers development or anomalous accumulations of heavy metals were detected, while seeds oil characterization revealed a favorable composition in view of the biodiesel production. The use of a vertical flow unit, followed by an horizontal one, resulted to be effective in removing nitrogen due to nitrification and denitrification, as confirmed by the nitrogen mass balance. Removal efficiencies of nitrogen and phosphorous, provided by the landfill leachate, were excellent, while COD removal efficiency was affected by the leaching of organic substances from the medium (mainly from compost). The mathematical model, developed to study the kinetics nitrogen removal in vertical flow units, revealed that nitrification was the fastest process. However, the high level of uncertainty and the large number of assumptions limited the ability of the model to describe the full set of processes occurring in the experimental units. In this optic, the development of a more complex model could be a feasible option, even if the increase of the complexity may lead to a further increase of the level of uncertainty.

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Chapter 6: Leachate phytotreatment with P*ennisetum Purpureum* (elephant grass) in view of its cultivation on the top of closed landfills

Based on:

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Abstract

Despite the gradual decreasing of waste landfilling in most of industrialized countries, leachate treatment is still considered one of the main issues in landfills management. Economically and environmentally sustainable solutions are in growing demand and leachate phytotreatment with energy crops seems to be a suitable solution. This study evaluated the phytotreatment capacity of *Pennisetum Purpureum* (elephant grass). The plants were grown on reactors which were designed to assure a vertical sub-superficial irrigation flow. Reactors were irrigated with landfill leachate produced by a landfill in operation; the pollutants loads were increased over time. The removal efficiencies were in the range 95-99% for all the investigated contaminants (TKN, ammonia, COD). The vertical sub-surface flow led to a total nitrification, as expected, but in addition a partial denitrification was detected. A simple model was developed to study the kinetics of nitrogen removal, which confirmed the occurrence of a fast nitrification. *Pennisetum Purpureum* growth seemed to be stimulated by leachate irrigation; no significant accumulation of heavy metals was observed in the biological tissues: the cultivation of this crop on the top of closed landfills with the aim of renewable energy generation is a feasible option for in-situ sustainable leachate treatment.

Keywords: landfill leachate phytotreatment; *Pennisetum Purpureum*, kinetics of nitrogen removal

6.1. INTRODUCTION

Landfill leachate is generated by the degradation of landfilled waste and the excess rainwater percolating through the waste layers (Christensen and Kjeldsen, 1989). If not properly treated and disposed, leachate could be a source of soil and groundwater contamination.

Phytotreatment is defined as the exploitation of plants and associated microflora for environmental cleanup (Salt et al., 1998). Among others, the advantages include easy installation, low operating costs, acceptance by the population due to the low environmental impact (Farraji et al., 2016; Pivato et al., 2018), making it a widely investigated system for wastewater treatment and soil remediation. And more, phytotreatment appears to be one of the most promising in-situ landfill leachate treatment options (Lavagnolo et al., 2016).

In the last years, economic and political guidelines and the diversification of energy supply sources are leading to a significant increase of energy crops production (Mañas et al., 2014). The utilization of energy crops to treat landfill leachate matches the growing demand of economically and environmentally sustainable solutions (Garbo et al., 2017). Several studies on the phytoremediation of municipal wastewater with different energy crops have been successfully carried out (Dimitriou and Aronsson, 2010; Zalesny et al., 2009; Zupančič Justin and Zupančič, 2009). The leachate phytotreatment ability of some oily crops (sunflower, soybean, rapeseed) was investigated with excellent results by Garbo et al. (2017) and Lavagnolo et al. (2016).

Pennisetum Purpureum is an abundant and fast growing perennial plant, mainly used for grazing and fodder (Xu et al., 2015; Strezov et al., 2008). It is characterized by a high biomass production rate and can be harvested up to four times per year (Cittadino et al., 2016; Wilawan et al., 2014; Khan et al., 2007; Xie et al., 2011). The growth rates up to 40 tons of dry biomass per hectare per year makes it one of the most emergent crop for biogas or bioethanol production (Wilawan et al., 2014; Ra et al., 2012; Strezov et al., 2008; Stanley et al., 2017). Due to its strong resistance to pollutants, the use of elephant grass for phytoremediation purposes is a promising solution (Hei et al., 2016; Xu et al., 2015): the application of elephant grass in a site contaminated by heavy metals showed a higher efficiency in terms of bioethanol production (Ko et al., 2017).

Top covers of closed landfills are often considered to be devalued areas and currently are not exploited at all (Pivato et al., 2018). The growth of energy crops on such available areas is an interesting option which may contribute to reduce the competition of land for food and energy production.

This paper aims to describe the phytotreatment performances of *Pennisetum Purpureum* irrigated with diluted landfill leachate. The goal of this research was to investigate the elephant grass biomass development, the wastewater volume reduction due to evapo-transpiration, the removal efficiencies of the main contaminants (COD and nitrogen), the potential accumulation of heavy metals in the substrate and in the plant tissues and to study the kinetics of nitrogen removal.

6.2. MATERIALS AND METHODS

6.2.1. Research program

The research was carried out at the Laboratory of Environmental Engineering of the University of Padua (Italy). Five columnar reactors were placed in a controlled greenhouse. All the experimental units were characterized by vertical sub-superficial irrigation. *Pennisetum Purpureum* plants were grown in three units called P1, P2, and PC (three plants in each unit). P1 and P2 were irrigated with diluted landfill leachate while PC was irrigated with tap water and synthetic nitrogen. Two additional reactors without plants were irrigated with landfill leachate (CL) and tap water (CW). CL was used as control to investigate the role of the substrate in the leachate treatment, CW to detect potential leaching of substances from the substrate itself (Table 6.1)

Table 6.1. Set up of experimental units

Experimental unit	Irrigation	Column description	Plant species
P1	Leachate	Experimental column	*Pennisetum P.*
P2	Leachate	Experimental column	*Pennisetum P.*
PC	Tap water + synthetic nitrogen	Plant control column	*Pennisetum P.*
CL	Leachate	Soil control column	-
CW	Tap water	Soil control column	-

All the plants were irrigated with tap water during the initial 15 days. Then, the reactors P1, P2 and CL were fed with increasing diluted leachate dosages up to 300 mgN/L (nitrogen was the reference parameter) (Table 6.2). This value was set in order to avoid phyto-toxicity phenomena, which may occur on plants exposed to excess nitrogen (Garbo et al., 2017).

The control column with plants (PC) was irrigated with tap water with the addition of synthetic nitrogen, provided as NH_4Cl: the nitrogen supplied was equal to the amount provided by the leachate in the irrigation of reactors P1 and P2.

The feeding volume of 5 L/week was maintained constant for the duration of the experiment in each unit.

Table 6.2. Leachate dosages and contaminants concentration in the feeding of P1, P2, and CL

Phase	Leachate dose (%)	TKN conc. (mgN/L)*	TKN load (mgN/week)	COD conc. (mgO_2/L)	COD load (mgO_2/week)
Acclimation	0	-	-	-	-
Week 1	10	50	250	49	245
Week 2	20	100	500	98	490
Week 3	30	150	750	147	735
Week 4	40	200	1000	196	980
Week 5	50	250	1250	245	1225
Weeks 6-8	60	300	1500	294	1470

* the same concentrations of input nitrogen (as NH_4Cl) were applied to PC control unit

Each column was drained once per week and the liquid samples were stored at -20°C and subsequently analyzed for the following parameters: Total Kjeldhal Nitrogen (TKN), ammonia, nitrate, nitrite, and COD. Plants of *Pennisetum Purpureum* were harvested at their maximum growth (about 10 weeks after planting). Plant biomass, after roots separation from stems, was dried at 60 °C in the oven and weighed. Dry mass, total nitrogen and heavy metals contents in the biological tissues were determined. After plant harvesting, substrate soil was sampled at three different depths in each reactor (Top: 0-25 cm, Intermediate: 25-50 cm; Bottom: 50-75 cm). Total nitrogen and heavy metals contents were determined in the substrate samples.

6.2.2. Equipment

Columnar PVC pipes, with a diameter of 25 cm and a height of 100 cm, were used. The reactors, arranged in vertical position to assure a vertical flow, were sealed at the bottom. A drainage layer, 15 cm thick, made up of gravels with a diameter of 20-30 mm, was placed at the bottom. The columns were then filled with 75 cm of growing substrate to simulate the layout of a final top cover of a landfill. A fine plastic net was installed horizontally, between the two layers, to avoid the occurrence of intermixing phenomena. A flexible tube was installed at the bottom of the columns to allow the collection of the effluents. The column's scheme is reported in Fig. 6.1. The experimental units were placed in a controlled climatic chamber in which the temperature was maintained in the range 16-36 °C (26 °C on average). A 10-hour photoperiod with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity was imposed.

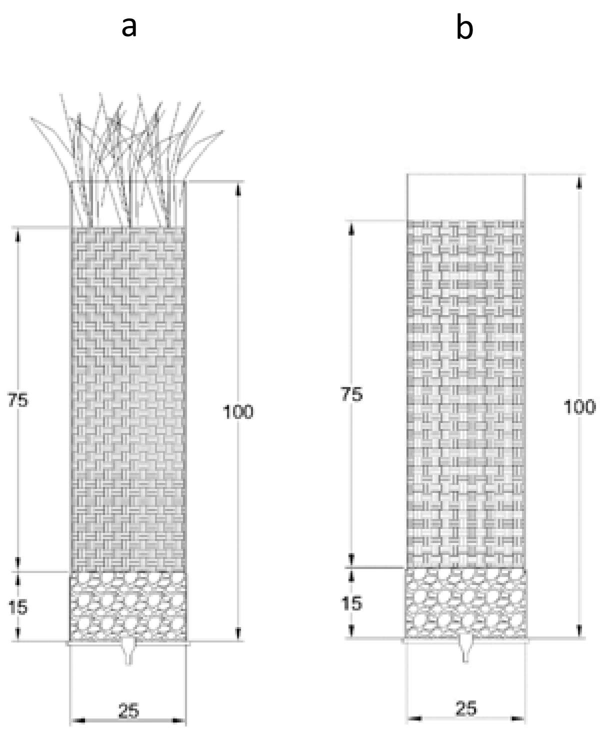

Fig. 6.1. Reactors used for the experiment (a: reactors in which *Pennisetum Purpureum* was grown - P1, P2, and PC; b: reactors without plants - CL and CW). Measures expressed in cm.

6.2.3. Landfill leachate

The leachate used in this experiment was sampled from a currently operated MSW landfill, located in the North of Italy, in which stabilized residual municipal solid waste is disposed of. Its composition is reported in Table 6.3 and the results are consistent with the kind of waste landfilled.

Table 6.3. Landfill leachate chemical characterization

Parameter	Unit	Values
pH		7.8±0.3
TKN	(mgN/L)	500±25
NH_4^+	(mgN/L)	453±22
P_{TOT}	(mgP/L)	2.3±0.2
TS	(mg/L)	2451±113
VS	(mg/L)	495±45
COD	(mgO_2/L)	490±32
BOD	(mgO_2/L)	<50
Cl^-	(mg/L)	685±36
NO_3^-	(mgN/L)	-
$NO2^-$	(mgN/L)	-
SO_4^{2-}	($mgSO_4$/L)	-
Ca	(mg/L)	142±11
K	(mg/L)	225±21
Mg	(mg/L)	88±10
Na	(mg/L)	353±18
Cd	(µg/L)	< 10
Cr	(µg/L)	64±3
Cu	(µg/L)	91±5
Fe	(µg/L)	5733±56
Mn	(µg/L)	313±20
Ni	(µg/L)	168±8
Pb	(µg/L)	46±3
Zn	(µg/L)	269±6

6.2.4. Substrate soil properties

A mixture made up of 50% of quartz sand and 50% of locally available soil (on volume basis) was used as substrate. Studies on artificially constructed wetlands recommend mixtures of natural soil and sand to provide the best conditions in terms of hydraulic conductivity and contaminant removal. Sand guarantees enough macro-porosity for air recirculation and roots development, avoiding water stagnation, while the agricultural soil is a substrate rich in micro-nutrients, fundamental for the plants growth (Garbo et al., 2017; Jones et al., 2006).

Substrate texture was determined with the Bouyoucos method (Bouyoucos, 1962) and, according to the soil taxonomy proposed by the USDA (USDA-NRCS, 1999), the substrate soil used was classified as sandy loam (14% clay, 10% silt, and 76% sand). Its chemical characterization is reported in Table 6.4.

Table 6.4. Chemical characterization of the substrate used in the experiment (TS=Total Solids).

Parameter	Unit	Value
Volatile Solids	mg/kg_{TS}	2.2
Total Carbon	mgC/kg_{TS}	56900
Total Nitrogen	mgN/kg_{TS}	150
Cd	mg/kg_{TS}	0.38
Cr	mg/kg_{TS}	20
Pb	mg/kg_{TS}	19
Cu	mg/kg_{TS}	27
Ni	mg/kg_{TS}	18
Zn	mg/kg_{TS}	65
Fe	mg/kg_{TS}	4924
Mn	mg/kg_{TS}	308

6.2.5. Hydraulic retention time

The Hydraulic Retention Time (HRT) is defined as the average time that a fluid remains inside a reactor:

$$HRT = \frac{V_{reactor}}{Q} \tag{6.1}$$

where:

$V_{reactor}$ = volume of the reactor

Q = flow rate

This formula is based on the hypothesis that the volume remains constant during the process, that is not the case under investigation because of the presence of a strong evapotranspiration factor which reduces the volume of the liquid phase. This is typical of constructed wetlands, as already reported by Bialowiec et al. (2014). Anyhow, in order to provide an average value, an indicative HRT* was estimated with the following empirical formula:

$$HRT^{*} = \frac{\sum(V_i \cdot t_i)}{\sum V_i} \tag{6.2}$$

where:
V = volume of water provided in the *i* day
t = number of days until the following total drainage
$_i$ = day of the week

This formula takes into account the residence time of every liter poured in the column until the weekly total drainage, corresponding to the day zero for the HRT assessment.
Therefore, being all the reactors fed with the same amount of water and drained at the same time, the retention time was the same in each reactor and resulted to be 3.4 d.

6.2.6. Analytical methods

Analysis on liquid samples were performed according to the IRSA-CNR methods for water quality analysis (CNR-IRSA, 29/2003). Analysis on plants and substrate samples were carried out according to the IRSA-CNR guidelines for solid samples (CNR-IRSA, 64/1986).

6.2.7. Nitrogen mass balance

At the end of the experimental trial, the nitrogen mass balance of vegetated experimental units was performed to assess the role of the different systems components (plants, substrate) in removing nitrogen. It was based on the following equation:

$$N_{in} = N_{out} + \Delta N_P + \Delta N_S + \Delta N_L \tag{6.3}$$

where:

N_{in} = total amount of nitrogen entering each unit (mgN)

N_{out} = total amount of nitrogen in the outflow (mgN)

ΔN_P = amount of nitrogen accumulated in the plant tissue (mgN)

ΔN_S = nitrogen accumulated in the substrate (mgN)

ΔN_L = nitrogen gaseous losses due to nitrification and denitrification phenomena (mgN)

6.2.8. Kinetics of nitrogen removal

The Matlab software was used to calibrate the kinetic parameters of nitrogen removal in leachate irrigated units (P1 and P2). According to Kadlec and Wallace, (2009), treatment wetland hydraulics is best represented by the Tank-in-Series (TIS) model,which is an intermediate case between the ideal plug-flow and the continuous flow stirred tank reactor (CSTR). The representation of one wetland by a series of CSTR units is just for mathematical convenience and the exact number of *N* tanks to be used in the representation should be estimated by tracer tests. However, according to Boog et al. (2014), for vertical flow reactors the number of TIS is approximately 1. Therefore, in this study, the units were assumed to be operated as single CSTR to keep the complexity of the system at minimum. The general mass balance equation of a CSTR is:

$$V\frac{dC}{dt} = Q_{in}\,C_{in} - Q_{out}\,C \pm rV \qquad (6.4)$$

where:

V: liquid volume (L)

C: concentration inside the reactor and in the effluent (mg/L)

C_{in}: concentration in the influent (mg/L)

Q_{in}: influent flow rate (L/d)

Q_{out}: effluent flow rate (L/d)

r: reaction rate (mg L^{-1} d^{-1})

The nitrogen removal processes were described using a first order kinetic (Saeed and Sun, 2011; Jorgensen and Bendoricchio, 2001):

$$r = \mathrm{k}\,C \qquad (6.5)$$

where:

C = concentration in the reactor (mg/L)

k = reaction rate constant (d^{-1})

The reaction rate constant (k) varies with the temperature according to the Arrhenius equation:

$$k_T = k_{20}\,\Theta^{(T-20)} \tag{6.6}$$

where:

k_T: reaction rate constant (d^{-1}) at the operating temperature T (°C);

k_{20}: reaction rate constant at 20° C (d^{-1});

ϴ: dimensionless parameter (>1)

6.2.8.1. Mathematical model conceptualization

A mathematical model was developed to describe the nitrogen transformations occurring in the experimental reactors P1 and P2 and to calibrate the values of the reaction rate constants. The modelled processes were:

- Mineralization of organic nitrogen to ammonia
- Oxidation of ammonia to nitrate (nitrification)
- Reduction of nitrate to atmospheric nitrogen (denitrification)
- Plants uptake of ammonia and nitrate (assuming that the uptake of ammonia and nitrate are similar and could be jointly modelled)
- Settling/adsorption/biological degradation of organic nitrogen and ammonia in the medium

The forcing functions which were modeled were:

- Influent volumes
- Effluent volumes
- Influent concentrations
- Effluent concentrations
- Temperature of the liquid volumes

The modelled state variables were:

- Organic nitrogen concentration (Norg)
- Ammonia concentration (NH_4^+)
- Nitrate concentration (NO_3^-)

6.2.8.2. Mathematical formulation

Eq. 2 and Eq. 3 were combined to link the inlet and outlet concentrations of the state variables across the V units (Saeed and Sun, 2011). The resulting mass balances of the state variables were expresses as Ordinary Differential Equations (ODE):

$$\frac{dNorg}{dt} = \frac{Q_{in}\,Norg_{in}}{V} - \frac{Q_{out}\,Norg_{out}}{V} - k_1\,Norg_{out} - k_5\,Norg_{out} \tag{6.7}$$

$$\frac{dNH4}{dt} = \frac{Q_{in}NH4_{in}}{V} - \frac{Q_{out}\,NH4_{out}}{V} - k_2\,NH4_{out} - k_4\,NH4_{out} + k_1\,Norg_{out} - k_5\,NH4_{out} \tag{6.8}$$

$$\frac{dNO3}{dt} = -\frac{Q_{out}\,NO3_{out}}{V} - k_3\,NO3_{out} - k_4\,NO3_{out} + k_2\,NH4_{out} \tag{6.9}$$

The reaction rate constants (k_1, k_2, k_3, k_4, k_5) refer to the transformations reported in Table 6.5.

Table 6.5. Indexes and description of the reaction rate constants calibrated in the model

Process	Reaction rate constants
Mineralization of organic nitrogen	k_1
Nitrification	k_2
Denitrification	k_3
Ammonia and nitrate uptake by plants	k_4
Organic nitrogen and ammonia removal due other processes (settling, adsorption, etc)	k_5

6.2.9. Statistical analysis

The concentrations of heavy metals in the biological tissues of plants grown on the different units (P1, P2 and PC) were compared by one-way analysis of variance. The F-test was used to assess whether there were significant differences amongst the means at the 95.0% confidence level ($p<0.05$); pairwise comparisons were assessed with the Tukey's honestly significant difference (HSD) procedure. Statistical analysis was performed using Statgraphics software.

6.3. RESULTS AND DISCUSSION

6.3.1. *Pennisetum Purpureum* growth

Elephant grass grew uniformly in all the units, as shown in Fig. 6.2. Plants grown in reactors P1 and P2 reached maximum heights ranging between 120 and 140 cm. Comparable heights were reached by the plants in the control unit PC, suggesting that leachate irrigation did not inhibit the growth in P1 and P2. The maximum height was reached during week 7.

Plants dry weights confirmed that leachate did not limit plant growth, on the contrary it seemed to stimulate the biomass growth: average weight of plants cultivated in leachate irrigated reactors (12.87 gTS/plant in P1 and 11.41 gTS/plant in P2) was even higher than the average weight in the control column PC (9.95 gTS/plant) (Fig. 6.3).

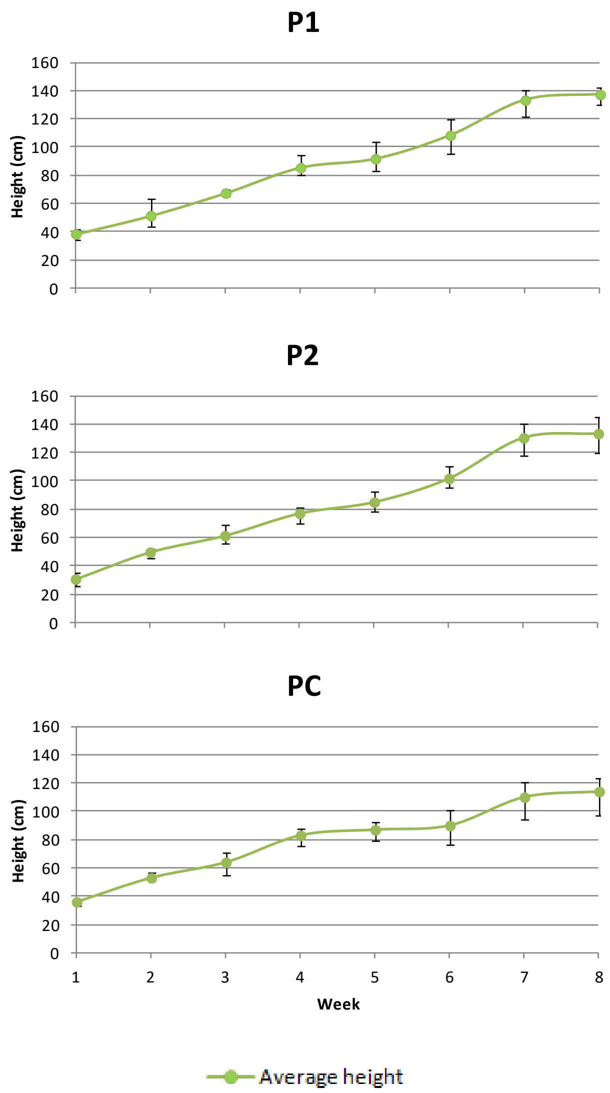

Fig. 6.2. Above-ground height of *Purpureum Purpureum* in reactors P1, P2, and PC over the whole experimental period.

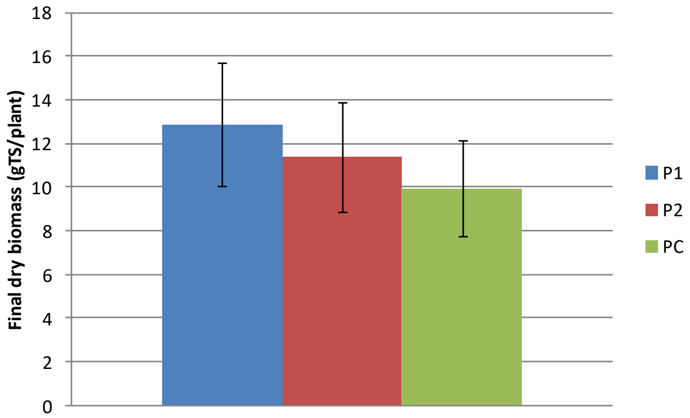

Fig. 6.3. Final dry weights of *Pennisetum Purpureum* in units P1, P2, and PC

6.3.2. Evapotranspiration rates

Evapo-transpiration (ET) is a desirable effect in phytotreatment systems to reduce the outlet volumes, especially in areas in which the discharge limits can make treatments extremely expensive (Headley et al., 2012).

Analysis on the weekly irrigation rate (V_{IN}) and total drainage (V_{OUT}) allowed the estimation of the evapotranspiration rates, calculated as:

$$ET = \frac{V_{IN} - V_{OUT}}{V_{IN}} \tag{6.10}$$

where:

V_{IN} = inlet volume (L/week)

V_{OUT} = outlet volume (L/week)

The evapotranspiration rates in vegetated reactors tended to increase over time (Table 6.6), in parallel with the plant growth. ET reached the maximum values during week 7 and then decreased, when plants growth stopped. As reported by Lavagnolo et al. (2016) and Garbo et al. (2017), evapotranspiration rates in lab-scale leachate phytotreatment tests with oily crops reached the maximum values just before blooming; thereafter ET decreased as a result of reduced plant requirements. In reactors P1 and P2, average ET was 30% of the inlet volume, the same of the control column PC. As expected, evaporation occurred also in control columns without plants but its contribution was limited: 23% of the influent in CL; 18% in CW. The direct comparison between CL and CW showed that substrate soil irrigated with leachate presented an increased evaporation efficiency with respect to substrates irrigated only with tap water, as already observed by Białoweic et al. (2007).

Table 6.6. Influent and effluent volumes, and evapotranspiration rates (ET %) in the experimental units over the whole experimental period

Week	V_{IN} (L/week)	P1 V_{OUT} (L/week)	P1 ET (%)	P2 V_{OUT} (L/week)	P2 ET (%)	PC V_{OUT} (L/week)	PC ET (%)	CW V_{OUT} (L/week)	CW ET (%)	CL V_{OUT} (L/week)	CL ET (%)
1	5	4.0	20	4.6	8	3.6	28	3.5	30	2.9	42
2	5	3.6	28	3.5	30	3.7	26	4.1	18	3.7	26
3	5	3.7	26	3.9	22	3.7	26	3.9	22	3.4	32
4	5	3.5	30	3.6	28	3.6	28	4.2	16	4.6	8
5	5	3.0	40	3.0	40	3.1	38	4.1	18	3.7	26
6	5	2.9	42	3.0	40	3.2	36	4.4	12	4.1	18
7	5	3.2	36	2.8	44	3.0	40	3.7	26	3.8	24
8	5	4.0	20	3.4	32	3.7	26	4.7	6	4.4	12
Average	5	3.5	30	3.5	30	3.5	30	4.1	18	3.8	23

6.3.3. Contaminant removal

Due to a strong evapotranspiration effect, evaluation of contaminant removal should take into account the reduction of the effluent volumes therefore Removal Efficiency (RE) should be based on weekly loads:

$$RE = \frac{V_{IN} \cdot C_{IN} - V_{OUT} \cdot C_{OUT}}{V_{IN} \cdot C_{IN}} \tag{6.11}$$

where

V_{IN} = influent volume (L/week)

V_{OUT} = effluent volume (L/week)

C_{IN} = influent concentration of the considered contaminant (mg/L)

C_{OUT} = effluent concentration of the considered contaminant (mg/L)

Input and output weekly loads, and average removal efficiencies of the investigated contaminants in leachate irrigated units are reported in Table 6.7. NO_2^- are not included because they were always below the detection limits. Columns were subjected to increasing contaminants loads over time, with maximum values reached in week 6 and kept constant till the end of the experimental trial (Table 6.2). RE of TKN and ammonia were always in the range 95-99%, even in the control column without plants (CL). Nitrate was not present in the influent water, but was detected in the effluent of each column, suggesting the occurrence of nitrification process. Its formation was significant: the output loads were much higher than output loads of TKN and ammonia in each leachate irrigated reactor. COD removal was always above 92%, with the best performances observed in the experimental units with the essences (P1 and P2). Summarizing, P1 and P2 showed excellent performances with no differences among the replicas but RE were excellent also in the control column CL, highlighting the primary role of the substrate in contributing in the removal of the investigated contaminants.

Table 6.7. Input and output weekly loads, and average Removal Efficiency (RE) in leachate irrigated units

Parameter	Week	Load in (mg/week)	P1 Load out (mg/week)	P1 RE (%)	P2 Load out (mg/week)	P2 RE (%)	CL Load out (mgN/week)	CL RE (%)
TKN	1	250	20	92	15	94	34	86
	2	500	22	95	14	97	55	89
	3	750	23	97	16	98	50	93
	4	1000	22	98	15	98	68	93
	5	1250	18	98	12	99	55	95
	6	1500	18	99	12	99	61	96
	7	1500	20	99	12	99	56	96
	8	1500	20	99	11	99	65	96
	Average	*1031*	*21*	*98*	*14*	*98*	*55*	*95*
NH_4^+ (as N)	1	226	8	96	5	98	13	94
	2	453	9	98	5	99	22	95
	3	679	9	99	5	99	20	97
	4	906	9	99	5	99	27	97
	5	1132	7	99	4	99	22	98
	6	1359	7	99	4	99	24	98
	7	1359	8	99	4	99	22	98
	8	1359	8	99	4	99	26	98
	Average	*934*	*8*	*99*	*5*	*99*	*22*	*98*
NO_3^- (as N)	1	-	168	-	186	-	55	-
	2	-	162	-	145	-	133	-
	3	-	145	-	135	-	149	-
	4	-	179	-	179	-	266	-
	5	-	174	-	192	-	274	-
	6	-	237	-	248	-	417	-
	7	-	339	-	280	-	578	-
	8	-	464	-	380	-	816	-
	Average	-	*234*	-	*218*	-	*336*	-
COD	1	245	35	86	51	79	110	55
	2	490	35	93	45	91	185	62
	3	735	29	96	31	96	78	89
	4	980	49	95	18	98	82	92
	5	1225	27	98	21	98	48	96
	6	1470	39	98	24	98	32	98
	7	1470	32	98	33	98	34	98
	8	1470	22	97	24	97	26	98
	Average	*937*	*32*	*96*	*32*	*96*	*75*	*92*

6.3.4. Nitrogen effluent concentrations

Nitrogen in the irrigation reached values up to 300 mgN/L, well above the limit set by the Italian legislation (D. Lgs. 152/2006) for the discharge in water bodies: 15 mgN/L for TKN, 20 mgN/L for NO_3^-. Therefore analysis of effluent concentrations was useful to evaluate whether the phytotreatment process allowed the compliance with legal requirements.

The analysis of TKN and NO_3 concentrations in the effluents (Fig. 6.4) showed an almost total conversion of TKN into NO_3, as TKN concentrations in the effluent were negligible in all the experimental units. This was expected, as vertical flow reactors are engineered to promote the nitrification process. The replicas with leachate irrigated plants behaved in a very similar way: trends in P1 were similar to P2. Nitrate concentrations never exceeded 150 mgNO_3-N/L in both P1 and P2 reactors.

The gap between the influent TKN and the effluent NO_3^- concentrations might be due to a higher nitrate accumulation in the plants or in the soil, but the nitrogen mass balance (Table 6.8) showed that it could not be just related to the role of plants and substrate. Therefore, the occurrence of denitrification could not be excluded. Although vertical flow enhanced the presence of aerobic conditions inside the columns, proved by the occurrence of an almost total nitrification, probably some parts of clay-rich soil, along the columns, limited the air intrusion, thus promoting the denitrification. In similar experiments with vertical flow reactors fed with synthetic wastewater, Fan et al. (2013a, c) observed the occurrence of both nitrification and denitrification, but only if forced aeration was applied.

On the contrary, nitrate effluent concentrations in control column with plants (PC) were higher than P1 and P2; in some cases even close to the influent concentration, suggesting the occurrence of a complete nitrification but the lack of denitrification.

The lack of denitrification in control columns PC is confirmed looking at the TKN and nitrate effluent concentrations in CL: in the latter, denitrification was observed as the NO_3^- concentration did not exceed 200 mgNO_3-N/L. Denitrification is performed by heterotrophic bacteria naturally present in the soil in presence of bio-available organic substances (Pajares and Bohannan, 2016). Therefore, denitrification occurred only in the experimental units irrigated with leachate, which provided the organic matter needed for the process. Excellent removal performances were achieved even if the COD/N ratio in the influent was 0.98: Fan et al. (2013b) reported that for similar efficiencies the COD/N should be higher than 10, and that forced aeration should be applied. Nitrogen leaching from the substrate was negligible, as shown by the unit CW.

Considering the leachate irrigated reactors, only TKN concentrations in the outflow always met the Italian discharge limit of 15 mg/L (D. Lgs. 152/2006) during the experiment. On the contrary, nitrate concentrations in the outflow did not accomplish the Italian regulation, which sets a discharge limit of 20 mgN/L. In order to improve the nitrogen removal, an horizontal sub-surface flow could be provided after the vertical one (Garbo et al., 2017; Lavagnolo et al., 2016; Cheng and Chu, 2011).

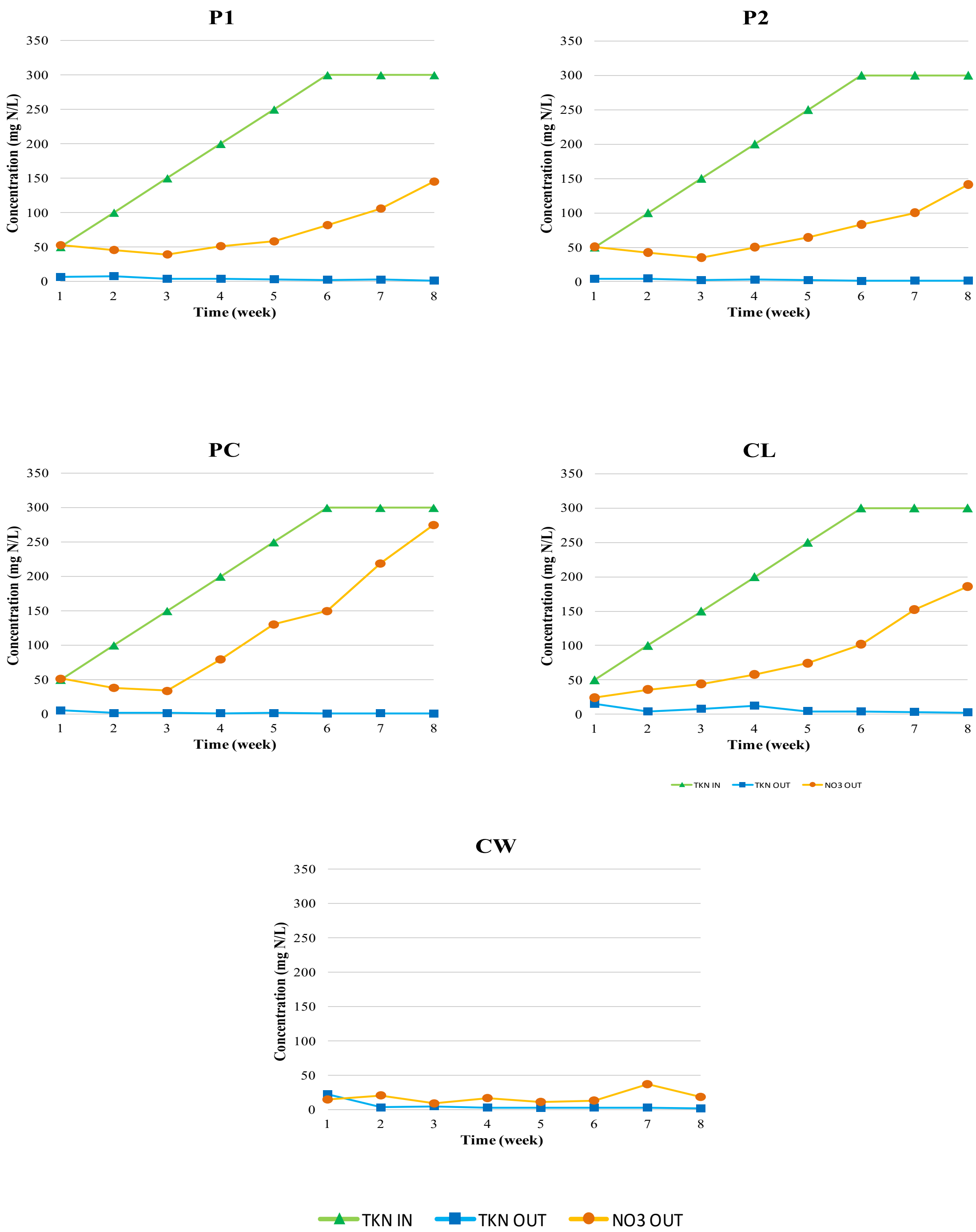

Fig. 6.4. TKN influent and effluent concentrations, and nitrate effluent concentrations in the experimental units

6.3.5. COD effluent concentrations

As for nitrogen, COD in the influent was higher than the legal limit value of 160 mgO_2/L for the discharge in water bodies (D. Lgs. 152/2006). Outlet COD concentrations were always below 50 mgO_2/L (Fig. 6.5), even if the influent concentration reached the maximum value of 300 mgO_2/L: all the experimental units were able to remove most of the influent organic matter and comply with the Italian discharge limit. Trends of effluent concentrations in leachate irrigated reactors were comparable to the trend detected in CW, in which there was no addition of external organic matter: wastewater did not produce any additional increase in the effluent concentration. Likely, a relevant fraction of the influent COD was consumed by heterotrophic microorganism living in the substrate (e.g.: to perform the denitrification process) or adsorbed by the substrate itself. The lowest output concentrations were detected in reactors with elephant grass (P1 and P2): plants are known to favor the growth of microorganisms, which play a dominant role in the degradation of organics, by conveying the oxygen to the rhizosphere (Akinbile et al., 2012). Anyhow, it was a further confirmation that phytotreatment is a combination of different phenomena, rather than the isolated action of plants (Jones et al., 2006).

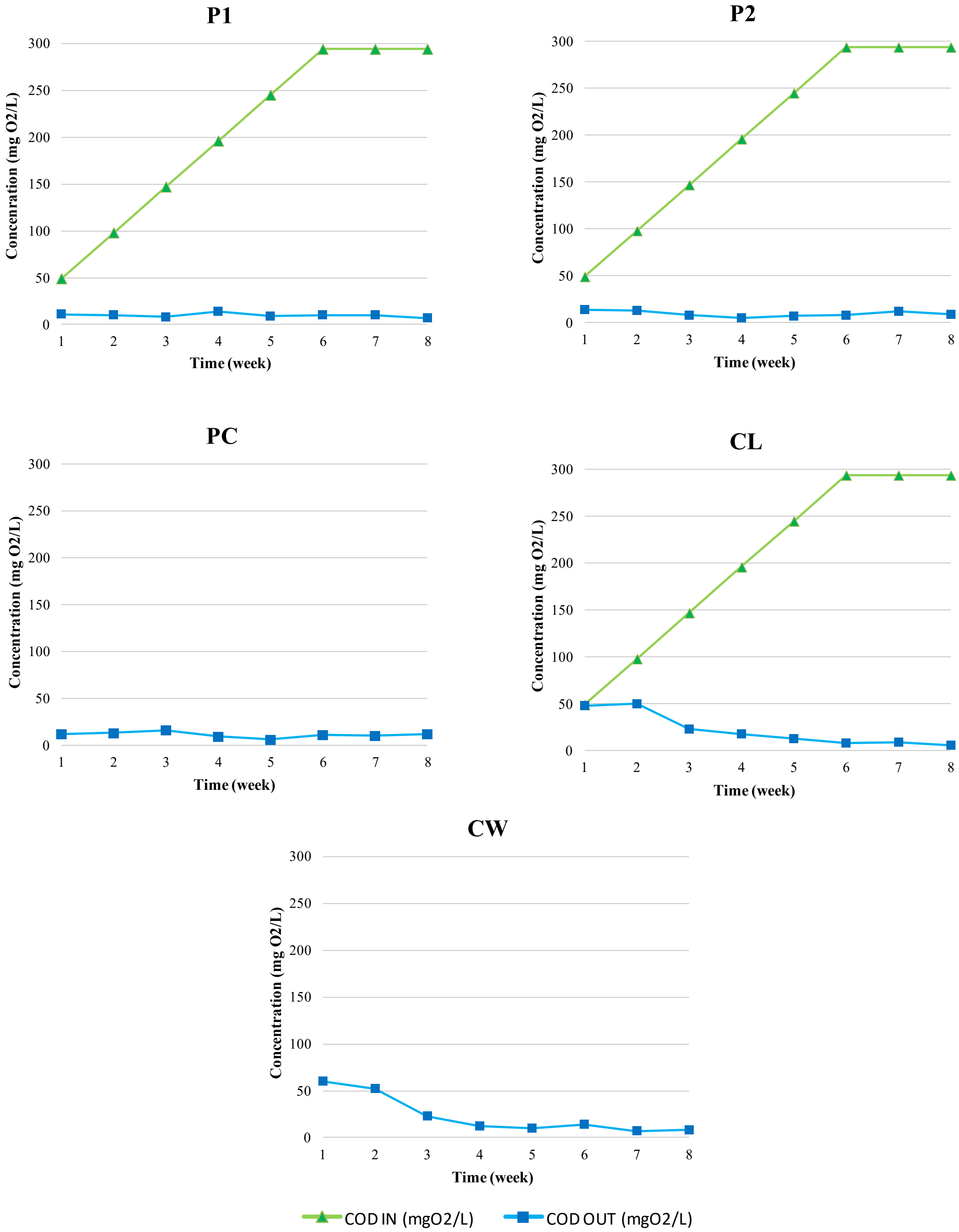

Fig. 6.5. COD influent and effluent concentrations in the experimental units

6.3.6. Nitrogen mass balance

At the end of the experimental period, fate of nitrogen in leachate irrigated columns and control column with *Pennisetum Purpureum* was investigated. Nitrogen mass balance for the system components (effluent, substrate soil and plants), calculated according to equation (6.3), is reported in Table 6.8. Inlet nitrogen was added to the system by irrigation mainly as ammonium form (Table 6.4). Part of the influent was found in the effluent (range: 23-46%), a fraction was adsorbed by the substrate soil (range: 12-23%), and another small fraction (range: 8-15%) was taken up by the plants. Sum of nitrogen accumulation in substrate and plants and effluent was always lower compared to the influent nitrogen, revealing the occurrence of nitrogen losses, likely due to the nitrification and denitrification processes which were already discussed. During denitrification, nitrogen was converted into gaseous form as N_2 and released in the atmosphere, as already observed by Garbo et al. (2017), Lavagnolo et al. (2016), Cheng and Chu (2011).

P1 and P2 shown similar values in the final mass balance, with approximately 25% of the influent nitrogen found in the effluent. The combined role of *Pennisetum Purpureum* uptake and substrate accumulation accounted for another 25%; the main fraction of the influent nitrogen (approximately 50%) was removed by nitrification and denitrification. Control column PC, although subjected to the same nitrogen load in the influent, was characterized by the lowest nitrogen loss (24%) and the highest release of N in the effluent (42%): the absence of external COD addition due to leachate irrigation likely limited the development of the bacteria populations involved in the denitrification process. Nitrogen loss in CL was comparable to P1 and P2, while the fraction found in the effluent (36 %) exceeded the values found in P1 and P2 (25 and 23%, respectively): this difference could be related to the presence of the plants, which contributed in the removal of nitrogen, otherwise released with the outflow.

Table 6.8. Final nitrogen mass balances in the leachate irrigated reactors with *Pennisetum Purpureum*

Reactor	Unit	N_{tot} in influent - N_{in}	N_{tot} in effluent - N_{out}	Plant uptake - ΔN_P	Substrate accumulation - ΔN_S	Nitrogen loss - ΔN_L
P1	mg	7950	1977	1162	938	3873
	% (on N_{tot} in influent)	-	25	15	12	48
P2	mg	7950	1815	659	1173	4303
	% (on N_{tot} in influent)	-	23	8	14	55
PC	mg	7950	3328	821	1876	1925
	% (on N_{tot} in influent)	-	42	11	23	24
CL	mg	7950	2881	-	1055	4014
	% (on N_{tot} in influent)	-	36		13	50

6.3.7 Kinetics of nitrogen removal

The calibrated values of the reaction rate constants of nitrogen removal are reported in Table 6.9. Values detected in reactors P1 and P2 were similar. Nitrification (k_2) was the fastest process, while nitrogen removal due to other processes (e.g.: settling, adsorption, etc), mainly related to the role of the substrate (k_5), seemed to be the slowest one. Even if the occurrence of denitrification was observed, k_2 was much greater than k_3 ($k_2/k_3 \approx 10^5$) confirming that vertical flow reactors are optimized for the occurrence of nitrification. The calibrated values are out of the ranges reported in literature for k_1, k_2 and k_3, but a direct comparison is not feasible as literature value are referred to free flow wetlands in which hydrophytes were grown (Kadlec and Knight, 1996; Jorgensen and Bendoricchio, 2001; Kadlec and Wallace, 2009; Saeed and Sun, 2011). Anyhow, the ratios between the parameters are consistent with the data available, with $k_2 > k_1 \approx k_3$.

The values of k_4 confirm the minor role of plants in terms of nitrogen removal, as already pointed out in Table 6.8. The value of k_5, 10^{-16} d^{-1} in both P1 and P2, is contradictory: according to the modeling procedure the role of the substrate seems to be negligible; while the nitrogen mass balance revealed that the medium played a primary role. Probably the model was an oversimplification of the reality, and was not able to fully describe all the undergoing processes. Indeed, some assumptions (e.g. CSTR, 1° order reactions) might be too strong and could limit the capacity of the model to mimic the behavior of the investigated experimental units.

Table 6.9. Calibrated values of the reaction rates constants and comparison with literature data

	Process	P1	P2	Literature data*
k_1 (d^{-1})	Mineralization of N_{org} to ammonia	1.99	0.83	0.01 – 0.03
k_2 (d^{-1})	Nitrification	11.5	7.9	0.1 – 0.2
k_3 (d^{-1})	Denitrification	0.0001	0.0001	0.01 – 0.1
k_4 (d^{-1})	Ammonia and nitrate uptake by plants	0.0001	0.0001	-
k_5 (d^{-1})	Organic nitrogen and ammonia removal due other processes occurring in the medium	10^{-16}	10^{-16}	-

* Kadlec and Knight, 1996; Jorgensen and Bendoricchio, 2001; Kadlec and Wallace, 2009; Saeed and Sun, 2011

6.3.8. Heavy metals profile in the substrate soil and concentration in *Pennisetum Purpureum* biological tissues

Heavy metals concentrations in the substrate soil of leachate irrigated reactors P1, P2 and CL, and in controls PC and CW, detected at the end of the experiment, are reported in Fig. 6.6. Analyzing the trends along the depth, an increase of heavy metals concentration with the increasing depth was detected in each experimental unit. This phenomenon could be ascribed to the vertical flow pattern, which tended to accumulate the elements at the bottom of the columns. The lowest concentrations were detected in controls PC and CW while the highest concentrations were detected in CL, as a result of the combined effect of leachate irrigation, which provided heavy metals, and the absence of the plants uptake contribution. The only exception was represented by Cd, whose values, on the contrary, slightly decreased with the depth in all the reactors.

Anyhow, final concentrations were below the initial values of the substrate soil in all the columns (with again the exception of Cd). This means that leachate irrigation did not lead to an accumulation of heavy metals in the substrate soil, thus minimizing the risk of contamination related to the phytotreatment process, which could potentially result in the generation of a contaminated soil (which then needs to be remediated). The reason is that the heavy metals concentration in the leachate is on the order of μg/L (Table 6.4), while in the initial substrate soil is on the order of mg/kg_{TS} (Table 6.3). Therefore, for short and medium-cycle phytotreatment tests, the role of leachate irrigation could be considered negligible in the build-up of dangerous heavy metal concentrations.

The analysis of substrate soils in which leachate phytotreatment has been applied for long times (e.g.: years) would be interesting to assess the occurrence of heavy metals accumulation phenomena. Heavy metals concentration in *Pennisetum Purpureum* tissues was investigated to assess whether leachate irrigation resulted in an accumulation of such chemical elements. Essences growing in reactors P1 and P2 were analyzed and the resulted compared with those of plants grown in PC (Fig. 6.7). Some metals (Cd, Cr, Pb, Ni) are not visible because their value was below 1 mg/kg_{TS}. A higher concentration of Fe and Mn was detected in tissues of plants grown in P1 and P2, but the statistical analysis did not reveal any significant increase compared to plants grown in the control PC. These results suggest that conversion of elephant grass into bio-energy is a feasible option and that risks related to the potential presence of contaminants into the biological tissues were not revealed by the experimental data.

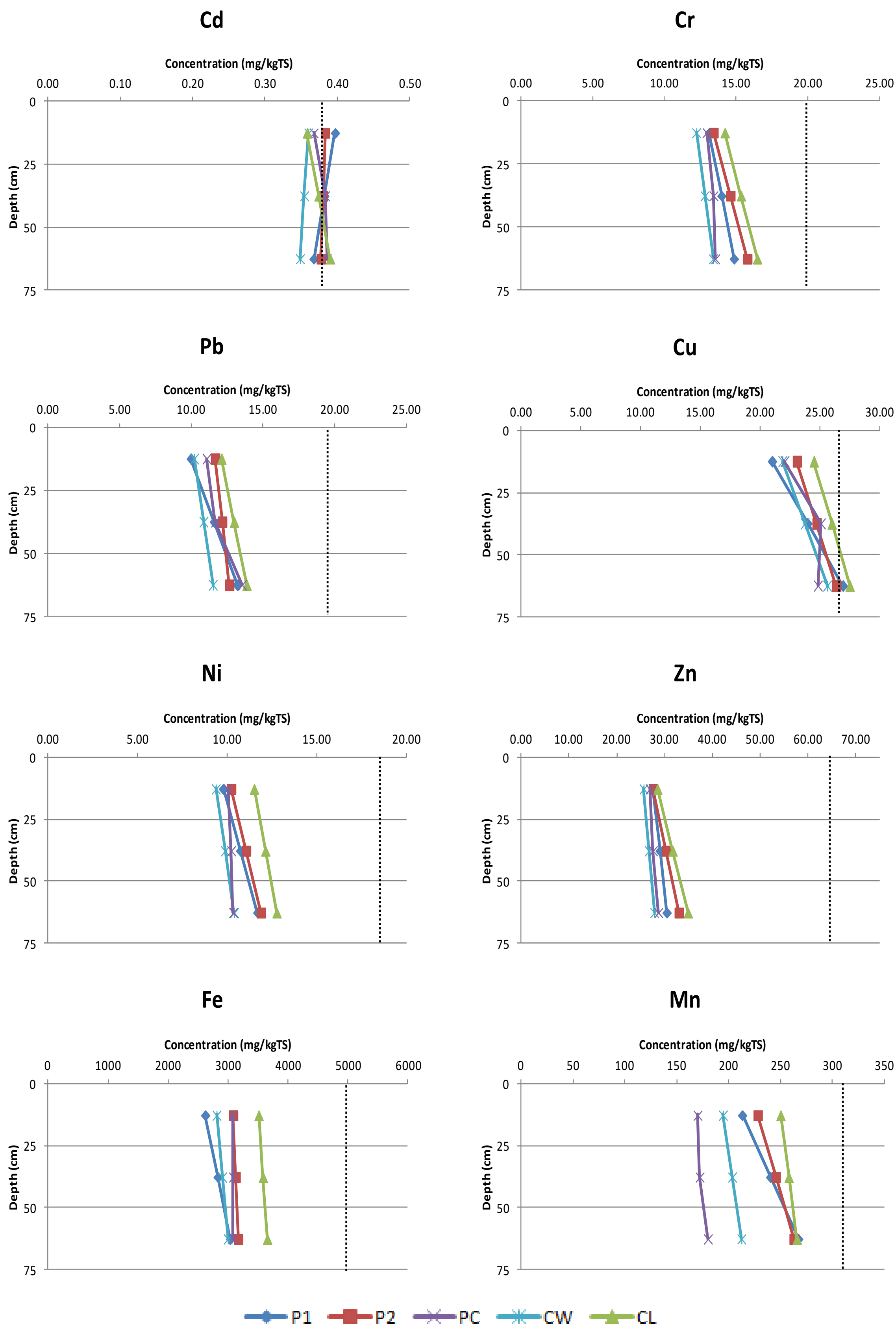

Fig. 6.6. Heavy metals distribution in soil along the columns depth (Top: 0-25 cm; Intermediate: 25-50 cm; Bottom: 50-75 cm). Vertical dotted lines represent the initial concentrations of the investigated chemical species

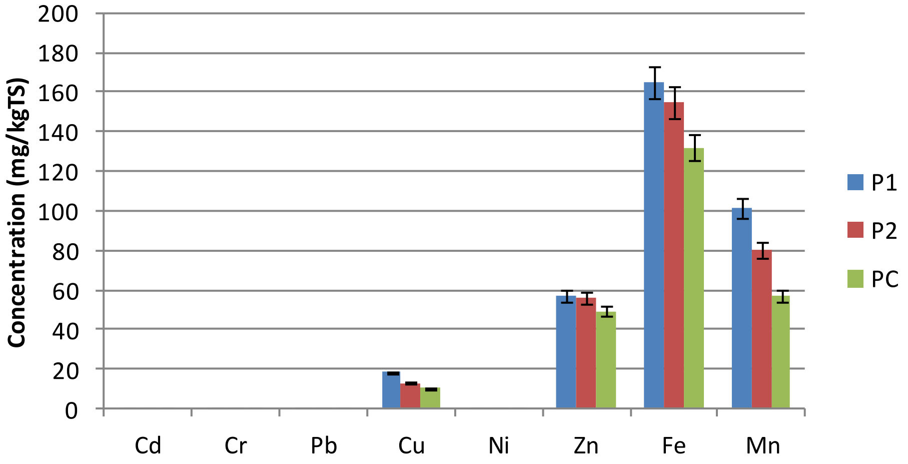

Fig. 6.7. Heavy metals concentration in the biological tissues of *Pennisetum Purpureum*

6.4. CONCLUSIONS

Landfill leachate phytotreatment using elephant grass proved to be feasible under lab-scale conditions. To our knowledge, this is the first time that elephant grass was used in landfill leachate phytotreatment. Plants growth was not affected by leachate irrigation, which on the contrary seemed to stimulate the biomass development. Evapotranspiration played an important role on leachate volume reduction; removal efficiencies of the investigated contaminants were excellent: more than 95% for TKN, ammonia and COD. Complete nitrification was observed in all the units in which nitrogen addition was applied, but also a partial denitrification in leachate irrigated reactors, which was confirmed by the final nitrogen mass balance. Final concentration of heavy metals along the columns at the end of the experiment showed an increase from the top to the bottom (except for Cd), but final concentrations were below the initial values of the substrate soil, indicating that for short and medium-cycle phytotreatment tests, the role of leachate irrigation could be considered negligible in the build-up of dangerous heavy metal concentrations. Final heavy metals concentration in the tissues of *Pennisetum Purpureum* showed an acceptable, not significant increase. Further investigations, performed on a longer time span, are required to assess the long term performances of the system, and the production of renewable energy (e.g. bio-ethanol) which could be achieved.

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Chapter 7: Assessment of the ecotoxicity of phytotreatment substrate soil as landfill cover material for in-situ leachate management

Garbo, **F.**, Pivato, A., Manachini, B., Moretto, C.G., Lavagnolo, M.C., 2019. Assessment of the ecotoxicity of phytotreatment substrate soil as landfill cover material for in-situ leachate management. J. Environ. Manage. 231, 289-296. https://doi.org/10.1016/j.jenvman.2018.10.014
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Abstract

Phytotreatment capping in closed landfills is a promising, cost-effective, in situ option for sustainable leachate treatment and might be synergistically coupled with energy crops to produce renewable energy (e.g. biodiesel or bioethanol). This study proposes to use 0.30 m of soil as growing substrate for plants cultivated on the temporary cover of closed landfills. Once the leachate phytotreatment process is no longer required, 0.70 m of the same soil would be added to attain the final top cover configuration. This solution would entail saving the costs of excavation and backfilling. However, worsening of the initial soil quality due to potential contaminant transfer from the liquid to the solid matrix must be avoided because EU legislation (such as that in Italy) fixes concentration limits for contaminants in soil. In this research, samples of soil used as substrate in a lab-scale leachate phytotreatment test with sunflowers were analysed to provide chemical characterization before, during, and at the end of the experiment. The results showed that the phytotreatment activity did not increase initial contaminant concentrations. These results are reinforced by those from ecotoxicological bioassays in which *Eisenia fetida* (earthworms), *Lepidium sativum* (cress), *Folsomia candida* (collembola), and *Caenorhabditis elegans* and *Steinernema carpocapsae* (nematodes) were used. It was observed that, by the end of the experiment, the substrate soil did not affect the earthworms, collembola and nematode behaviour, or the growth of cress.

Keywords: landfill leachate phytotreatment; closed landfills; substrate soil chemical characterisation; ecotoxicological bioassays; environmental legislation

7.1. INTRODUCTION

Landfilling is still considered the final element of most waste management strategies, so as to close the material usage loop. However, among others, the main problems linked to landfills are leachate management and the damage to the landscape that these waste masses can create (Cossu and Williams, 2015). In fact, one of the most onerous items of expenditure is the leachate management (Oloibiri et al., 2017), which is stored and then, most of times, treated ex situ often using highly sophisticated technologies such as reverse osmosis, evaporation systems and membrane bioreactors (Di Maria et al., 2018; Saleem et al., 2018). In addition, landfills are not typically accepted by citizens: following the "NIMBY" (Not In My Back Yard) principle (Achillas et al., 2011; Ma and Hipel, 2016), they consider them dangerous accumulations of waste. These oppositions could be minimized by the utilization of energy crops growing on the top of closed landfills, not only for leachate phytotreatment purposes but also for renewable energy generation, offering a pleasant view of the site (Lavagnolo et al., 2016) and enhancing the process of environmental restoration (Pivato et al., 2018a) at the same time. The landfill leachate, which is collected and re-circulated to the top of the closed landfill, could be phytotreated on a portion of the surface area with little slope. Additionally, this would make it possible to save the huge amounts of water necessary to irrigate these types of plants (Garbo et al., 2017). Energy crops can be used effectively to treat landfill leachate because they are able to resist the organic and inorganic contaminants (Agostini et al., 2003; Brunetti et al., 2011; January et al., 2008; Marchiol et al., 2007; Schnoor et al., 1995; Tang et al., 2016). These plants were tested by several authors (Akinbile et al., 2012; Fraser et al., 2004; Hasselgren, 1992; Ma et al., 2016) who demonstrated their high efficiency in contaminant removal due to the synergic effects of the plants and the microorganisms living in the soil. The final objective of energy crop cultivation is the production of renewable energy: bioethanol from ligneous biomass, biodiesel from oleaginous crops and biogas from the biomass feedstock (Di Maria and Sisani, 2017; Lavagnolo et al., 2017; Pandey et al., 2016). Garbo et al. (2017) and Lavagnolo et al. (2016) have already considered the use of oleaginous crops (e.g. sunflower, soybean, rapeseed) on the top of a landfill for leachate phytotreatment and biodiesel production. They reported good results, achieving efficiencies higher than 80% for Chemical Oxygen Demand (COD) reduction, and removal of more than 70% of total nitrogen (N) and more than 95% of total phosphorous (P_{tot}). Moreover, a significant fraction of the leachate volume was removed by natural evapo-transpiration (Garbo et al., 2017; Lavagnolo et al., 2016).

The EU Directive 1999/31/CE mandates the competent authority (region or province) to prescribe surface sealing of the landfill only if a potential hazard to the environment is recognized. On the other hand, the Italian transposition (D. Lgs. 36/2003) of the EU Directive and some regional regulations (e.g. DGR Lombardia n. X/2461/2014) prescribe a mandatory impermeable final top cover, aimed at minimizing the infiltration of liquids into the landfill body. Therefore, a phytotreatment basin built on the landfill final top cover is discouraged by the current Italian laws and regulations. To comply with the current national legislation, the following scenario was proposed (Fig. 7.1): the plants, irrigated with the leachate, are cultivated during the temporary cover period in 0.30 m of substrate soil, which is required for root development. At the end of the phytotreatment process, an additional layer of soil (0.70 m) is added to reach the final top cover configuration called for in D. Lgs. 36/2003 (at least 1.00 m of natural soil as superficial layer). In this manner, the costs of excavation and backfilling can be limited because the substrate soil used for phytotreatment is simply covered with the same type of soil. Moreover, to minimize leachate infiltration in the landfill body, an additional 0.50 m thickness of clay, for a total of 1.00 m, is also considered; in fact, the legislation (D. Lgs. 36/2003) requires a minimum thickness of 0.50 m. Therefore, the proposal for the final configuration is – from bottom to top – a 0.15 m compensation layer, 0.50 m of gravel to permit landfill gas drainage and collection, a 1.00 m layer of clay (instead of 0.50 m), with a permeability k less than 10^{-9} m/s, a High-Density PolyEthylene (HDPE) geomembrane, a geotextile, 0.50 m of gravel to drain the water and 1.00 m of natural soil. Figure 1 shows that the proposed final cover has the same configuration as the final cover now prescribed by law, except for the clay layer.

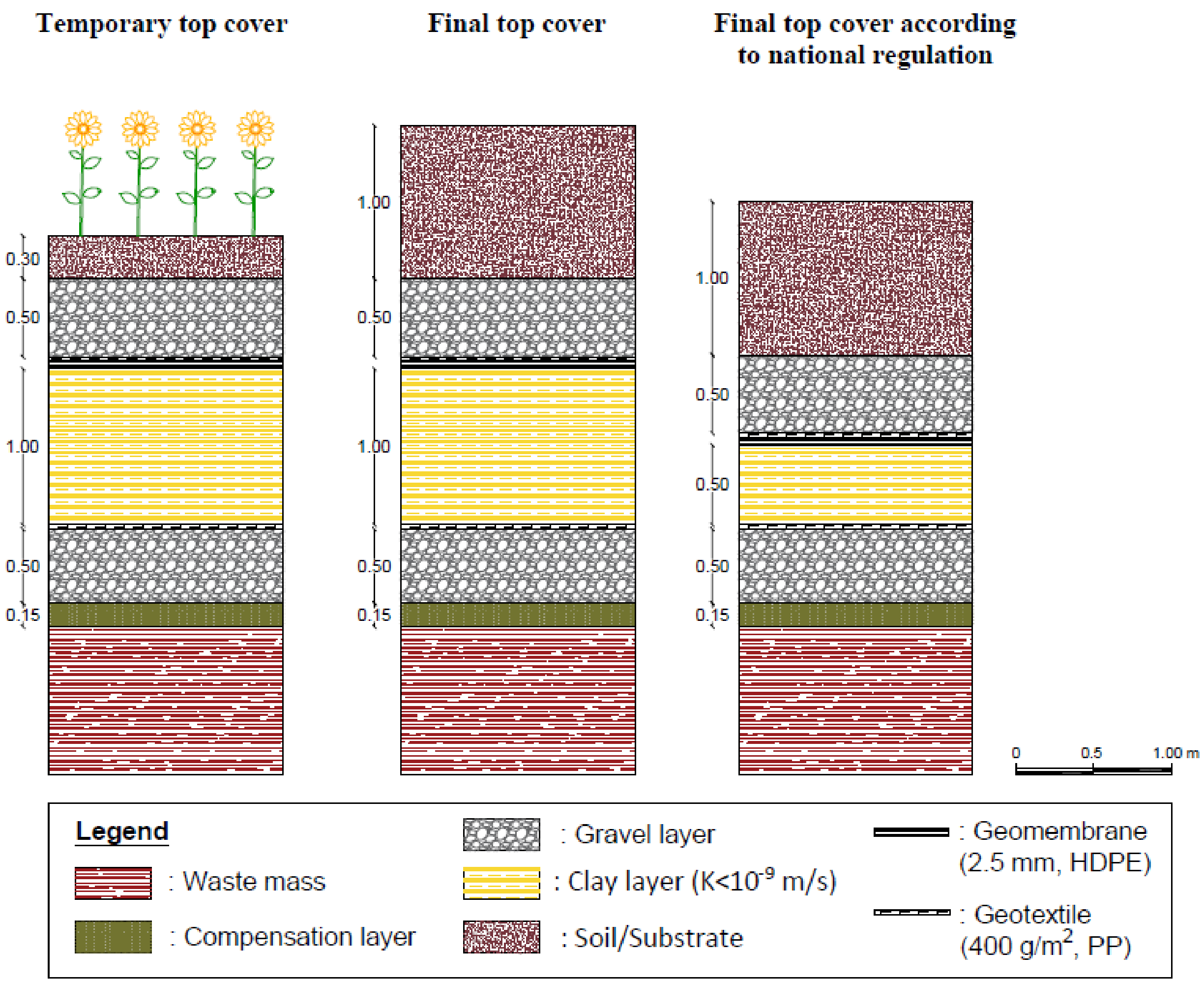

Fig. 7.1. Comparison between temporary and final top cover proposed in this article and final top cover prescribed by the current Italian legislation

In this research, experiments were performed using the substrate soil on which sunflowers were cultivated. Sunflowers were irrigated with leachate to represent the scenario of leachate phytotreatment on the top of the landfill. One of the critical points of full-scale application could be the substrate soil quality at the end of phytotreatment period. Based on our literature review, there are no studies in which the wetlands growing medium has been chemically characterized and compared with the reference values set by the current legislations. However, a chemical analysis for a substance-based approach is not sufficient because the soil is a very complex living matrix including soil fauna along with microorganisms (EFSA, 2017; Manachini et al., 2009). These can absorb elements such as carbon and nitrogen, to degrade organic compounds and to amass stock substances in the form of humus (EFSA, 2017; Jacomini et al., 2000). Thus, it is necessary to consider also ecotoxicological analysis for a matrix-based approach (Pivato et al., 2017).

Ecotoxicological testing involves the study of the effects of toxic compounds present in the soil on representative organisms (APAT, 2004; Hennebert, 2017).

In the past, some studies considered the use of earthworms, nematodes, and the germination of seeds as bio-indicators to determine the toxicity of a soil. For example, Dawson et al. (2007) considered earthworms and seed germination assays as indicators to assess the ecological health of soils from a former gas-works site undergoing various remediation treatments. Holmstrup et al. (2010) considered the effects of natural stresses during ecotoxicological analysis using earthworms and nematodes. Pivato et al. (2018b; 2016; 2014) utilized *Eisenia fetida* earthworms and *Folsomia candida* collembola to investigate the quality of compost and digestate for possible use in agriculture. There are no references, however, reporting the ecotoxicological characterisation of a substrate soil used for landfill leachate phytotreatment with energy crops.

In this work, chemical and ecotoxicological characterisations were conducted on the substrate soil before, during and after the leachate phytotreatment to determine if the substances contained in the leachate, or formed during the phytotreatment process, cause significant worsening of the soil quality. The concentrations of contaminants in the substrate soil were compared with reference values (screening values) for potentially contaminated sites defined in Table 1 of Annex 5 to Part IV of D. Lgs. 152/06 (soil for public, private and residential green areas in column A; soil for commercial and industrial activities in column B) to check if contamination occurred.

Chemical characterization was combined and reinforced by a series of ecotoxicological tests that were conducted using the following suitable vulnerable model species (EFSA, 2017): *Lepidium sativum* (cress), *Eisenia fetida* (earthworms), *Folsomia candida* (collembola) and the nematodes *Caenorhabditis elegans* and *Steinernema carpocapsae*, in which the potential toxicity of the substrate soil samples was assessed based on the growth and biological development of the organisms.

7.2. MATERIAL AND METHODS

7.2.1. Experimental design

The tested samples were collected from a lab-scale phytotreatment test, performed according to the experimental design described by Lavagnolo et al. (2016) and Garbo et al. (2017). Four 45 L polyethylene tanks, with a surface area of 0.16 m^2, were used. All tanks were placed in a controlled climatic chamber in which a 14 h photoperiod with 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity was imposed. The mean air temperature was maintained at 24 °C (MIN=17 °C, MAX=35 °C). To the four tanks were added – from the bottom to the top – 8 cm of gravel (20–30 mm diameter) for drainage, a small net to avoid clogging of the drainage system and 30 cm of substrate soil (Fig. 7.2). Four sunflowers were planted in each experimental unit. Based on previous experiences (Garbo et al., 2017), the number of plants was considered to be sufficient. After an initial acclimation period, lasting for 14 days, in which tap water was used, sunflowers were irrigated with a mixture containing water and an increasing amount of landfill leachate, as reported in Table 7.1. The applied Hydraulic Loading Rate (HLR) was 4.5 mm d^{-1}. The irrigation was spread uniformly over the entire surface of each reactor. The leachate dose was increased gradually to adapt the plants to the increasing concentration of contaminants and to avoid sudden failure from potential phytotoxicity. The nitrogen concentration in the feed was used as a reference parameter in setting the irrigation timetable; previous studies had revealed that nitrogen exceeding 400 mg-N/L could produce a negative effect on plants (Garbo et al., 2017; Lavagnolo et al., 2016). Once a week, the tanks were drained through a valve at the bottom.

The substrate soil was the same in all the experimental units. The initial sample (initial substrate soil) was analysed before the start of the phytotreatment tests. After 35 days from the beginning, the substrate soil was excavated from two tanks, mixed, and analysed (intermediate substrate soil). The remaining two reactors were run until clear senescence of the sunflowers was reached (70 days from the beginning of the phytotreatment): then the plants were harvested, reactors were excavated, and the substrate soils were mixed and analysed (final substrate soil).

Table 7.1. Main characteristics of the irrigation water over the whole experimental period and the sampling timetable

Week	HLR ($mm \cdot d^{-1}$)	Leachate percentage	COD inlet ($mg \cdot L^{-1}$)	P inlet ($mg \cdot L^{-1}$)	TKN inlet ($mg \cdot L^{-1}$)
Collection of initial substrate soil samples					
1	4.5	10%	49	0.23	50
2	4.5	20%	98	0.46	100
3	4.5	30%	147	0.69	150
4	4.5	40%	196	0.92	200
5	4.5	50%	245	1.15	250
Collection of intermediate substrate soil samples (35 days from the beginning of the test)					
6-10	4.5	60%	294	1.38	300
Collection of final substrate soil samples (70 days from the beginning of the test)					

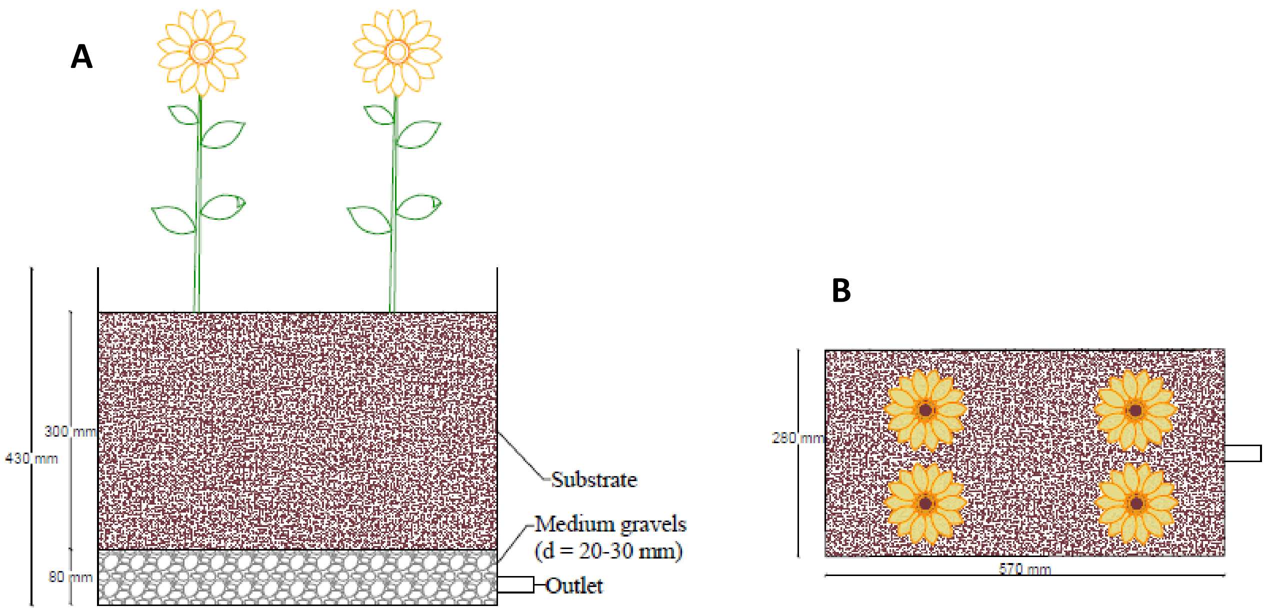

Fig. 7.2. Scheme of the lab-scale phytotreatment test: cross section (A) and plan (B)

7.2.2. Leachate characterization

The leachate used in the experiment was collected from a sector of an operating landfill located in the North of Italy, in which residual waste from separate collection of Municipal Solid Waste (MSW) is disposed of. It was sampled once and analyzed four times during the experiment to check whether the main parameters (e.g.: nitrogen) were changed over time. It was analysed according to the CNR-IRSA standard Italian analytical methods for liquid samples (CNR-IRSA, 29/2003). Its composition is reported in Table 7.2 and the results consistent with the kind of waste landfilled.

Table 7.2. Characteristics of the leachate utilized for the experiment

Parameter	Values
pH	7.8±0.3
TKN (mg-N·L^{-1})	500±25
NH_4^+ (mg-N·L^{-1})	453±22
P_{TOT} (mg-P·L^{-1})	2.3±0.2
TS (mg·L^{-1})	2451±113
VS (mg·L^{-1})	495±45
COD (mgO_2·L^{-1})	490±32
BOD (mgO_2·L^{-1})	<50
Cl^- (mg·L^{-1})	685±36
NO_3^- (mg-N·L^{-1})	-
SO_4^{2-} (mg-SO_4·L^{-1})	-
Ca (mg·L^{-1})	142±11
K (mg·L^{-1})	225±21
Mg (mg·L^{-1})	88±10
Na (mg·L^{-1})	353±18
Cd (µg·L^{-1})	< 10
Cr (µg·L^{-1})	64±3
Cu (µg·L^{-1})	91±5
Fe (µg/L)	5733±56
Mn (µg·L^{-1})	313±20
Ni (µg·L^{-1})	168±8
Pb (µg·L^{-1})	46±3
Zn (µg·L^{-1})	269±6
Se (µg·L^{-1})	121±12

7.2.3. Substrate soil characterization

7.2.3.1. Texture characterization

The substrate soil utilized for the lab-scale phytotreatment system was a locally available soil rich in sand. It was collected in the proximity of the research centre in which the experiments were performed, in the North-East of Italy. Long-term studies indicate that mixtures of soil and sand provide an optimal combination for phytotreatment systems (Lavagnolo et al., 2016; Stottmeister et al., 2003; Verakoon et al., 2013) because they provide sufficient air circulation, while at the same time guaranteeing proper root development. The texture was determined using the Bouyoucos Method (Bouyoucos, 1962) and, according to the soil taxonomy proposed by the USDA (USDA-NRCS, 1999), the substrate soil was classified as sandy loam (14% clay, 10% silt, and 76% sand).

7.2.3.2. Chemical characterization

The chemical characterization determined the presence of chemical compounds in the three substrate soil samples, which were analysed in triplicate. The compounds analysed were compared to the reference values (columns A and B) reported in Table 1 of Annex 5 to Part IV of D. Lgs. 152/06, already mentioned in the Introduction. The chemical analysis was performed according to the EPA Hazardous Waste Test Methods (SW-846).

7.2.3.3. Ecotoxicological characterization

7.2.3.3.1. Lepidium sativum (cress) tests

Soil quality can be evaluated using plants as bio-indicators. In this case, *Lepidium sativum* (cress) was used, according to the APAT guidelines (APAT, 2004), due to its ability to reveal quickly the potential toxicity of the soil. The tests were performed using Petri dishes (Ø = 9 cm). A mixture of 10 g of test-substrate soil (e.g. final substrate soil) and artificial soil (quartz sand with more than 50% of particles between 50 and 200 microns) was added to each dish. Increasing concentrations of the test-substrate soil were used: 0 (control), 2, 3, 5, 7, 10, 20, 30, 50, 70, and 100% (w/w referred to dry matter) to which deionized water was added to reach 100% of the Water Holding Capacity (WHC) of the mixture, plus 5 mL. As suggested by the USEPA (2005), the test concentrations were chosen to follow a geometrical series, with an average ratio of 1.5. Two controls were used: one with just 5 mL of deionized water (as prescribed by the APAT guidelines) and another with 10 g of artificial soil and 5 mL deionized water. The latter was used to be consistent with the testing procedure, which is based on the use of 10 g of material. Ten seeds were placed in each dish on a filter paper on top of the media, and the dishes were covered using parafilm. Seeds available in the market for bioassays were used. The tests were conducted under standardized conditions: 25 °C and complete darkness (0 lux). After 72 h, the elongation of the emerged roots was measured. As prescribed by the APAT guidelines, each concentration (including the controls) was tested using four replicas. The results were expressed as percentage Germination Index (GI%); each Germination Index (GI) was calculated by multiplying the number of germinated seeds with the mean root length of each plant, as follows:

$$GI = n.germinated\ seeds \cdot mean\ roots\ length \tag{7.1}$$

The mean GI was calculated for each substrate soil sample ($\overline{GI}$) and control $\overline{(GI_C)}$ and the percentage GI (GI%) was calculated as ratio between $\overline{GI}$ and ($\overline{GI_C}$), as follows:

$$GI\% = \frac{\overline{GI}}{\overline{GI_C}} \cdot 100 \tag{7.2}$$

7.2.3.3.2. Earthworms tests

The method adopted was a chronic test performed according to the OECD Guideline 222/2004. Ten *Eisenia fetida* adult earthworms were put in plastic containers (volume 1.2 L) filled with 500±5 g of a mixture of artificial soil and test-substrate soils, at different concentrations (the same concentrations used for the cress tests). The artificial soil was composed of 70% sand, 20% clay and 10% peat (w/w), as prescribed by the OECD Guideline 222/2004. Its WHC was adjusted to 40%. The maximum WHC of the artificial soil was determined in accordance with the procedures described in Annex 2 of ISO 11274 (1998). The initial weight of the earthworms ranged from 0.3 to 0.9 g. Soil mixtures and earthworms were placed in the containers and closed with holed plastic lids to prevent the worms from escaping, to permit air passage and to limit evaporation. The earthworms were fed weekly with 5 g of dried cow manure. The test was performed in a thermostatic room with a monitored temperature of 20±2 °C, light-dark cycles L:D 16:8 (L = 400–800 lux). After 28 days (Day 28), earthworms were counted and weighted. As prescribed by the OECD guidelines, each concentration was tested in triplicate. The results were expressed as percentage Relative Survival (RS%) and percentage Relative Growth (RG%). They were both defined as the average variation between the final and the initial earthworm conditions and were normalized using the values found in the controls (with 0% test-substrate soil), as follow:

$$RS = \frac{\text{final n. of earthworms}}{\text{initial n. of earthworms}} \tag{7.3}$$

$$RG = \frac{\text{final earthworms weight}}{\text{initial earthworms weight}} \quad (7.4)$$

The mean RS was calculated for each substrate soil sample ($\overline{RS}$) and control ($\overline{RS_C}$) and the percentage RS (RS%) was calculated as ratio between $\overline{RS}$ and ($\overline{RS_C}$), as follows:

$$RS\% = \frac{\overline{RS}}{\overline{RS_C}} \cdot 100 \quad (7.5)$$

The mean RG was calculated for each substrate soil sample ($\overline{RG}$) and control ($\overline{RG_C}$) and the percentage RG (RG%) was calculated as ratio between $\overline{RG}$ and ($\overline{RG_C}$), as follows:

$$RG\% = \frac{\overline{RG}}{\overline{RG_C}} \cdot 100 \quad (7.6)$$

7.2.3.3.3. Collembola tests

The collembola chronic bioassay was carried out using the common springtail (*Folsomia candida*) according to the ISO 17512-1 (2008) guideline. After preliminary bioassays that did not result in differences according to the concentrations, it was decided (also for practical and economic reasons) to use 100% substrate soil concentration for all test samples (initial, intermediate, and final substrate soil). The test was carried out in glass containers with 10 g of test-substrate soil (dry weight). Ten specimens of *F. candida* were introduced into each container. At the beginning, deionized water and 10 mg of dried baker's yeast were added to each container. Test containers were closed with parafilm and incubated at 20±2 °C, in the dark, for 28 days. At the end, exposure mortality of adults was determined. As prescribed by the ISO 17512-1 (2008) guideline, four replicates were used. The survival percentage (Su) at Day 28 was considered the endpoint.

7.2.3.3.4. Nematodes tests

The bacterial feeding nematode *Caenorhabditis elegans* was maintained as a stock of dauer larvae (juvenile stage that occurs with a lack of food) on nematode growth medium agar (Brenner, 1974), according to standard procedures (Lewis and Fleming, 1995; Sulston and Hodgkin, 1988). The nematode bioassay with *C. elegans* was carried out according to standard methods (ASTM guidelines E2172, 2014 and to the principles of ISO 10872, 2010). For the test, 0.5 g of each test-substrate (air-dry weight) was moistened with 0.35 mL of medium (containing Na_2HPO_4, KH_2PO_4, NaCl, and $MgSO_4$) in test wells and then mixed with *Escherichia coli* as the food supply. Ten first-stage juvenile nematodes were transferred to each test well (total of 160 nematodes). Their mean initial body length was 260±38 µm. Four replicates were set up for each test-soil substrate (initial, intermediate, and final) and the control. Even in this case only the concentration of 100% test-substrate soil was considered. In fact, as for *F. candida*, preliminary bioassays indicated no difference in the lower concentrations, thus for practical and economic reasons it was decided to use only the highest soil concentration. After 96 h of incubation at 20 °C, the test was stopped by heat killing the nematodes at 50 °C, after checking the vitality of the specimens. The samples were then mixed with 0.5 mL of an aqueous solution of Rose Bengal to stain specimens for counting. Four different endpoints were considered: survival, growth, fertility, and reproduction. Survival percentage was considered also as the endpoint and was checked considering as alive the motile nematodes. Nematode growth was determined by measuring the body length at 100-fold magnification using a light microscope. Growth was calculated by subtracting the mean initial body length of the test organisms from the mean body length after incubation. Nematode fertility was quantified by calculating the percentage of gravid organisms. Nematode reproduction was quantified by counting the number of eggs under a dissecting microscope at 75-fold magnification.

The second nematode toxicity test examined the direct exposure of one of the entomopathogenic nematodes (EPN) most used in biological control, which is also one of the most common species living in agricultural soil, *Steinernema carpocapsae*. Monoxenic infective juveniles in a *S. carpocapsae* culture (Becker Underwood, Ltd) were used for the bioassay. For the test, 0.5 g of each test-substrate soil (air-dry weight) was moistened with 0.35 mL of medium (containing Na_2HPO_4, KH_2PO_4, NaCl, and $MgSO_4$). The toxicity test was carried out according to ASTM guidelines E2172, 2014 and ISO 10872, 2010. The results were expressed as Su at 24 hours and at 48 hours.

7.2.4. Statistical analysis

Statistical analysis was performed using Statgraphics® software. The responses to different substrate soil samples were compared by one-way analysis of variance. The F-test was used to assess whether there were significant differences amongst the means at the 95.0% confidence level ($p<0.05$); pairwise comparisons were assessed with the Tukey's honestly significant difference (HSD) procedure.

7.3. RESULTS AND DISCUSSION

7.3.1. Substrate soil chemical characterization

The results of the chemical characterization are reported in Table 7.3 and were compared with the reference values from Italian legislation for soil contamination (Table 1 of Annex 5 to Part IV of D. Lgs. 152/2006). Statistical analysis revealed a statistically significant increase (from the initial substrate soil samples to the final ones) of the following chemical species: total chromium, lead, copper, zinc. However, treatment-related overall build-up of heavy metals spanned conditions from negligible to acceptable because concentrations remained well within the limits for residential soil. The concentration of each chemical element was always below the reference values, even in the final substrate soil, with the only exception being selenium. The concentration of this element exceeded the reference value of column A (screening values for public, private, and residential green areas), but remained below the corresponding reference value of column B (screening values for commercial and industrial activities). But it must be noted that selenium was above the reference value of column A even in the initial substrate soil and no change of concentration was recorded throughout the experiment. The initial substrate soil samples were collected before the beginning of the phytotreatment tests; therefore, the abnormal concentration of this element cannot be related to the leachate irrigation procedure, but rather to the characteristics of the locally available soil utilized in the experiment.

Table 7.3. Chemical characterization of substrate soil samples. Comparison with reference values of Table 1 of Annex 5 to Part IV of D. Lgs. 152/2006. * denotes a statistically significant difference. Different apical characters indicate statistically significant differences among the samples

	Reference values column A (mg/kgTS)	Reference values column B (mg/kgTS)	Initial substrate soil (mg/kgTS)	Intermediate substrate soil (mg/kgTS)	Final substrate soil (mg/kgTS)	p-value
Cadmium	2	15	0.2±0.0	0.3±0.1	0.3±0.1	0.296
Cobalt	20	250	8±1	9±2	11±1	0.098
Total Chromium	150	800	$20{\pm}3^{X}$	$25{\pm}2^{XY}$	$28{\pm}2^{Y}$	0.017^{*}
Chromium VI	2	15	< 0.2	< 0.2	< 0.2	-
Mercury	1	5	< 0.05	< 0.05	< 0.05	-
Nickel	120	500	18±3	21±2	23±2	0.105
Iron ^	-	-	28884±758	26718±969	24903±1352	0.072
Manganese ^	-	-	176±5	170±7	163±11	0.422
Lead	100	1000	$19{\pm}2^{X}$	$25{\pm}3^{XY}$	$29{\pm}3^{Y}$	0.011^{*}
Copper	120	600	$27{\pm}5^{X}$	$41{\pm}5^{Y}$	$46{\pm}3^{Y}$	0.004^{*}
Zinc	150	1500	$65{\pm}9^{X}$	$81{\pm}9^{XY}$	$89{\pm}7^{Y}$	0.032^{*}
Antimony	10	30	< 1	< 1	< 1	-
Arsenic	20	50	12±3	13±3	17±2	0.113
Beryllium	2	10	0.7±0.2	0.7±0.1	0.8±0.1	0.629
Selenium	3	15	14±2	14±3	15±2	0.842
Thallium	1	10	< 0.2	< 0.2	< 0.2	-
Vanadium	90	250	31±3	35±3	37±4	0.164
Cyanides	1	100	< 0.10	< 0.10	< 0.10	-
Fluorides	100	2000	< 10	< 10	< 10	-
Hydrocarbons C<12	10	2000	< 0.05	< 0.05	< 0.05	-
Hydrocarbons C>12	50	2000	< 10	< 10	< 10	-
Aromatic hydrocarbons	0.1 - 1	2 - 100	< 0.001	< 0.001	< 0.001	-
Aromatic polycyclic hydrocarbons	0.5 - 5	5 - 50	< 0.01	< 0.01	< 0.01	-
Aliphatic chlorinated carcinogenic hydrocarbons	0.01 - 1	0.1 - 20	< 0.001	< 0.001	< 0.001	-
Aliphatic chlorinated non-carcinogenic hydrocarbons	0.3 - 1	5 - 50	< 0.001	< 0.001	< 0.001	-
Aliphatic halogenated carcinogenic hydrocarbons	0.01 - 0.5	0.1 - 10	< 0.001	< 0.001	< 0.001	-

Nitrobenzene	0.1 - 0.5	10 - 30	< 0.01	< 0.01	< 0.01	-
Chlorobenzene	0.05 - 1	10 - 50	< 0.001	< 0.001	< 0.001	-
Phenol	1	60	0.039±0.018	0.018±0.012	0.017±0.011	0.175
Methylphenol (o-, m-, p-)	0.1	25	0.0079±0.001 X	0.0051±0.001 Y	0.0059±0.001 XY	0.034*
Chlorinated phenols	0.01 - 0.5	12 - 50	<0.001	<0.001	<0.001	-
Aromatic amines	0.05 - 0.5	13 - 50	< 0.01	< 0.01	< 0.01	-
Esters of phtalic acid	10	2000	< 1.0	< 1.0	< 1.0	-

^ Table 1 of Annex 5 to Part IV of D. Lgs. 152/2006 does not specify any reference value for Iron and Manganese.

7.3.2. Substrate soil ecotoxicological characterization

7.3.2.1. Lepidium sativum bioassay

The GI% of *L. sativum* is shown in Figures 7.3 and 7.4. Focusing on the results referred to deionized water as control (Fig. 7.3), similar trends were detected for the three substrate soils for concentrations between 2 and 10%, characterised by peaks of the GI% up to 180% (Fig. 7.3B). For concentrations higher than 10%, the GI% of the initial substrate soil presented a slightly decreasing trend but remained always above 80% (Fig. 7.3A). The other samples presented some fluctuations, which were more marked for the intermediate substrate soil. For the intermediate sample, GI% ranged between 140 and 160% (Fig. 7.3B), while for the final sample, the GI% ranged between 100 and 120% (Fig. 7.3C). The trends of the GI% referred to controls in which artificial soil and deionized water were used (Fig. 7.4) are similar to those reported in Fig. 7.3, especially for concentrations higher than 10%. In fact, the GI% decreased but remained above 50% for the initial soil (Fig. 7.4A), between 75 and 100% for the intermediate soil (Fig. 7.4B), and between 90 and 110% for the final substrate soil (Fig. 7.4C). Statistical analysis was performed on the results of the bioassays in which 100% test-soil substrate was used, in order to mimic the real scale conditions in which the substrate is not mixed with artificial soil (Table 7.4). It revealed a statistically significant increase of the GI% between initial and intermediate substrate soil, and between initial and final substrate soil, respectively. The higher values of the GI% of intermediate and final samples could be due to an increased concentration of nutrients (especially nitrogen) in the substrate soil, compared to the initial sample. The increase of nitrogen and phosphorous content during the phytotreatment lab-scale tests is reported in Table 7.5. The maximum increase of the nitrogen content was 13% (Δ Final-Initial): it seemed to have a great influence on the cress development, although a statistically significant increase was not detected. The phosphorus concentration result was always below the detection limits. Summarizing, it is possible to affirm that the germination of *Lepidium sativum* did not present anomalies (e.g.: phytotoxicity phenomena) induced by leachate application and the phytotreatment process.

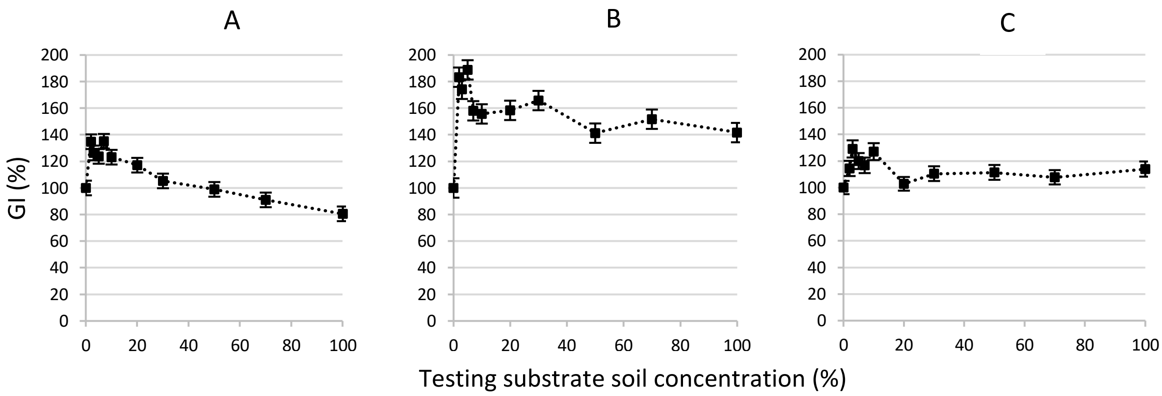

Fig. 7.3. Results of the percentage Germination Index (GI%) for initial (A), intermediate (B), and final (C) substrate soil (deionized water as control). Deviation bars refer to the 95% confidence level.

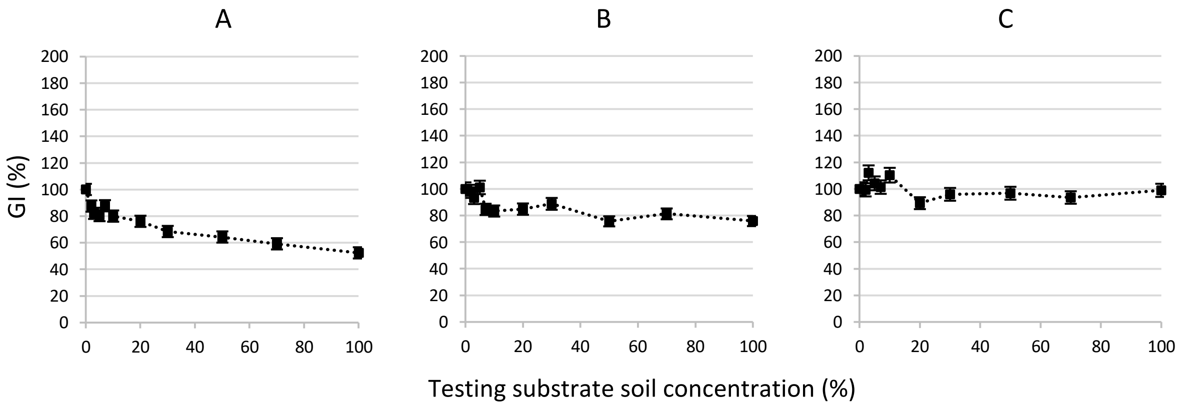

Fig. 7.4. Results of the percentage Germination Index (GI%) for initial (A), intermediate (B), and final (C) substrate soil (artificial soil and deionized water as control). Deviation bars refer to the 95% confidence level.

Table 7.4. Summary of the GI% referred to the bioassay with 100% test-substrate soil. * denotes a statistically significant difference. Different apical characters indicate statistically significant differences among the substrate soil samples

	100% initial substrate soil	100% intermediate substrate soil	100% final substrate soil	p-value
GI% (deionized water as control)	80.60±5.6 ^{X}	141.60±18.7 ^{XY}	113.90±11.3 ^{Z}	0.0003*
GI% (artificial soil and deionized water as control)	52.30±3.6 ^{X}	75.90±10.1 ^{YZ}	99.00±9.8 ^{Z}	0.0001*

Table 7.5. Nitrogen and phosphorous content in the substrate soils (initial, intermediate, and final) used for the experiments

	Initial (%)	Intermediate (%)	Final (%)	p-value	Δ Intermediate-Initial (%)	Δ Final-Initial (%)
Nitrogen content	0.15±0.1	0.16±0.1	0.17±0.1	0.1250	7	13
Phosphorous content	<0.05	<0.05	<0.05	-	-	-

7.3.2.2. Earthworm bioassay

Results of *E. fetida* earthworms percentage Relative Survival (RS%) are reported in Figure 7.5. Relative Survival close to 100% was detected for all three substrate soils, independent of the concentrations, meaning that almost all the earthworms remained alive in the initial, intermediate, and final substrate soils. Focusing on the lowest values, a minimum 90% of Relative Survival was observed with 5% of initial substrate soil (Fig. 7.5A), a minimum 85.7% of RS% with 2% of intermediate substrate soil (Fig. 7.5B) and a minimum 92.9% of RS% with 3% and 70% of final substrate soil (Fig. 7.5C). In the assays with the final substrate soil, some values exceeded 100%, indicating that survival of earthworms was even higher than in the controls, in which artificial soil, described in the OECD Guideline 222/2004 as optimal for the earthworms, was used.

Statistical analysis was applied to the results of the bioassays in which 100% test-soil substrate was used (Table 7.6) and did not reveal any significant difference among the different substrate soil samples (initial, intermediate, final).

Results of the earthworm percentage Relative Growth (RG%) are presented in Figure 7.6. The RG% increased with increasing concentrations of initial substrate soil, reaching a maximum value equal to approximately 200% (Fig. 7.6A) for concentrations of test samples with greater than 30% test-substrate soil. With regards to the intermediate and final substrate soils (Fig. 7.6B and 7.6C), after an initial increase, the trends of the RG% decreased with increasing concentration of the test substrate soils but were never below 100%, which is the value of the control. Statistical analysis was applied again to the results of the bioassays in which 100% test-soil substrate was used, revealing a statistically significant decrease of the RG% between initial and intermediate substrate soil, and between of initial and final substrate soil, respectively, clearly visible also in Fig. 7.6. However, RG% was always above 100%, the value of the control, in which artificial soil, specifically prepared the ensure optimal growing conditions, was used. Therefore, similarly to the *L. sativum* bioassays, the tests performed with *E. fetida* earthworms did not reveal anomalies which could be related to the applied process of phytotreatment.

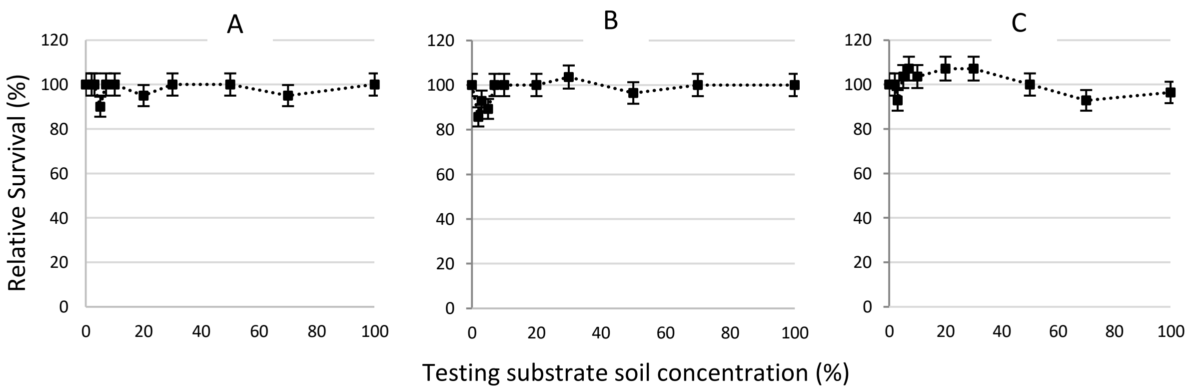

Fig. 7.5. Earthworm percentage Relative Survival (RS%) for initial (A), intermediate (B), and final (C) substrate soil. Deviation bars refer to the 95% confidence level.

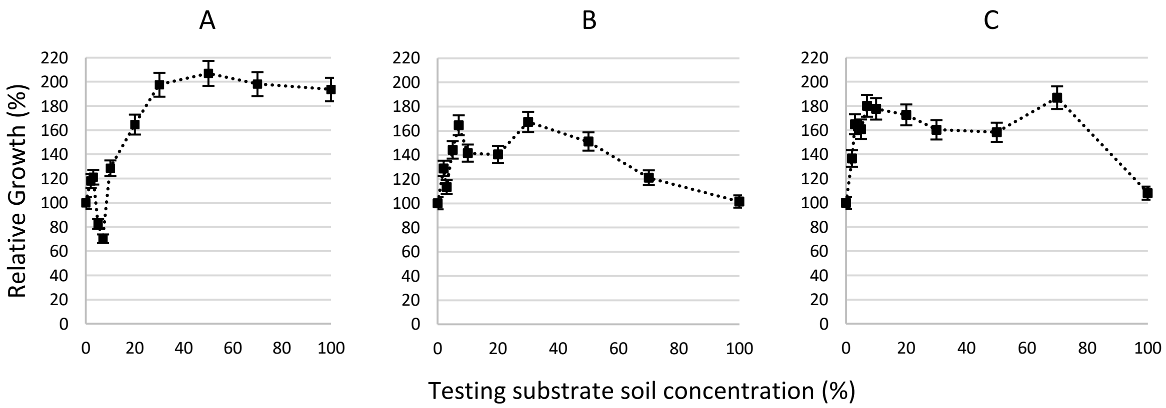

Fig. 7.6. Earthworm percentage Relative Growth (RG%) for initial (A), intermediate (B), and final (C) substrate soil. Deviation bars refer to the 95% confidence level.

Table 7.6. Summary of RS% and RG% referred to the bioassay with 100% test-substrate soil. * denotes a statistically significant difference. Different apical characters indicate statistically significant differences among the substrate soil samples

	100% initial substrate soil	100% intermediate substrate soil	100% final substrate soil	p-value
RS%	100.00±0.0	100.00±6.2	96.40±10.7	0.8274
RG%	193.70±15.8 ^{X}	101.60±5.1 ^{YZ}	107.00±16.8 ^{Z}	0.0012*

7.3.2.3. Collembola bioassay

Endpoints results of toxicity tests on *F. candida,* expressed as Survival (Su), are reported in Table 7.7.

The average Su in the intermediate substrate soil (94.75%) was higher than the Su for the initial soil sample (92.50%); Su decreased to 90.50% in the final sample. These minimal variations of the Su were not statistically significant and were not likely related to the applied phytotreatment process: the values of the intermediate and final samples are very close to the Su of the initial substrate soil, but the latter was sampled before the start of the test.

Table 7.7. Survival rates (Su) of *F. candida* bioassays

	Control (Su %)	Initial substrate soil (Su %)	Intermediate substrate soil (Su %)	Final substrate soil (Su %)	p-value
F. Candida	100±0	92.50±3.5	94.75±2.8	90.50±1.7	0.156

7.3.2.4. C. elegans and S. carpocapsae nematode bioassays

Results of the ecotoxicity tests on the nematodes *C. elegans* and *S. carpocapsae* are reported in Table 7.8. As already noticed for the *F. candida* assays, minimal variations (not statistically significant) were detected for all the endpoints considered (survival, growth, fertility, and reproduction) among the three substrate soils. Again, these minimal variations were not likely related to the applied phytotreatment process.

Table 7.8: *C. elegans* and *S. carpocapsae* nematode average endpoint results

		Control	Initial substrate soil	Intermediate substrate soil	Final substrate soil	p-value
C. elegans	Survival (%)	100±0	99.25±0.9	99.75±0.5	99.50±1	0.716
	Growth (µm)	1325±64	1275±28	1313±62	1350±57	0.181
	Fertility (%)	100±0	91.50±5.9	96.75±5.1	96.50±5.1	0.250
	Reproduction (N° egg/female)	22.25±1.7	19.00±1.4	21.25±1.7	20.75±0.9	0.108
S. carpocapsae	Survival at 24 h (%)	100±0	95.25±3.3	94.00±3.5	95.00±3.6	0.900
	Survival at 48 h (%)	100±0	91.00±4.2	89.00±3.5	92.00±3.1	0.608

7.4. CONCLUSIONS

The aim of this study was to provide a contribution to the current Italian legislation regarding the properties of the substrate soil used for the leachate phytotreatment process on the top of closed landfills. The results of the chemical analyses were compared to the reference values for soil contamination. Almost all the parameters were below the reference values, except for selenium, which exceeded the reference even in the initial sample. The tests on earthworms did not present any critical results; in fact, the survival percentages remained close to 100% and the growth results were equal or even higher than the control value, especially in intermediate and final substrate soil samples. The same consideration is valid for the bioassays in which *L. sativum* was used, which did not show significant variations in the Germination Index trend. The four endpoints of the nematode *C. elegans* (survival, growth, fertility, and reproduction) and the survival percentage results of the springtail *F. candida* and nematode *S. carpocapsae* also demonstrated that the three sample types did not affect the behaviour of these invertebrates.

The minimal quantity of contaminants detected in the substrate soil at the end of the test could be linked to phytotreatment activity by the sunflowers but further studies are required to understand the pathways of contaminants removal (e.g.: plants uptake, microbial degradation).

The results of this research indicate that phytotreatment on the top of closed landfills is a feasible option for in-situ leachate management. However, it is important to implement additional researches, for example by changing the quality of the leachate, the quality of the substrate soil, and by increasing the number of model and focal species in the ecotoxicological tests.

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Chapter 8: Energy crops on landfills: functional, environmental, and costs analysis of different landfill configurations

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Abstract

The cultivation of energy crops on landfills represents an important challenge for the near future, as the possibility to use devalued sites for energy production is very attractive. In this study, four scenarios have been assessed and compared with respect to a reference case defined for northern Italy. The scenarios were defined taking into consideration current energy crops issues. In particular, the first three scenarios were based on energy maximisation, phytotreatment ability, and environmental impact, respectively. The fourth scenario was a combination of these characteristics emphasised by the previous scenarios. A Multi-Criteria Analysis, based on economic, energetic, and environmental aspects was performed. From the analysis, the best scenario resulted to be the fourth, with its ability to pursue several objectives simultaneously and obtain the best score relatively to both environmental and energetic criteria. On the contrary, the economic criterion emerges as weak, as all the considered scenarios showed some limits from this point of view. Important indications for future designs can be derived. The decrease of leachate production due to the presence of energy crops on the top cover, which enhances evapotranspiration, represents a favourable but critical aspect in the definition of the results.

Keywords: energy crops; landfills management; scenarios evaluation; multi-criteria analysis; energy maximisation; phytotreatment ability; environmental impact

8.1. INTRODUCTION

Nowadays, advanced industrial societies still depend on fossil fuels. However, in the last decades, industrial and scientific efforts have been made toward renewable sources (Lavagnolo et al., 2011). The European Union strongly supports the production of renewable energy (EC, 2009). Specifically, Directive 2009/28/EC sets targets for State Members to reach the so-called '20-20-20 objectives' by 2020: 20% reduction of greenhouse gas emissions (compared to 1990 levels); 20% reduction of energy consumption; 20% of energy consumption from renewable sources. The new EU Framework for climate and energy (EC, 2014) sets even more stringent targets to be reached by 2030, including a 40% reduction in greenhouse gas emissions (compared 1990 levels) and at least a 27% share of renewable energy consumption.

The use of energy crops, fast-growing plants, aimed to produce biofuels or generate energy (e.g. electric energy from biomethane), represents an important alternative to traditional sources of energy, with important advantages also with respect to environment and agricultural and economic development (Garbo et al., 2017; Koçar and Civaş, 2013; Mench et al., 2009).

However, the cultivation of energy crops to produce significant amounts of biomass may lead to the use of not-sustainable irrigation rates, resulting in potential shortages in the supply of fresh water (Zema et al., 2012).

Nowadays, more than 70% of water consumption is due to agricultural activities (FAO, 2015; Oviedo-Ocaña et al., 2017; United Nations, 2015), and an additional 19% increase by 2050 has been estimated (United Nations, 2015). The exploitation of unconventional water resources (like landfill leachate) could be considered an optimal compromise between different needs: simple and low-cost wastewater treatment (Carvalho et al., 2014; Mench et al., 2009; Vangronsveld et al., 2009), plants growing for energetic purposes, and conservation of water storages (Garbo et al., 2017). Irrigation with diluted landfill leachate may support plants growing, with such plants contributing to the removal of contaminants (Garbo et al., 2017; Lavagnolo et al., 2016). In this work, the cultivation of energy crops on landfills was analysed. Several scenarios were defined and compared with a reference case, a representative average landfill in northern Italy. The comparison was carried out through a multi-criteria analysis (MCA), based on three criteria: economic, correlated with the cost of each intervention; energetic, linked to the potential energy production during the whole landfill life cycle; and environmental, related to the impacts of the intervention on the environmental components near the landfill site.

8.2. MATERIALS AND METHODS

The research activity was performed in the following steps:

- Criteria definition: economic, energetic, and environmental criteria were defined in order to analyse and compare different scenarios of landfill configurations.

- Scenarios definition: a reference scenario (scenario "zero") and other four scenarios were defined. Current energy crops issues such as energetic potential, the possibility of leachate phytotreatment, as well as environmental impact were considered.
- Multi-Criteria Analysis: the scenarios were compared by means of an MCA, with equal weight to each criterion.

8.2.1. Criteria definition

The definition of the criteria was fundamental for the analysis of scenarios in the MCA. Three criteria were considered: economic, which evaluated the total landfill cost (€); energetic, which considered the energy net gain for the whole landfill cycle (GJ) and environmental, which defined the mean biopotentiality index for the landfill site ($Mcal/m^2/y$).

8.2.1.1. Economic criterion

The total landfill cost was defined using quantity bills and drafting financial plans for each scenario, from official price lists and market surveys. Costs were evaluated through the whole landfill life cycle, thus considering the phases of design and authorisation, construction, operation, and aftercare. The last phase consists of monitoring and maintenance activities, which mainly relate to: top cover, leachate collection system, landfill gas collection system and migration control, groundwater and surface water, as well as security and ground stability. The economic analysis for the determination of the landfill costs was based on the technical procedures used in the bill of quantities, in which a generic item of cost is determined applying the following expression:

$$Cost[€] = Quantity\ [reference\ unit]\ * Unit\ cost\ \left[\frac{€}{reference\ unit}\right] \tag{8.1}$$

The cost of the item refers to the cost of the specific intervention or operation considered (e.g. activity, equipment, material) and the reference unit of each item was selected each time as the most suitable unit of measure (e.g. m^3, m^2, m, t, body). Unit prices were found on the official price lists of the Veneto region (Regione Veneto, 2013), Lombardia region (Regione Lombardia, 2011) and from market analysis.

The calculation of the final total landfill cost for each scenario allowed to define the values to fill in the evaluation matrix for the MCA ($x_{economic}$). The following expression was used:

$$x_{economic} = C_{reference} - C_i \quad (8.2)$$

where:

- $x_{economic}$ includes the additional costs or savings compared to the reference scenario;
- $C_{reference}$ is landfill cost of the reference scenario;
- C_i is the cost of the $_i$-th scenario.

8.2.1.2. Energetic criterion

The energetic criterion evaluated the cumulative energy net gain as the difference between energy input and output. The operational phase duration was assumed to be 10 years, that is, the mean duration resulting from a statistical investigation of 15 landfills located in northern Italy, while the aftercare duration was set by EU regulation to 30 years. Therefore, the criterion was evaluated on a time scale of 40 years.

The Joule was adopted as the unit of measurement (Angelini et al., 2009; Fiala et al., 2010; Nassi o Di Nasso et al., 2010).

Inputs were estimated for each vegetal species, considering both direct and indirect factors and correlating them with the surface. Direct energy inputs were calculated by multiplying the energy equivalent of fuel, fertilizers, herbicides, seeds, and manpower by their quantities, defined according to the needs of each species (Table 8.1). Seeds, manpower, and other productive inputs were directly estimated using experimental data depending on the specific crop considered (Angelini et al., 2009; Fiala et al., 2009; Nassi o Di Nasso et al., 2010; Venturi and Venturi, 2003). Indirect energy, which is the fossil energy consumed for production manufacturing (Nassi o Di Nasso et al., 2010) and not often taken into account due to its difficult quantification, is reported to have a moderate impact on the total energy input value (Fiala and Bacenetti, 2009). For the sake of caution, in this work it was assumed such an impact to be 10% of direct energy. The choice of using simplified assumptions could be limiting but is due to the complexity of the analyzed system, in which specific values of inputs and outputs cannot be estimated a priori.

Table 8.1. Direct energy inputs values adopted in the calculations (Venturi and Venturi, 2003).

Direct input	Energy value
Fuel (use of machines, etc.)	47.8 MJ/L
Nitrogen fertilization (Urea)	76 MJ/kg
Phosphorous fertilization (P_2O_2),	14 MJ/kg
Potassium fertilization (K_2O),	10 MJ/kg
Herbicides	202 MJ/kg

Instead, energy outputs values were determined coupling agricultural production data (and thus crop yield, expressed as t/ha) with specific energetic characteristics (Lower Heating Value, LHV) of final crop products (grain, oil, or biomass) (Venturi and Venturi, 2003).

It must be noted that, in this energy analysis, output and input were not strongly correlated: indeed, it is not always true that a final low (or high) energy input results into low (or high) energy output (Venturi and Venturi, 2003). For this reason, an experimental data collection would be useful to minimize such uncertainties.

The energy net gain of the i-th scenario (E_i) was calculated as:

$$E_i[GJ] = \sum_j Energy\ output_j - \sum_k Energy\ input_k(direct\ and\ indirect) \quad (8.3)$$

After the calculation of the energy net gain for each scenario, the values to be included in the MCA evaluation matrix ($x_{energetic}$) were assessed using the following formula:

$$x_{energetic} = E_i - E_{reference} \quad (8.4)$$

where:

- $x_{energetic}$ is the energy gain or loss compared to the reference scenario;
- E_i is the energy net gain of the i-th scenario;
- $E_{reference}$ is the energy net gain of the reference scenario.

8.2.1.3. Environmental criterion

The environmental criterion took into consideration the effects on the environment caused by energy crops. In this work, the indicator adopted was a common index in the field of Landscape

Ecology: the biopotentiality index or biological territorial capacity (BTC) of vegetation. The BTC is measured in Mcal/m^2/year and represents the latent energy of a given site, that is, the energy that a vegetative system has to dissipate in order to maintain the degree of organisation (Fig. 8.1) (Pivato et al., 2013). The time-evolution analysis of BTC for a specific site allows to assess the landscape transformation. In particular, a decrease in the BTC value generally corresponds to a degradation of the site, because of a net loss in its self-rebalancing capabilities. On the contrary, an increase in the BTC value results in an improvement of the site quality (Ingegnoli, 2015).

The procedure followed for the calculation of the mean BTC values can be summarised in three fundamental points (Pivato et al., 2013):

- Establishment of the proper scale for the analysis (spatial-temporal);
- Definition of BTC_i for each landscape element;
- Evaluation of the mean BTC through a Monte Carlo method.

A proper choice of the spatial-temporal scale is crucial, as it determines the limits for the applicability of the analysis itself (Pivato et al., 2013). The time scale must allow the comparison of the state of the landfill site before the operational phase with that at the end of the aftercare phase, avoiding longer horizons that could make the results unrealistic. Moreover, the spatial scale must not be too narrow to avoid errors in the assessment specificity. The choice of a spatial scale of 300 m (measured from the landfill perimeter) allowed to obtain an average response of the landfill site and its surrounding areas. In this case, the surroundings were assumed to be mainly agricultural areas.

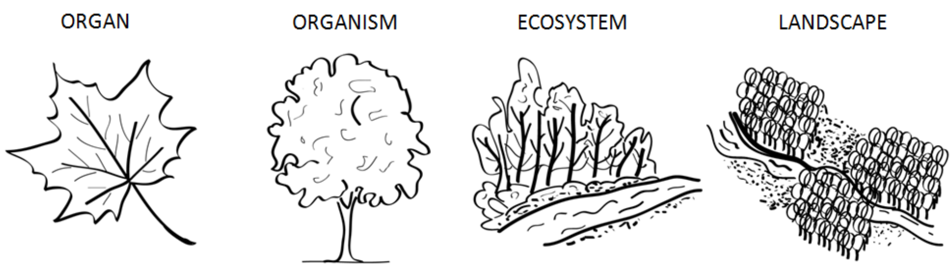

Fig. 8.1. Different levels of organization of the ecological systems (adapted from Ingegnoli, 2015)

Specifically, the BTC was defined for each landscape element, considering data from Ingegnoli (2011), Ingegnoli and Giglio (1999), and Pivato et al. (2013) (Table 8.2).

The mean BTC (BTC_{mean}) was calculated using (Pivato et al., 2013):

$$BTC_{mean} = \frac{(\sum_i S_i * BTC_i)}{S_{domain}}, \tag{8.5}$$

where BTC_{mean} (Mcal/m^2/year) is the mean Biopotentiality related to the spatial domain considered, S_{domain} (m^2), and BTC_i (Mcal/m^2/year) is the biopotentiality of the *i*th landscape element characterised by a surface S_i (m^2).

A probabilistic approach to minimise uncertainty and potential errors in the BTC_i value definition was considered. A Monte Carlo method was adopted, which is based on the random sampling from each distribution probability of the variables and their successive combinations, starting from an analytical formulation fixed by the user (Eq. (8.5) in this case). The variables corresponded to the BTC_i values, and were defined by a log-normal Probability Density Function (PDF). The variance of each variable was assumed to be equal to 10% of its average value: this seems to be realistic since, in this way, an increase of landscape ecological complexity would imply a higher uncertainty about the variable itself. The variables were assumed to be independent. Although unrealistic, the high quantity of data needed did not allow a different assumption (Pivato et al., 2013).

As for the previous criteria, the values to be included in the MCA evaluation matrix ($x_{environmental}$) were calculated using the formula:

$$x_{environmental} = BTC_i - BTC_{reference} \tag{8.6}$$

where:

- $x_{environmental}$ is the environmental benefits or degradation in terms of biopotentiality compared to the reference scenario;
- BTC_i is the biopotentiality of the i-th scenario;
- $BTC_{reference}$ is the biopotentiality of the reference scenario.

Table 8.2. BTC_i values assumed for the landscape elements used in the analysis

Landscape elements	BTC_i (Mcal/m^2/year)
Landfill in operation	0.40
Service area	0.30
Artificial water channel	0.20
Leachate and LFG treatment, temporary storage, wastewater treatment	0.30
Roads	0.40
Annual crop field	0.80
Simple crop field	1.30
Grass	0.70
Shrubs and grass associations	2.40
Woods plantation	3.10

8.2.2. Scenario definition

Each scenario should represent a possible solution for energy crops cultivation on landfills. In particular, the scenarios were chosen according to the energetic characteristics, the phytotreatment efficiency and environmental impact of energy crops. Factors as climatic conditions and relationship between crop and site characteristics were considered (Venturi and Venturi, 2003; Zegada-Lizarazu and Monti, 2011). The assumptions underlying each scenario have been itemized in Annex 8.1.

8.2.2.1. The reference scenario (scenario "zero")

The reference scenario was based on the design of a modern landfill model representing the main geometry (volume and surface) and the constructive characteristics of landfills in northern Italy. The landfill was assumed to collect non-hazardous waste (Municipal Solid Waste and Special Waste). The design was performed according to Italian legislation (D.Lgs. 36/2003), following national landfills guidelines (CTD, 1997; DGR X/2461/2014) and best practices.

The model landfill is underground (60% of the investigated landfills are underground), realised in a gravel pit, with a total waste volume of 800000 m^3 and a surface, at the ground level, of 50000 m^2. The height of the waste mass (excluding daily, temporary, and top cover system) is about 23 m. The landfill is rectangular, with a top cover characterised by two parts: the upper central part with a slope of 4% and a surface of 21417 m^2, and the remaining part with a slope of 24% and a surface of 29412 m^2. The landfill is subdivided into 4 sectors.

The leachate collection system was designed according to previous calculations (Canziani and Cossu, 1989). Specifically, calculations on leachate production (Blakey, 1992; Canziani et al., 1989) show a maximum of 5500 m^3/year in the operational phase and a constant production of 2027 m^3/year in the aftercare. The total leachate produced in 40 years is calculated to be 103366 m^3 (42540 m^3 in the operational phase and 60826 m^3 in the aftercare). The leachate is collected and stored in four fibreglass tanks of 100 m^3 located within a concrete-made containment basin of 420 m^3, representing a safety measure in case of failure. The full procedure for the calculation of leachate produced is described in Annex 8.2.

The landfill gas extraction system was designed according to the quantity produced (about 16 million Nm^3 of biogas in 40 years), estimated with the model suggested by Cossu et al. (1992). The full procedure for the calculation of biogas produced is described in Annex 8.2.

Energy recovery was not considered in the analysis, as in modern landfills the disposal of inert and stabilised wastes minimises the biogas emission potential (DGRV n. 995/2000). A torch was included, designed in accordance to D.Lgs. 36/03 and CTD (1997).

The service area has a surface of 3950 m^2, including temporary storage, tire washing system, truck scale, office building, and vehicles parks.

The landfilled waste was assumed to be 30% Municipal Solid Waste (MSW) and 70% Special Waste, coming from separate collection systems. The waste characterisation assumed was as follows: paper (1.5%), cardboard (1.5%), glass and inert (52%), plastic (12%), metals (3%), stabilised inert (15%), and sludge (15%). The waste density was 1.1 t/m3, resulting in a daily waste inflow of 240 t/day (approximately 88000 t/year); therefore a total amount of 880000 t of waste was disposed during the operational phase, assumed to last for 10 years. All the values were assumed in accordance to the data from the statistical investigation on 15 MSW landfills located in northern Italy.

The application of a simple grass cover over the landfill allows to easily compare the interventions planned in the other scenarios. The reference scenario is represented in Fig. 8.2.

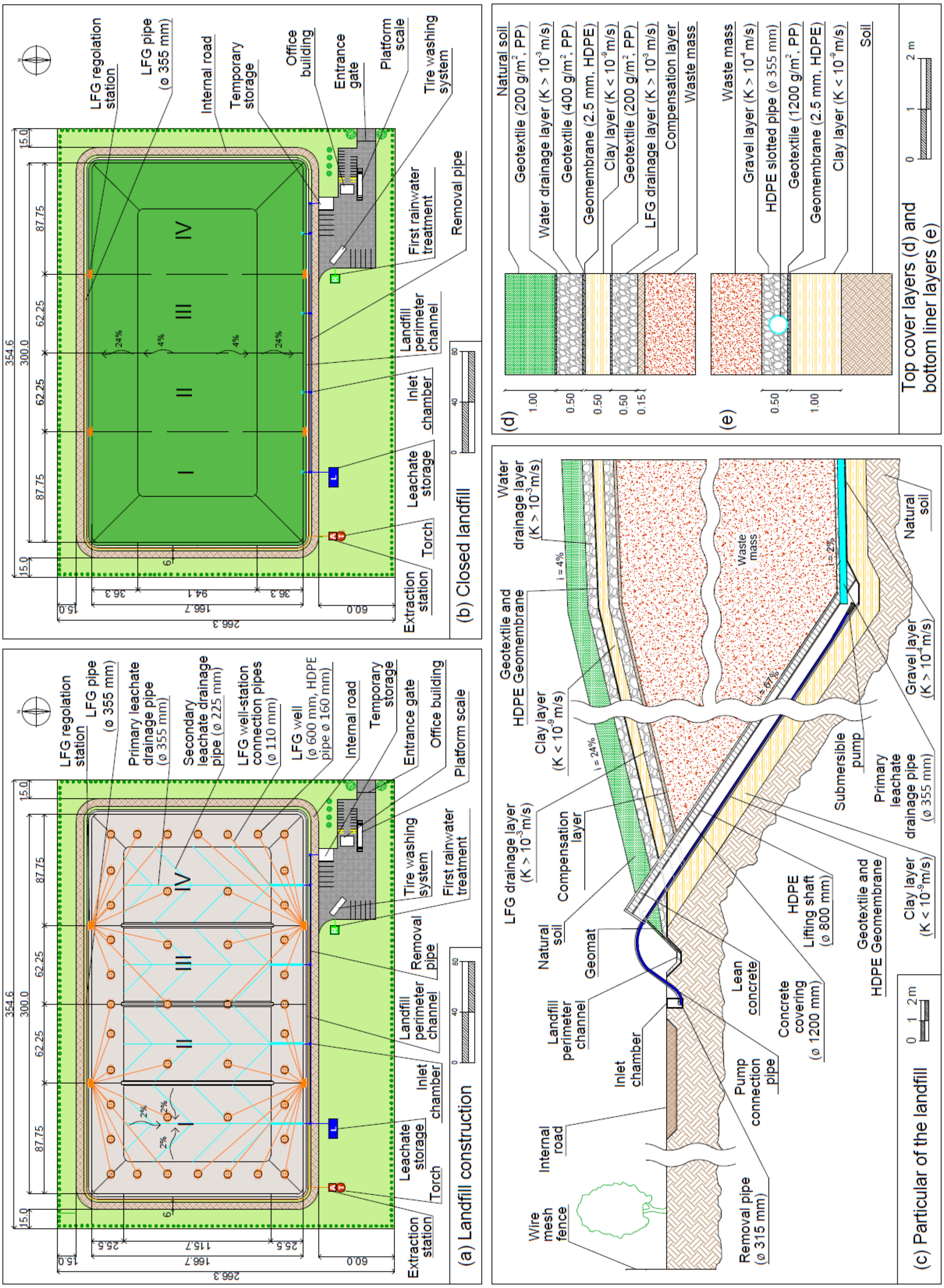

Fig. 8.2. Reference scenario: planar view of the landfill site during the construction phase (a); planar view of the landfill site after the closure (b); section of the landfill after the closure in which the leachate drainage system is emphasized (c); particular of the final top cover (d) and bottom liner (e) of the landfill

8.2.2.2. The first scenario (energy maximisation scenario)

This scenario was defined considering the energetic potential of a promising crop, Miscantus (*Miscanthus x giganteus*). In particular, the choice aimed to guarantee a positive energy balance between inputs and outputs, thus characterised by a significant energy net gain. Miscanthus seems to be very promising from this point of view (Venturi and Venturi, 2003), as it is a herbaceous plant with low nutrients requirements, low weeds and pests risk, and very high biomass yield. It is a perennial essence, harvested yearly between autumn and late winter. The product can be managed similarly to hay grass, with a reduction of the biomass produced in mown bales.

In this scenario, Miscanthus plantation on the landfill top cover was planned during the aftercare phase (Fig. 8.3). A Miscanthus lifetime of 15 years was assumed. After that, the whole Miscanthus plantation was assumed to be removed and then reinstalled, thus allowing another cycle until the end of the aftercare (30 years).

The whole top cover surface with small slope (4%) was assumed to be cultivated, for a total of 21417 m^2. In order to prevent potential problems of liners damaging due to roots penetration, an additional 0.5 m thickness of natural soil was considered to be added to the final top cover. Indeed, Miscanthus plants can have a deep root mat: depths of 2 m are not unusual. However, the high density of the roots system can prevent water leaching through the top cover system, thus decreasing leachate production (Lewandowski et al., 2000). The leachate production was estimated with the commercial software "Visual HELP", which can include the different thickness and composition of the top cover. Moreover, an increased evapotranspiration was considered. The results, after proper software calibration, showed a decrease in the leachate production by about 91 m^3/year (total reduction of 2745 m^3 in 30 years) in the aftercare as compared to the reference scenario.

It was assumed no biomass production in the first year of installation, 10 tonnes in the second, 20 in the third, and 25 from the 4^{th} to the 15^{th} year (Lewandowski et al., 2000; Veneto Agricoltura, 2010).

A small, artificial water pond of 550 m^3 was considered in order to guarantee the water self-supply and irrigation independence of the landfill site.

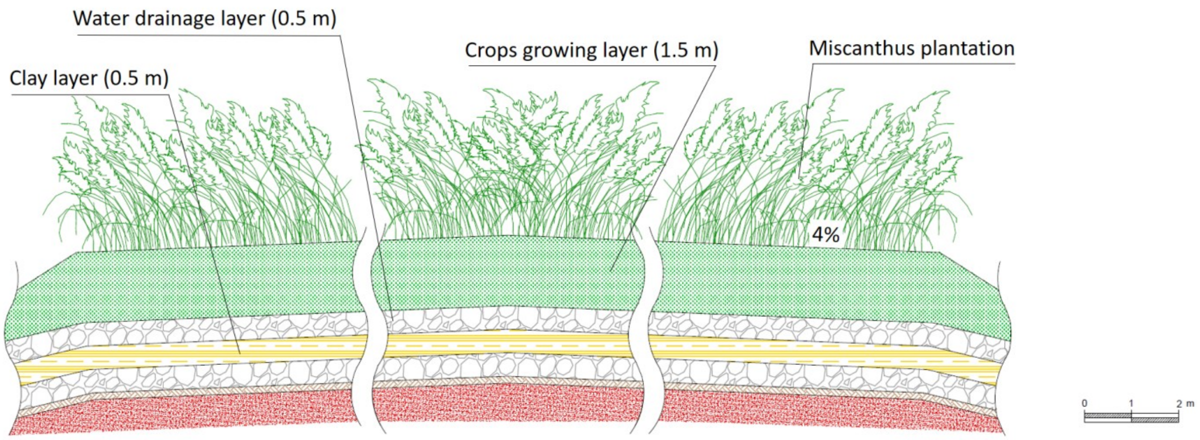

Fig. 8.3. First scenario. Miscanthus plantation on the landfill top cover during the aftercare phase

8.2.2.3. The second scenario (phytotreatment scenario)

The scenario was defined taking into consideration the leachate phytotreatment capacity of some oily energy crops. In this respect, several authors highlighted their effectiveness on wastewater and leachate contaminant abatements (Garbo et al., 2017; Jones et al., 2006). In this scenario, the application of Sunflower (*Helianthus annuus L.*), Rapeseed (*Brassica napus)*, and Soybean **(***Glycine max***)** was considered. Garbo et al. (2017) and Lavagnolo et al. (2011, 2016, 2017) reported good performances, achieving efficiencies higher than 80% for COD removal, higher than 70% for total N, and higher than 95% for total P removal, due to the soil and plant synergic effects.

The rotation was assumed to be biannual: Sunflower in spring-summer and Rapeseed seeding in autumn in the first year, Rapeseed harvesting at the end of the spring and Soybean application in summer in the second year. The yearly seed productions were assumed to be 2 t/ha years for Sunflower, 2.2 t/ha for Rapeseed, and 2.3 t/ha for Soybean (Karmakar et al., 2010).

Crops were assumed to be cultivated on phytotreatment basins created by proper surrounding clay levees, in order to avoid the dispersion of the phytotreatment outflow. These basins were installed on the top cover of the landfill, in the area with lower slope (Fig. 8.4). According to Garbo et al. (2017) and Lavagnolo et al. (2016), basins require at least two horizontal layers: a bottom drainage layer to collect the treated leachate and a crops-growing layer. A value of 0.3 m of thickness of crops growing layer was considered sufficient to permit the contact between the plant's roots system and the leachate to be treated, as well as the correct performance of soil tillage works. The phytotreatment basin cannot be built on the final top cover (D.Lgs. 36/2003; DGR X/2461/2014), therefore it was realised on the temporary cover, characterised by the same composition of the final top cover but with a natural layer of 0.3 m instead of 1 m. Then, this temporary top cover could be easily transformed into a final top cover at the end of agricultural activities, by simply adding 0.7 m thickness of natural soil. The layer's composition for the temporary and the final top cover is shown in Fig. 8.5. Note that, by comparison with the scheme adopted for the reference scenario (Fig. 8.2), an additional 0.5 m thickness of clay is also considered, in order to minimise potential infiltrations to the landfill body.

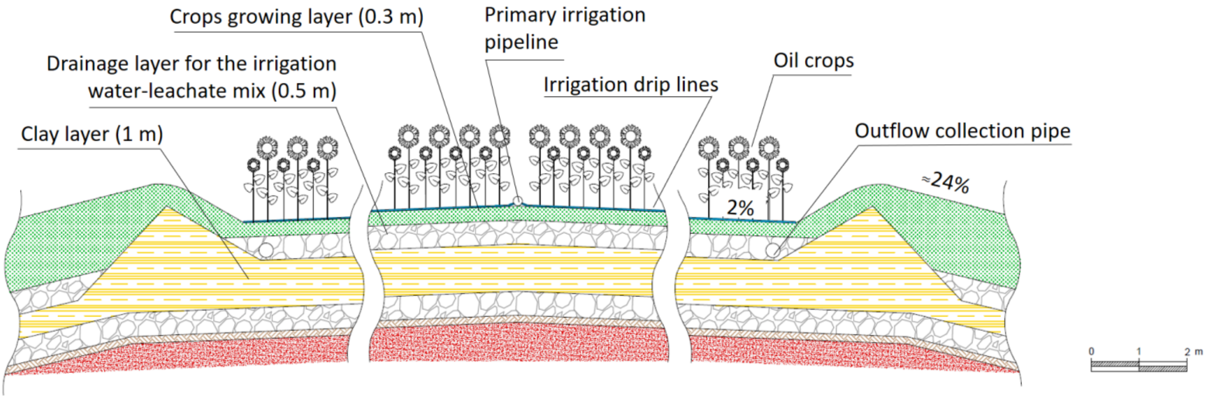

Fig. 8.4. Second scenario. Phyotreatment basins installed on the top of the temporary cover

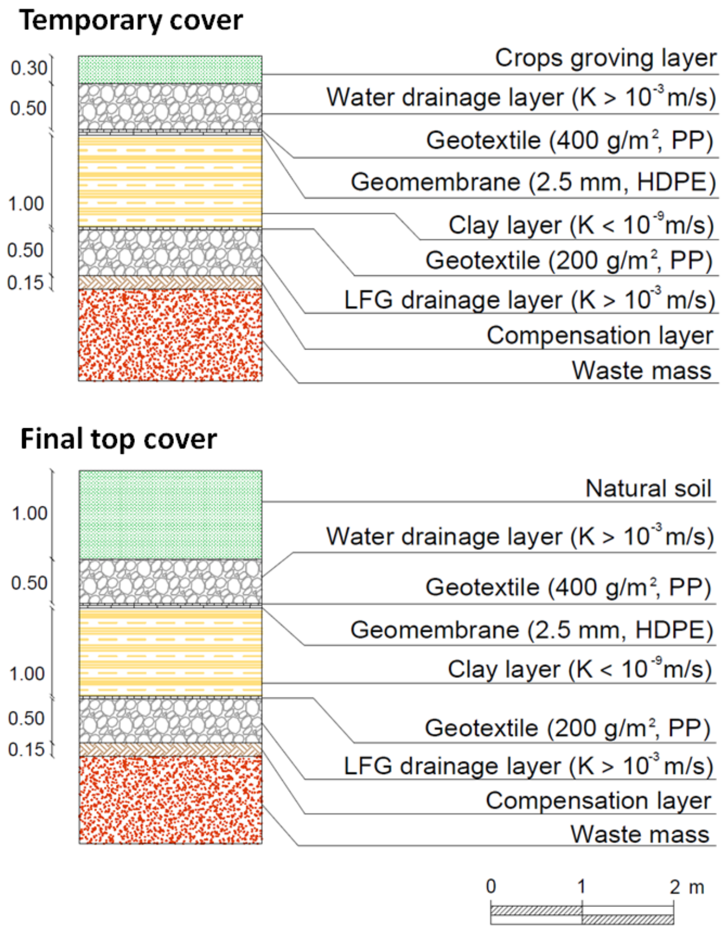

Fig. 8.5. Temporary and final top cover adopted for the phytotreatment basins in the second scenario

The assumption on the use of a temporary cover also affects the period length for energy crops cultivation, since it is directly correlated with the landfill operation phase. Indeed, the aftercare phase can only start once the final top cover is installed. A proper preliminary analysis shown that the best solution was the use of the first two closed sectors for the phytotreatment basin. A surface of 6425 m^2 was estimated, allowing the treatment of about 4440 m^3 of leachate in four years of activities. The leachate quantity introduced directly into the phytotreatment system should be diluted with rain water. This quantity was calculated as the percentage of the total inflow flux of the phytodepuration basin and was not fixed a priori, since it mainly depends on the response of the plants. In the current analysis, a percentage of 30% was considered, as in Garbo et al. (2017). The inflow flux was calculated assuming a mean porosity of 30% within the phytotreatment basin (range 20-50%) and an average Hydraulic Retention Time (HRT) of 15 days (range 7 days-1 month). The mean outflow from the phytotreatment process was assumed to be 50% of the inflow, as in Garbo et al. (2017) and Lavagnolo et al. (2016). Therefore, the total outflow was 7401 m^3 in four years of activities. This rough estimation is affected by many uncertainties: an accurate hydrological balance should be developed, which would allow to take into consideration seasonal variations in precipitations, irrigation, and plant water requirement, as well as other important aspects such as evapotranspiration, evaporation, or soil humidity regulation processes.

A small pond of 500 m^3 collecting rainwater was considered, in order to ensure the complete water self-supply of the site even during drought periods. Due to the presence of the phytotreatment basins on the top cover, the software Visual HELP showed a decrease in leachate production with respect to the reference, for a total of about 1336 m^3 in the 4 years of phytodepuration in the operation phase (total cumulative of 42540 m^3 in the reference versus 41204 m^3 of this case).

Additional monitoring investigations were considered during the phytotreatment period. The most important one regarded the periodic soil sampling (concentrations of contaminants in the soil should not exceed the screening values defined by the D. Lgs. 152/2006 for potentially contaminated soils) and the chemical analysis of the inflow and outflow liquid of the phytotreatment.

8.2.2.4. The third scenario (environmental compensation scenario)

The scenario was defined by focusing on the environmental impacts of energy crops. However, in the analysis, it must be noticed that energy crops were grown in a territory already transformed and damaged (by the landfill construction, but also previously by the gravel pit). In this sense, the introduction of energy crops may help to reduce the negative effects of the intervention. For instance, the use of wood plantations for biomass production is a common practice, with well-known advantages on ecological (biodiversity increase, CO_2 adsorption, wildlife habitat, etc.), protective (soil protection from erosion, etc.), sanitary (defence from noise and contaminants, etc.), and aesthetic (recreational and touristic activities) aspects (Santacroce et al., 2007).

In this scenario (Fig. 8.6), the following interventions were assumed:

- Creation of a green belt mainly made of poplars, around the landfill perimeter. A medium cutting frequency of 5 years was planned (Medium Rotation Forestry, MRF). This type of installations is characterised by a life time of 15 years: for this reason, two installations were planned during the 30 years of the aftercare phase. After that, plants were not removed but left as a frame to the recreational area. A planting system with plants spaced 3 m each to the others (both on rows and intrarows) allowed the installation of 870 plants on a surface of 7020 m^2. The value is in accordance with the ranges defined in the literature for MRF (1100 and 1500 plants/ha, Bergante and Facciotto (2006)). The crop yield was assumed to be 80 t/ha and constant for every cycle of cuttings (range around 60 -120 t/ha, Santacroce et al. (2007) and Fiala et al. (2010)).
- Creation of a wood plantation located along the south side of the landfill. The plantation has the same characteristics as the green belt in the previous point. In this case, 756 Poplar plants were assumed to be installed on a surface of 6175 m^2.
- Shrubs were considered to be planted on the landfill top cover, by creating shrubs spots of about 20 plants each (about 40 spots/ha). The installation of shrubs allows a better landfill inclusion in the landscape, while promoting the development of a cenosis and improving the ecological value of the area. Several shrubs species, belonging to the autochthonous vegetation heritage of the site, could be grown. The introduction of shrubs is accompanied by the creation of walk paths, allowing the exploitation of the surface as a recreation area.

The choice of shrubs instead of poplars on the top cover is due to the risk of roots penetration: although poplars can be considered the type of plants that are the closest match for a compensatory measure, their roots can damage the landfill top cover liners.

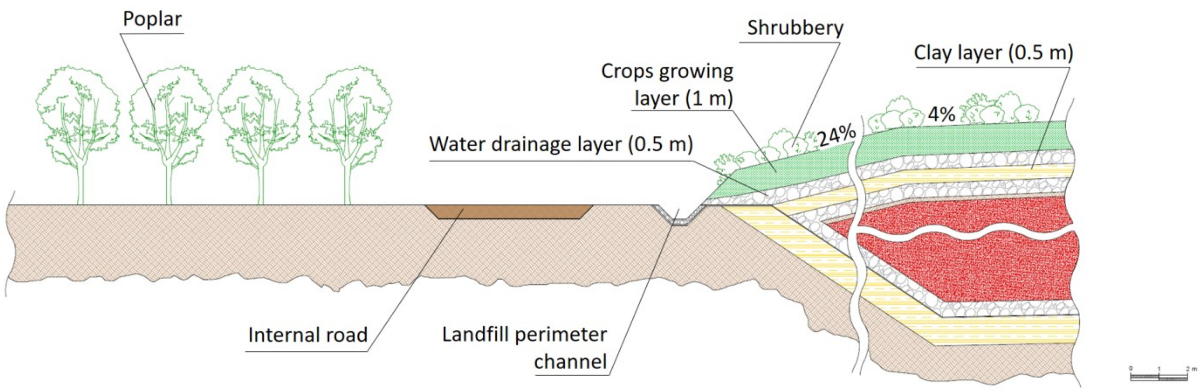

Fig. 8.6. Third scenario. Shrubs and poplars plantations on the landfill top cover and around the landfill perimeter, respectively

8.2.2.5. The fourth scenario (combination scenario)

This scenario combined the solutions adopted in the other three scenarios (Figs. 8.3, 8.4, 8.5, and 8.6). In particular, it assumed an oil crops phytotreatment field during operation in the first two closed sectors (as in the leachate phytotreatment scenario), the Miscanthus cultivation on the top cover in the aftercare (as in the energetic maximisation scenario), and poplars and shrubs plantation (as in the compensation scenario). The characteristics of these interventions are similar to those previously explained in detail for each scenario. However, in this case, shrubs installation on the top was planned only at the end of the aftercare phase, after the removal of Miscanthus. This scenario allowed to combine the specific characteristics of energy crops emphasised by each of the other scenarios (Fig. 8.7).

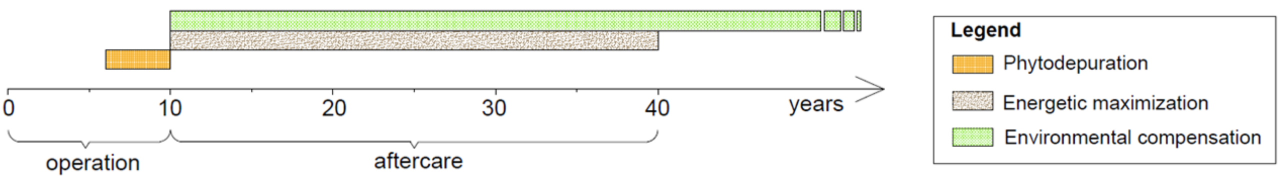

Fig. 8.7: The fourth scenario, combination of the other scenarios over time

8.2.3 Multicriteria analysis

A multicriteria analysis (MCA) was performed in order to compare the different scenarios. In this analysis, how criteria are weighted is very important, since it defines criteria priorities. However, different actors can express contrasting views (even if legitimate) about a certain topic and the solution can sometimes reflect their preferences. The weighting process is thus highly discussed, with frequent misunderstandings. In this case, it was assumed the same weight for all the three criteria considered.

The evaluation matrix was composed by the x_i values (with i = economic, energetic, or environmental according to the criterion) as previously defined (Eqs. (8.2), (8.4), and (8.6)). The built matrix was then linearised using a simple interval standardisation:

$$\frac{x_i - x_{min}}{x_{max} - x_{min}} \tag{8.7}$$

where x_{min} and x_{max} are, respectively, the minimum and the maximum values in the scenarios for the i-th criterion. This linearisation allowed to refer to the values of each criterion by assigning “0” to the minimum value and “1” to the maximum one. The criteria were assumed to have the same weight (w_i). Then, the best scenario was represented by the scenario satisfying the following equation:

$$\text{Best scenario} = \text{Max} \sum_i x_i * w_i = \text{Max} \sum_i x_i * 1/3 \tag{8.8}$$

8.3. RESULTS AND DISCUSSION

The results for economic, energetic and environmental criteria are reported in Tables 8.3, 8.4 and 8.5, respectively. Additional information on the calculations are available in Annex 8.3.

The total cost for the reference scenario was calculated to be 68833045.81 € (Table 8.3).

In the first scenario, the landfill cost was 446060.98 € more than the reference scenario. The main difference arises from the construction phase costs, where the increase is due to the addition of 0.5 m thickness of natural soil in the cultivated field, and from the investment in an irrigation system and water pound construction.

The operational costs of this scenario do not substantially differ from the reference. Savings mainly derive from the agricultural activity and the leachate management during the aftercare. The agricultural works considered in the calculations varied across years. Specifically, in the first year, great efforts were required for the Miscanthus installation (soil tillage operations and transplantation of Miscanthus roots). The prices considered for agricultural works mainly refer to the agromechanical works price list of the province of Verona (A.P.I.M.A. Verona, 2011). It must be pointed out that the economic values of agricultural activities and their revenue are subject to significant annual fluctuations, depending on the market request; such fluctuations are not considered in the present work.

In the second scenario, the landfill cost was 119452.35 € higher than the reference. Differences in landfill management costs emerged mainly in the operational phase. The reduction in the quantities of leachate produced, due to the placement of the basins on the top cover, allowed savings for 53447.90 € on leachate management costs. Moreover, savings for 29606.40 € were obtained from the leachate phytotreatment process. In this case, a unit cost of 20 €/m^3 for the outflow treatment was considered (half of what commonly assumed for leachate treatment), as the effluent exiting the phytotreatment basins not always meets the standards for direct discharge in water bodies (D. Lgs. 152/2006, Garbo et al., 2017; Lavagnolo et al., 2016).

The agricultural works considered were the same for each crop, including soil tillage operations, seeds sowings, chemical and mechanical weeding, harvesting, and transport of products. Fertilisation was not taken into because of the high nutrients content of the water-leachate mix.

As for the third scenario, the total landfill cost was 306025.78 € more than the reference. In this case, the total construction phase cost includes the cost for Poplar trees and shrubs species installation (132390.00 €). During the landfill aftercare phase, the agricultural works needed for the use of Poplar as energy crop (fertilisation, phytosanitary control, cuttings, transport, etc.) and those for the maintenance of shrubs and walk paths were included in the cost calculation. Revenues from the biomass sale were 49877.10 € in 30 years of aftercare.

In the fourth scenario, the total landfill cost was 356144.59 € more than the reference. The calculation was based on considerations made for the other scenarios and opportunely combined. The most important costs were in the construction phase. The operation and the aftercare phases allowed significant savings.

Note that, in all scenarios, the calculations of the revenues from agricultural activity were related only to the sale of final products, without considering public subsidies, land benefits, and other revenue sources.

The energy input (Table 8.4) was defined by calculating the values of the input variables during the production of Miscanthus and poplar. A constant input was calculated for sunflower, rapeseed, and soybean, respectively. The assumed values are listed in Annex 8.1.

As for the output calculation, the LHVs assumed are reported in Annex 8.1. This analysis did not include by-products. All the values used in the energetic analysis are consistent with the literature (Venturi and Venturi, 2003).

The energy net gain of the reference scenario was zero, as no energy crops were applied. The first and fourth scenarios, which were optimized for the energy generation, shown the highest values in terms of net gain in 40 years while the second scenario was characterized by a negative net gain (i.e. the inputs exceeded the outputs), mainly due to the energy effort required by the processes related to the oil production. The mean biopotentiality was calculated for all scenarios via Monte Carlo method implemented with the commercial software Crystal Ball. The calculated BTC_{mean} was the same for each scenario before the landfill operation, while at the end of the 40 years it was slightly higher for the third and fourth scenarios (Table 8.5). The results could have been strongly affected by possible miscalculation of the BTC_i values, especially with regard to the agricultural land, representing the predominant area (between 88.5% and 91.1% of the total area, depending on the scenario). Besides, the spatial scale assumed highlighted an important aspect. By considering either a larger or a smaller scale, the changes in BTC_{mean} could be less or more visible, respectively. From a practical point of view, this means that, despite the proposed interventions being important from an ecological perspective, they are usually really site specific and represent a local improvement. To achieve this perspective, the compensation applied to the landfill should be seen as part of a larger compensation measure, realised, for instance, through ecological corridors or green buffer zones (Johnson and Calhoun, 1999; Lineah et al., 1995).

The linearised evaluation matrix based on x_i values defined previously is shown in Table 8.6.

From the MCA, the fourth scenario resulted first in the final score among the cases considered, as it maximised both the energetic and the environmental criteria.

The cheapest solution corresponded to the reference scenario, where there were no interventions with energy crops. All the others presented additional costs. In particular, none of the solutions, where energy crop fields were placed on the top cover, seemed to be able to cover the additional investment costs required, especially if an increase in the top cover layer thickness was considered. For instance, the energetic maximisation scenario, without considering this cost, may also return a positive economic balance. The use of energy crops fitting energetic and environmental criteria without affecting the top cover composition should be better considered. However, from an economic point of view, good perspectives emerged in the leachate phytotreatment scenario. In this case, an effective solution may result from the consideration of longer time horizons for the leachate treatment, for instance by extending the application of the phytotreatment process in the aftercare phase. In this respect, the current normative restrictions related to the composition of the final top cover are limiting. A better integration of legislation with applications related to new technologies should be studied. Indeed, without these limitations, the final score obtained in the leachate phytodepuration scenario could be different, since some important economic savings could be possible.

The energetic and environmental criteria results were maximised when lignocellulosic crops were considered. As for the energetic criterion, the first scenario showed better results thanks to the higher crop yield offered by Miscanthus. The environmental criterion, instead, was better in the third and fourth scenarios, when evaluated after the end of the aftercare phase. It must be underlined that these considerations are based on data which could be subjected to many variations.

The introduction of the social impact as criteria of the MCA could be also interesting. The thought is that interventions, aimed to apply energy crops in sites such as those of landfills, should improve the social acceptance of the site, by creating new works places (agricultural activities), by improving the aesthetic vision of the site and by the creation of recreational areas.

Table 8.3. Results obtained from the analysis of the scenarios according to the economic criterion. The evidenced row represent the categories directly affected by the energy crops application.

Items		Cost (€)				
		Reference scenario	First scenario	Second scenario	Third scenario	Fourth scenario
1	Design and Authorization Phase	870303.61	897111.28	879417.67	881785.30	903059.05
1.1	Design and Authorization Cost	870303.61	897111.28	879417.67	881785.30	903059.05
2	Construction Phase	18031437.69	18486725.57	18186226.28	18226437.01	18587739.60
2.1	Area Acquisition	1417500.00	1417500.00	1417500.00	1417500.00	1417500.00
2.2	Construction Cost	10503795.11	10838890.95	10617720.86	10647316.31	10913238.16
2.2.1	Preliminary Works	164987.20	164987.20	164987.20	164987.20	164987.20
2.2.2	Morphological Shaping	271455.00	271455.00	271455.00	271455.00	271455.00
2.2.3	Bottom Liner System	2849076.45	2849076.45	2849076.45	2849076.45	2849076.45
2.2.4	Top Covers System	4671169.43	4903115.54	4697521.94	4671169.43	4872542.55
2.2.5	Leachate System	409841.30	409841.30	409841.30	409841.30	409841.30
2.2.6	Landfill Gas System	458982.86	458982.86	458982.86	458982.86	458982.86
2.2.7	Monitoring	32135.10	32135.10	32135.10	32135.10	32135.10
2.2.8	Landfill Hydraulic Settlement	25386.66	25386.66	25386.66	25386.66	25386.66
2.2.9	Underground Utilities	170621.27	170621.27	170621.27	170621.27	170621.27
2.2.10	Internal Road and Service Area	274463.58	274463.58	274463.58	274463.58	274463.58
2.2.11	Facilities	179000.00	179000.00	179000.00	179000.00	179000.00
2.2.12	Environmental Restoration Works	137746.59	137746.59	137746.59	137746.59	137746.59
2.2.13	Final Works	80870.77	159198.59	160005.07	213760.77	258611.59
2.2.14	Safety	778058.90	802880.81	786497.84	788690.10	808388.01
2.3	Machinery Purchase	1350000.00	1350000.00	1350000.00	1350000.00	1350000.00
2.4	Financial Expenses	4760142.58	4880334.62	4801005.42	4811620.71	4907001.45
3	Operation Phase	29325233.72	29327089.28	29258643.36	29325233.72	29260043.52
3.1	Operation Cost	10039565.86	10039565.86	9973294.85	10039565.86	9973294.85
3.1.1	Staff	4598350.00	4598350.00	4598350.00	4598350.00	4598350.00
3.1.2	Consumptions and Materials	400000.00	400000.00	401200.00	400000.00	401200.00
3.1.3	Leachate Management	1917973.00	1917973.00	1834596.98	1917973.00	1834596.98
3.1.4	Landfill Gas Management	458982.86	458982.86	458982.86	458982.86	458982.86
3.1.5	Daily Top Cover	464760.00	464760.00	464760.00	464760.00	464760.00
3.1.6	Monitoring	344500.00	344500.00	355000.00	344500.00	355000.00
3.1.7	Maintenance	750000.00	750000.00	750405.01	750000.00	750405.01
3.1.8	Other Services (technical costs, etc.)	1105000.00	1105000.00	1110000.00	1105000.00	1110000.00
3.2	Pollution Liability Protection in Operation	180000.00	180000.00	180000.00	180000.00	180000.00
3.3	Financial Guarantees in Operation	118787.86	120643.42	118468.51	118787.86	119868.67
3.4	Contribution for Environmental Annoyance and Landfill Tax	18986880.00	18986880.00	18986880.00	18986880.00	18986880.00
3.4.1	Contribution for Environmental Annoyance	5807120.00	5807120.00	5807120.00	5807120.00	5807120.00
3.4.2	Landfill Tax	13179760.00	13179760.00	13179760.00	13179760.00	13179760.00
4	Aftercare Phase	7149570.29	7024477.56	7148358.03	7189288.60	6912223.31
4.1	Aftercare Cost	6557113.38	6433013.45	6555910.75	6596675.20	6321650.11
4.1.1	Staff	2035570.00	2035570.00	2035570.00	2035570.00	2035570.00
4.1.2	Consumptions and Materials	244000.00	244000.00	244000.00	244000.00	244000.00
4.1.3	Leachate Management	3068401.95	2957635.58	3067199.32	3068401.95	2839732.21
4.1.4	Landfill Gas Management	229491.43	229491.43	229491.43	229491.43	229491.43
4.1.5	Monitoring	422250.00	422250.00	422250.00	422250.00	422250.00
4.1.6	Maintenance	512400.00	499066.44	512400.00	531961.82	505606.46
4.1.7	Other Services (technical costs)	45000.00	45000.00	45000.00	65000.00	45000.00
4.2	Pollution Liability Protection in Aftercare	540000.00	540000.00	540000.00	540000.00	540000.00
4.3	Financial Guarantees in Aftercare	52456.91	51464.11	52447.29	52613.40	50573.20
5	General Expenses and Net Income	13456500.51	13543703.10	13479852.82	13516326.95	13526124.91
5.1	General Expenses	7198950.89	7245602.48	7211443.89	7230956.80	7236198.51
5.2	Net Income	6257549.62	6298100.62	6268408.92	6285370.14	6289926.40
TOT	TOTAL COST - NO VAT (22%)	68833045.81	69279106.78	68952498.16	69139071.58	69189190.40
	$X_{economic}$	0	-446060.98	-119452.3490	-306025.78	-356144.59

Table 8.4. Results obtained from the analysis of the scenarios according to the energetic criterion.

	Reference scenario	First scenario	Second scenario	Third scenario	Fourth scenario
Energy input in 40 years (GJ)	0	1199.94	107.35	674.08	1981.37
Energy output in 40 years (GJ)	0	24877.99	101.86	11414.73	36394.57
Net gain (output – input) in 40 years (GJ)	0	23678.05	-5.49	10740.65	34413.20
$x_{energetic}$ (GJ)	0	23678.05	-5.49	10740.65	34413.20

Table 8.5. Results obtained from the analysis of the scenarios according to the environmental criterion.

	Reference scenario	First scenario	Second scenario	Third scenario	Fourth scenario
t = 0, before the landfill construction (Mcal/m^2/year)	0.89	0.89	0.89	0.89	0.89
t > 40 years, after the closure of landfill (Mcal/m^2/year)	0.90	0.90	0.90	1.06	1.06
$x_{environmental}$ (Mcal/m^2/year)	0	0	0	0.16	0.16

Table 8.6. Evaluation matrix linearized.

Criteria	Reference scenario	First scenario (Energetic maximization scenario)	Second scenario (Leachate phytotreatment scenario)	Third scenario (Environmental compensation scenario)	Fourth scenario (Combination scenario)
Economic criterion	1.0000	0.0000	0.7322	0.3139	0.2016
Energetic criterion	0.0002	0.6854	0.0000	0.3149	1.0000
Environmental criterion	0.0000	0.0000	0.0000	1.0000	1.0000
Final score	0.3334	0.2285	0.2441	0.5430	0.7339

8.4. CONCLUSIONS

This work suggested some design possibilities for the cultivation of energy crops on landfills. In the first and second scenarios, the results showed that the benefits related to the presence of energy crops on the top cover are not cost-effective. In general, the study shows that landfill costs were greater than in the reference scenario for all the scenarios considered, and therefore not economically favourable. However, difficulties in the evaluation of a reduction in the leachate production, due to presence of energy crops on the top cover, could have significantly affected the results.

From an economic point of view, the consideration of longer time horizons for the leachate phytotreatment in the second scenario is an option, as extending the period in the aftercare phase could allow important economic savings. The obstacles generating from legal restrictions to the implementation of new technologies to landfill sites, should be taken into more careful consideration by the legislator.

The energetic and environmental criteria were maximised by lignocellulosic crops in the third and fourth scenarios. The higher crop yield of Miscanthus and the higher biopotentiality of Poplar and shrubs species had a fundamental role in the analysis.

The MCA determined the fourth scenario as the best solution among those considered, as it produced the best results for the energetic and environmental criteria, while keeping the cost close to that obtained in the other scenarios.

The evaluation of potential scenarios could assist decision makers, providing preliminary information on the alternatives under examination. However, the high level of uncertainty and the large number of assumptions could affect the entire analysis and, consequently, the final ranking. In this optic, the MCA, even the most comprehensive, should be based on real, site-specific data and should extend the uncertainty analysis (e. g. Monte Carlo method) to the whole assessment.

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Chapter 9: General conclusions

Wastewater phytotreatment using energy crops proved to be feasible, yielding a series of favourable outputs both in terms of biomass growth and pollutant removal rates.

Several experimental researches were performed under different experimental conditions (e.g.: different wastewaters, plant species and substrates were used; different flow systems were tested; etc.).

The following general conclusions can be drawn:

- High pollutant removal rates have been obtained in all the experimental phases
- Sunflower plants were characterized by the highest performances
- The removal mechanisms involve complex interactions between chemical, physical and biological processes
- Efficiencies started to decrease when plants reached their maximum development and began their senescence period, suggesting that plants are actively involved in the removal of contaminants, up until flowering. This could be ascribed to the reduction of oxygen transfer from the leaves to the root zone, which occurs during senescence and limits activity of the bacterial population living in symbiosis in the root zone
- Clayey soil proved to be more suitable for COD removal, while nitrification was better in sandy soil
- The substrate acted as "sink" for nitrogen, as revealed by the mass balances
- The use of vertical flow units connected in series with horizontal ones was effective in removing nitrogen due to nitrification and denitrification
- Wastewater volume reduction was significant: up to more than 80% of inlet wastewater was removed by evapotranspiration. The limited volume of effluents was characterized by low concentrations of contaminants, demonstrating the efficiency of the systems in abating both volumes and pollutant concentrations
- The oil composition of seeds (especially of sunflowers) seemed to be particularly favourable to biodiesel production as the content of unsaturated long chain free fatty acids was lower than values commonly detected
- Leachate irrigation did not produce an accumulation of heavy metals in the biological tissues of the analyzed plants. At the end of the experiments, concentrations in the biological tissues were comparable (or even lower) than values found in the corresponding controls

- The initial quality of the substrate was not affected by the applied leachate phytotreatment processes. The innovative scientific approach used, in which the outputs of chemical analyses were assessed in combination with the effects on target organisms, revealed that the substrate soil used for leachate phytotreatment was not contaminated (e.g.: no heavy metals accumulation)
- According to the Multi-Criteria Analysis, the benefits related to the presence of energy crops for leachate phytotreatment on the top of landfills could be not economically favourable and might not be fully compensated by energetic and environmental gains. However, the use of several assumptions could have significantly affected the results.

FUTURE DEVELOPMENTS

Future developments should elucidate the mechanisms of contaminants adsorption and transport from the root zone to the aerial part, focusing in particular on sunflowers, which demonstrated to have the greatest potential in terms of decontamination capacity and biomass growth. The new experiments should be performed using tracer substances, not subjected to microbiological degradation in the substrate. Alternatively, hydroponic systems could be used to minimize the disturbances due to the presence of the substrate.

The projects described in this Thesis were performed in a climatic chamber to keep under control the experimental conditions; the plants were harvested just after clear senescence was reached to complete the set of scheduled analysis. A scale-up of the current experiences should be implemented to analyze system dynamics in a close-to-reality context and assess the effects of climatic conditions on the processes. Moreover, the experiments, performed on a larger scale, should be run for a longer time span to evaluate the long-term performances and make sure that pollutants degradation processes have been stabilized and steady-state conditions have been reached.

The choice of testing sunflower, soybean and rapeseed was based on the observation that these plants are considered optimal oily crops for Mediterranean and Continental areas. If phytotreatment is going to be applied in different areas, the use of locally-available energy crops should be considered (e.g.: *Pennisetum Purpureum* in developing countries), together with the production of alternative biofuels (e.g.: bioethanol or biomethane).

Ascertained that phytotreatment with energy crops is effective in removing organics and nutrients, future researches should also focus on emerging pollutants. Emerging contaminants include pesticides, industrial compounds, pharmaceuticals and personal care products. Although detected in low concentrations (e.g.: μg/L) in both municipal wastewaters and landfill leachates, they pose a serious threat to the environment and the human health.

The use of models to simulate the fate of pollutants removal might be useful in the design phase of a pilot or real scale phytotreatment unit and should be better developed in future studies. Phytoremediation, however, is a unique process, consisting in a combination of different phenomena, and the knowledge of each single contribution is still matter of research. Therefore "black-box" models should be preferred to descriptive models, although the large number of assumptions, typical of black-box approach, may limit the ability of the model itself to describe the full set of processes.

In view of the real scale application of phytotreatment with energy crops, the use of more comprehensive decision models (e.g.: SWOT analysis instead of Multi-Criteria Analysis) should be further implemented to help decision makers to take the best decision, keeping into account different priorities and needs. It is also highly recommended a strong cooperation with the legislator and/or the competent authority to develop new guidelines on sustainable solid waste management, promoting the use of landfill top covers for phytotreatment purposes, as this solution is currently discouraged by the European and Italian regulations.

Annexes

Annex 5.1: Detailed characterization of the seeds oil. Results expressed as % on the oil content

Free fatty acid concentration	V1	V2	H1	H2	VC	HC
Hexanoic (C6:0)	0.000	0.000	0.000	0.000	0.000	0.009
Caprilic (C8:0)	0.000	0.013	0.030	0.027	0.025	0.020
Capric (C10:0)	0.000	0.003	0.024	0.022	0.019	0.016
Lauric (C12:0)	0.009	0.003	0.027	0.028	0.025	0.022
Myristic (C14:0)	0.090	0.097	0.125	0.172	0.119	0.136
Pentadecanoic (C15:0)	0.048	0.047	0.055	0.044	0.047	0.031
Palmitic (C16:0)	6.754	6.853	6.629	6.900	6.512	7.031
14-methylhexadecanoic (C17:0 anteiso)	0.000	0.000	0.000	0.004	0.000	0.000
Margaric (C17:0)	0.088	0.082	0.100	0.051	0.110	0.093
Stearic (C18:0)	2.620	2.794	2.863	2.575	2.363	2.169
17-methyloctadecanoic (C19:0 iso)	0.007	0.000	0.016	0.019	0.007	0.012
Arachidic (C20:0)	0.265	0.302	0.305	0.271	0.240	0.217
Heneicosanoic (C21:0)	0.000	0.000	0.003	0.000	0.000	0.004
Behenic (C22:0)	0.710	0.752	0.752	0.686	0.692	0.621
Tricosanoic (C23:0)	0.076	0.075	0.093	0.058	0.062	0.049
Lignoceric (C24:0)	0.627	0.661	0.638	0.618	0.598	0.642

Myristoleic (C14:1c n5)	0.000	0.000	0.000	0.000	0.000	0.012
(Z)-hexadec-7-enoic (C16:1n9c)	0.075	0.076	0.068	0.066	0.067	0.071
Palmitoleic (C16:1 n7c)	0.225	0.239	0.237	0.225	0.195	0.263
(Z)-heptadec-10-enoic (C17:1n7c)	0.086	0.073	0.095	0.068	0.117	0.110
Elaidic (C18:1n9t)	0.062	0.063	0.067	0.070	0.051	0.056
Oleic (C18:1n9c)	34.762	33.935	45.285	37.941	37.448	41.082
Vaccenic (C18:1n7c)	1.001	1.117	0.950	1.032	0.923	1.061
(Z)-nonadec-10-enoic (C19:1n9c)	0.027	0.007	0.019	0.018	0.025	0.028
(Z)-nonadec-enoic (C19:1c)	0.015	0.004	0.016	0.021	0.027	0.015
(Z)-eicos-8-enoic (C20:1n12)	0.000	0.009	0.003	0.000	0.004	0.004
Gondoic (C20:1n9)	0.217	0.229	0.223	0.230	0.223	0.222
(E,Z)-octadeca-9,12-dienoic (C18:2 t9,c12)	0.121	0.084	0.120	0.107	0.071	0.107
Linoleic acid (C18:2c9c12-n6)	51.987	52.338	41.128	48.610	49.923	45.797
Pinolenic (C18:3n6)	0.003	0.000	0.000	0.003	0.004	0.000
Alpha-linolenic (C18:3 n3)	0.121	0.130	0.127	0.127	0.100	0.098
(Z11,Z14)-eicosadienoic (C20:2n6)	0.003	0.014	0.000	0.007	0.003	0.003

Annex 8.1: List of assumptions for each scenario

Table 1. Assumptions of the reference scenario (scenario "zero")

FACTORS	ASSUMPTIONS
Landfill geometrical/volumetric properties	
Model landfill	Underground
Landfill shape	Rectangular
Total waste volume (m^3)	800,000
Surface (at ground level) (m^2)	50,000
Landfill height (m)	23 m
Number of sectors	4
Landfilled waste inflow (t/year)	88,000
Landfilled waste composition	Paper (1.5%), cardboard (1.5%), glass and inert (52%), plastic (12%), metals (35%), stabilised inert (15%), sludge (15%)
Landfilled waste density (t/m^3)	1.1
Top cover surface (ha)	2.14 (4% slope) + 2.94 (24% slope)

Table 2. Assumptions of the first scenario (energy maximisation scenario)

FACTORS	ASSUMPTIONS
1) Scenario configuration	
Landfill geometrical/volumetric properties	Same of scenario "zero"
Energy crop considered	Miscanthus (*Miscanthus x giganteus*)
2) Energy crop	
Crop lifetime (year)	15 (1st cycle) + 30 (2nd cycle)
Cultivation area (ha)	2.14 (4% slope)
Crop yield (t/ha/year)	0.00 (1st year) 10.00 (2nd year) 20.00 (3rd year) 25.00 (other years)
Energy input (GJ/ha/year)	43.79 (1st year) 15.84 (from 2nd to 14th year) 30.46 (other years)
Low Heating Value - Energy output (GJ/t)	17.60
Landfill phase	Aftercare

Table 3. Assumptions of the second scenario (phytotreatment scenario)

FACTORS	ASSUMPTIONS
1) Scenario configuration	
Landfill geometrical/volumetric properties	Same of scenario "zero"
Energy crops considered	Sunflower (*Heliantus annuus L*), Rapeseed (*Brassica napus*), Soybean (*Glycine max*)
2) Energy crops	
Crops lifetime (years)	4
Crops rotation	Biannual: Sunflower in spring-summer and Rapeseed seeding in autumn in the first year, Rapeseed harvesting at the end of the spring and Soybean cultivation in summer in the second year
Cultivation area (ha)	0.64 (4% slope)
Crop yield (t/ha/year)	2.0 (seeds) - 0.90* (Sunflower) 2.2 (seeds) - 0.77* (Rapeseed) 2.3 (seeds) - 0.44* (Soybean)
Energy input (GJ/ha/year)	29.02** (Sunflower) 21.33** (Rapeseed) 27.18** (Soybean)
Low Heating Value - Energy output (GJ/t)	38.40* 37.40* 36.40*
Landfill phase	Operation

* values referred to the final oil product

**values including the oil production

Table 4. Assumptions of the third scenario (environmental compensation scenario)

FACTORS	ASSUMPTIONS
1) Scenario configuration	
Landfill geometrical/volumetric properties	Same of scenario "zero"
Energy crops considered	Poplars (green belt around the landfill perimeter and plantation along the south side of the landfill) and shrubs on the landfill top
2) Energy crops	
Crops lifetime (years)	>30
Cultivation area (ha)	0.70 (poplar green belt) + 0.62 (poplar plantation) + 5 (shrubs)
Crop yield (t/ha/year)	16 (80 t/ha every 5 years)
Energy input (GJ/ha/year)	45.66 (1st year) 23.36 (5th and 10th years) 29.55 (15th year) 7.99 (other years)
Low Heating Value - Energy output (GJ/t)	17.80
Landfill phase	Aftercare

Table 5. Assumptions of the fourth scenario (combination scenario)

FACTORS	ASSUMPTIONS
1) Scenario configuration	
Landfill geometrical/volumetric properties	Same of scenario "zero"
Energy crops considered	Miscanthus, Sunflower, Rapeseed, Soybean, poplars, shrubs
Crops lifetime (years)	4 (Sunflower, Rapeseed, Soybean); 30 (Miscanthus), >30 (poplars and shrubs)
Cultivation area (ha)	2.14 (Miscanthus); 0.64 (Sunflower, Soybean, Rapeseed); 0.70 + 0.62 (poplars); 5 (shrubs)
2) Energy crops	
The same values reported in Tables 2, 3, and 4 of this Annex were used	

Annex 8.2: Calculation of biogas and leachate generation

1. Reference Scenario: leachate production

Leachate production (L) model is based on a hydrological balance model (Canziani et al., 1989), which is a simplified water mass balance drawn around the physical boundaries of the landfill body (Fig. 1).

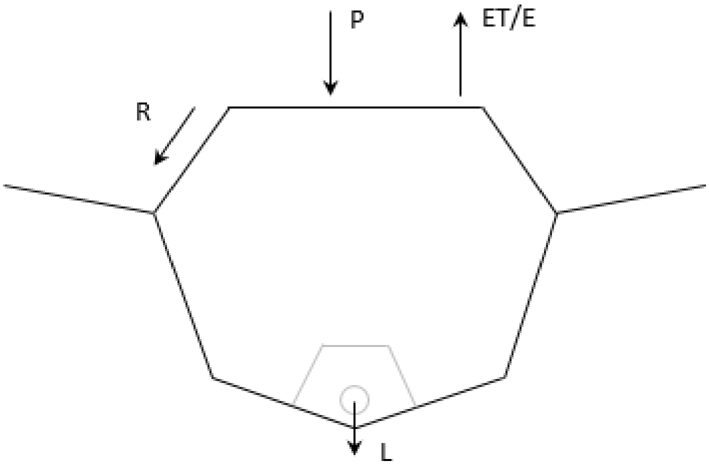

Fig. 1. Landfill hydrological balance

The use of a low-permeability liner on the bottom allows the assumption that groundwater cannot infiltrate into the landfill body and that there are no leachate uncontrolled leaks. A landfill perimeter channel avoids the runoff of surrounding rainwater into the landfill. During the operational phase and before the final closure, the lack of an impervious top cover promotes the infiltration of precipitation (P). The aim is improving biological degradation and flushing of waste, but water release and consumption due to biochemical reactions were neglected. In the warm months, however, evaporation (E) consistently limits the net amount of precipitation that infiltrates. During the aftercare phase, the scarce infiltration is further enhanced by the vegetation on the final top cover, in this case the phenomenon is known as evapotranspiration (ET). Besides, a certain degree of imperviousness in the final top cover causes a runoff (R) of rainwater into the landfill perimeter channel.

According to this consideration, two scenarios were evaluated:

$$L = P - E \qquad (1)$$

for the operational phase, when no R occurs and the only obstacle to infiltration is E;

$$L = P - R - ET \qquad (2)$$

for the aftercare phase after closure, when R is introduced and E was substituted by ET.

The terms were estimated by the implementation of appropriate formulas. In particular, the evaporation term (E) relies on the application of Turk formula:

$$E_{10} = \frac{P_{10} + a}{\sqrt{1 + \frac{P_{10} + a}{L}}} \qquad (3)$$

where:

E_{10} = evaporation in 10 days (mm/10d);

P_{10} = average precipitation in 10 days (mm/10d);

a = amount of water that can evaporate in 10 days without precipitation; given by the following formula:

$$a = 10 - 0.01 * t^{0.5} \qquad (4)$$

t= time since the last precipitation (s);

L= heliothermic factor, given by the following formula:

$$L = \frac{1}{16}(T + 2)\sqrt{I_g} \qquad (5)$$

where:

T= monthly average temperature (°C);

I_g= solar radiation (cal/cm^2/d), given by the following formula:

$$I_g = I_o \left(0.18 + 0.62\,\frac{n}{N}\right) \quad (6)$$

where:

I_o= theoretical maximum solar radiation (cal/cm^2/d), depending on month and on latitude (Table 1);

n = effective hours of incoming solar radiation (h);

N= theoretical maximum hours of incoming solar radiation (h), depending on month and on latitude (Table 1).

Table 1: Values of I_o and N for a latitude of 45°.

Months	Io (cal/cm^2/d)	N (h)
January	293.00	9.24
February	425.00	10.56
March	600.00	11.88
April	783.00	13.44
May	928.00	14.76
June	983.00	15.60
July	955.00	15.36
August	838.00	14.16
September	665.00	12.60
October	485.00	10.92
November	330.00	9.60
December	255.00	8.76

Remembering that leachate production in operational phase is L = P – E, precipitation data, estimated evaporation values and calculated leachate production (monthly) are shown in Table 2.

Table 2: Leachate production in operational phase

Months	P (mm/month)	E (mm/month)	Operation L = P – E (mm/month)
January	43.42	19.82	23.60
February	22.30	15.70	6.60
March	33.10	21.45	11.65
April	70.30	45.68	24.62
May	54.53	40.57	13.96
June	74.82	55.01	19.80
July	63.57	48.32	15.25
August	76.63	56.19	20.45
September	94.58	64.13	30.45
October	88.02	51.95	36.07
November	75.13	37.40	37.73
December	63.82	26.16	37.65

The amount of runoff (R) on the top cover could be calculated as a fraction of precipitation using a multiplicative coefficient:

$$R = C * P \tag{7}$$

where:

R= surface runoff (mm/d);

P= precipitation (mm/d);

C= runoff coefficient.

The runoff coefficient is different according to top cover slope: it was assumed 0.70 for the area with slope 24% and 0.55 for the area with slope 4%.

After closure, when vegetation starts to grow on the final top cover, evaporation is substituted by evapotranspiration. The evapotranspiration depends on the potential evapotranspiration (PE), which is the maximum evapotranspiration occurring in case of optimal sunlight rays and optimal soil moisture conditions. As s consequence, the evapotranspiration is different in wet and dry seasons. For the calculation of the potential evapotranspiration, the Thorntwaite formula was used:

$$PE = 16\left(\frac{10 * T}{I_T}\right)^a C \tag{8}$$

where:

PE= potential evapotranspiration [mm/month];

T= monthly average temperature [°C];

C= depends on hours of sunlight and on latitude (Table 3);

I_t= annual thermal index, given by the following formula:

$$I_T = \sum_{1}^{12}\left(\frac{T}{5}\right)^{1.514} \tag{9}$$

a = given by the following formula:

$$a = 6.75 * 10^{-7} {I_T}^3 - 7.71 * 10^{-5} {I_T}^2 + 1.79 * 10^{-2} I_T + 0.49239 \tag{10}$$

Table 3. Values of C for a latitude of 45°.

Months	C ($cal/cm^2/d$)
January	0.77
February	0.88
March	0.99
April	1.12
May	1.23
June	1.30
July	1.28
August	1.18
September	1.05
October	0.91
November	0.80
December	0.73

In particular, for the landfill described in scenario "zero", I_t resulted to be 58.35 and a resulted to be 1.41.

The evapotranspiration (ET) was calculated as follows:

- For wet season, characterized by P – R > PE:

$$ET = PE \tag{11}$$

- For dry season, characterized by P – R < PE:

$$ET = PE * \frac{U}{FC} \tag{12}$$

where:

U= average moisture content of the cover, estimated to be 35%;

FC= field capacity, given by the following formula:

$$FC = 0.6 - 0.55\left(\frac{W}{4535 + W}\right) \quad (13)$$

where:

W= waste density, estimated to be 1,100 kg/m^3.

The value obtained for FC was 0.49.

Remembering that leachate production in the aftercare phase is L = P – R – ET (Eq. (2)); precipitation data, estimated runoff, evapotranspiration values, and calculated monthly leachate production are reported in Tables 4 and 5, for top cover slope of 24% and 4%, respectively. Negative leachate production values were set equal to zero.

Table 4: Leachate production in aftercare phase (top cover slope of 24%)

Months	P (mm/month)	R (mm/month)	ET (mm/month)	L = P - R - ET (mm/month)
January	43.42	30.39	3.98	9.04
February	22.30	15.61	6.87	0.00
March	33.10	23.17	20.48	0.00
April	70.30	49.21	36.60	0.00
May	54.53	38.17	70.03	0.00
June	74.82	52.37	93.01	0.00
July	63.57	44.50	102.59	0.00
August	76.63	53.64	95.91	0.00
September	94.58	66.21	57.88	0.00
October	88.02	61.61	32.14	0.00
November	75.13	52.59	19.24	3.30
December	63.82	44.67	5.99	13.15

Table 5: Leachate production in aftercare phase (top cover slope of 4%)

Months	P (mm/month)	R (mm/month)	ET (mm/month)	L = P - R - ET (mm/month)
January	43.42	23.88	3.98	15.56
February	22.30	12.27	9.66	0.37
March	33.10	18.21	20.48	0.00
April	70.30	38.67	36.60	0.00
May	54.53	29.99	70.03	0.00
June	74.82	41.15	93.01	0.00
July	63.57	34.96	102.59	0.00
August	76.63	42.15	95.91	0.00
September	94.58	52.02	57.88	0.00
October	88.02	48.41	32.14	7.47
November	75.13	41.32	19.24	14.57
December	63.82	35.10	5.99	22.72

The cumulated leachate production over time is reported in Fig. 2.

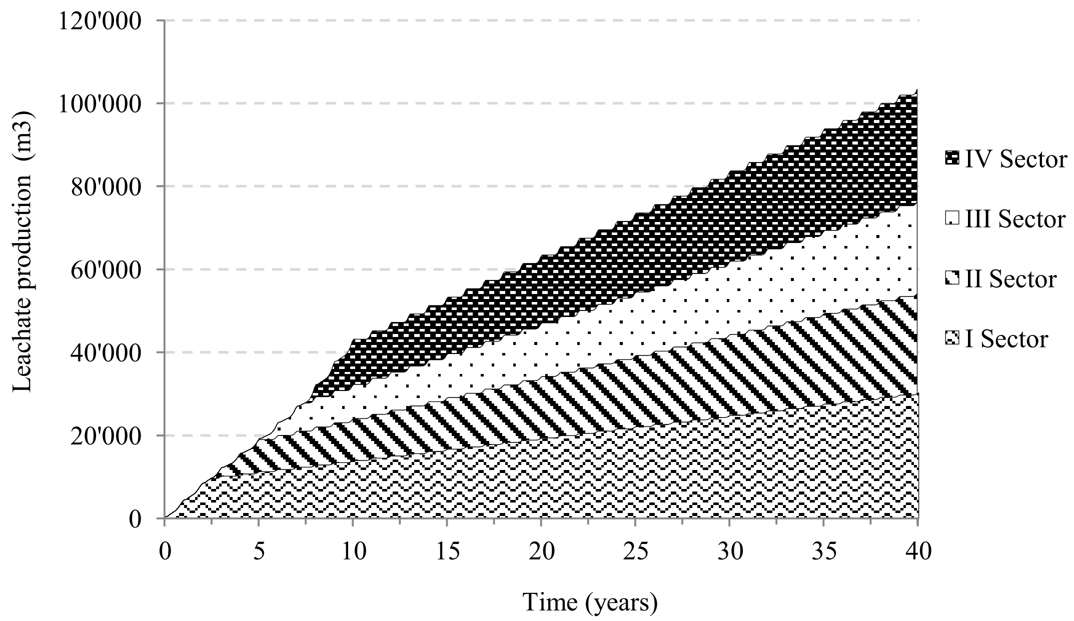

Fig. 2 Cumulated leachate production for the reference scenario over time

2. Reference Scenario: biogas production

For the estimation of the landfill gas (LFG) production, the model suggested by Cossu et al., 1996 was used. The procedure consists of the following main phases:

- waste characterization and estimation of the biodegradable organic carbon;
- estimation of the maximum LFG yields of waste components;
- application of the LFG production yields to the input waste quantity;
- estimation of the LFG volume production through a LFG generation model.

LFG production depends on the anaerobic degradation of the organic fraction of waste, thus the model is based on the following stoichiometric reaction:

$$C_aH_bO_cN_d + nH_2O \rightarrow xCH_4 + yCO_2 + wNH_3 + zC_5H_7O_2N + \text{energy} \qquad (14)$$

Where $C_aH_bO_cN_d$ represents the biodegradable fraction in solid waste and $C_5H_7O_2N$ the composition of bacteria cells. The biodegradable organic carbon in the waste body was estimated through the following formula:

$$(OC_b)_i = OC_i \cdot (f_b)_i \cdot (1 - u_i) \cdot p_i \qquad (15)$$

where:

$(OC_b)_i$= biodegradable organic carbon in component i of waste ($kg_{biodegradableC}$/ kg_{wetMSW});

OC_i= organic carbon content in the dry component i of waste (kg_C / $kg_{dry\ i\ component}$);

$(f_b)_i$= biodegradable fraction of OC_i ($kg_{biodegradableC}$/ kg_C);

u_i= moisture content of the i component of waste (kg_{H2O} / $kg_{wet\ i\ component}$);

p_i= wet weight of the i component of waste ($kg_{i\ component}$/ kg_{MSW}).

Waste fractions disposed in the landfill, as well as the assumed values for each of them, are summarized in Table 6.

Table 6. Waste fractions and assumed values of OC_i, $(fb)_i$ and u_i (Cossu et al., 1992).

Waste components	OC_i (kg_C / $kg_{dry\ i\ comp.}$)	$(f_b)_i$ ($kg_{biod\ C}$/ kg_C)	u_i (kg_{H2O} / $kg_{wet\ i\ comp}$)	p_i ($kg_{i\ comp}$/ kg_{MSW})
Paper	0.44	0.50	0.08	1.5
Cardboard	0.44	0.50	0.08	1.5
Glass and inerts	0.00	0.00	0.03	52.0
Plastic	0.70	0.00	0.02	12.0
Metals	0.00	0.00	0.03	3.0
Stabilized inerts	0.00	0.00	0.03	15.0
Sludge	0.02	0.05	0.33	15.0

The estimated amount of inflow waste was 88,000 t per year.

LFG specific yield (Y_{LFG}) was estimated using the following formula, representing the common theoretical basis for the majority of LFG generation models:

$$Y_{LFG} = 1.867 \cdot OC_i \cdot (f_b)_i \cdot (1 - u_i) \cdot p_i \qquad (16)$$

where 1.867 comes from the following equivalence: 1g C = 1.867 NL (of CH_4+CO_2).

The results are shown in Table 7.

Table 7. LFG specific yields for waste components of model landfill.

Waste components	Y_{LFG} (NL/g_{MSW})
Paper	0.0057
Cardboard	0.0057
Glass and inerts	0.00
Plastic	0.00
Metals	0.00
Stabilized inerts	0.00
Sludge	0.0002

LFG specific production rate is described by a first order kinetic model:

$$g = \frac{dG_t}{dt} = \sum_{i=1}^{3} 1.867 * (OC_b)_i * (1 - e^{-k_i t}) \tag{17}$$

where:

g = annual LFG specific yield ($Nm^3/kg_{MSW}/y$);

G_t = specific LFG production (Nm^3/kg_{MSW});

$(OC_b)_I$ = biodegradable C ($kg_C/kg_{wet\ MSW}$);

k_i = decay time constant [y^{-1}], given by the following formula:

$$k_i = \frac{ln2}{t_{50}} \tag{18}$$

where: t_{50} = time for half of total LFG generation to occur.

The degradation constant is different for each fraction. For seek of simplicity, only three fractions were considered (Table 8), and it is the reason why the sum defined for i ranged from 1 to 3.

Table 8. Fractions for LFG degradation constant.

Fractions	t_{50} (y)	k_i (y^{-1})
Highly biodegradable	1	0.693
Moderately biodegradable	5	0.139
Slowly biodegradable	15	0.046

Cumulated LFG production of each sector over time is reported in Fig. 3.

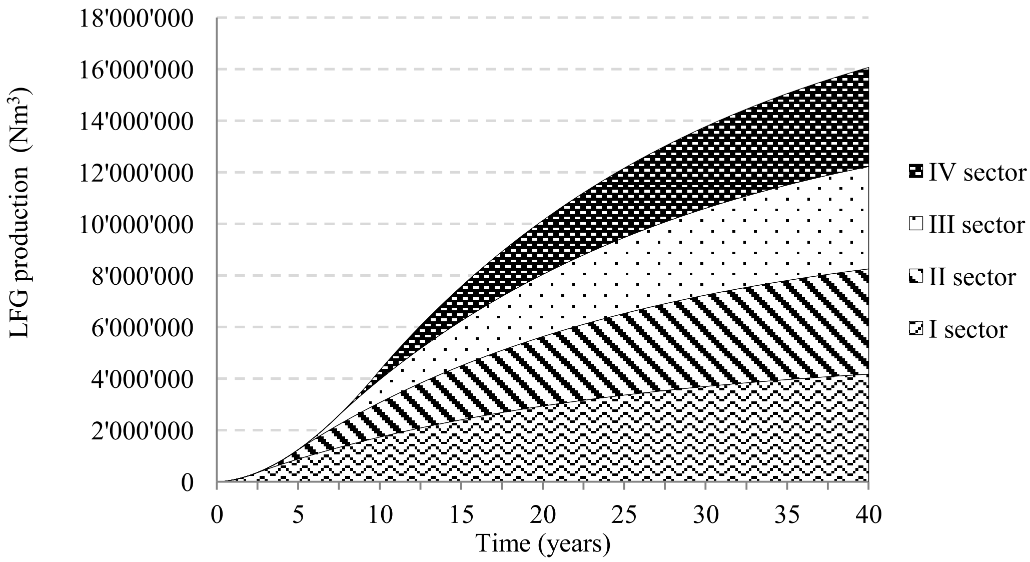

Fig. 3. Cumulated LFG production for the reference scenario over time

References

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Cossu, R., Andreottola, G., Muntoni, A., 1996. Modelling landfill gas production. In: Christensen, T.H., Cossu, R., Stegmann, R., 1996. Landfilling of Waste: Biogas. E & FN SPON, London, pp 237-268.

Annex 8.3: Calculations of energetic and environmental criteria for each scenario

Table 1: Energetic criterion. Energy input and energy output for each scenario

Year	First scenario (energy maximisation)		Second scenario (phytotreatment)					
	Miscanthus		Sunflower		Rapeseed		Soybean	
	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	20.24	22.21	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	15.21	18.50	18.23	10.22
9	0.00	0.00	20.24	22.21	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	15.21	18.50	18.23	10.22
11	93.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	33.92	376.94	0.00	0.00	0.00	0.00	0.00	0.00
13	33.92	753.88	0.00	0.00	0.00	0.00	0.00	0.00
14	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
15	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
16	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
17	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
18	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
19	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
20	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
21	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
22	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
23	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
24	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
25	65.25	942.35	0.00	0.00	0.00	0.00	0.00	0.00
26	93.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	33.92	376.94	0.00	0.00	0.00	0.00	0.00	0.00
28	33.92	753.88	0.00	0.00	0.00	0.00	0.00	0.00
29	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
30	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
31	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
32	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
33	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
34	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
35	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
36	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
37	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
38	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
39	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
40	65.25	942.35	0.00	0.00	0.00	0.00	0.00	0.00
Total	**1,199.94**	**24,877.99**	**40.48**	**44.41**	**30.42**	**37.01**	**36.45**	**20.44**

(continued)

(continued)

Year	Third scenario (environmental compensation)			
	Poplar plantation		Poplar green belt	
	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
11	28.19	0.00	32.05	0.00
12	4.93	0.00	5.61	0.00
13	4.93	0.00	5.61	0.00
14	4.93	0.00	5.61	0.00
15	14.43	890.31	16.40	1,012.14
16	4.93	0.00	5.61	0.00
17	4.93	0.00	5.61	0.00
18	4.93	0.00	5.61	0.00
19	4.93	0.00	5.61	0.00
20	14.43	890.31	16.40	1,012.14
21	4.93	0.00	5.61	0.00
22	4.93	0.00	5.61	0.00
23	4.93	0.00	5.61	0.00
24	4.93	0.00	5.61	0.00
25	46.44	890.31	52.79	1,012.14
26	28.19	0.00	32.05	0.00
27	4.93	0.00	5.61	0.00
28	4.93	0.00	5.61	0.00
29	4.93	0.00	5.61	0.00
30	14.43	890.31	16.40	1,012.14
31	4.93	0.00	5.61	0.00
32	4.93	0.00	5.61	0.00
33	4.93	0.00	5.61	0.00
34	4.93	0.00	5.61	0.00
35	14.43	890.31	16.40	1,012.14
36	4.93	0.00	5.61	0.00
37	4.93	0.00	5.61	0.00
38	4.93	0.00	5.61	0.00
39	4.93	0.00	5.61	0.00
40	46.44	890.31	52.79	1,012.14
Total	**315.46**	**5,341.87**	**358.63**	**6,072.86**

(continued)

(continued)

Year	Fourth scenario (combination)							
	Miscanthus		Sunflower		Rapeseed		Soybean	
	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	20.24	22.21	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	15.21	18.50	18.23	10.22
9	0.00	0.00	20.24	22.21	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	15.21	18.50	18.23	10.22
11	93.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	33.92	376.94	0.00	0.00	0.00	0.00	0.00	0.00
13	33.92	753.88	0.00	0.00	0.00	0.00	0.00	0.00
14	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
15	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
16	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
17	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
18	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
19	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
20	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
21	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
22	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
23	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
24	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
25	65.25	942.35	0.00	0.00	0.00	0.00	0.00	0.00
26	93.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	33.92	376.94	0.00	0.00	0.00	0.00	0.00	0.00
28	33.92	753.88	0.00	0.00	0.00	0.00	0.00	0.00
29	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
30	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
31	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
32	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
33	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
34	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
35	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
36	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
37	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
38	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
39	33.92	942.35	0.00	0.00	0.00	0.00	0.00	0.00
40	65.25	942.35	0.00	0.00	0.00	0.00	0.00	0.00
Total	**1,199.94**	**24,877.99**	**40.48**	**44.41**	**30.42**	**37.01**	**36.45**	**20.44**

(continued)

(continued)

Year	Fourth scenario (Combination)			
	Poplar plantation		Poplar green belt	
	Input (GJ)	Output (GJ)	Input (GJ)	Output (GJ)
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
11	28.19	0.00	32.05	0.00
12	4.93	0.00	5.61	0.00
13	4.93	0.00	5.61	0.00
14	4.93	0.00	5.61	0.00
15	14.43	890.31	16.40	1,012.14
16	4.93	0.00	5.61	0.00
17	4.93	0.00	5.61	0.00
18	4.93	0.00	5.61	0.00
19	4.93	0.00	5.61	0.00
20	14.43	890.31	16.40	1,012.14
21	4.93	0.00	5.61	0.00
22	4.93	0.00	5.61	0.00
23	4.93	0.00	5.61	0.00
24	4.93	0.00	5.61	0.00
25	46.44	890.31	52.79	1,012.14
26	28.19	0.00	32.05	0.00
27	4.93	0.00	5.61	0.00
28	4.93	0.00	5.61	0.00
29	4.93	0.00	5.61	0.00
30	14.43	890.31	16.40	1,012.14
31	4.93	0.00	5.61	0.00
32	4.93	0.00	5.61	0.00
33	4.93	0.00	5.61	0.00
34	4.93	0.00	5.61	0.00
35	14.43	890.31	16.40	1,012.14
36	4.93	0.00	5.61	0.00
37	4.93	0.00	5.61	0.00
38	4.93	0.00	5.61	0.00
39	4.93	0.00	5.61	0.00
40	46.44	890.31	52.79	1,012.14
Total	**315.46**	**5,341.87**	**358.63**	**6,072.86**

Table 2: Environmental criterion. BTC_i values and surfaces adopted in the BTC_{mean} calculation for each scenario

Scenario	Crop	Crop Yield (t/ha)	Input (GJ/ha)	LHV (GJ/t)	Surface (ha)	Landfill phase
First scenario (Energy maximisation)	Miscanthus	0.00 (1st year) 10.00 (2nd year) 20.00 (3rd year) 25.00 (from 4th to15th year)	43.79 (1st year) 15.84 (from 2nd to14th year) 30.46 (15th year)	17.60	2.14	Aftercare
Second scenario (Phytotreatment)	Sunflower	0.90*	29.02**	38.40*	0.64	Operation
	Rapeseed	0.77*	21.33**	37.40*	0.64	
	Soybean	0.44*	27.18**	36.40*	0.64	
Third scenario (Environmental compensation)	Poplar plantation	16.20 (81 t/ha every 5 years)	45.66 (1st year) 23.36 (5th and 10th years)	17.80	0.62	Aftercare
	Poplar green belt	16.20 (81 t/ha every 5 years)	29.55 (15th year) 7.99 (other years)	17.80	0.70	
Fourth scenario (Combination)	Sunflower	0.90*	29.02**	38.40*	0.64	Operation
	Rapeseed	0.77*	21.33**	37.40*	0.64	
	Soybean	0.44*	27.18**	36.40*	0.64	
	Miscanthus	0.00 (1st year) 10.00 (2nd year) 20.00 (3rd year) 25.00 (from 4th to15th year)	43.79 (1st year) 15.84 (from 2nd to14th year) 30.46 (15th year)	17.60	1.29	Aftercare
	Poplar plantation	16.20 (81 t/ha every 5 years)	45.66 (1st year) 23.36 (5th and 10th years)	17.80	0.62	Aftercare
	Poplar green belt	16.20 (81 t/ha every 5 years)	29.55 (15th year) 7.99 (other years)	17.80	0.70	

* values referred to the final oil product
** values including the oil production

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Francesco

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Francesco