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CICLO XXVI

PHYTOREMEDIATION OF DIFFERENTS WASTEWATERS USING ENERGY CROPS

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ABSTRACT

The sources of renewable energy acquire considerable interest, if accompanied by a more rational use of energy, to facilitate the transaction by a high use of fossil fuels to a sustainable use of renewable energy. There are many alternative energy source such as wind, solar, geothermal and biomass that fulfil the criteria of sustainability and economic feasibility. Biomass refers to all the vegetable matter that can be obtained from photosynthesis. Biodiesel can be produced from a variety of feedstock; they are renewable, sustainable, biodegradable, and environmentally friendly.

Decentralized wastewater treatment systems are designed to operate at small scale; they not only reduce the effects on the environment and public health but also increase the ultimate reuse of wastewater depending on the community type, technical options and local settings. Used effectively, it promote the return of treated wastewater within the watershed of origin. Aquanova is a flexible system of decentralized processing in which every single supply in term of water resources, organic substance and energy and its subsequent disposal, once it has finished its function (wastewater sewerage, solid waste) is considered as a part of the close loop of the sustainable management. Aquanova provides for the source separation of municipal wastewater in three separate streams (brown waters, yellow waters and grey water) through the use of special toilet. The yellow and grey waters are treated in a wetland system, considered a low energy demand and limited environmental impact technology; these phytotreated waters can be reused for toilet flushing.

Landfill leachate can be defined as the liquid produced from the decomposition of waste and infiltration of rainwater in a landfill; it contains heavy metals, salts, nitrogen compounds and various types of organic matter. Generation of leachate occurs when moisture enters the refuse in a landfill, dissolves the contaminants into liquid phase and produces moisture content sufficient to initiate liquid flow. Leachate varies from one landfill to another, and over space and time in a particular landfill with fluctuations that depend on short and long-term periods due to variations in climate, hydrogeology and waste composition.

Phytoremediation is characterised by biological type treatments, in which the plants growing in water-saturated soil develop a key role for direct action of the bacteria that colonize the root system and rootstock. Practically, it consists of mitigating pollutant concentrations in contaminated soils, water or air with plants able to contain, degrade or eliminate contaminants. It has the advantage to be an in situ technology, but on the other hand it is a quite slow process as it is dependent on a plant's ability to grow in a stressed environment that is not ideal for the normal plant growth. The

use of phytoremediation is one possibility to develop an economically and environmentally sustainable management of waste and polluted sites, which is raising interest in recent years.

The present research tested different wastewater streams, by the use of the decentralized Aquanova systems for the domestic wastewaters and landfill leachate. The wastewaters were treated through phytoremediation facilities; for each case of study it has been proposed oleaginous plants- known as energy crops- as species for the phytotreatment; subsequently the cultivated seeds were considered as suitable biomass for the production of biodiesel in a short time. All these aims were developed in four experimental phases; a greenhouse was used to control the temperature and the light exposition of the plants.

The ***first phase*** proceed with the phytotreatment using six 300 liters tanks filled with 10 cm coarse gravel and 30 cm of mixture soil, chosen the following crops: *Helianthus annus* (H), *Glycine max* (G) and *Brassica napus* (B). The wastewater components (grey and yellow waters) have been separated through the toilet facilities of the Aquanova project implementation at the LISA laboratory, as mentioned before. Half of the tanks were irrigated with increasing percentage of grey and yellow waters (0.1-3.5% YW and 99.9- 96.5% GW), and the other half with tap water as control units.

In the ***second phase***, old landfill leachate was used as irrigation water. Coarse gravel was arranged in 10 cm drainage layer on the bottom of each pot; pure sand in half pots and a mixture of sand and clayey soil in the other half pots were used to build up a 30 cm deep growing layer. Half of the pots were irrigated with increasing leachate concentrations (2-30 % Leachate; 90 – 70% tap water), and the other half with tap water as control units. *Brassica napus* grew slowly compared to the other vegetal essences and it did not produce any flower and it was favoured by sand, rather than soil substrate; their response ca be attributed at the captivity inside the greenhouse.

According to the results obtained in the two previous phases, in the ***third phase*** *Brassica napus* was not used anymore. Here, the seeds (H & G) were germinated in LISA laboratory under controlled conditions, using different kinds of substrate and different leachate dilution in order to test the maximum leachate percentage to be used in the irrigation. *Glycine max* seeds presented better germination at 5% of diluted leachate and in sand substrate, while *Helianthus annus* seeds had a better germination on soil mixed with concentrated solutions at 10% to 20% of leachate. The irrigation water was decided as a mixture of 20% leachate and 80% grey water. The same greenhouse and the same pots were used, half of those irrigated only with tap water as control.

Last experimental phase was performed in the same (eight) tanks used in phase 1 (four for each species, *Helianthus annus and Glycine max*) inside the greenhouse. Six tanks were irrigated with the leachate mixture (10 - 60% leachate and 90 - 40% tap water) and the other two with tap water as control.

The results of the whole research can be summarized as removal efficiencies of each tested analytical parameter. Analysis was performed in double. Mass balance of the two representative parameters as nitrogen and phosphorus was performed. The phytotreatment did not inhibit the growth of the species: in fact the energy crops produced bigger biomass and roots length with wastewater feeding rather than with tap water in each experimental phase, in similar way with the production of seeds. Nutrient removal by the plants was fully effective until the flowering point and after that, removal rates started decreasing. As grey water revealed lacking in nutrients, the increasing percentage of urine until 3.5 % in the feeding (phase 1), was crucial for the growth of the plants. The percentage of 20% leachate in the mixture has showed the best results in terms of growth of the plants and Nitrogen & Phosphorus removal efficiencies. The sand was not a good substrate for the growth of the plants, even if the irrigation water was leachate, rich in nutrients, except for *Brassica napus*. With mixed soil, better performances in removal rates were obtained.

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1 INTRODUCTION

Over the years, we are facing a very strong reality, it shows us how are we (and even we will be) able to develop with regard to new technologies in innovative creations that give us greater pleasure and comfort to live on this planet; so we define it today: more material things we possess (latest electrical and electronic equipment, clothing brand, furnishings, etc.), better our lifestyle; that it to say, our level of consumerism has become part of a priority, and therefore accumulate more residues that are becoming more difficult to degrade, and do not forget to mention the world of industry that is growing rapidly. Unfortunately all this lifestyle carries very serious consequences from the environmental point of view, putting in real danger our natural resources: flora, fauna, and our own health. “More things we acquire, more waste we produce”.

In addition, with the rapid population growth (including the growth of our needs), the high-energy demand in the industrialised world, as much in the domestic sector, as in transport and industry its increase, and the derived problems of the widespread use of fossil fuels, make increasingly necessary the development of renewable energy resources of limitless duration and smaller environmental impact than the traditional ones (G. Antolin et al, 2002).

The rapid depletion of non-renewable fossil resources has not continue; coal, oil and gas, which will certainly be of great value to future generations, are non-renewable natural resources.

For all these, there are made (and continue doing) research to minimize these aspects that directly impact our environment, manly, based on savings, reuse and optimization of our resources studies: water, soil, vegetation, energy, reuse of waste (solid, liquid and gaseous).

Water is becoming an increasingly scares resources that are forced to consider any sources of water which might be used economically and effectively to promote further development. At the same time, with population expanding at a high rate, the need for increased food production. As long as good quality water is scarce, water of certain type of quality will have to be considered for use in agriculture. With the usual emphasis on environmental health and water pollution issues, there is an increasing conscience of the need to dispose this wastewater safely and beneficially.

Apart from the natural scarcity of freshwater in various regions and countries around the world, the quality of the available freshwater is also deteriorating due to pollution. It is estimated that today throughout the world, more than 5 million people die annually from illnesses caused by drinking poor quality water. The number of people lacking access to safe drinking water, mainly in developing countries, will increase more and more (Kivaisi, 2001).

Fresh water resources become more and more contaminated with micro-quantities of many man-made pollutants (different chemicals introduced for the benefit of daily life, medicine, food production and industrial purposes); besides, some of these pollutants may possess the undesirable property of exerting estrogenic activity on various higher organisms (P. Schoder et al, 2007). Due to steadily improving capabilities for environmental analysis, we are able to detect compounds in very low concentration ranges in water bodies and sediments.

Wastewater reuse is an important strategy for conserving water resources, particularly in areas suffering from water shortage (A.K. Kivaisi, 2001). The reduction of pollution in wastewater will depend on what a given community or an industrial area allows into the effluent stream, and on the efficiency and effectiveness with which these effluents are treated.

Industrial and municipal wastewaters discharged into aquatic ecosystems either directly because of inadequate treatment of process water can lower water quality of a region by increasing concentrations of pollutants such as organic matter, suspended particulates, micro pollutants, nutrients (phosphorus and nitrogen) or heavy metals, thereby causing adverse effects on human health and undesirable changes in the composition of aquatic biota.

Properly planned use of municipal wastewater alleviates surface water pollution problems and not only conserves valuable water resources but also takes advantage of the nutrients contained in sewage to grow crops. Many countries, like Australia, Usa, etc., have included wastewater reuse as an important dimension of water sources, using wastewater in agriculture, releasing high quality water supplies for potable use.

It is generally accepted that wastewater use in agriculture is justified on agronomic and economic grounds but care must be taken to minimize adverse health and environmental impacts.

Decentralized wastewater treatment systems collect; are the main scope to treat and reuse and/or dispose treated wastewater at or near the generation point, is an effective method to optimise the environmental sustainability of the system. (T.A. Larsen and M. Maurer, 2011).

Advantage of decentralized wastewater treatment:

- ✓ are designed to operate at small scale (USEPA, 2004), they not only reduce the effects on the environment and public health but also increase the ultimate reuse of wastewater depending on the community type, technical options and local settings.
- ✓ used effectively, promote the return of treated wastewater within the watershed of origin.

- ✓ can be installed on as needed basis, therefore evading the costly implementation of centralized treatment systems.
- ✓ are generally more profitable for managing in rural areas than the centralized systems. (USEPA, 1997).

Disadvantages of decentralized wastewater treatment:

- ✓ it cannot treat and discharge large quantities of wastewater (West, 2001).
- ✓ Mechanical systems are efficient in terms of spatial requirements compared with natural systems.

Considering the main problems cited before, population increases and a higher consumption of energy; resources of oil, natural gas and so on, will be exhausted within a few decades. In this context, important researches and development of technologies are being conducted, allowing the production of *clean energy* through the use of renewable resources (water, sun, wind, biomass, geothermal, hydrogen), to contribute to a global sustainable development and that is, while preserving the environment (A.M. Omer, 2008).

An ever-increasing demand could put considerable pressure on the current energy infrastructure and potentially damage world environmental health by the emission of CO, CO_2, CH_4, SO_2, NO_x effluent gas emissions and the consequent global warming. According to Omer (2008), the world population is growing fast with an average of 2%, which increases the needed for more energy (Figure 1.1.).

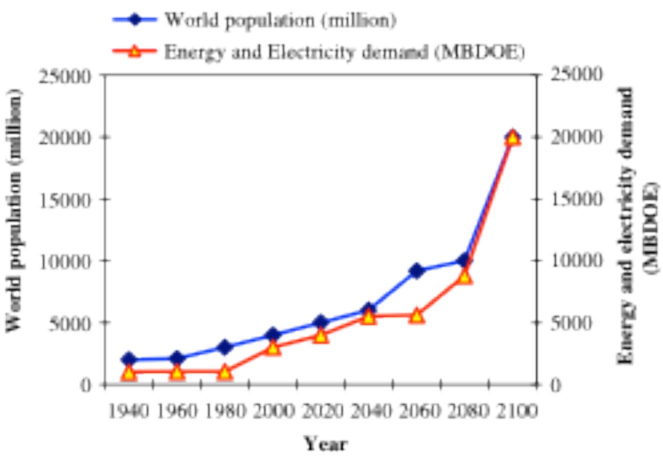


Figure 1.1. Annual and estimated world population and energy demand in [Million of barrels per day of oil equivalent (MBDOE)] (A.M. Omer, 2008)

Improved lifestyle and energy demand rise together and the wealthy industrialised economics, which contain 25% of the world's population, consume 75% of the world's energy supply. The world's energy consumption today is estimated to 22-billion kWhyr -1. About 6.6 billion metric tons carbon equivalent of greenhouse gas (GHG) emission are released in the atmosphere to meet this energy demand. Approximately, 80% is due to carbon emissions from the combustion of energy fuels, concern values to be considered by the competent authorities.

The energy consumption can be categorized according to usage:

- ✓ Traditional sector- industrial, transportation, etc.
- ✓ End-use- space heating, process steam, etc.
- ✓ Final demand- total energy consumption related to automobiles, to food, etc.
- ✓ Energy source- oil, coal, etc.
- ✓ Energy form at point of use- electric drive, low temperature heat, etc.

Problems with energy supply and use are related not only to global warming that is taking place, due to effluent gas emission mainly CO_2 (see table 1.1.), but also to such environmental concerns as air pollution, acid precipitation, ozone depletion, forest destruction and emission of radioactive substances.

Rank	Nation	CO_2	Rank	Nation	CO_2	Rank	Nation	CO_2
1	USA	1.36	5	India	0.19	9	Mexico	0.09
2	Russia	0.98	6	UK	0.16	10	Poland	0.08
3	China	0.69	7	Canada	0.11	11	S. Africa	0.08
4	Japan	0.30	8	Italy	0.11	12	S. Korea	0.07

Table 1.1. Global emissions of the top twelve nations by total CO2 volume (billion of tons). (A. M. Omer, 2008)

Renewable energy sources acquire considerable interest, since appear to be the most efficient and effective solutions for the intimate relationship between renewable energy and sustainable development, accompanied by a more rational use of energy. Renewable energy is the term used to describe a wide range of naturally occurring, replenishing energy sources; this is particularly true, technically and economically feasible to supply all of man's needs from the most abundant energy source of all, the sun. The sunlight is not only inexhaustible, but it is the only energy source, which is completely non-polluting.

To reducing and controlling CO2, which is the major contributor to global warming, today are being used and / or exploring alternative methods of power generation and could be used in the future as *green energy* sources.

There are many alternatives for energy source such as wind, solar geothermal and biomass that fulfil the first criterion: sustainability, and second criterion: economic feasibility. The best option of both is biofuel, particularly that made from readily available biomass feedstock.

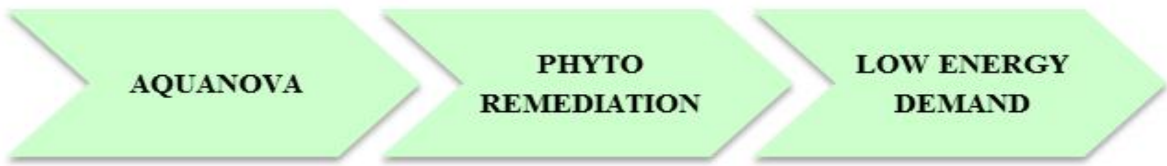
Biomass refers to all the vegetable matter that can be obtained from photosynthesis. Biofuel can be produced from a variety of bio-feed stocks; they are renewable sustainable, biodegradable, carbon neutral for the whole life cycle and environmentally friendly; biodiesel is an alternative fuel, produced by chemically reacting a vegetable oil or animal fat with an alcohol, is biodegradable and non-toxic, and it significantly reduces toxic and other emissions when burned as a fuel (Yusuf et al, 2011).

The main advantages are related to energy, agriculture and environment problems, its can be summarised as follows:

- ✓ Reduction of dependence on import of energy and related products.
- ✓ Reduction of environmental impact of energy production (greenhouse effect, air pollution, waste degradation).
- ✓ Substitution of food crops and reduction of food surpluses and of related economic burdens. Utilisation of marginal lands and of set aside lands and reduction of related socio-economic and environmental problems (soil erosion, urbanisation, landscape deterioration, etc.).
- ✓ Development of new know-how and production of technological innovation.

Until now are mentioned three fundamental aspects to be treated; decentralized wastewater systems, reuse wastewater and renewable energy; achieving the wastewater treatment and production of energy at the same time; saving potable water for crops irrigation; and not competing between land for food and for energy. All this can be achieved by creating a decentralized system: low energy demands, that reutilize the materials to be treated, instead of polluting decontaminate the environment, thus allowing to close the cycle.

In this way, the following concepts are proposed:



The Aquanova Project;

The Aquanova project is inspired by the growing need for management of water resources that yields water saving through its reuse and reduce energy consumption in the early stages of procurement and final treatment.

The Aquanova project is based on a system of decentralized processing and flexible, in which every single supply in term of water resources, organic substance and energy) and its subsequent disposal, once it has finished its function (wastewater sewerage, solid waste) is considered the interior of a sustainable management. It is based on the study, implementation and application in different areas and different scales, of a system of integrated management of municipal wastewater and putrescible organic fraction of municipal solid waste.

The Aquanova system provides for the separation of the origin of municipal wastewater in three separate streams (brown waters yellow waters and grey water) through the use of special toilet. Also planned is the introduction of a quarter effluents obtained by crushing through a heatsink domestic putrescible organic fraction. The four streams are treated in a purification system with which you can achieve water savings, energy to the recovery and reuse of water resources. The four streams are treated in a purification system with which you can achieve water savings, energy to the recovery and reuse of water resources.

The yellow and grey waters are instead treated in a wetland system: the use of this facility allows the killing of nutrients present in the grey water and yellow with the use of a technology with low energy demand and limited environmental impact; also the use of plants for ornamental vegetation plant phytoremediation determines in overall enrichment and an embellishment of the landscape context in which the system inserts. The water exiting the wetland can be reused for toilet flushing.

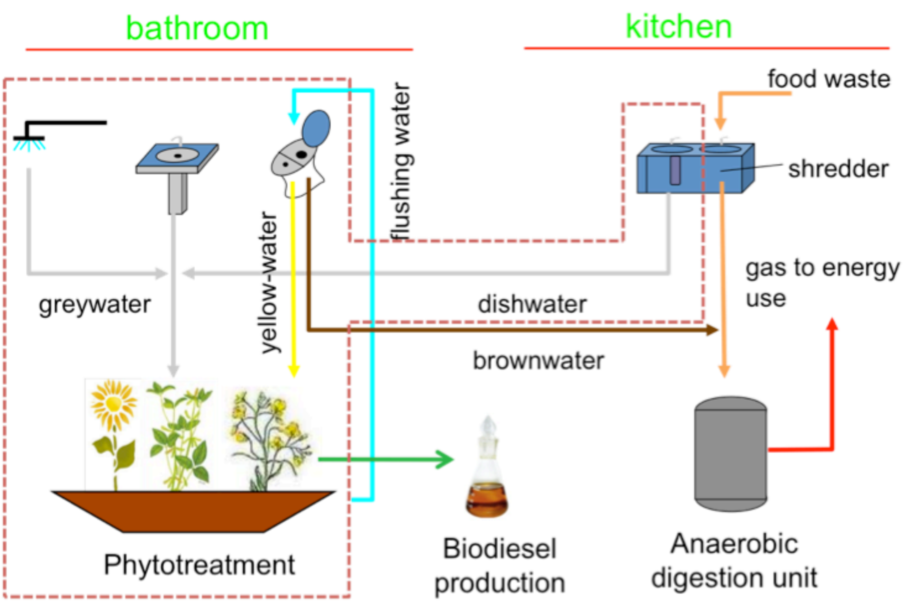


Figure 1.2. Separation of substances through Aquanova system and possible utilizations

The principal aim of the Aquanova project is to evidence the environmental sustainability of the integrated management system of waste and wastewater proposed by limiting the consumption of water resources, the energy production through anaerobic digestion of the organic fraction putrescible, brown water, and the use of techniques and low energy demand for the purification and the way they blend with the surrounding landscape.

The reduction of water consumption is obtained through the use of toilet flow separation in which it is possible to measure the amount of water required for the expulsion of the flushing. The division of waters yellow and brown makes it possible to use much less than a litre for the expulsion of yellow waters and a few litres (4-5) for the expulsion of brown water.

Figure 1.3. Toilet in flow separation

The opportunity to use the phytopurified water for replenishment of toilet flushes further reduces the need for new water resources by maximizing the reuse of the same. The use of the management for the anaerobic treatment of water and brown organic fraction putrescible allows the production of usable biogas for energy purposes directly on site with a technology with low energy demand.

The use of species for phytoremediation also equipped with aesthetic value, as well as purifying, allows optimum incorporation of such Implant in the surrounding landscape. The innovative aspects of the system, are integrated waste management and liquid, their separate collection and treatment systems for the detection of low-cost energy, chosen specifically to the intrinsic characteristics of individual streams to be treated and yet the decentralization of wastewater treatment plants which allows for greater awareness and empowerment of the population served, a reduction of the environmental impacts of large centralized treatment plants and a reduction in the cost of transport and management of liquid and solid waste.

Phytoremediation

Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. Over the past 20 years, this technology has become increasingly popular and it has been employed at sites with soils contaminated; it has the advantage that environmental concerns may be treated in situ; one major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on a plant's ability to grow and thrive in an environment that is not ideal for normal plant growth. Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering on going pollution; this refers to the natural ability of certain plants called hyperaccumulators to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air.

Phytodepuration is characterised by biological type treatments, in which the plants growing in water-saturated soil develop a key role for direct action of the bacteria that colonize the root system and rootstock. The ability of wetlands to transform and store organic matter and nutrients has resulted in wetlands often being described as (Mitsch & Gosselink, 1993) and being exploited for water quality improvement.

Phytoremediation is best applied at sites with shallow contamination of organic compounds and metal pollutants that are amenable to:

- ✓ phytoextraction: utilization of plants to remove inorganic compounds, principally metals, from contaminated soils without destroying the soil structure and its fertility; the removal of pollutants happens through the gathering of the aerial part of the plants grown in contaminated substrates.
- ✓ rizofiltration: this technique exploits terrestrial or aquatic plants to adsorb, concentrate and precipitate in the roots the polluted substances present in water streams characterized by a low level of contamination; in addiction, the plants have the capacity to release in the rizosphere exuded radicals that favour the precipitation of many metals; the contaminants immobilized or accumulated in the plants or near them are removed with the gathering of plants;
- ✓ phytostabilization: it foresees the immobilization of soil contaminants through absorption and accumulation in the roots, adsorption on the roots, precipitation in the rizosphere, complexation or convertion to chemical forms less toxic; the exploitation of plants allows to prevent the dispersion of pollutants caused by lisciviation, hydric or eolic erosion, and from the dispersion of the soil, reducing the bioavailability of metals for the animal organisms and their entrance in the food chain;
- ✓ phytovolatilization: it is a specialized form of phytoextraction that can be employed only for contaminants highly volatile, such as mercury and selenium; the plants used absorb the contaminants from the soil and convert them, through the action of enzymes, in volatile compounds less toxic that can be released in the atmosphere through the transpiration of plants.

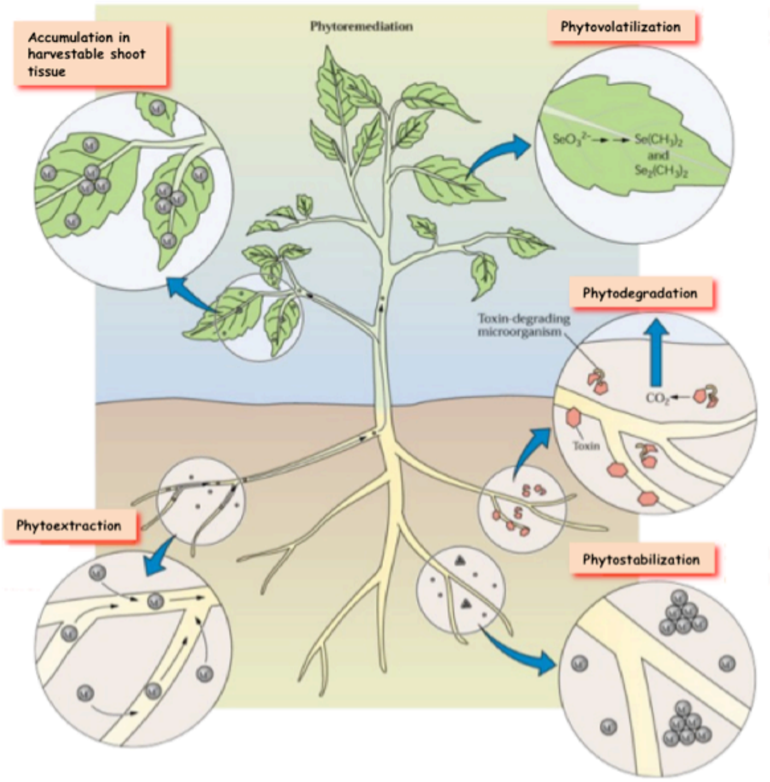


Figure 1.4. Principal processes involved in phytodepuration (Pilon-Smits E., 2005)

Generally, the characteristics a plant should embody to be ideal for phytodepuration are the following:

- wide radical apparatus;
- efficient translocation from the rizosphere to the aerial part;
- noticeable biomass;
- quick growth.

The phytoremediation techniques (green bioremediation) currently being developed and applied in constructed wetlands and barrier systems seem rather poor. However, they have been demonstrated to be very effective in numerous cases and especially in small systems, even if they might appear somewhat primitive; they guarantee a stable effluent quality with low nutrient content, so affording high hygienic levels (P. Schoder et al, 2007).

The design of a system of phytoremediation is complex and requires multidisciplinary skills, relating to:

- the characterization of the soil and the influence of its characteristics on plant growth;
- modelling the flow of groundwater and surface water at the site to study the dangers and transport of contaminants in the groundwater;
- to plant physiology to identify the effects produced by the contaminant on the plants and the type of plants most suitable to the site in question;

- identification of microorganisms influential on the process and mechanisms of influence;
- the management system and the optimization of its performance.

Constructed Wetlands (CW's)

A constructed wetland (CW) is an artificial wetland created as a new or restored habitat for native and migratory wildlife, for anthropogenic discharge such as wastewater, storm water runoff, or sewage treatment, for land reclamation after mining, refineries, or other ecological disturbances such as required mitigation for natural areas lost to a development; it act as a biofilter, removing sediments and pollutants such as heavy metals from the water, and constructed wetlands can be designed to emulate these features.

These areas are now widely used to improve the quality of point and nonpoint sources of water pollution, including storm water runoff, domestic wastewater, agricultural wastewater, and coal mine drainage. Systems for treatment of wastewater by artificial wetlands are engineered systems that have been designed and realized with the aim of reproducing the natural auto-depurative processes in a more controllable environment. The CW system could be used to achieve a better removal of nutrients and pathogens from wastewater prior the final release into the water supply (A.K. Kivaisi, 2001).

Constructed wetlands (CW's) are designed to mimic natural wetland systems, utilizing wetland plants, soil, and associated microorganisms to remove contaminants from wastewater effluent. CW's are artificial marsh or swamp, which have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial assemblages to assist in waste treatment. It usually consists of a number of individual rectangular and/or irregularly-shaped basins connected in series and surrounded by clay, rock, concrete or other materials. Three types of cells may be used in a constructed wetland system (CWS): free water surface (FWS) cells; sub-surface flow (SSF) cells, and hybrid cells that incorporate surface and subsurface flows.

Several discussed about the advantages of using constructed wetlands for wastewater treatment were done, especially compared to conventional treatment systems, CW as more natural system, is effective, relatively cheap, more easily operated, more efficient to maintain and have low environmental impacts. Minimal fossil fuel is required and no chemicals are necessary (Kadlec and Knight, 1996, T.A. Larsen and M. Maurer, 2011). However, there are space intensive, and thus in competition with other space-consuming activities.

An additional benefit gained by using wetlands for wastewater treatment is the multi-purpose sustainable utilization of the facility for uses such as swamp fisheries, biomass production, seasonal agriculture, water supply, public recreation, wild life conservation and scientific study. Being low-cost and low-technology systems, wetlands are potential alternative or supplementary systems for wastewater treatment.

CW's systems reduce or remove contaminants including organic matter, inorganic matter, trace organics and pathogens from the water, due to a high rate of primary productivity and a reduced rate of decomposition due to anaerobic conditions; this reduction is to be accomplished by diverse treatment mechanisms including sedimentation, filtration, chemical precipitation and adsorption, microbial interactions and uptake by vegetation (Watson et al., 1989; Brix 1993a).

Incoming nutrients support the growth of vegetation, which converts inorganic chemicals into organic materials, the basis of the wetland food chain. As a result of ample light, water and nutrient supply, the primary productivity of wetland ecosystems is typically high (Brix, 1993a). The hydrology of the place, vegetation and soil has been reported to be the main factors influencing water quality in wetlands. The hydrological cycle is the main factor, which influence the type of vegetation, microbial activity, and biogeochemical cycling of nutrients in soil (Mitsch & Gosselink, 1993, Kivaisi, 2001). Plants in a natural wetland provide a substrate (roots, stems, and leaves) upon which microorganisms can grow as they break down organic materials and uptake heavy metals (Zhang et al, 2010).

CW's have proven successful for remediating a variety of water quality issues, with advantages over the natural wetlands, but still have some disadvantages: performance of CWS may be less consistent than in conventional treatments due to the environmental changes at different seasons; the biological components are sensitive to toxic chemicals (e.g., ammonia and pesticides); and flushes of pollutants or surges in water flow may temporarily reduce treatment effectiveness.

Then, below are cited the most important advantages and disadvantages of the Constructed wetlands (CW) – secondary treatment (M.A. Massoud et al, 2009).

<u>Advantages :</u>

- ✓ Very minimal operation needed
- ✓ The lower organic and suspended solids content of the effluent may allow a reduction of land area requirements for substrate disposal systems.
- ✓ Inexpensive to operate and construct
- ✓ Reduced odours
- ✓ Able to handle variable wastewater loadings

- ✓ Reduces land area needed for wastewater treatment.
- ✓ An additional benefit by using wetland for wastewater treatment is the multi-purpose sustainable utilization of the facility for uses such swamp fisheries, biomass production, seasonal agriculture, wildlife conservation and scientific study (A.K. Kivaisi, 2001).

Disadvantages:

- ✓ The area of a site occupied by the wetland would have very limited use
- ✓ Require a continuous supply water
- ✓ Affected by seasonal variations in weather conditions
- ✓ Can be destroyed by overloads of ammonia and solids levels
- ✓ Remove nutrients for use of crops

In synthesis, constructed wetlands (and similar green technologies) are of high sustainability require a low input in energy and manpower, and offer possibilities of carbon sequestration in biomass, as well as the recycling of materials and matter (P. Schoder et al, 2007). In other words, they are effective in treating organic matter, nutrients and pathogens and are used worldwide to treat different qualities water. Compared to conventional technical solutions for water treatment, CW's are relatively easy to maintain and operate, resulting in low operative costs.

Besides water quality improvement and energy savings, CW's have other features related to the environmental protection such as promoting biodiversity, providing habitat for wetland organism and wildlife, and serving climatic (less CO2 production, and hydrological functions, and heavy metal accumulation and biomethilation.

There are two basic types of constructed wetlands according to the type of flow: surface flow and subsurface flow systems; species of plants, conception of the system (dimensions and number of beds) and structure of substratum (soil or gravel). Surface flow wetlands are similar to natural wetlands (figure 5.1a), with shallow flow of wastewater over saturated soil substrate.

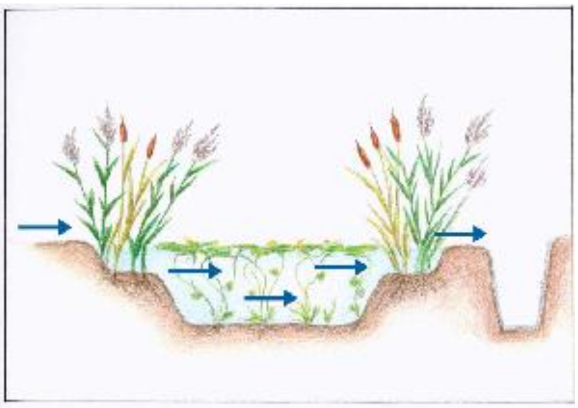


Figure 1. 5(a). Constructed wetland surface free flow

Subsurface flow wetlands mostly employ gravel as the main media to support the growth of plants; wastewater flows vertically (figure 1.5b) or horizontally (figure 1.5b) through the substrate where it comes into contact with microorganisms, living on the surfaces of plants roots and substrate, allowing pollutant removal from the bulk liquid (T. Saeed and G. Sun, 2012).

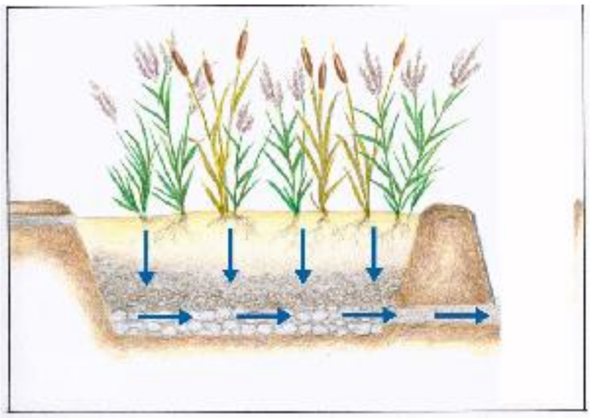


Figure 1. 5(b). Constructed wetland subsurface vertical flow

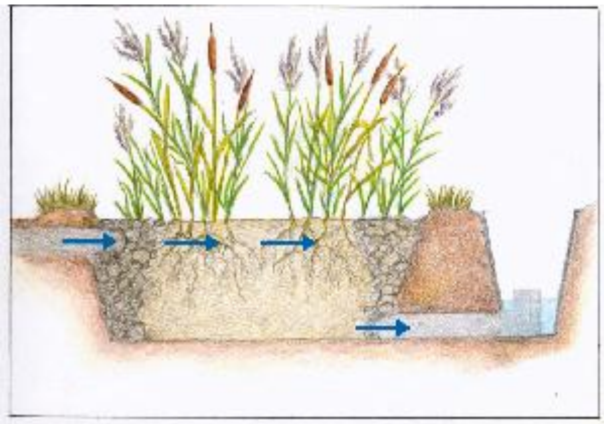


Figure 1. 5(c). Constructed wetland subsurface horizontal flow

A comparison between vertical subsurface flow (SSVF) and subsurface flow horizontal flows (HF) systems is found in Table 1.2.

Type	Advantages	Disadvantages
Vertical flow wetlands	Smaller area demand.	Short flow distances.
	Good oxygen supply, good nitrification, better organics and SS removal, simple hydraulics.	Poor denitrification, higher technical demand, low nitrate removal.
	Higher purification from the beginning, better than HF beds as water flows from surface to bottom, which enhances oxygen mixing.	Loss of performance especially P removal.
Horizontal flow wetlands	Long flowing distance, nutrients gradients can be established, efficient in the removal of SS, organics.	Higher area demand, clogging problem is observed, sulphur transformation can affect nitrification sensitivity, loss of P removal performance.
	Possible Denitrification.	Careful calculation of hydraulics necessary for optimal oxygen supply, low ammonium oxidation.
	Formation of humic acids for N, P removal.	Uniform passage of wastewater throughout the packed media is complicated (due to possible presence of

		dead zones).

Table 1.2. Advantages and disadvantages of VF and HF wetlands (T. Saeed, G. Sun, 2012)

The steps to be followed for a correct design of the CW's system are:

- Site characterization;
- Selection of plant species;
- Assessing the possibilities for remediation of the site;
- Density and form of planting;
- Irrigation, chemical additives and conservation;
- Possibility of seepage into the groundwater and transpiration rate;
- Rate of absorption of contaminants and time required for remediation.

2 STATE OF THE ART

2.1. Domestic Wastewaters

Wastewater collection systems, centralized and decentralized treatment systems are designed and managed primarily to protect human and environmental health. A positive aspect of the sewer network is the collection and transport of wastewater to appropriate treatment facilities, whereby pathogens and chemical constituents such as oxygen- depleting organic matter and phosphorus are removed before the treated water is returned to the environment; a negative aspect of such a network is that it can create an imbalance in water and nutrient fluxes and therefore distort natural hydrological and ecological regimes.

In figure 2.1, is described a typical classification of wastewater, considering the separate flux at one it was explained in the previous chapter; with yellow colour are selected the wastewaters considered for this research.

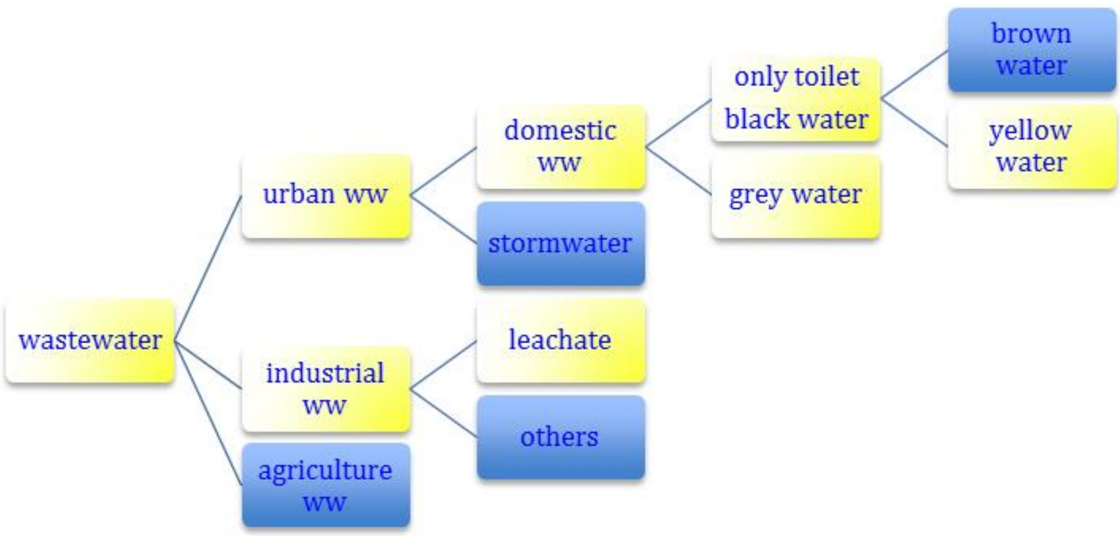


Figure 2.1. Wastewater classification

Wastewater is mainly comprised of water (99.9%), and often contains a variety of organic and inorganic compounds of anthropogenic and natural origin; the naturally occurring constituents in wastewater were present in the source that was supplied to the user. The constituents in wastewater can be divided into main categories (see table 2.1.) and the contribution of constituents can vary strongly.

Wastewater constituents		
Microorganisms	Pathogenic bacteria, virus and worms eggs	Risk when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers, lakes and fjords	Fish death, odours
Other organic materials	Detergents, pesticides, fat, oil and grease, colouring solvents, phenols, cyanide	Toxic effect, aesthetic inconveniences, bio accumulation in the food chain
Nutrients	Nitrogen, phosphorus, ammonium	Eutrophication, oxygen depletion, toxic effect
Metals	HG, Pb, Cd, Cr, Cu, Ni	Toxic effect, bioaccumulation
Other inorganic materials	Acids, for example hydrogen sulphide bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora e fauna
Odour (and taste)	Hydrogen sulphide	Aesthetic inconvenience, toxic effect
Radioactivity		Toxic effect, accumulation.

Table 2.1. Constituents present in domestic wastewater (M. Henze et al, 2008).

Transport of water and wastewater across watershed boundaries not only increases the embodied energy of a material and requires extensive infrastructure needs, but it may also result in adverse changes in an ecosystem's hydrology. In addition, the treatment facilities, while they treat wastewater to a quality deemed safe for discharge, also consume considerable energy during their operational life, and consequently contribute to atmospheric carbon dioxide emission.

The wastewater treatment technologies include mechanical systems, lagoons systems, and land treatment systems. Mechanical systems such as activated sludge utilize physical, chemical and biological mechanisms to remove nutrients, pathogens, metals and other toxic compounds. Lagoon systems primarily use physical and biological processes to treat wastewater, while land treatment systems utilize soil and plants, without significant need for reactors and operational labour, energy, and chemicals (Metcalf and Eddy, 2003).

Domestic wastewater is the discharge from domestic residences, commercial or industrial premises into the public sewer, originated from all aspects of human sanitary water usage. Constitutes from a combination of flow derivate from bathroom, toilets, floor traps, kitchen sinks, dishwasher and washing machines. In table 2.2, shows the compositions of typical domestic/municipal wastewater where concentrated wastewater (high) refers to low water consumption and/or infiltration; and diluted wastewater (low) represents the opposite of the first.

Parameter	High	Medium	Low
COD	1200	750	500
BOD	560	350	230
N total	100	60	30
Ammonia-N	75	45	20
P total	25	15	6
Ortho-P	15	10	4
TSS	600	400	250
VSS	480	320	200

Table 2.2. Composition of raw municipal wastewater (M. Hense et al, 2008)

The separation and draining of household waste into grey water and black water is becoming more common in the developed world, with grey water being permitted to be used for watering plants or recycled for flushing toilets. In domestic wastewater, around 80% of the nitrogen and 50% of the phosphorus stem from urine. Separating these nutrients efficiently at the source would result in a fairly balanced C:N:P ratio at the treatment plant and thus eliminate the need of advanced nutrient elimination. The requirements for domestic wastewater treatment are typically associated with the removal and conversion of dissolved organic matter to biomass, the removal and recovery of nutrients, and the inactivation of pathogens (A.G. Werker et al, 2002).

The concentrations found in wastewater are a combination of pollutant load and the amount of water with which the pollutant is mixed. The composition of the municipal wastewater varies from one location to another; on a given location the composition will vary with time, due to variations in the discharged amounts of substances.

2.1.1. Grey water.

Grey water is the urban wastewater that include the total volume that derives from washing food, clothes, dishware an also from bathing. It is difference from domestic waters in the absence of faecal matter and urine. The volume produced in a household is 50-80 % on the total wastewater (Buttler, 1995; Li et al, 2009); is generated as a result of the living habits of the people involved the products used and the nature of the installation, that’s why it characteristics are highly variable. Grey water is considered to be of no major hygienic concern, although it gives the largest portion of wastewater volumetrically.

In other words, grey water refers to combined domestic wastewater without toilet waste, is the most obvious target for source separation because of its value as an alternative to drinking water, especially for non-potable purposes. Reclaimed grey water is typically intended for toilet flushing, cleaning purposes, car washing and irrigation. Toilet flushing is the typical example for indoor use and irrigation the typical example for outdoor use, and the decentralized reuse of these two application can be present the most advantages and risks.

A decentralized approach to grey water recycling for non potable purposes is often taken it is attractive to avoid a second distribution net, and for water, which is not of drinking water quality, a shorter residence time in the system is favourable.

In general, grey water is high in S, Ca, K and Al, and the concentration levels of the trace nutrients are closed to the reported requirements. Due to the low levels of contaminating pathogens and nitrose, reuse and recycle of grey water is receiving more and more attention.

Water reuse is the dominating reason for decentralized grey water treatment; it is generally considered more attractive for reuse than combined wastewater from the point of view of both aesthetics and pathogen organism. The amount of grey water produced greatly varies from 15 l/person/day to more than 100 l/person/day. (T.A. Larsen & M. Mauren, 2011; E.Erikson, 2002 and Li et al, 2009). The major target of grey water reclamation and reuses is to reduce the suspended solids, the organic strength and the microorganism due to its relationship with the aesthetic and health characteristic of the product water and directly through legislative requirements.

Grey water is generated by the use of soap products or soap products for body washing and as such, varies in quality according to geographical locations, demographics and level occupancy (Table 2.3). Grey water has a similar organic strength to domestic wastewater but is relatively low in suspended solids and turbidity, indicating that a greater proportion of the contaminants dissolved; however, their chemical nature is quite different than those from domestic wastewater (B. Jefferson et al., 1999).

	BOD_5 (mg/l)	COD (mg/l)	Turb (NTU)	NH_3 (mg/l)	P (mg/l)	Total coliforms
Hand basin	109	263	–	9.6[a]	2.58	–
Combined	121	371	69	1	0.36	–
Synthetic grey water	181	–	25	0.9	–	1.5×10^{8}
Single person[b]	110	256	14	–	–	–
Single family[c]	–	–	76.5	0.74	9.3	–
Block of flats[b]	33	40	20	10	0.4	1×10^{6}
College[b]	80	146	59	10	–	–
Large college[d]	96	168	57	0.8	2.4	5.2×10^{6}

[a] Total nitrogen.

Table 2.3. Typical composition of Grey Water from various resources (B. Jefferson et al, 1999).

The most common application for GW reuse in urban areas is toilet flushing, which can reduce water demand within dwellings by up to 30%. However, other applications such as irrigation of green areas in parks, schoolyards, cemeteries, golf areas, car wash, and fire protection are practiced. The use of GW for irrigation is one of the methods that is currently widely used. This is particularly important in arid zones, where water is scarce and reuse of GW for irrigation could reduce potable water use by up to 50%, also because, this fraction of waste water is less polluted urban wastewater not differentiated due to the absence of faeces, urine and toilet paper. Hygienic aspects, related to the presence of microorganisms, and the dynamics of organic substances and metals are aspects to monitor closely. (Eriksson et al., 2002). The nutrients found in domestic sewage and organic waste are nearly sufficient to fertilize the plantations for the production of food to feed the world's population. Is about 20-40% of water consumption in cities with sewer systems is used for flushing the toilets; to make this possible, it is necessary to recycle nutrients, reduce water consumption, and minimize the energy requirements to operate the waste treatment processes.

The characterization of the water takes on an important significance in the evaluation of the possibility of reuse, including the need for proper treatment; the typical flow characteristics reveal that the urine contains the highest percentage of nutrients: their separation in the first analysis can reduce the cost of nitrification - denitrification in treatment plant (Gabrel, 2001). Besides the high content of nutrients (phosphorus and nitrogen) of the urine makes excellent fertilizer, since the artificial production of nitrate and phosphate-mining processes are extremely expensive (in terms of economic and energy). Faeces, with high organic content and rich in pathogens, can be stabilized and sterilized, and used in agriculture along with the urine. The following table (2.4) summarizes the flow characteristics of grey, yellow and brown.

	Grey water	*Yellow water*	*Brown Water*
Volume (l/p/yr)	25000 - 100000	500	50
COD	41 %	12 %	47 %
N	3 %	87 %	10 %
P	10 %	50 %	40 %
K	34%	54 %	12 %

Table 2.4. Characteristics of GW, YW and BW. (Otterphol, 1999)

2.1.2. Yellow water.

Yellow water is a domestic wastewater stream originating in new sanitation systems focusing on decentralized treatment. These systems are especially relevant for areas without existing wastewater treatment, with water scarcity or with high fertilizer prices. Urine is a possible alternative fertilizer for agriculture as it contains relatively high concentrations of the macronutrients nitrogen, phosphorus, and potassium. It is used as fertilizer in agriculture in countries all around the world. However, this usage of urine is associated with a risk of transfer of pharmaceutical residues to agricultural fields (Lienert et al., 2007; Winker et al., 2008b).

Traditionally, urine has been collected and used as a fertilizer in agricultural applications. The largest part of nutrients in waste waters stem from urine, with about 80% of the nitrose and 50% of the phosphor in wastewater originate from urine. Urine source separation will be the choice where treatment plants do not exist and nutrient removal is required for water pollution control; at the same time, the nutrients in urine could be used beneficially in agriculture as a fertilizer; this is favourable especially in view of the limited phosphorus resources (T.A. Larsen and M. Mauren, 2011).

Nitrogen is present in urine, in the form of ammoniac or urea, which is a valuable material for use as a fertilizer. This would replace a chemical synthesis of these compounds, which require energy and auxiliary materials. Nitrogen recovery from urine can result in a lower ecological burden in comparison to the use of chemically produced fertilizer. The nutrients in urine reflect the components necessary for plant growth, which makes it suitable as a fertilizer in agricultural applications (W. Pronk, D. Koné, 2009). Some urine- separating toilets also give rise to water saving and can therefore be seen as an alternative to grey water recycling for toilet flushing.

The urine containing a large fraction of the plant nutrients if is collected separately, simultaneously, flush water is saved (B. Vinnera°s & H. Jonsson, 2002). If al the toilet waste is recirculated to agriculture, between 75% and 85% of the nitrogen, phosphorus and potassium from the households will be used as a resource instead of being a potential pollutant to the environment. The heavy metal content in the urine fraction is generally very low. Due to the high concentration of ammonia and the high pH in the urine, metals used in the urine sewer pipes are easily corroded and thereby contaminate the urine (yellow water).

The volume of yellow water collected depends on the model or urine separating toilet using; and the difference between excreted and collected amount of urine depends upon time spent at home and amount of misplaced urine.

The amount of organic waste and nutrients produced in households is showed in table 2.5, one can note that the urine (yellow water) is the main contributor to nutrients in household wastes, thus separating out the urine will reduce nutrient loads in wastewater significantly. Urine separation will reduce nitrogen content in domestic wastewater to a level where nitrogen removal is not needed.

Parameter	*Unit*	*Toilet*		*Kitchen*	*Bath/laundry*	*Total*
		Total *(incl. urine)*	Urine			
Wastewater	m3/yr	19	11	18	18	55
COD	kg/yr	27.5	5.5	16	3.7	47.2
BOD	kg/yr	9.1	1.8	11	1.8	21.9
N	kg/yr	4.4	4.0	0.3	0.4	5.1
P	kg/yr	0.7	0.5	0.07	0.1	0.87
K	kg/yr	1.3	0.9	0.15	0.15	1.6

Table 2.5. Source for householder components -especial attention in Urine-(M. Hense et al, 2008)

2.2. Landfill Leachate

Landfill leachate can be defined as the liquid produced from the decomposition of waste and infiltration of rainwater in a landfill; it contains heavy metals, salts, nitrogen compounds and various types of organic matter. Generation of leachate occurs when moisture enters the refuse in a landfill, dissolves the contaminants into liquid phase and produces moisture content sufficient to initiate liquid flow. Leachate varies from one landfill to another, and over space and time in a particular landfill with fluctuations that depend on short and long-term periods due to variations in climate, hydrogeology and waste composition (Keenan et al., 1984). Generally, leachate possesses high concentrations of ammonia and organic contaminants (measured in terms of chemical oxygen demand COD and biochemical oxygen demand BOD), halogenated hydrocarbons and heavy metals. In addition, leachate usually contains high concentrations of inorganic salts (mainly sodium chloride, carbonate and sulphate) and is dependent on the composition of the landfill waste.

Landfill leachate is a high strength wastewater generated as a result of percolation of the rainwater and moisture through the waste resulting in the solubilisation of nutrients and the contaminants within a landfill. Generally, the quantity of leachate is a direct function of the amount of external water entering the landfill.

The variation in leachate quality can be due to a variety of reasons based on the four aspects of landfills, which are:

- Waste type - its grade of decomposition with possible seasonal variance in its deposal;
- Landfill environment - waste degradation phase, humidity, precipitation, temperature, etc.;
- Filling technique - waste compaction, landfill cover and height of landfill layers; and
- Sampling - method of analysis and point of sample collection.

The leachate from landfills of wastewater is complex and highly polluted; that pollution is the result of biological processes, physical and chemical taking place all 'inside of landfills, together with the composition of the waste and the water regime of the landfill. .

Due to anaerobic conditions and long retention time prevailing in sanitary landfills, landfill leachate normally contains high concentrations of organic matters, nutrients, pathogens and heavy metals which, if not properly collected and treated, can cause serious pollution to nearby surface and groundwater sources. The presence of heavy metals at high concentrations in landfill leachate usually causes toxic effects to microbes, making it difficult to be treated biologically (V. Sawaittayothin and C. Polprasert, 2007).

Over the years it has been tried to use techniques for the treatment of leachate is more efficient, a possibility that is taking interest in recent years is the use of phytoremediation, or the purification of the soil from polluting substances, while maintaining a good quality status for cultivation farm.

Leachate is one of many external load (input/flow) wastewaters in the sewerage that a treatment plant has to handle. Leachate can contain high concentrations of soluble inert COD which passes through the plant without any reduction or change. In same cases where regulations do not allow discharge of untreated leachate, separate pre-treatment of leachate is required on-site prior to its discharge to a public sewer.

MSW Landfill leachate composition;

Leachate from MSW landfills typically has high values for total dissolved solids and chemical oxygen demand, and a slightly low to moderately low pH. MSW leachate contains hazardous constituents, such as volatile organic compounds and heavy metals. Wood-waste leachate typically is high in iron, manganese, and tannins and lignins. The precipitation that falls into a landfill, coupled with any disposed liquid waste, results in the extraction of the water-soluble compounds and particulate matter of the waste, and the subsequent formation of leachate. A typical quality of municipal waste is showed in table 2.6a.

Parameter	[b] *Typical Range (mg/l)*	[b] *Upper Limit (mg/l)*	[a] *Range (mg/l)*
pH			4.5 – 9
Total Alkalinity (as CaCO 3)	730–15050	20850	2500-3500
Calcium	240–2330	4080	10-7200
Chloride	47–2400	11375	150-4500
Magnesium	4–780	1400	30-15000
Sodium	85–3800	7700	70-7700
Sulfate	20–730	1826	8-7750
Total Dissolved Solids	1000–20000	55000	2000-60000
Chemical Oxygen Demand	100–51000	99000	140-152000
Biological Oxygen Demand	1000–30300	195000	20-57000
Iron	0.1–1,00	5500	3-5500
Total Nitrogen	2.6–945	1416	14-2500
Total Phosphorus			0.1 – 2.3
Potassium	28–1700	3770	50-3700
Chromium	0.5–1.0	5.6	0.02 – 1.5
Manganese	Not detected – 400	1400	0.03 – 1400
Copper	0.1–9.0	9.9	0.005 – 10
Lead	Not detected – 1.0	14.2	0.001-5
Nickel	0.1–1.0	7.5	0.015-13

Table 2.6 (a). Typical leachate quality of Municipal Waste ([b]Lee &Jones 1991, and [a]Kjeldsen 2010)

When waste is buried in a landfill, a complex series of biological and chemical reactions occur as the refuse decomposes. Generally, it is accepted that landfills undergo at least four phases of decomposition: an initial aerobic phase, an anaerobic acid phase, an initial methanogenic phase, and a stable methanogenic phase. Once the waste is very well decomposed, the rate of oxygen diffusion into the landfill may exceed the rate of microbial oxygen depletion.

Thus, over time the anaerobic landfill is hypothesized to become an aerobic ecosystem. As waste is buried in landfills over many years in a series of cells and lifts, it is quite common for different parts of the landfill to be in different phases of decomposition. An understanding of leachate composition is critical for making projections on the long-term impacts of landfills. Even after a landfill stop accepting waste and a final cover is placed over the landfill, the waste will continue to decompose (Kjeldensen, 2010).

Typically, the leachate can be characterized into main four groups of pollutants:

- ✓ Dissolved organic matter, quantified as Chemical Oxygen Demand (COD) or Total Organic Carbon (TOC), volatile fatty acids (that accumulate during the acid phase of the waste stabilization, Christensen and Kjeldsen, 1989) and more refractory compounds such as fulvic-like and humic-like com- pounds.

- ✓ Inorganic macrocomponents: calcium (Ca2+), magnesium (Mg2+), sodium (Na+), potassium (K+), ammonium (NH4+), iron (Fe2+), manganese (Mn2+), chloride (Cl–), sulfate (SO42–) and hydrogen carbonate (HCO3–).
- ✓ Heavy metals: cadmium (Cd2+), chromium (Cr3+), copper (Cu2+), lead (Pb2+), nickel (Ni2+) and zinc (Zn2+).
- ✓ Xenobiotic organic compounds (XOCs): originating from household or in- dustrial chemicals and present in relatively low concentrations (usually less than 1 mg/l of individual compounds). These compounds include among others a variety of aromatic hydrocarbons, phenols, chlorinated aliphatics, pesticides, and plastizers.

Leachate quality is significantly influence by the waste age or length of time after waste fill; it is reported that leaching quality achieved at maximum after two or three years and decline subsequently (A.H. Lee et al., 2010).

The leachate generation in sanitary landfill is a complex combination of physical, chemical and biological processes whereby waste age has impact to performance of landfill that generate leachate. For this reason, is important to differentiate the several parameters according to this waste age (acetogenic and methanogenic phase), detailed below in table 2.6b:

Parameter	*Acetogenic phase (mg/l)*		*Methanogenic (mg/l)*	
	Average	*Range*	*Average*	*Range*
pH	6.1	4.5 – 7.5	8	7.5 – 9
BOD_5	13000	4000-40000	180	20-550
COD	22000	600-60000	3000	500-4500
BOD_5/COD (ratio)	0.58		0.06	
Sulfate	500	70-1750	80	10-420
Calcium	1200	10-2500	60	20-600
Magnesium	470	50-1150	180	40-350
Iron	780	20-2100	15	3-280
Manganese	25	0.3-65	0.7	0.03-45
Ammonia-N	740		740	
Chloride	2120		2120	
Potassium	1085		1085	
Sodium	1340		1340	
Total Phosphorus	6		6	
Cadmium	0.005		0.005	
Chromium	0.28		0.28	
Cooper	0.062		0.062	
Lead	0.09		0.09	
Nickel	0.6	0.1-120	0.6	0.3-4
Zink				

Table 2.6 (b). Leachate composition in terms of average values and ranges for Acid and Methanogenic Phase (Kjeldsen 2010)

Leachate composition varies relative to the amount of precipitation and the quantity and type of wastes disposed. In addition to numerous hazardous constituents, leachate generally contains non-hazardous parameters that are also found in most groundwater systems (see above table). These constituents include dissolved metals (e.g., iron and manganese), salts (e.g., sodium and chloride), and an abundance of common anions and cations (e.g., bicarbonate and sulfate). However, these constituents in leachate typically are found at concentrations that may be an order of magnitude greater than concentrations present in natural groundwater systems. The presence of heavy metals at high concentrations in landfill leachate usually causes toxic effects to microbes, making it difficult to be treated biologically. Although several physical, chemical and biological processes can be employed to treat landfill leachate.

2.2.1. Waste management

Landfills are a vital component of any well-designed Municipal Solid Waste Management System. They are the ultimate repository of a city's MSW after all other options have been exercised. The main differences among the landfills involve the degree of isolation, the means of accomplishing it and optimizing the landfill reactions. The rate and extent of decomposition of the landfilled wastes are dependent on the design of isolation. Innovative planning and design can accelerate the decomposition and facilitate productive reuse of the landfill property after the landfill is closed.

A landfill is any form of waste disposal land, ranging from an uncontrolled rubbish dump to a full containment site engineered with high standards to protect the environment. Figure 2.2, shows a cross-section of a typical sanitary landfill, which has provisions for containing leachate.

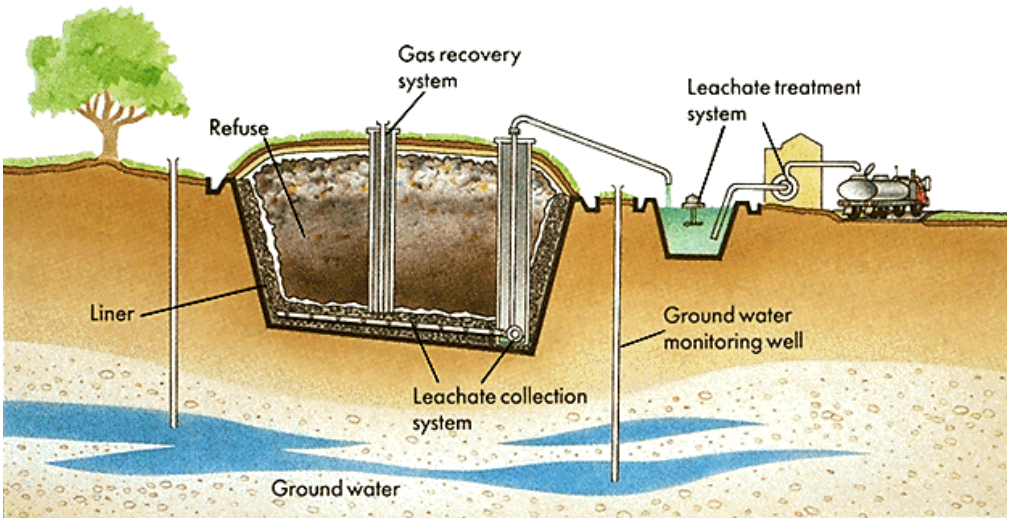


Figure 2.2. A Sanitary Landfill (W. Pronk and D. Kone, 2009)

The sanitary landfill play a most important role in the framework of solid waste disposal and will remain an integral part of the news strategies based on integrated solid waste management. Main designs (according to technical, social and economic development) are devoted towards ensuring minimal environmental impact in accordance with observation made concerning the operation of old landfills (T. Christensen et al., 2002).

To ensure the protection of our ecosystem, environmental health, and foster sustainable development, the waste generated by the increasing urban population requires treatment and disposal in an environmentally sound manner. The municipal solid waste (MSW) from the urban habitat is disposed of in dumpsites or in sanitary or engineered landfills. The constituents of the MSW undergo biological and chemical degradation after disposal resulting in emissions of landfill gas and discharge of leachate, which is a highly polluted form of wastewater when discharged into the environment, and would cause potential damages to environmental health and the ecosystem.

A landfill has an acceptable impact if emissions that come from it do not change in a considerable way quality of the surrounding air, ground and groundwater; therefore emissions that do not cause significant modifications to the surrounding environment can be considered as negligible. Several approaches to the environmental sustainability have been proposed, based either on the modification of the characteristics of the waste to be landfilled such as mechanical and biological pre-treatments, or on the modification of the landfill construction and operation procedures such as aerobic or semi-aerobic landfill, flushing, leachate recirculation.

2.2.2. The role of landfills

Among other disposal methods, landfill is the more significant method for final disposal of MSW. In spite of the low upfront capital and low expertise is needed when compared to other disposal or treatment methods, landfill can accept all sort of waste.

Solid waste landfills are a necessity in modern-day society, because the collection and disposal of waste materials into centralized locations helps minimize risks to public health and safety. Solid waste landfills, which are regulated differently than hazardous waste landfills, may accept a variety of solid, semi-solid, and small quantities of liquid wastes. Landfills generally remain open for decades before undergoing closure and post-closure phases, during which steps are taken to minimize the risk of environmental contamination.

In recent years, the driving principle of landfill management has been to prevent saturation of the waste to minimize the likelihood of leachate leaking into the surrounding ground. This has resulted in very slow rates of waste degradation, with projected stabilization times of the order of hundreds of years. Degradation can in principle be accelerated by circulating fluids through the waste in a controlled manner, and operating the landfill as an engineered wet bioreactor. This concept offers significant economic and environmental benefits and is more consistent with the aims of a sustainable waste management policy than previous approaches, which leaves landfilled wastes in a potentially polluting state for many generations.

Organic material present in the waste mainly comprise of kitchen waste while the inorganic constituents consist of plastic, glass, metal, ash, silt etc. The leachate composition depends upon the ratio of organic and inorganic components present in the waste disposed in the landfill. It is estimated that approximately one-half of the municipal solid waste is typically composed of cellulose and hemicellulose (Barlaz et al., 1989), which are considered readily degradable in the environment. The organic content leached is as a result of hydrolysis and degradation of higher molecular weight organic compounds by the microorganisms present in the waste.

2.2.3. Use of landfills in the world

In the past two decades, it has undertaken an environmental policy in the world focused on the development of the collection, aimed at reuse and recycling of materials found in municipal solid waste (MSW). But despite the efforts made to have an optimal management of the overall system for the disposal of municipal waste, the landfill is still the main method of disposal of MSW for many territories. The damage caused by this system to environmental matrices is essentially related to the dispersion of the degradation products of the organic component of the waste: biogas and leachate. This last is a liquid that originates from ' infiltration ' in the mass of wastewater, and the decomposition of these through the combination of physical, chemical and microbiological processes.

Over the years it has been tried to use techniques for the treatment of leachate is more efficient, a possibility that is taking interest in recent years is the use of phytoremediation, or the purification of the soil from polluting substances, while maintaining a good quality status for cultivation farm.

2.2.4. Problems of "old" landfills:

The main problems of pollution are the production of greenhouse gases (some potentially explosive such as hydrocarbons) and emissions of leachate from landfills, especially, because of their toxic impact when released into the environment in an uncontrolled manner, even for hundreds of years after their closure (Christensen et al., 1992).

After the closure of landfills, these are managed for relatively short periods of 20-30 years, thus bringing any case, if significant pollution problems (Robinson, 1995). During the percolation of rainwater and moisture through municipal solid waste (MSW) in a landfill, the liquid medium absorbs nutrients and contaminants from the waste and exudes as leachate from the landfills or dumps posing a hazard to the receiving water bodies. This leachate contains many substances which depend on the types of waste disposed into the landfill, and may be toxic to life or may simply alter the ecology of the stream or watercourse if not removed by treatment.

The major environmental impacts related to landfills, is related to discharge of leachate into the environment; polluting the groundwater and surface water, this risk is consider probably the most severe environmental impact from landfills because historically most landfills were built without engineered liners and leachate collection systems. More recently, regulations in many countries have required the installation of liners and leachate collection systems as well as a plan for leachate treatment. Surface water pollution caused by leachate has also been observed, although relatively few cases have been described in the literature. The major potential effects of a leachate release to surface water are expected to be oxygen depletion in part of the surface water body, changes in the stream bottom fauna and flora and ammonia toxicity.

Due to anaerobic conditions ad long retention time prevailing in sanitary landfills, landfill leachate normally contains high concentrations of organic matters, nutrients, pathogens and heavy metals, which, if not properly collected and treated, can cause serious pollution to nearby surface and groundwater sources. The presence of high concentrations of heavy metals in a landfill leachate usually causes toxic effects to microbes, making it difficult to be treated biologically (V. Sawaittayothin & C. Polprasert, 2007).

The differentiation of landfill age can be made based on dominating degradation phase and the composition of the resultant leachate. The climatic variation of leachate quality and quantity depends on its age. The BOD/COD ratio that depicts the biodegradability of the leachate has a decreasing trend with age from a readily biodegradable ratio of 0.5 to a higher fraction of poor degradability with a value of 0.1 or less.

The fresh leachate (from a young landfill) possesses high proportion of organic material, which can be removed by biological processes. Owing to biological reactions, fresh leachate is expected to produce an acidic pH and an unpleasant smell. The decrease in VS/TS ratio from fresh to old leachate also suggests that stabilized leachate is less amenable to biological treatment as the landfill age increases. The redox potential and pH increases with the stabilization process. A colour change from light yellow to dark brown or black is also observed due to iron oxidation from ferrous to ferric iron, resulting in an increase in turbidity.

Figure 2.3. Problems of untreated landfills

Landfill leachate in untreated form is unsuitable for direct discharge into surface watercourses, as the high BOD and NH3-N concentrations would have a severe impact on the ecology of the receiving water. Notwithstanding effective advance leachate treatment system exist, some landfill operator search alternatives because of their high costs and specialised management requirements. Land-based treatment systems are an attractive alternative for landfill operators since they utilise an existing land resource, there are cheap to build and operate and the better is that not need sophisticated management.

Reduction, recycling, and reuse of municipal waste are preferred practices solid waste disposal in landfills is still a common occurrence throughout the world. After being disposed in landfills, solid waste undergoes complex physicochemical and biological reactions. As a result, organic substances are degraded into leachable liquids or landfill gases (LFG).

The characteristics of cover soil and vegetation in a closed landfill play an important role in methane emission; methane concentration, according to Xiaoli et al., (2011), usually decreases with increasing depth of cover soil. The concentration of CH4 from vegetated cover soil is lower than of naked soil in a closed landfill. However, the water is still the most important ecological factor for the distribution of vegetation species and their coverage in the closed landfill. The vegetation coverage, species and their height increase with the stabilization process of closed landfill. In general, the characteristics of cover soil and vegetation condition are factors that heavily influence on the landfill gas component from the closed landfill, and they should be considered as a priority factor in the design of future landfills.

2.2.5. The phytoremediation of landfill leachate

The use of phytoremediation is one possibility to priority the development of economically and environmentally sustainable management of waste and polluted sites, which is taking interest in recent years, the purification of the soil from polluting substances, while retaining a good quality status for agricultural cultivation, through the use of a plant-system soil (Pathak et al., 2012). Many experiments have led interesting results, while others have not produced satisfactory data, due, above all, the use of an excessive amount of leachate, poor management of the system or a lack of understanding of the plant-soil (Jones, 2006).

Traditional approaches for landfill treatment: on-site treatment, transport to off-site facilities, are often undesirable because transport can be dangerous and expensive, while commonly used on-site facilities require high capital inputs and energy can generate large quantities of by-products. In spite of different views on the leachate treatment, many experts agree that on-site treatment facility is needed that requires little maintenance or power and it is financially less demanding. One low-cost method is the on-site treatment using green technology like CW, as a part of sustainable solution for landfill sites (T. Bulc, 2006).

Phytoremediation systems utilise the potential of the natural or actively managed soil-plant system to detoxify, degrade and inactive potentially toxic elements in the leachate.

Above ground process include:

- ✓ The foliar uptake of gaseous nutrients emitted from the applied leachate and their use in making new plant biomass (eg., NH3);
- ✓ The foliar uptake of soluble nutrients and metals from the applied leachate and their use for growth (e.g., NO3, Zn) or their sequestration in the leaves (e.g., Pb);
- ✓ The foliar uptake of volatile and soluble organic compounds within the applied leachate (e.g., chlorinated hydrocarbons) and their subsequent detoxification or sequestration;
- ✓ The enhanced evaporation of water from the leachate during and after irrigation, thereby reducing effluent volume.

Below ground processes include:

- ✓ The uptake of water from the soil to drive shoot transpiration, which first draws the components contained in the leachate towards the root, where they can be taken up by the plant, and secondly reduces leachate volume and consequently reduces downward migration of contaminants; this, however, may also cause the gradual accumulation of leachate constituents in soil (e.g., Na);
- ✓ The root uptake of inorganic nutrients (K, NH4) and other metals (e.g., Na, heavy metals) which can either be sequestered, used in growth or transported to the shoots;
- ✓ The uptake of organic compounds which can either be sequestered, degraded, used in growth or transported to the shoots;

- ✓ The stimulation of rhizosphere microorganisms (including mycorrhizas), which reduce the BOD, load of effluents, detoxify organic pollutants and sequester and render some metals non-toxic (e.g., Cu). This process is typically termed rhizoremediation. The roots also promote the development of microsites which favour specific soil chemical transformations (e.g., denitrification, NO^{-}_{3} to N_2);
- ✓ The sorption, complexation and fixation/precipitation of metals onto the soils solid phase; this includes immobilization onto both soil organic matter and mineral particles;
- ✓ The sorption and degradation (biotic and abiotic) of organic compounds present in leachate;
- ✓ The promotion of soil structure by plant roots, which enhances infiltration of leachate into the soil and reduces the risk of surface run-off. The enhancement of soil structure also stimulates better soil aeration that promotes a more efficient biodegradation of organic compounds.

As many landfill sites are located in rural, agricultural and wooded areas, spray irrigation of untreated or partially treated leachate onto vegetated land has been considered as a potential remediation option. Leachate remediation to land can provide the opportunity for closing the nutrient cycling loop and at the same tine, producing effluent of a suitable quality for discharge (N. Sang et al, 2010).

Reduction, recycling, and reuse of municipal refuse are preferred practices solid waste disposal in landfills is still a common occurrence throughout the world. The characteristics of cover soil and vegetation conditions in landfills can affect the efficiency of CH_4 oxidation and bring about the alteration of the LFG components. Cover soils with high porosity and large particle-size distribution can retain CH_4 and oxygen longer in the pores, resulting in a higher oxidation rate of CH_4. There is a close relationship between cover soil and vegetation condition, and the migration of CH_4 generally has a strong impact on the vegetation condition of landfill.

The success of this remediation option, is critically dependent on whether the leachate is introduced into the soil-vegetation system can tolerate the environmental stress from landfill leachate, in such a way as to maximise the degree of inactivation or attenuation of contaminants, before re-entering the hydrological cycle.

Landfill leachate is a significant pollution factor of municipal landfill sites generated by decomposition of landfill organic waste and precipitations, which percolates through the waste material. Due to the low biodegradability, high nitrogen content and other possible toxic components, the co-treatment of leachate on conventional municipal wastewater is undesiderable. One of the low-cost on-site treatment possibilities is constructed wetlands (CWs), which have been widely practiced for many years in a number of countries with varying degrees of success (M.Z. Justin and M. Zupancic, 2009).

The role of energy crops in landfill areas

Energy crops have the potential to utilise agricultural and municipal wastes, and to stabilise or clean up contaminated land (C. Britt et al, 2002). From the perspective of waste disposal/utilisation, energy crops offer the following potential benefits:

- ✓ they are not going to enter the human food chain;
- ✓ they are perennial crops, thus allowing long-term breakdown of organic matter in soils prior to renovation to food cropping;
- ✓ they produce large quantities of biomass that, theoretically, requires large quantities of nutrients, and thus are a sink for the nutrients in waste.

From the perspective of bioremediation of contaminated sites, they offer the following potential solutions:

- ✓ they utilise land that would otherwise have no agricultural value;
- ✓ they are non-food crops that will not enter the human food chain;
- ✓ they are perennial crops which may act as ‘excluders’ of contaminants in the soil;
- ✓ alternatively, they may act as ‘tolerators’ of the contaminants, actively taking up the elements which, in some instances, can then be recovered during biomass combustion;
- ✓ the crops can also act as bioremediators of liquid leachate produced from rainfall onto landfill and other contaminated sites;
- ✓ in these situations, they may also act as recipients of agricultural and municipal wastes.

The practices, therefore, have the potential of negating some benefits derived from saving fossil fuels by growing energy crops. There is a current lack of evidence on the thresholds of application that are acceptable in different situations and this is, in part, due to a lack of primary research in many areas.

2.3. Biodiesel from energy crops

Any wastewater can be linked to a different kind of culture, land and irrigation. As an example: leachate from an old landfill with no impermeabilization on top, can be phytotreated by the use of energy crops, as *Arundo donax* (normal cane), *Saccharum officinarum* (sugar cane) or other kind of plants to produce bioethanol. The treatment plant can be installed on the top of the same old landfill and, depending to the quality of the leachate and of the environmental situation of the old landfill, the leachate can be re-circulated more than one time to irrigate the energy crops (M.C. Lavagnolo et al, 2011).

Biodiesel production was intended to mainly address the issue of fuel supply security, but recently more attention has been centred on the use of renewable fuels in order to minimize the overall net production of carbon-dioxide (CO_2) from non-renewable fossil fuel combustion. Furthermore, biodiesel does not increase greenhouse gas levels in the atmosphere because of its closed cycle. (Ferella F. et al., 2010). There are several other advantages in using biodiesel. The main advantages are that it is biodegradable, can be used without modifying existing engines, and produces less harmful gas emissions such as sulphur oxide (Gerpen et al., 2005).

Demirbas (Demirbas A., 2008) stated that biodegradable fuels such as biodiesels have a wide range of potential applications and they are environmentally friendly. Biodiesel is biodegradable and more than 90% biodiesel can be biodegraded within 21 days. The ability of biodiesel to be highly biodegradable and its superb lubricating property when used in compression ignition engines makes it to be an excellent fuel. It was stated that biodiesel is non-toxic and degrades about four times faster than petro-diesel. In addition, biodiesel reduces net carbon-dioxide emissions by 78% on a life-cycle basis when compared to conventional diesel fuel (Gunvachai K et al., 2007). Also its renewability and similarities in physicochemical properties to petro-diesel, revealed its potentials and practical usability as fuel for the replacement of petro-diesel in the nearest future. These properties include among others engine power, increase in calorific value, reduced emission of nitrogen oxides (NO_x), and low temperature properties improvement.

2.3.1. Biodiesel feedstocks

Globally, there are more than 350 oil-bearing crops identified as potential sources for biodiesel production. As much as possible the feedstock should fulfil two main requirements: low production costs and large production scale. The availability of feedstock for producing biodiesel depends on the regional climate, geographical locations, local soil conditions and agricultural practices of any country. Therefore, selecting the cheapest feedstock is vital to ensure low production cost of biodiesel. In general, biodiesel feedstock can be divided into four main categories as below (A.E. Atabani et al, 2012):

- ✓ Edible vegetable oil: rapeseed, soybean, peanut, sunflower, palm and coconut oil.
- ✓ Non-edible vegetable oil: jatropha, karanja, seamango, algae and halophytes.
- ✓ Waste or recycled oil.
- ✓ Animal fats: tallow, yellow grease, chicken fat and by-products from fish oil.

It is very important to consider some factors when comparing between different feedstocks. Each feedstock should be evaluated based on a full life-cycle analysis. This analysis includes:

- ✓ Availability of land,
- ✓ Cultivation practices,
- ✓ Energy supply and balance,
- ✓ Emission of greenhouse gases,
- ✓ Injection of pesticides,
- ✓ Soil erosion and fertility,
- ✓ Contribution to biodiversity value losses,
- ✓ Logistic cost (transport and storage),
- ✓ Direct economic value of the feedstocks taking into account the co-products,
- ✓ Creation or maintain of employment,
- ✓ Water requirements and water availability,
- ✓ Effects of feedstock on air quality.

Renewable oils are derived from widely available crop seeds depending on the agro climatic region; rapeseed (brassica napus) in northern Europe, soybean (glycine max) in the USA, canola oil in Canada, palm oil, coconut and sunflower (helianthus annus) in tropical regions; Ireland uses frying oil and animal fats. Among the animal fats, bovine fat, the fish oils, the pig fat, duck and beef tallow, lard are being considered as renewable oil.

Edible oils like soybean, sunflower, rapeseed and palm are used as main biodiesel feedstocks throughout the world. Non-edible oils like jatropha, pongamia, neem, etc. have been found to be promising feedstocks in developing countries where edible oils are in short supply. Oil from rapeseed has been the great choice in the early days and is still leading with a share of over 80% as a raw material source with highly suitable properties; sunflower oil takes second place with over 10%, followed by soybean oil.

Emerging feedstocks being considered as potential are mustard, hemp, castor oil, peanut oil, coconut oil, cotton seed oil, corn oil, rice bran oil, coffee ground, mahua oil, neem oil, tobacco oil, sesame oil, pongamia oil, passion seed oil, babassu oil, grape oil, algae oil and waste vegetable oil (Karmamar et al, 2010).

1.3.1.1. Commonly used edible oils

- ✓ Sunflower oil (Helianthus annuus); Sunflower with high oil content is one of the more prominent oilseed crops for biodiesel production. At one point, it was considered to be the second primary source of edible oil next to soybean. Sunflower can grow in a variety of climatic conditions but it is considered to be an inefficient user of nutrients. Average yield is approximately reported to be lower than soybean yields, and necessary inputs are greater (Pimental and Patzek, 2005).

- ✓ Rapeseed oil (Brassica napus L.); is a cruciferous crop that is harvested for oil production. Rapeseed oil is the most significant raw material for biodiesel producing industry in EU and Canada. Rapeseeds are small and hot dry conditions can limit their oil content. About 1.1 l of rapeseed oil is necessary to produce 1 l of diesel substitute. The greatest experience with regard to biodiesel usage has been gained with rapeseed oil methyl ester (RME). However, there is great concern for the use of rapeseed oil for biodiesel production because rapeseed is presently grown with a high level of nitrogen containing fertilizer and the manufacture of these generates N2O, a potent greenhouse gas with 296 times the global warming potential of CO2. It has been estimated that 3–5% of N2 provided as fertilizer for rapeseed is converted to N2O (Lewis, 2007).
- ✓ Soybean oil (Glycine max); Soybean oil is used as a major source of edible oil throughout the world. With about 222 million tonnes, soybean is the most important oil bearing plant cultivated worldwide and its production is seeing a further expansion, particularly in the USA, Brazil and Argentina. Soybeans can be produced without or nearly zero nitrogen. This makes soybeans advantageous for the production of biodiesel as nitrogen fertilizer is one of the most energy costly inputs in crop production. (Pimental and Patzek, 2005) studied the energy estimation for producing soybean biodiesel. They reported that 5546kg of soybeans were required for producing 1000 kg of oil and biodiesel production using soybean required 27% more fossil energy than the biodiesel fuel produced.
- ✓ Palm and palm kernel oil (Elaeis guineensis); Oil is derived from both the flesh and the seed of the palm fruit. The fruit consists of an outer pulp, which is the source of crude palm oil and two or three kernels, which are the source of another oil type palm kernel oil. Crude palm oil is semisolid at room temperature. Palm kernel oil is rich in lauric and myristic fatty acid with an excellent oxidative stability and sharp melting. Palm oil has been proved to be an efficient biodiesel source. The average yield of approximately 6000 l of palm oil/ha can produce 4800 l of bio- diesel (Addison and Hiraga, 2010 in Karkamar et al, 2010) It has been stated that palm oil can have high levels of fatty acids, which require extra methanol transesterification before it can be used as biodiesel, thus increasing the cost of production somewhat (Crabbe et al., 2001). Farmers in Ghana are producing biodiesel from palm kernel oil for powering their farm vehicles and generators.

- ✓ Peanut oil (Arachis hypogeae); The peanut or groundnut is native to South America, Mexico and Central America. Rudolf Diesel, the inventor of compression ignition engines, used first time peanut oil in 1900. The physico- chemical characteristics of peanut oil biodiesel (POB) closely resemble to those of diesel fuel (Clark et al., 1984; Mittetbach and Tritthart, 1988). But the production of biodiesel from peanut oil is not economically viable as peanut oil is more valuable than soy oil in the world market. Studies are going on at the University of Georgia to develop non-edible peanut varieties that are high in oil, but will not compete with peanuts grown for food or cooking oil purposes (Roberson, 2006).

Figure 2.4. Principal edible oils feedstocks (A.E. Atabani et al, 2012)

1.3.1.2. Commonly used non-edible oils

Around the world, there are a number of sources that do not rely on usual food oils or do not rely on annual field crops. Examples include oil from the Honge (Indian beech or pongamia) tree, and Jatropha curcas (physic nut). Hence prices of edible oils are higher than that of petro-diesel. Due to this, these are not viable and use of non-edible oils is suggested as biodiesel feedstock. Even though the consumption of edible oils in some countries like India is high, the availability of used cooking oil is very small as it is used till the end. Hence, focus needs to be shifted to non-edible resources.

Non-edible oils like Jatropha (J. curcas); Pongamia oil (Pongamia pinnata); Neem (Azadirachta indica); Mahua oil (Madhuca indica); Jojoba oil (Simmondsia chinensis), etc. have been found to be promising feedstocks especially in developing countries where edible oils are in short supply.

To consider any feedstock as a biodiesel source, the oil percentage and the yield per hectare are important parameters. Table 2.7, shows the estimated oil content and yields of different biodiesel feedstocks.

Feedstock	Oil content (%)	Oil yield (L/ha/year)
Castor	53	1413
Jatropa	Seed: 35-40 Kernel: 50-60	1892
Linseed	40-44	-
Neem	20-30	-
Pongamia pinnata (karanja)	27-30	225-2250a
Soybean	15-20	446
Sunflower	25-35	952
Calophyllum inophyllum L.	65	4680
Moringa oleifera	40	-
Euphorbia lathyris L.	43	1500-2500a
Sapium sebiferum L.	Kernel: 12-29	-
Rapeseed	38-46	1190
Tung	16-18	940
Pachira glabra	40-50	-
Palm oil	30-60	5950
Peanut oil	45-55	1059
Olive oil	45-70	1212
Corn (Germ)	48	172
Coconut	63-65	2689
Cottomseed	18-25	325
Rice bran	15-23	828
Sesame	-	696
Jojoba	45-50	1818
Rubber seed	40-50	80-120a
Sea mango	54	-
Microalgae (low oil content)	30	58700
Microalgae (medium oil content)	50	97800
Microalage (high oil content)	70	136900

a (kg oil/ha).

Table 2.7. Estimated oil content and yields of different biodiesel feedstock (A.E Atabani et al, 2012)

2.3.2. Biodiesel production

Biodiesel is one of the best available sources to fulfil the energy demand of the world. The petroleum fuels play a very important role in the development of industrial growth, transportation and agricultural sector and to meet many other basic human needs. However, these fuels are limited and depleting day by day as the consumption is increasing very rapidly. Biodiesel is gaining more importance as an attractive fuel due to the depleting nature of fossil fuel resources. The main drawback of vegetable oil is their high viscosity and low volatility, which causes poor combustion in diesel engines. The sequence of biodiesel production is better explained in the next flow chart (figure 2.5):

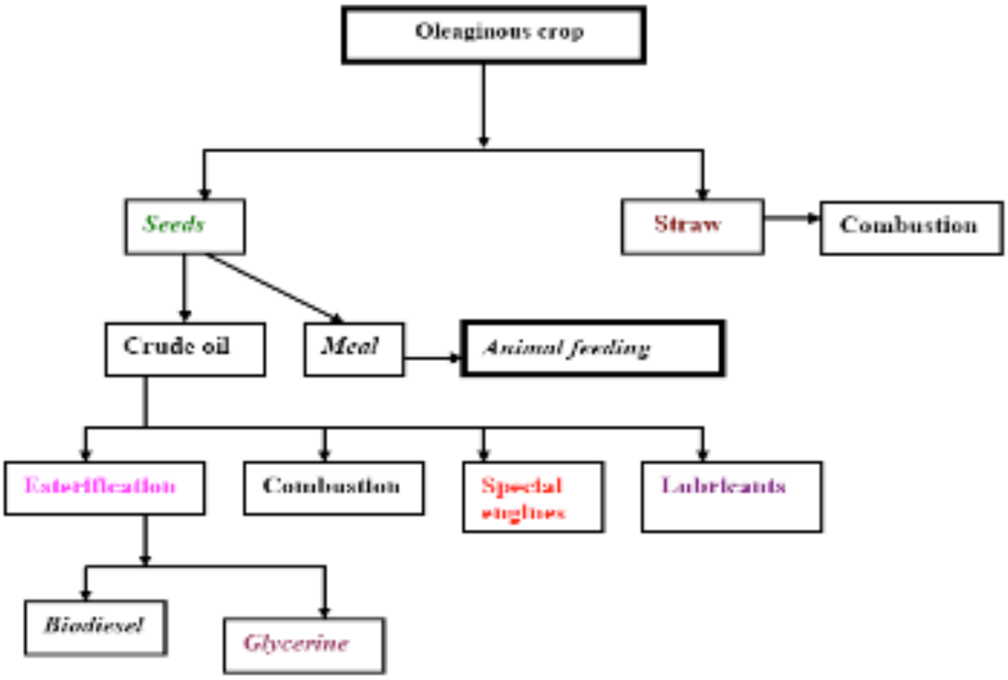


Figure 2.5. Flow chart of biodiesel production (Omer, 2006)

Based on the positive energy balance or life cycle analysis, biodiesel is shown to be sustainable. However, the competition of feed source with food, and destruction of natural habitats resulting from energy crop plantation are some inevitable issues, which require attention (J. Janaun and N. Ellis, 2010).

Biodiesel obtained from energy crops produce favourable effects on the environment, such a decrease in acid rain and in the greenhouse effect caused by combustion; due to this factor and to its biodegradability, the production of biodiesel is considered an advantage to that of fossil fuels. In addition, it also shows a decrease in the emission of CO2 and SOx, and unburned hydrocarbons during the combustion process. Energy crops have been considered as one of the best alternatives in the agricultural sector, whose production for food purposes has been limited by the PAC, thus allowing the development of new industries such as the agro-energy industry with employed creation and regional development (G. Antolin et al, 2002).

Biodiesel is made from vegetable oils, animal fats or recycled greases (see figure 1.11). Biodiesel can be used as a fuel for vehicles in its pure form, but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles. Biodiesel is the most common biofuel in Europe. Biofuels provided 1.8% of the world's transport fuel in 2008. Investment into biofuels production capacity exceeded $4 billion worldwide in 2007 and is growing (U.N.E.P., 2009-10-16).

Biodiesel is a synthetic diesel-like fuel produced from vegetable oils, animal fats or waste cooking oil. It can be used directly as fuel, which requires some engine modifications, or blended with petroleum diesel and used in diesel engines with few or no modifications. At present, biodiesel accounts for less than 0.2% of the diesel consumed for transport. Biodiesel has become more attractive recently because of its environmental benefits. The cost of biodiesel, however, is the main obstacle to commercialization of the product. (A. Dermibas, 2009).

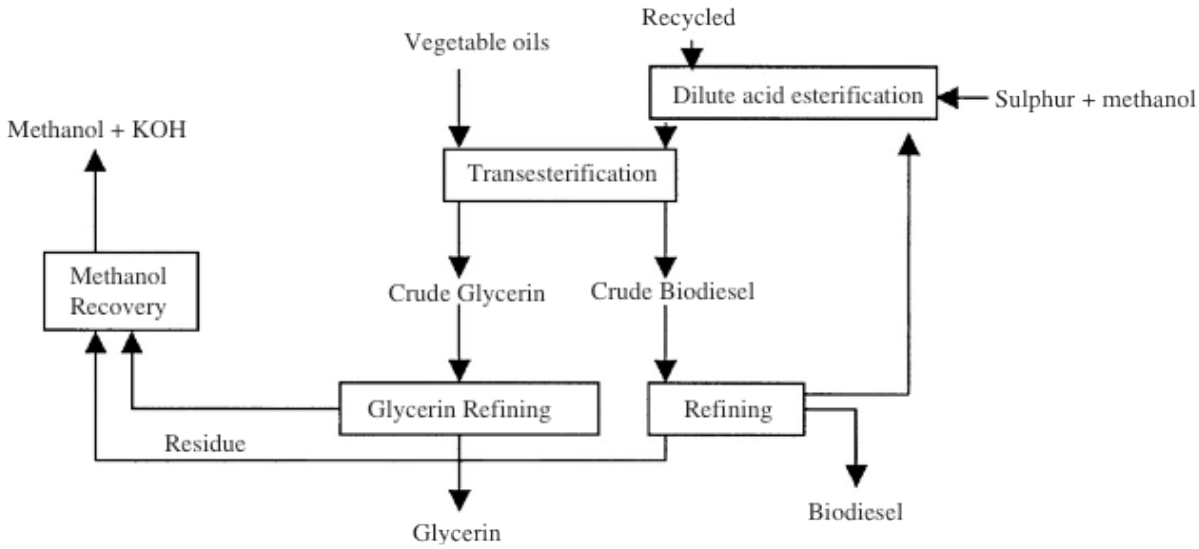


Figure 2.6. Basic scheme for biodiesel production (J.M. Marchetti et al, 2007)

The purpose of transesterification process to lower this high viscosity of the oil; consist of removing the glycerides and combining oil esters of vegetable oil with alcohol, this process reduces the viscosity to a value comparable to that of diesel and hence improves combustion. Biodiesel emits fewer pollutants over the whole range of air–fuel ratio when compared to diesel. The scientists tested a number of different raw and processed vegetable oils like rapeseed oil, sunflower oil, palm oil, soybean oil (S.A. Basha et al, 2009). In a transesterification or alcoholysis 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 process is a sequence of three reversible reactions, in which the triglyceride molecule is converted step by step into diglyceride, monoglyceride and glycerol (Mittelbach and Remschmidt, 2004). The simplified form of its chemical reaction is presented in equation (see figure 2.7):

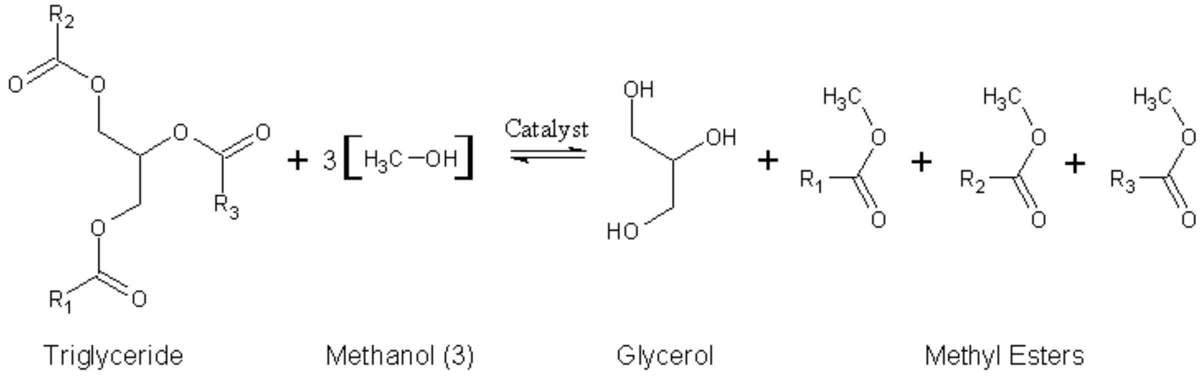


Figure 2.7. Chemical reaction of transesterification process

Where R_1, R_2, R_3 are long-chain hydrocarbons, sometimes called fatty acid chains.

The major drawbacks inhibiting commercial production of biodiesel include the cost of raw materials and the presence of free fatty acids, water in the oils and the use of higher alcohol molar ratios. The presence of water molecules reduces the catalytic effectiveness while free fatty acids lead to the formation of soap when alkali catalyst is used during transesterification reaction. This process decreases the yields of esters and renders purification of crude biodiesel difficult and expensive. The production of soap, sometimes called alkaline hydrolysis, converts trialcylglycerols to glycerol and form a mixture of salts of long chain carboxylic acids. The purity and quality of biodiesel is determined by the amounts of free and bonded glycerine. Combustion of these substances in compression ignition engines can enhance the formation of undesirable substances such as acrolein, a photochemical smog ingredient (Li-Hua C. et al., 2009).

Highly purified biodiesel is necessary to achieve the stringent standard specifications, which could be either the American standards for testing materials (ASTM 6751-3) or the European Union (EN 14214, reported in Table 2.8.) for biodiesel fuel. According to the European Union (EU) standard specifications for biodiesel fuel water content, free fatty acids, and free and bound glycerine, must be kept to a minimum level and the purity of the fuel must exceeds 96.5% (Karaosmanog et al., 1996). The main objective in the purification of crude biodiesel is to remove the fatty acid alkyl esters from the mixture, and maintain lower cost of production and also ensure a highly purified biodiesel product. Refined vegetable oils tend to ease the difficulties encountered during separation and purification of the transesterified products (biodiesel) and provide biodiesel with better physicochemical properties such as viscosity, flash point and densities, etc. The technologies applied to refine the feedstock and convert it to fatty acid alkyl esters (biodiesel) determine whether the fuel produced will meet the designed specification standards.

Property	Units	Lower limit	Upper limit	Test-method
Ester content	% (m/m)	96.5	-	Pr EN 14103 d
Density at 15 °C	kg/m³	860	900	EN ISO 3675/EN ISO 12185
Viscosity at 40 °C	mm²/s	3.5	5.0	EN ISO 3104
Flash point	C	>101	-	ISO CD 3679e
Sulfur content	mg/kg	-	10	-
Tar remnant (at 10% distillation remnant)	% (m/m)	-	0.3	EN ISO 10370
Cetane number	-	51.0	-	EN ISO 5165
Sulfated ash content	% (m/m)	-	0.02	ISO 3987
Water content	mg/kg	-	500	EN ISO 12937
Total contamination	mg/kg	-	24	EN 12662
Copper band corrosion (3 h at 50 °C)	rating	Class 1	Class 1	EN ISO 2160
Oxidation stability at 110 °C	h	6	-	pr EN 14112 k
Acid value	mg KOH/g	-	0.5	pr EN 14104
Iodine value	-	-	120	pr EN 14111
Linoleic acid methyl ester	% (m/m)	-	12	pr EN 14103d
Polyunsaturated (P4 double bonds) methylester	% (m/m)	-	1	-
Methanol content	% (m/m)	-	0.2	pr EN 141101
Monoglyceride content	% (m/m)	-	0.8	pr EN 14105m
Diglyceride content	% (m/m)	-	0.2	pr EN 14105m
Triglyceride content	% (m/m)	-	0.2	pr EN 14105m
Free glycerine	% (m/m)	-	0.02	pr EN 14105m/pr EN 14106
Total glycerine	% (m/m)	-	0.25	pr EN 14105m
Alkali metals (Na+K)	mg/kg	-	5	pr EN 14108/pr EN 14109
Phosphorus content	mg/kg	-	10	pr EN14107p

Table 2.8. International standard (EN 14214) requirements for biodiesel (Demirbas A. et al. 2009).

In Table 2.9 are reported the results of the quality of several synthesized biodiesels which were tested according to the European Standard EN 14214. The degree of compliance of the reported parameters (ester content, methanol content, kinematic viscosity, acid value, triglycerides, diglycerides, monoglycerides and glycerol content -free and total- and flash point) depends on the degree of oil refinement (previous pre-treatment step), the transesterification process (conversion) and the quality of phases purification step.

Property	Units	Test method	Limits		Palm	Olive	Peanut	Rape	Soybean	Sunflower	Grape	H.O. Sunflower	Almond	Corn
			Min.	Max.										
Ester content	wt.%	EN 14103	96.5		97.7	99.0	99.5	99.5	96.9	97.2	97.8	99.5	99.7	99.8
Kinematic viscosity, 40 °C	mm²/s	EN ISO 3104	3.5	5.0	4.5	4.5	4.6	4.4	4.2	4.2	4.1	4.4	4.2	4.4
Flash point	°C	EN ISO 3679	120		176	178	176	170	171	177	175	174	172	170
Cetane number	-	-[a]	51		61	57	53	55	49	50	48	53	57	53
Oxidative stability, 110 °C	h	EN 14112	6.0		4.0	3.3	2.0	2.0	1.3	0.8	0.5	1.2	3.0	1.2
Acid value	mg KOH/g	EN 14104		0.50	0.12	0.13	0.10	0.16	0.14	0.15	0.27	0.21	0.17	0.15
Iodine value	g I_2/100 g	EN 14111		120[b]	57	84	97	109	128	132	138	102	92	101
Linolenic acid content	wt.%	EN 14103		12.0	0.2	0.6	0.3	7.9	6.3	0.2	0.4	0.1	0.8	0.1
CFPP	°C	EN 116		–[c]	10	−6	17	−10	−5	−3	−6	−6	−6	−12
Methanol content	wt.%	EN 14110		0.20	0	0	0	0	0	0	0	0	0	0
Monoglycerides content	wt.%	EN 14105		0.80	0.17	0.67	0.32	0.41	0.21	0.37	0.28	0.31	0.27	0.40
Diglycerides content	wt.%	EN 14105		0.20	0.06	0.09	0.07	0.08	0.10	0.07	0.08	0.05	0.08	0.06
Triglycerides content	wt.%	EN 14105		0.20	0.04	0.03	0.03	0.03	0.07	0.04	0.03	0.06	0.02	0.03
Free glycerol	wt.%	EN 14105		0.02	0.01	0.00	0.01	0.01	0.07	0.00	0.00	0.00	0.00	0.00
Total glycerol	wt.%	EN 14105		0.25	0.06	0.19	0.11	0.09	0.00	0.11	0.09	0.13	0.07	0.09

Table 2.9. Properties of biodiesel from all vegetable oils (UNE-EN 14214)

$_a$ Internal procedure.

$_b$ RD 61/2006 (Spain) iodine value, 140 max. (g I2/100 g).

$_c$ RD 61/2006 (Spain) CFPP, 0 °C max. in summer time and -10 °C max in winter time.

To ensure safe operation in diesel engines, the most important aspects of the biodiesel product are the completion of the transesterification reaction: if it is not complete then triglycerides, diglycerides, or monoglycerides may be left in the final product. Chemically, each of these compounds contains a glycerol molecule. Fuel with excessive free glycerol may plug the fuel filters and cause combustion problems in the diesel engine. The data in table show that all the biofuels respected these limits. An adequate cetane number is required for good engine performance. High cetane numbers help ensure good cold start properties and minimize the formation of white smoke. It is well known that biodiesel cetane number depends on the feedstock used for its production. The longer the fatty acid carbon chains and the more saturated the molecules, the higher the cetane number; this cetane number for biodiesel should be a minimum of 51 (UNE-EN 14214). According to this, biodiesels of soybean, sunflower and grape seed oil were out of specification. Palm biodiesel, rich in these compounds, gave the highest cetane number. Olive, almond and rapeseed biodiesels presented a cetane number near to palm biodiesel. Finally, peanut, high oleic sunflower and corn biodiesels, those which were richer in unsaturated ester of linoleic acid (C18:2), present a cetane number in the medium range.

Iodine value is a measure of total un-saturation within a mixture of fatty acid. It is expressed in grams of iodine, which react with 100 g of the respective sample when formally adding iodine to the double bonds. Iodine value is limited to 120 g I2/100 g in the European biodiesel standard UNE-EN 14214. The limit of 120 g I2/100 g demanded by the European biodiesel standard excludes several promising oil sources such as soybean or sunflower seed oil, as well as grape seed oil, from serving as raw materials for biodiesel production. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides; this can lead to the formation of deposits or to deterioration of the lubricating. The more unsaturation is present in the oil, the higher the iodine value; Lin et al., 2006). Soybean, sunflower and grape seed oil were located between the limit imposed by the European Standard and the limit imposed by the Spanish law.

All the biodiesels obtained did not achieve the minimum limit of six hours for oxidation stability. This limit corresponds to the period of time passing before fatty acid methyl esters, aged at 110 °C under a constant air stream, are degraded to such an extent that the formation of volatile acids can be recorded through a conductivity increase. One feasible solution for increasing resistance of biodiesels against autoxidation is to treat them with oxidation inhibitors (antioxidants) (Rodríguez et al., 2006). Vegetable oils rich in linoleic and linolenic acids, such as soybean, sunflower and grape seed oil, tend to give methyl ester fuels with poor oxidation stability, whereas non polyunsaturated fuels, such as palm, olive and almond oil methyl esters, generally show improved stability. The groups together those biodiesel with similar properties given by similar methyl ester compositions, and summarizes the main characteristics and standards agreement of different biodiesel sources (María J. R. et al., 2009).

Low cetane numbers have been associated with more highly unsaturated components (C18:2 and C18:3). Polyunsaturated fuels that contain high levels of these components include soybean, sunflower and grape seed oils. In addition, these biodiesels showed high iodine values. Furthermore, the oxidation stability decreased with the increase of the content of polyunsaturated methyl esters. This was not the case for the cold filter plugging point: biodiesels rich in long carbon chain saturated methyl esters showed the worst CFPP values. Biodiesel of almond, olive, corn, rapeseed and high oleic sunflower oils had the global better properties because they have the greater monounsaturated content.

- ✓ ***Advantages of biodiesel***:

Biodiesel is the only alternative fuel with the property that low-concentration biofuel petroleum fuel blends will run well in unmodified conventional engines. Biodiesel can be made form domestically produced, renewable oilseed crops such as soybean, rapeseed and sunflower. Biodiesel is safe to handle and transport because it is as biodegradable as sugar and has flash point compared to pretoleum diesel fuel. Mixed: 20% biodiesel with 80% petroleum diesel. Biodiesel emits carbon monoxide, carbon dioxide, and oxides of nitrose, sulfure oxides and smoke. Biodiesel is non-toxic and degrades about four times faster than petroleum diesel, its oxygen content improve the biodegradation process, leading to an increate level of quick biodegradation. Environmental benefits: reduces carbon dioxides, carbon monoxides, PAH emissions; the use of biodiesel decreases the solid-carbon fraction of PM and reduce the sulphate fraction.

Economic impacts	Sustainability Fuel diversity Increased number of rural manufacturing jobs Increased income taxes Increased investments in plant and equipment Agricultural development International competitiveness Reducing the dependency on imported petroleum
Environmental impacts	Greenhouse gas reductions Reducing of air pollution Biodegradability Higher combustion efficiency Improved land and waste use Carbon sequestration
Energy security	Domestic targets Supply reliability Reducing use of fossil fuels Ready availability Domestic distribution Renewability

Table 2.10. Major benefits of biofuels

Another advantages of biofuels are the following:

- ✓ Biofuels are easily available from common biomass sources,

- ✓ They represent a carbon dioxide-cycle in combustion,
- ✓ Biofuels have a considerable environmentally friendly potential,
- ✓ There are many benefits to the environment, economy and consumers in using biofuels and
- ✓ They are biodegradable and contribute to sustainability.

The benefits include greenhouse gas reductions including reduced carbon dioxide emissions, which will contribute to domestic and international targets, the diversification of the fuel sector, biodegradability, sustainability, and an additional market for agricultural products. Biofuels help to protect and create jobs.

2.3.3. Costs and economic estimation of Biodiesel

Biofuels production costs can vary widely by feedstock, conversion process, scale of production and region. For biofuels, the cost of feedstock (crops) is a major component of overall costs. Total biofuel costs should also include a component representing the impact of biofuels production on related markets, such as food. In particular, the cost of producing oil-seed-derived biodiesel is dominated by the cost of the oil and by competition from high-value uses like cooking. At present, biodiesel accounts for less than 0.2% of the diesel consumed for transport. Biodiesel (biofuel) has become more attractive recently because of its environmental benefits. The cost of biodiesel, however, is the main obstacle to commercialization of the product. (A. Dermibas, 2009).

Policy drivers for renewable liquid biofuels have attracted particularity high levels of assistance in some countries given their promise of benefits in several areas of interest to governments, including agricultural production, greenhouse gas emissions, energy security, trade balances, rural development and economic opportunities for developing countries. In table 2.8 is given the total biofuel support estimates for US and EU in 2006.

	Biodiesel	Ethanol	Total biofuels
The United States (US)	0.60	5.96	6.70
Europe Union (EU)	3.11	1.61	4.82
Total of world	3.65	7.85	11.79

Table 2.11. Total biofuel support estimates in 2006 (billions of US$) (A. Demirbas, 2009).

The main economic factors of biodiesel production are: capital costs, plant capacity, process technology, raw material cost and chemical cost (labour, methanol and catalyst must be added to the feedstock). The major economic factor to consider for input costs of biodiesel production is the feedstock, which is about 75-80% of the total operating cost. Using an estimated process cost, exclusive of feedstock cost, of US$ 0.158/l for biodiesel production, and estimating a feedstock of US$ 0.539/l for refined oil, an overall cost of US$ 0.70/l for the production of soy-based biodiesel.

The oil in the vegetable seeds is converted into biodiesel through oil extraction, oil refining, and transesterification. Increasing feedstock yields, increasing economic return on glycerol production by finding other uses of this by-product, can lower the cost of biodiesel, etc. biofuels production cost vary widely by feedstock, conversion process, scale of production and region.

Average international prices in 2007 for common biocrude, fat, crops and oils used as feedstock for biofuel production are given in table 2.9; the cost of feedstock is a major economic factor in the viability of biodiesel production.

Biocrude	167
Maize	179
Sugar	223
Wheat	215
Crude palm oil	543
Rapeseed oil	824
Soybean oil	771
Refined cottoseed oil	782
Crude corn oil	802
Crude peanut oil	891
Crude tea seed oil	514
Waste cooking oil	224
Yellow grease	412
Poultry fate	256

Table 2.12. Average Int. prices for biocrude, fat, crops and oilseed as feedstock for biofuel production in 2007 (US$/tom) [A. Dermibas, 2009]

3 RESEARCH PROGRAMME

3.1. Aims

It has been shown that irrigation of plants with wastewater such sewage, as well as landfill leachate, generally increased biomass production in case of its appropriate management. Apart from the increased biomass growth after irrigation with wastewater, the concentrations of nutrients in plant tissue increase; this show on high phytoremediation potential for the elimination of the pollutants as well as on the necessity for caution at further use of plant material.

Several successful demonstrations exists in leachate application by irrigations of energy crops, due that it composition is characterised by high concentration of several parameters as N, K, Mg, Ca, Zn and B, which indicates the possibility of leachate as a fertilizer addition for the growth of energy crops (M.Z. Junstin and M. Zupancic, 2009).

So after reviewed all the concepts, ideas and processes mentioned, the fundamental aim is to articulated the three main research areas, mentioned in the introduction: the reuse of wastewater, decentralized systems for wastewater treatment, and renewable energy: reusing and saving the wastewaters, making use in one hand of the decentralized Aquanova systems for domestic wastewater and to another hand of landfill leachate (industrial wastewater), both of them treated through the phytoremediation facilities; in every cases of study (that were called phases) we purposed to using oleaginous plants, known as energy crops, and subsequently with the seeds cultivated, it could be possible to obtain biomass suitable for the production of biodiesel in a short time.

3.2. Specifics aims,

In the phytotreatment facilities, the objectives are:

- ✓ Obtain an experience of phytodepuration with energy crops in order to facilitate the knowledge of the procedure to follow in the futures researches, which will be conducted in a greater scale, or better directly in open site (in-situ treatment).
- ✓ Calculate and evaluate the removal capacity of nutrients of the facility; the loads of nutrients applied to the plants and compare them with data available from other experimentations; the growth of vegetation and to study any possible inhibition; taken into account the comparison with their respective controls.

- ✓ Using mass balance approach to identify the main removal pathways of nitrogen and phosphate with treated domestic and industrial wastewater.
- ✓ Identifying the role and importance of plants in removing nutrients, how these kinds of plants (energy crops) respond to the feeding with different types of wastewaters combinations.
- ✓ Treatment of wastewater and production of energy at the same time

In the Aquanova context, the aims are to:

- ✓ Contribute to the study and implementation of sustainable water management concepts.
- ✓ Evaluate the implementation of an aesthetic approach in the phytotreatment of grey water & yellow water.
- ✓ Assess the role of different vegetation in the phytotreatment of grey water and yellow water, to determinate if it possible to reuse.
- ✓ Save potable water for the irrigation of the crops.

In the landfill leachate treatment the objectives are:

- ✓ Offer another alternative for treated leachate, without compromising transport technologies and very expensive treatment process.
- ✓ Implementation in-situ of the phytotreatment plant for leachate treated using the energy crops, enabling the recirculation of the leachate in the entire system.
- ✓ Cover soil surface with vegetation in a closure landfill, in a way to remediate the substrate, give it a better aesthetic appearance and utilize the cultivated seeds, in this case for non-edible scopes, for a biodiesel production.
- ✓ Reutilisation of derelict area (closed landfill, contaminated soil, etc.); no competing between land for food and for energy.

3.3. Research program

All the experimental phases were developed at LISA laboratory (Laboratorio d'Ingegneria Sanitaria Ambientale: Laboratory of Environmental Engineering), Department of Civil, Environmental and Architectural Engineering (DICEA) from the University of Padua, located at Voltabarozzo PD.

Different mixed combination of the materials were tested to identify the best performance for the phytotreatment units in terms of pollutants removal and plant growth for:

- ✓ Different wastewater streams
    - o Yellow water,
    - o Grey water,
    - o Landfill leachate

- ✓ Different types of energy crops
  - o *Helianthus annus* [H],
  - o *Glycine max* [G],
  - o *Brassica napus* [B])
- ✓ Different substrate:
  - o Sand
  - o Agricultural soil

To establish the different types of every component of the processes, we based in the local conditions, to obtained or collected all the wastewater streams, collected the sample of the substrate in the local area around our facilities, and the types of energy crops that are cultivated in this region, which exhibit better performance in the condition weather of the North of Italy.

The research programme was structured in phases, development in different seasonal period during the three years of the doctoral courses (see Table 3.1). For every case we tried to simulated real conditions with the essentials parameters of control for a good development of an experimental research.

Phase	*Wastewaters*	*Seasonal Period*	*Species*	*Reactors*	*Feeding water composition (%)*	*Substrate*	*Feeding Regime (results)*
1	Aquanova Project	June to August, 2011.	*Helianthus annus* *Glycine max* *Brassica napus*	Tanks	YW 0,1-3,5% GW(a) 99,9 - 96,5%	Soil	2,7 – 3,3 l/tank/day
2	Leachate A	November, 2011 to Jannuary, 2012.	*Helianthus annus* *Glycine max* *Brassica napus*	Pots	2-30 % leachate 90 – 70% tap water	Sand Soil	0,2 – 0,4 l/pot/day
3	Leachate B	May to August, 2012.	*Helianthus annus* *Glycine max*	Pots	20% leachate 80% GW(b)	Soil	0,2 – 0,4 l/pot/day
4	Leachate C (a,b)	May to Jun, 2013.	*Helianthus annus* *Glycine max*	Tanks	10-60% leachate 90 - 40% tap water	Soil	2,7 – 3,3 l/tank/day

Table 3.1. Research experimental programme (*YW = yellow water; GW = grew water).*

After to reviewed all the literature concernment and organized the material needed for the different phases, we could determinate the best options to proceed with the experimentations, taking into account all the parameters that can influences our work, starting with the irrigation of culture associated with the phytotreatment of the different wastewaters, the quality of the substrate and the choice of the plants species. In this context, we started with the Aquanova systems in order to verify and extend and lead to a larger scale of a previous study conducted in this department; the other phases were exclusively dedicated to the treatment of the leachate, in order to assess the system behaviour under different loading conditions, every phases was detailed below; all this was possible making use of the existing material in the laboratory, which has already been tested and used in previous experiments.

3.4. Materials

As described in paragraphs before, the most important for the selection of the adapted material is the clear definition of the aims programme, utilization of material according to the requirements of the place and its easy accessibility. The following describes each of the materials used in the different experimental phases, that it to say: Plants-substrates.

3.4.1 Plants – Substrate

3.4.1.1. Sunflower (H)- Helianthus annus L, (Asteraceae)

Sunflower is an annual plant about 2m in the natural state, with large capitula that support yellow flowers and produce large, black achenes. These capitula tend to orient themselves towards the rising sun. Sunflowers are chiefly used to produce edible vegetable oil, which is extracted from its seeds. The residues, rich in nitrogen compounds, are used for animal feed in the form of oilseed cakes. They can be cultivated as green forage, and have market potential as a biological fuel. Sunflower showed high oil content with high concentration of oleic acid. During sunflower cultivation farmers are able to take advantage of the low fertility lands and it can be grown with limited water availability. So far sunflower is cultivated in 23 European countries (Skolou et al, 2011).

Figure 3.1. Sunflowers (H) cultivated in the greenhouse

3.4.1.2. Soybean (G) – Glycine max, (Fabaceae)

Soybean is an annual legume forming a tuft 50 to 60 cm in height, alternating leaves of 3 leaflets. The small white or violet flowers are grouped in racemes underneath the leaves. It is cultivated for its oleaginous seeds rich in protein. It may also be used as green forage or green fertilizer. The largest seeds are washed, ground and cooked to form soya milk, the origin of margarine. Oil may be extracted from the seeds; the residues are used to make oilseed cakes, an important part of animal feed. Soybean oil reaches the 50% of internationally produced vegetable oils, while soybean cake is widely used for feed. The major soybean producers are USA (33%), Brazil (27%), Argentina (21%) and China (7%). Soybean absorbs N2 from the air and requires low N fertilization; No extra machinery is required for growing soybeans. Protein content of soybean is quite high (38%ww). The content of oil is about 18–22%ww (Skolou et al, 2011).

Figure 3.2. Soybean (G) cultivated in the greenhouse

3.4.1.3. Rapeseed (B) – Brassica napus, oleifera (Brassiceae)

Rapeseed is an annual plant with racemes of yellow flowers. The fruits are siliques (pods) enclosing small seeds, rich in lipids that are used in oil production. Rapeseed is also cultivated for green forage. Rapeseed is sown in the autumn and develops, before winter, a taproot and a rosette of about 20 leaves. Stem extension begins with the return of the growing season (spring). Flowering begins before stem extension has finished and continues for more than one month. Oilseed rape: the winter varieties are the most cultivated. After oil extraction, the protein-rich residues are made into oilseed cakes and used as animal feed. Oilseed rape has a potential market as a biological fuel. Rapeseed has high oil yield is attributed to its high cellulosic content and, consequently, rapeseed is at the moment the first option for biodiesel production, while it may be the only, at the moment, able to be used without prior treatment as liquid biofuel (Skolou et al, 2011).

Figure 3.3. Rapeseed (B) cultivated in the greenhouse

3.4.1.4. Structure of Substrate (substratum)

The substrate is the main supporting material for plant growth and microbial film. Moreover, the soil matrix has a decisive influence on the hydraulic processes. Both chemical composition and physical parameters such as grain-size distribution, interstitial pore spaces, effective grain-size, degrees of irregularity and the coefficient of permeability are all important factors influencing the biotreatment system. (U. Stottmeister et al, 2003). The physical parameters indicate certain states of the soil and considerable influence the flow of wastewater in constructed wetlands and the removal of contaminants. Long-term studies of the hydraulics of constructed wetlands with different soil parameters indicate that the mixture of sand and gravel produces best results in terms of both hydraulic condition and the removal of contaminants. So, we could test soil pollutants retention over time, which is a crucial aspect in phytoremediation application (Jones et al., 2005)

The land has a very important role in plant development, as it has to retain water and nutrients, with a good balance between macroporosity, for air circulation and root development while avoiding stagnant water, and microporosity for a suitable water retention, which must be available for plant growth. The rhizosphere must also allow the life of the microorganisms useful to the plant, able to provide nutrients, but also to facilitate removal processes useful to the phytoremediation (phytostimulation).

- ✓ **Sand Substrate:** Sand is a poor substrate with no nutrients for plants growth so that all the nutrients are provided by feeding water; sand was choice to avoid the interference of leaching of nutrients from the soil, utilized in the second phase and differentiating as H_sand, G_sand and B_sand.
- ✓ **Soil substrate:** agricultural soil is rich in organics and nutrients and some leaching of substances can occur during the treatment, so in general we used agricultural soil to collected

from areas close to our facilities. For the second we differentiated the species-soil combination as: H_soil, G_soil and B_soil. The soil substrate for the three first phases was the same, while for the last phases was taken for another place; anyway both soils presented similar characterizations; is in the classification of sandy clay loam, see figure 3.4. In Table 3.2 we can observe how the different range of envelope expressed according to carbon content, depending on the texture of the soil.

	Class grouping		
	Coarse	Media	Fine
	Textural classes USDA		
Endowment	sandy loamy - sand sandy - clay	loam sandy-clay- loam silty - clay- loam sandy clay	clay clay -loam silty-clay silty-clay-loam
	Organic carbon (g/Kg)		
low	less than 7	less than 8	less than 10
normal	between 7 and 9	between 8 and 12	between 10 and 15
good	between 9 and 12	between 12 and 17	between 15 and 22
very good	greater than 12	greater than 17	greater than 22

Table 3.2. Textural class of soil substrate for all the phases.

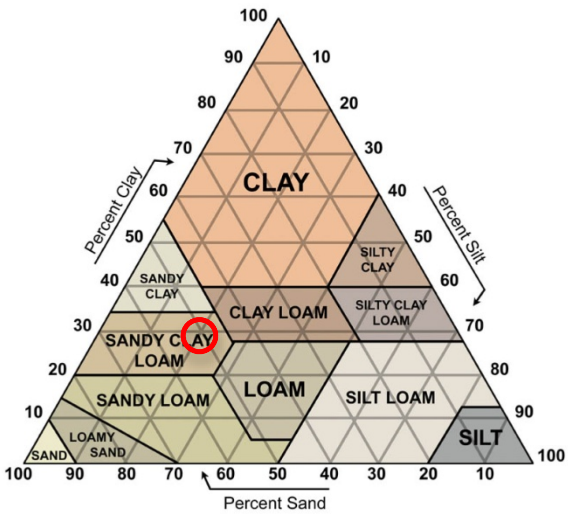


Figure 3.4. Triangle of the textural class for the substrate.

The main characteristics of the substrates are reported in Table 3.3, all the parameters were determinate according the Italian Analytical Standards (CNR-IRSA, 64/1986). The medium used as a substrate for the experiments consists mainly of sand, while it has a homogeneous supply of silt and clay. From the analysis it was possible to assess how the pH is close to neutrality, suitable for the growth of the plant, a low presence of organic carbon and a very low percentage of organic substance, less than 1%.

	Phase 1		*Phase 2*				*Phase 3*		*Phase 4*	
Parameters	*Soil*		*Soil*		*Sand*		*Soil*		*Soil*	
(mg_{DRY}/Kg)	Before trial	After trial	Before trial	After trial	Before trial	After trial	Before trial	After trial	Before trial	After trial
TS	90.0 %	94.9%	98.0%	94.9%	92.3%	90.5%	90.0%	82.3%	91%	83.6%
VS			1.20%	2.1%	2.1%	2.5%	4.1%	5,8%		
TOC	1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%
TKN	380	458	77.6	111	392	449	1900	1799	320	662
NH_4-N			55.1	60.4	79.4	73.0	608	485.7		
NO_3-N	21.5	95.9	< 10.0	48.2	63.8	73.0	76.2	82.1	5.6	68.8
TP	466	428	111	114	313	321	782	513	256	

Table 3.3. Substrate composition before and after the research phases

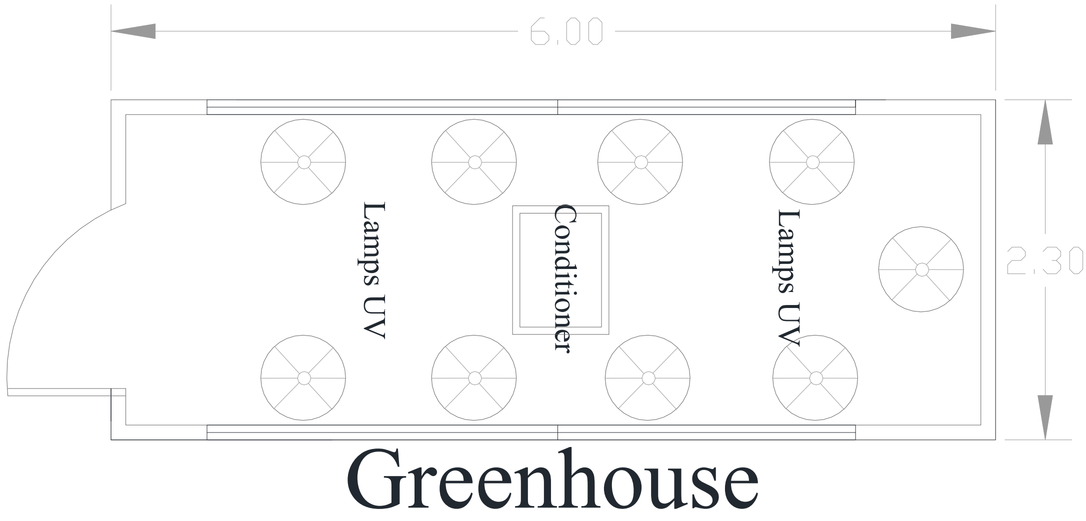
3.4.2. Reactors

Special reactor were needed in the experimental trials, in the facilities of the laboratory we had a disposition these reactors who made possible the development of every phase.

3.4.2.1. Greenhouse

All the trials were performed in the greenhouse (a commercial container) disposed with controlled climate conditions, and the pots and tanks, come from previously experimental research in the laboratory. The necessary equipment like lamps and conditioner were installed, showed in the scheme 1, making attention to profited all the interior space:

External view.



Scheme 3.1: The greenhouse facilities.

The greenhouse (showed in scheme 3.1), prefabricated of sized 6.0 x 2.3 m., provided a greenhouse conditions in terms of lighting provision, air conditioning and heating, temperature and the frequency of the irrigation.

- ✓ The lighting factor was exclusively controlled by the time switcher, which activated the lamps. The latter would provide light consecutively for 14 hours every day, from 6:00 am to 20:00 pm. Full darkness was achieved for the 10 following hours in order to assure the ideal plant growth. Darkness conditions were applied by covering the windows with a black plastic, not letting outside conditions affects the inside of the facility. Photometric tests were realized during the plants development to assure the well going of the experiment.
- ✓ The temperature was controlled by the thermometer and set as wanted by the air conditioning system on the roof of the facility and varied from 24-27 ◦C during the day and 21-24 ◦C at night. Temperature conditions may not have been entirely isolated from those of the outer environment.
- ✓ In the operation of the phytotreatment plant, daily monitoring included the following aspects: air temperature (maximum and minimum), water temperature (inflow and outflow), and the status of the vegetation. Air temperature was measured with a thermometer placed between the pots/tanks.
- ✓ To assess the status of vegetation each plant was continually monitored. Thus for each plant it was possible to follow its growing stages (i.e. sprout emission, growth, spread and blooming). A comprehensive photographic record was completed in which is possible to observe the growing stages throughout the two years of experimentation. All aspects

regarding vegetation are discussed in every phase. Inflow and outflow quality was weekly monitored.

Figure 3.5. Greenhouse facilities (internal view)

3.4.2.2. Pots

The pots (see Figure 3.6); individual pot of 50 cm high-truncated cone shaped pot (upper diameter of 30 cm, lower diameter of 20 cm) in plastic material. Each pot was holed on the bottom and connected to a flexible pipe, 1 cm thick. The pipe was kept in vertical position and used to monitor water level inside the pot for the whole irrigation of the period. The same pipe was regularly turned upside down to collect and determine drainage water volume, which was subsequently sent to the lab for analysis.

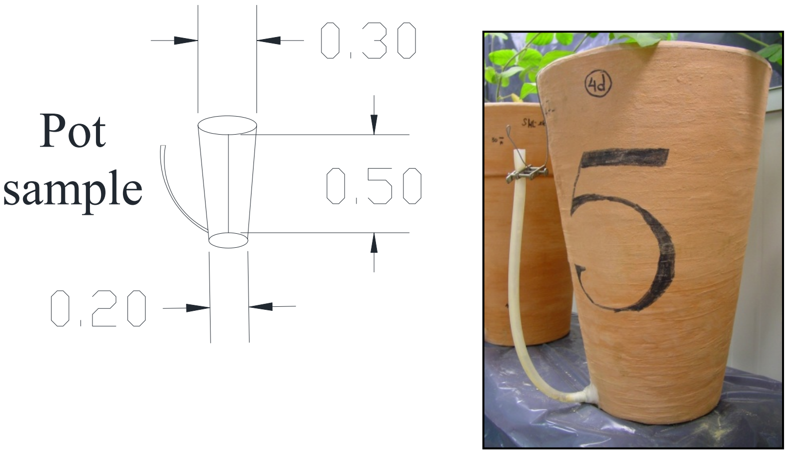


Figure 3.6. Pot sample

3.4.2.3. Tanks

The tanks, (see Figure 3.7); are rectangular containers in PVC plastic material of 95 x 64 x 50 cm., constructed with a tap at the bottom of each one, to take the outflow of the feeding samples.

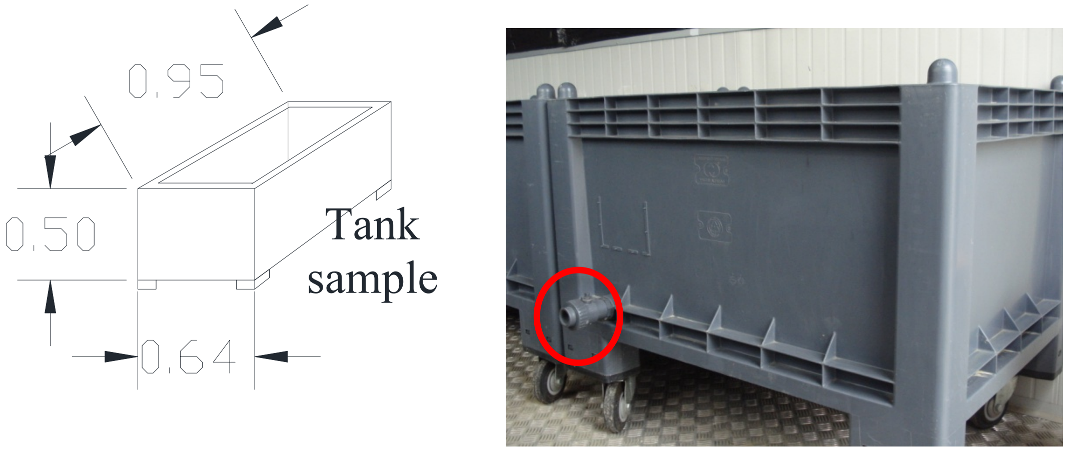


Figure 3.7. Tank sample

3.5. Methods

Data was then collected on the following set of environmental parameters: organic matter (COD, BOD5), solids (TS, SS, VS), nutrients (TKN, N-NH3, N-NO2, N-NO3, TP), metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn), pH, chloride and sulphide. For all analysis it was followed the Standard Methods methodology. Practically, the same methodology was adopted every experimental phases, here below, are described the principal parameters to be considered.

The compounds of nitrogen are among the most important constituents of wastewater for their role in eutrophication, for their effect on the oxygen present in the water, and for toxicity to aquatic species (Kadlec and Knight, 1996). The main forms of nitrogen present in wastewater are ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3-), nitrous oxide (N_2O) and dissolved nitrogen gas (N_2). Nitrogen is also present in organic form urea, amino acids, amines, purines, pyrimidines. Masotti (1999) suggests indicative percentages for these forms of nitrogen in wastewater: 60% ammonia form, 35% and 5% organic form of nitrites and nitrates.

Phosphorus is found in wastewater in the form of orthophosphate (PO43-) as a percentage of 40-50%, organic phosphorus as a percentage of 10-30%, and phosphorus or condensed polyphosphates (P2O74-or-P3O105) in the percentage of 40 - 60%. (Masotti, 1999). Important element in wet systems, phosphorus is essential both for the plant growth, both for the bacterial metabolism. A measure of the demand for P in the system may be given by the molar ratio of C: N: P = 106:16:1 and 41:7:1 weight. Often the tributaries do not meet these ratios with an excess of phosphorus (Kadlec and Knight, 1996).

The concentration of dissolved oxygen in water varies with the temperature, salinity and biological activity. The presence of C and N in the water inducing a biological oxygen demand, known as CBOD and NOD, which can be identified in four categories: oxygen demand of the sediment oxygen demand of breathing oxygen demand of the CBOD and for the NOD dissolved. The sediment oxygen required for the decomposition of detritus and organic compounds precipitated from the incoming wastewater. Breathing is the work of any animals that reside in the bed, but especially by plants: significant in this regard is the disappearance of dissolved oxygen during the night. NOD and BOD are respectively linked to the presence of ammonia and organic carbonaceous substance. The transfer of oxygen in the wetland is attributable to three processes: biochemical production by photosynthesis, the physical transfer by diffusion from the atmosphere and PAF (Plant Oxygen Transfer), or the passage of oxygen from the atmosphere to the substrate through the roots of plants. Photosynthesis requires sunlight and the presence of plankton and periphyton (typically can occur in systems SF).

The temperature is an important parameter in several respects. The receiving water body downstream of a phytopurification system may be very sensitive to temperature changes caused by a tributary, for example to the presence of particular species of fish. Some biochemical processes are very sensitive to temperature. The discharge of warm water in winter in damp areas can cause the formation of fog. The internal temperature of the wetland is to be expected by implementing the energy conservation equation, with appropriate modifications for the winter season if it were accompanied by frost and / or snow. (Kadlec and Knight, 1996).

For phytoremediaton plants, the pH is usually neutral or slightly acid, except those receiving acid wastewater from industrial processes. In aquatic systems the succession of respiration and photosynthesis between day and night produces a fluctuation in pH due to equilibrium carbon dioxide-carbonate affected by these processes. The organic substances circulating in the system are the basis of any weak acid. The non-ionised forms of humic substances are poorly soluble in water and precipitate in an acid environment. As a result of the wetland dabbing the possible entry of basic substances. Are less "protected" from the entrance of acidic substances due to the limited concentration of these humic solids content in the water column.

The suspended solids are the substances present in the water in the form of suspended particles or colloids (with dimensions beyond certain values), in practice they are not visible filterable substances, namely, that, in laboratory testing, remain captured in a particular filter (membranes or filter asbestos, 1, 0.45 or 0.2 microns) which is able to retain the parts coarse suspended and partly colloid, and also microorganisms of sufficient size. Suspended solids cause turbidity of the water - the called "visible pollution"- (Masotti, 1999). They also transmit diseases (may incorporate bacteria, protozoa and viruses) and carrying toxic elements such as heavy metals and organic compounds (PAHs, PCBs, etc). (Nazaroff and Alvarez-Cohen, 2001).

Of the hundreds of carbonaceous compounds that are found in wet environments, relatively few are inorganic. The dissolved inorganic carbon consists essentially of carbon dioxide, carbonate and bicarbonate. The pure water solution of the main species of carbonate are related to atmospheric CO_2 through the series of dissolution and dissociation employees from pH and temperature (Kadlec and Knight, 1996).

In addition to the pollutants discussed above, the wastewater typically contains many other substances, some of which can cause problems when discharged into the environment: their removal can be crucial and should therefore be considered in the design phase. These substances may be included in the following categories: salts, acids, bases, macronutrients (including C, N, P, already seen previously) micronutrients, and heavy metals (Kadlec and Knight, 1996).

The calcium (Ca) is biologically active because it is used as a nutrient by vertebrates and invertebrates and for his role in the carbon cycle. Is the main component of calcium carbonate, the basic element for shells, coral and bone. During photosynthesis, the calcium is removed from the water surface together with the carbon dioxide. During respiration, carbonate (and calcium bound) increases in concentrazioneman hand that CO2 is released as carbon dioxide. Since usually in surface water is present an excess of calcium, the concentration of this element does not vary significantly in wetland. Ca seems to be one of the least mobile elements in macrophytes.

The magnesium (Mg) is an essential micronutrient for it role in the phosphorus cycle and because it is a structural component of the chlorophyll molecule. Many natural wetlands serve more as producers as reservoirs of Mg, with removal efficiencies in the range from -300 to 36% has been verified a certain mobility of magnesium in the wetland, and demonstrated its pretty easy release from dead vegetation. (Kadlec and Knight, 1996).

The Chloride is an important element in biological systems for its role in photosynthesis. The application of organic chlorine is very low, however, and given its abundance in surface waters and its high solubility, its mass is fairly constant during the crossing system.

The heavy metals are present in the waste water in the ionized form, specifically iron, copper, zinc, cadmium, manganese, mercury, chromium, cobalt, nickel, lead; conventionally are indicated as such those with relative density greater than 6 g/cm3. In excessive concentration, present toxic action and inhibitory processes of biological treatment of wastewater. The maximum tolerable concentrations in biological treatment of wastewater are of the order of 5-10 mg / l of metals. Most of heavy metals in small concentrations, are not only not harmful, but even as trace elements are essential for the growth of bacteria and other organisms that govern the treatment. (Masotti, 1999).

3.5.1. Management of the experimental phases

Here, we proceeded to identify the different materials utilized in every phase. In the table 3.4, is summarized the composition of every type of wastewater utilized for every phase of study.

Parameters	*Yellow water*	*Grey water (a)*	*Grey water (b)*	*Leachate A*	*Leachate B*	*Leachate C(a)*	*Leachate C(b)*
pH	6.40	7.90	7.96	8.02	8.09	7.85	7.81
TS	36475	449	405	6315	7215	2605	3738
VS	20946	260	213	1548	2613	1010	1158
COD	6363	68	127	2255	3270	607	1005
BOD			29.4	75	203	24	38
TOC			15.4	1953	885	1002	1471
IC	-	-	-	140			
TKN	9765	4.30	5.60	1204	1285	627	1015
NH_4^+-N	1095	2.90	<0.5	1117	1176	588	1005
NO_3^--N	5.60	1.30	1.89	0.57	5.90	2.50	2.03
TP	3.00	0.30	0.50	22.0	23.0	7.60	2.90
PO_4^{3-}-P	0.30	0.20	<0.10	20.0	13.5	<10	<10
Cl^-	2208	43.7	46.1	1622	2057	594	886
SO_4^{2-}	1261	42.2	29.4	<10	<10	<10	<10

Note: All sample values are in mg l^{-1} except pH.

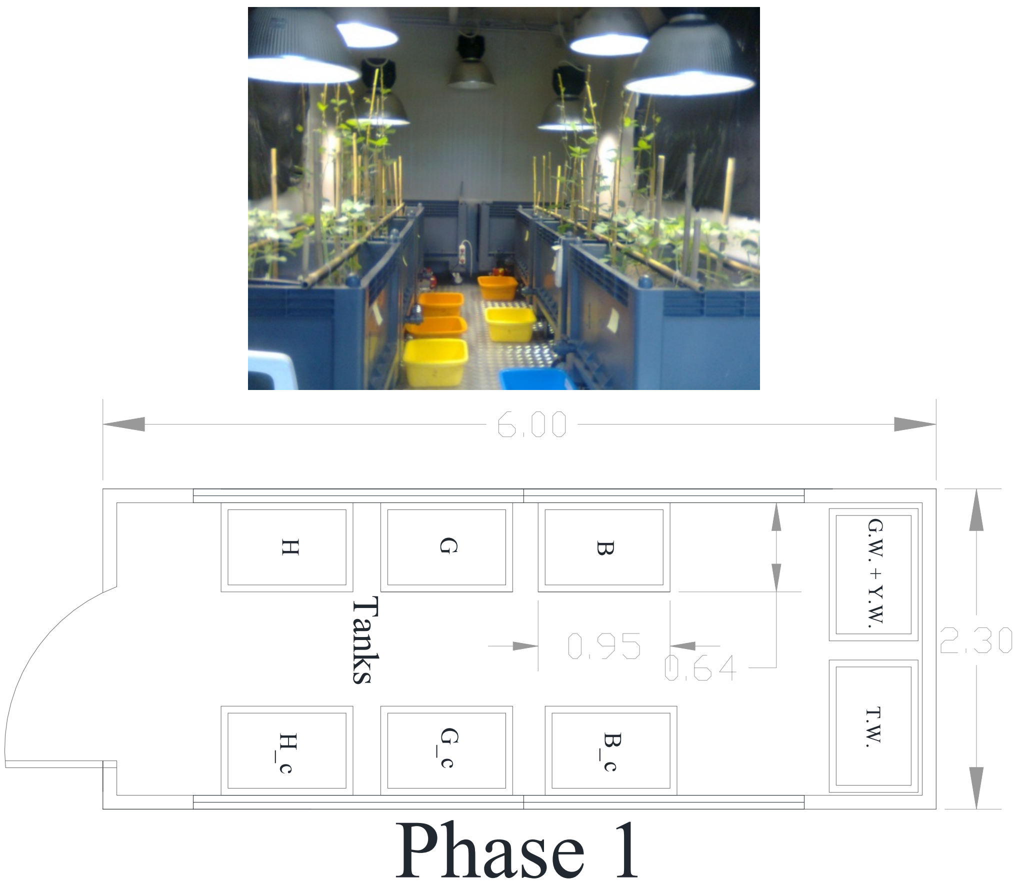
Table 3.4. Wastewaters initial composition

- ✓ Grey water (a,b) and Yellow water; were collected from derived towards from the bathrooms utilized in the department facilities, through the Aquanova system. These wastewaters were tested in the first phase.
- ✓ Leachate A and B; leachate samples was collected in a MSW landfill located in Ca 'Filissine in the municipality of Pescantina, Verona, in the North East of Italy; the site extends over a 12000 m2 surface. The first four lots, located to the west, operational from 1987 to 1999, represent the portion of the landfill empty and closed. The other four lots, located in the East, made extension, make up the portion of the new landfill, open and in operation at the time of the sequestration. The quality of leachate from the landfill is regularly monitored, with periodic sampling, either by the operator of the landfill is ARPAV of Verona. The samples are separately taken from storage tanks that collect separately the leachate generated in the landfill already closed. These leachate samples were tested in the second and third phase.
- ✓ Leachate C (a,b); the leachate samples was collected from the MSW of the landfill Ciliverghe lies in the southern part of the territory of the Municipality of Mazzano, in the municipality of Brescia: the area occupied by the landfill is 95,000 m2 with a volume of approximately 1,000,000 m3, the landfill has been used in several batches starting from the first phase in 1983 (employment of 20,000 m2) until you reach progressively the last phase (phase IV) with a tub of 9,200 m2 in 1989, the landfill is divided into 3 areas with design characteristics that do not provide sufficient sealing. The leachate samples were collected from the same place but in different periods for the fourth (last) phase.

- ✓ Hydraulic retention time affects phytotreatment performance as well as wastewater dosage. Feed and drainage operations should be analysed in order to evaluate the hydraulic residence time of the trial. Usually, we dosed the same feed for one week. The following week, we removed the water accumulated on the bottom of each pot and dosed another type of feed. Drained water was never recirculated back to the system. So, water fed to the system on day 1 stayed in the pot for 7 days. On the contrary, water fed to the system on day 7 stayed in the pot for 1 day. Assuming to feed the same water volume every day, and to neglect evapotranspiration process, the average hydraulic retention time is: HRT = 4 days. This is a quite short period to appreciate the effect of a a wastewater, specially with the leachate load on the experimental crops. Actually, scientific literature recommends 7 days, as the minimum HRT for phytotreatment applications (Kylefors, 1997).

3.5.1.1. Phase 1

In the first phase six plastics tanks (300L) were used placed inside the greenhouse, those tanks were filled with 10 cm – medium sized gravel at the bottom, and 30 cm of substrate soil. The disposition of the tank is illustrated in the scheme 3.2.



Scheme 3.2. Reactors dispositions - phase 1

The following crops have been chosen for phytotreatment: *helianthus annus (H), glycine max (G) and brassica napus (B),* each of them widely used especially in the local region- Nord of Italy, capable for the remediation of domestic waters, have the potential of increase the biomass by consuming nutrients that are included in this particular waters. The seeds germinated in the greenhouse of the Department of Agronomy Food Natural Resources Animal Environmental (DAFNE), then the seedlings were transplanted into the respective tanks, in quantity: eight seedlings for each tank.

During the phase 1, the concentrations of nutrients in the feeding increasing by the combination of different percentages of grew water (GW) and yellow water (YW). The wastewater components (GW and YW) have been separated through the toilet facilities of the Aquanova project implementation at the LISA laboratory, as mentioned before. Nitrogen and Phosphorus and the various microelements in the different streams are pollutant if discharged in the environment but represent a source of nutrients for the plants. So, we took into account the different levels of these contaminants in the wastewaters independently (Table 3.5). Three of the tanks were irrigated with the wastewater mixture and the other three were feed with tap water as control during the whole phase.

Parameters	*Yellow water*	*Grey water (a)*
pH	6.40	7.90
TS	36475	449
VS	20946	260
COD	6363	68.0
BOD		
TKN	9765	4.30
NH_4^+-N	1095	2.90
NO_3^--N	5.60	1.30
TP	3.00	0.30
PO_4^{3-}-P	0.30	0.20
Cl^-	2208	43.7
SO_4^{2-}	1261	42.2

Table 3.5. Wastewaters characterization - phase 1

*Feeding quality***,** the percentage of grey and yellow water was increasing from 0.1 to 3.5 % for YW and decreasing from 99.9% to 96.5% for GW. The irrigation volume was given as needed: as a result the dose was 3.0 L/tank/day more or less. Table 6 summarized the feeding characteristics through the different periods of the trial and emphasizes the progressive increase in concentrations of different parameters viewed as essential in the behaviour of phytoremediation.

Period	*Lasting days*	*Feed water composition (% v/v)*		*Main pollutants concentration (mg L^{-1})*		
		Grey water	*Yellow water*	*TKN*	*COD*	*TP*
1	11	100.0%	0.0%	4.3	68.2	0.30
2	21	99.9%	0.1%	14.1	74.5	0.30

3	30	99.5%	0.5%	53.1	99.7	0.31
4	35	99.0%	1.0%	101.9	131.1	0.33
5	49	98.0%	2.0%	199.5	194.1	0.35
6	66	97.5%	2.5%	248.3	225.6	0.37
7	79	97.0%	3.0%	297.1	257.0	0.38
8	88	96.5%	3.5%	345.9	288.5	0.39

Table 3.6. Feeding water characteristics - phase 1

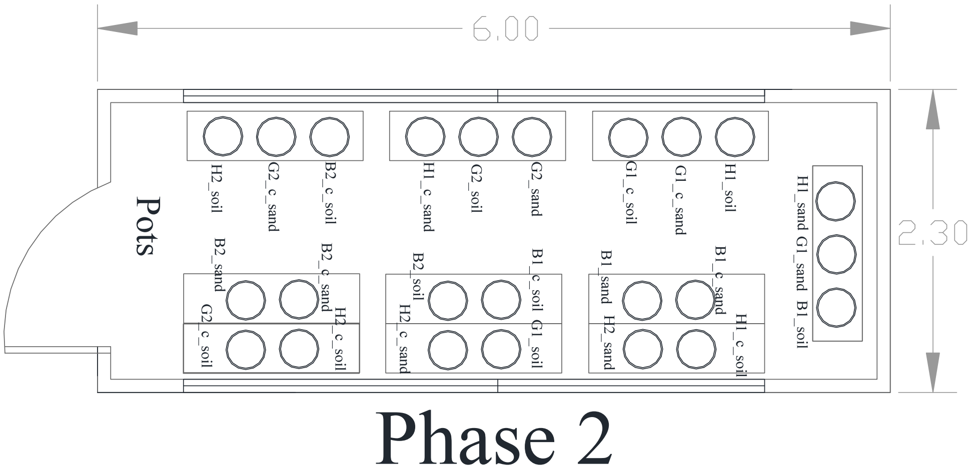
Flowering point was registered at the 72nd days for *Helianthus annus* and *Glycine max*, while *Brassica napus* never reached the point. During the irrigation period, occasional signs of stress of the plants were detected, old leaves desiccation, foliage spelling and abnormal production of flowers and pods.

Chemical and physical analysis were performed in the output waters according to the research program, analyses were performed in duplicate for each tank. Similar analyses were performed for the ground substrate, at the beginning and at the end of the operating phase.

With all data obtained, water, plants and soils could be determining the mass balance of the two representative parameters: nitrogen and phosphorus, for such purpose work with mean values, the final results are presented in tables and graphics, better explained in the next chapter.

3.5.1.2. Phase 2

In the second phase old landfill leachate was used as irrigation water. The experiment was performed in 24 pots (figure 3.6) inside the greenhouse. The dispositions of the pots are described in the Scheme 3. Coarse gravel was arranged in a 10 cm drainage layer on the bottom of each pot; subsequently, pure sand in half pots and a mixture of sand and clayey soil in the other half pots were used to build up a 30 cm deep growing layer. The choice of the different filling substrate was required for studying the influences of soil types on plants growth and the phytotreatment performance (M.C. Lavagnolo et al., 2011).



Scheme 3.3. Reactors disposition - phase 2

According to that six different combinations of soil and plat type were tested: H_sand, H_soil, G_sand, G_soil, B_sand and B_soil. The seeds germinated in the greenhouse of the department DAFNE (idem than before), then the seedlings were transplanted into the respective pot, one specie for each pot in the case of *Helianthus annus*, and two of each pot for *Glycine max* and *Brassica napus*. After an initial period to adaptation of the plants to their new habitat in which they all were watered with tap water, half of the pots were irrigated with increasing leachate concentration (2-30% leachate; 90-70% tap water), and the other half with tap water as control units, characteristics showed in table 3.7. Initiating increasingly the first day of each week and collecting the output samples at the beginning of the next week, that means, loading conditions were changed once per week. Controls growing on sand received a NPK rich solution (see Table 3.8), while controls growing on soil were judged nutrients self-nutrients and were maintained with tap water until the end of cycle.

Parameters	*Tap water*	*Leachate A*
pH	7.40	8.02
TS	-	6315
VS	-	1548
COD	<0.5	2255
BOD	-	75
BOD/COD (ratio)		0.03
TOC	-	1953
IC	-	140
TKN	<0.05	1204
NH_4^+-N	<0.05	1117
NO_3^--N	<0.05	0.57
TP	0.10	22.0
PO_4^{3-}-P	<0.10	20.0
Cl^-	8.0	1622
SO_4^{2-}	14.0	<10

Table 3.7. Leachate characterization - phase 2

Parameters	mg/L
KNO_3	101
$Ca(NO_3)_2$	164
$MgSO_4*7H_2O$	246
KH_2PO_4	136
$FeSO_4*7H_2O$	278

Table 3.8. Nutrient NPK Solution

Feeding quality, the irrigation volume was given as needed: as a result the dose of 0.3 L/pot/day more or less. Table 3.9 summarized the feeding characteristics through the different periods of the trial and emphasizes the progressive increase in concentrations of different parameters viewed as essential in the behaviour of phytoremediation.

Period	Lasting days	Feed water composition (% v/v)		Main pollutants concentration (mg l^{-1})		
		Tap water	Leachate	TKN	COD	TP
1	7	98%	2%	26.8	54.4	0.54
2	14	96%	4%	50.8	99.4	0.99
3	14	92%	8%	98.9	189	1.87
4	7	86%	14%	171	316	3.20
5	8	83%	17%	207	383	3.86
6	7	80%	20%	243	451	4.53
7	7	75%	25%	303	564	5.64
8	15	73%	27%	327	609	6.08
9	7	70%	30%	363	676	6.74

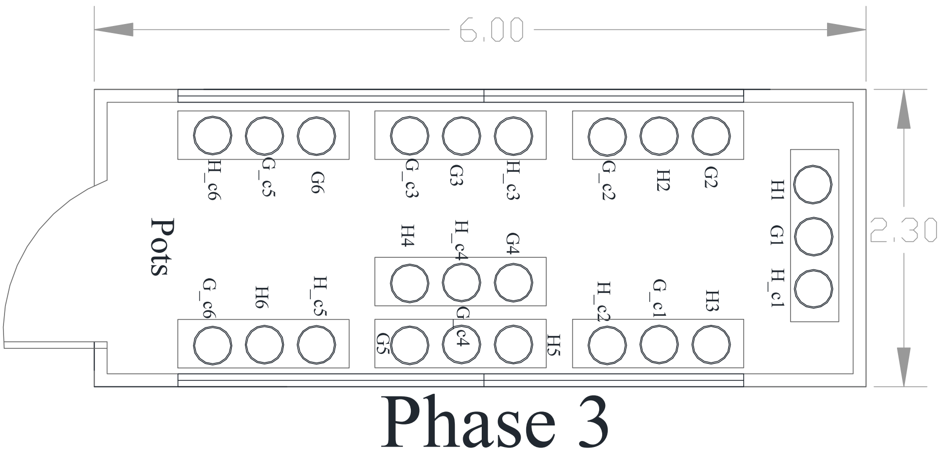
Table 3.9. Feeding water characteristics - phase 2

Plants growth day by day; *Glycine max* was the fastest to develop, after one month the first flowers appeared and soon after flowers were replaced by thin green pods. Control plants yellowed and defoliated before leachate irrigated plants; therefore, leachate stimulated, rather than to inhibit, the development of the crops. *Helianthus annus* development was deeply affected by the substrate of growth; plants growing on sand displayed early signs of stress, such as limited leaves production, thin stems development and progressive foliage dryness; on the contrary, *helianthus annus* growing on soil produced a lot of large leaves and very thick stems. We interpreted stress signals as a nutrient lack symptom. Considering the composition of the different feeding solutions (Table 8), we thought to a potential lack of phosphorus. Flowers appeared two months after planting, and both the samples associated to sand substrate and leachate irrigation displayed a multiple efflorescence. *Brassica napus* grew slowly compared to the other vegetal essences and it did not produce any flower and it was favoured by sand, rather than soil substrate. Its growth is temporarily suspended during the winter period and flowering is normally induced by the spring related temperature increase (Region of Umbria, 2000), thus the constant 24 °C greenhouse temperature limited plant activity to a continuous renewal of the leaves. Botanic handbooks report that plant growth can be inhibited by water stagnation (Region of Umbria, 2000). An intense irrigation activity, combined to a limited soil drainage capacity could be at the basis of rapeseed growth limitation in the soil filled pots.

Chemical and physical analysis were performed in the output waters according to the research program, analyses were performed in duplicate for each tank. Similar analyses were performed for the ground substrate, at the beginning and at the end of the operating phase. With all data obtained, water, plants and soils could be determining the mass balance of the two representative parameters: nitrogen and phosphorus, for such purpose work with mean values, the final results are presented in tables and graphics, better explained in the next chapter.

3.5.1.3. Phase 3

According to the results obtained in the previous phase, in the third phase *Brassica napus* was not used anymore. The experiment was performed in 24 pots -like before- inside the greenhouse.



Scheme 3.4. Reactors distribution - phase 3

The seeds were germinated in LISA laboratory under controlled conditions, using different kinds of substrate and different leachate dilution in order to test the maximum leachate percentage to be used in the substrate. So, 40 Petri dishes was used and divided depending on the substrate, of the culture and of the treatment solution with leachate following their initial characteristics (see table 3.10).

Parameters	*Grey water (b)*	*Leachate B*
pH	7.96	8.09
TS	405	7215
VS	213	2613
COD	127	3270
BOD	29.4	203
BOD/COD (ratio)		0.06
TOC	15.4	885
IC	-	
TKN	5.60	1285
NH_4^+-N	<0.5	1176
NO_3^--N	1.89	5.90
TP	0.50	23.0
PO_4^{3-}-P	<0.10	13.5
Cl^-	46.1	2057
SO_4^{2-}	29.4	<10

Table 3.10. Wastewater characterization, phase 3.

Subsequently were placed in incubation, in an oven at 25 ° C, to evaluate the effect of solution concentration and the type of substrate on germination. The germinated seeds were then planted in pots containing places mixture of soil, which will then be used in pots in the greenhouse (Fig. 2.5).

Figure 3.8. Seeds germinated - phase 3

Glycine max' seeds presented better germination at 5% of diluted leachate and in sand substrate, while *Helianthus annus'* seeds had a better germination on soil mixed with concentrated solutions at 10% and 20% of leachate (see figure 3.6). Subsequently, more vigorous seedlings were transplanted into pots in the greenhouse.

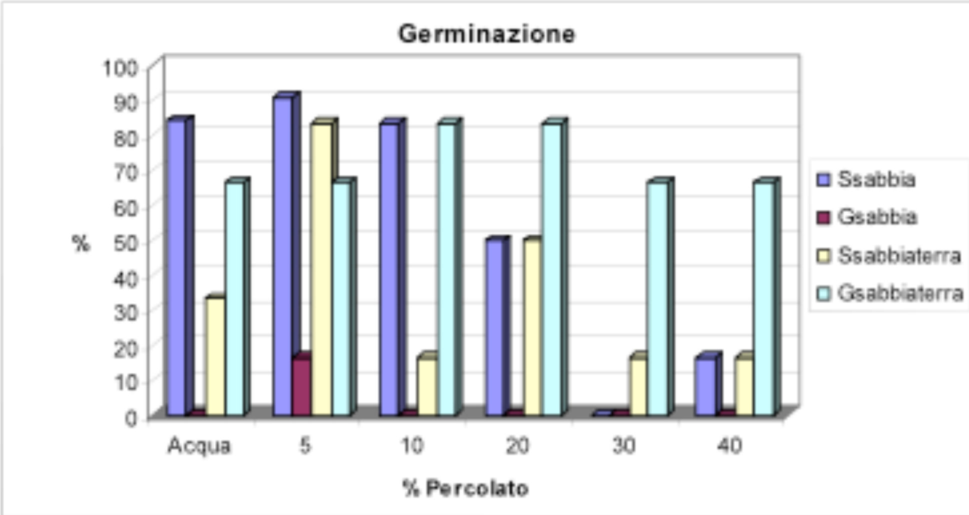


Figure 3.9. Germinations crops in different soil substrate and leachate dilutions – phase 3 (S: soybean, G:sunflower)

Feeding quality, the irrigation water was decided as a mixture of 20% leachate and 80% of grey water; half of pots were irrigation with the mixed of wastewater and the other half with tap water as control. The irrigation volume was as needed and as a result the quantity 0.3 L/pot/day. Table 3.11 summarized the feeding characteristics through the different periods.

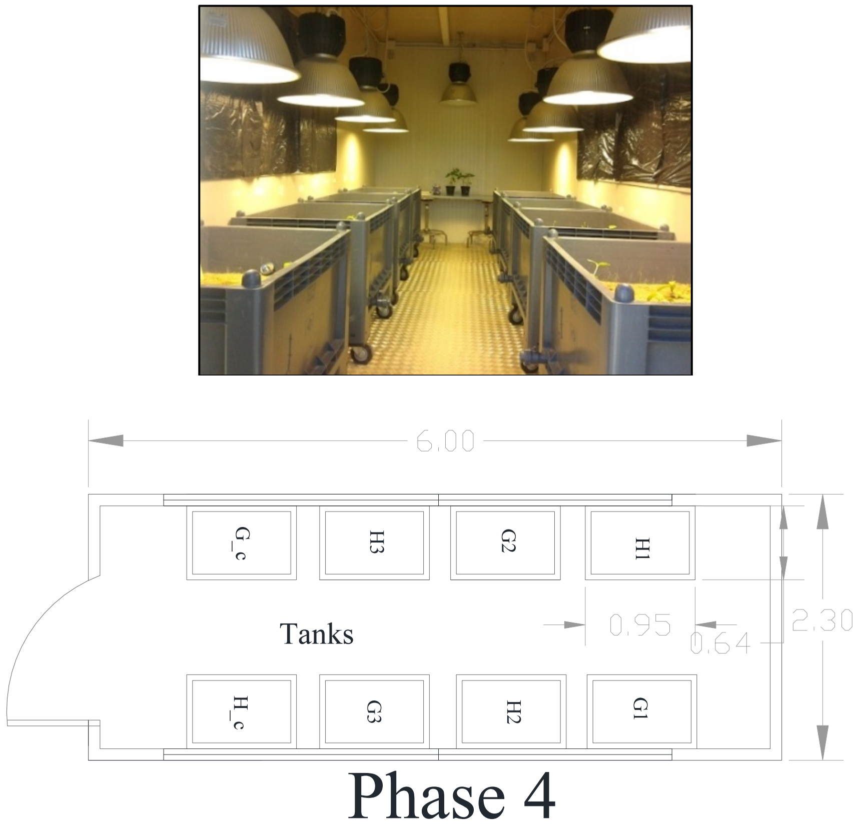
Flowering occurred at the 55th day after planting for both species; some signals of initial stress and inhibitions were registered. Chemical and physical analysis were performed in the output waters according to the research program, analyses were performed in duplicate for each tank. Similar analyses were performed for the ground substrate, at the beginning and at the end of the operating phase. With all data obtained, water, plants and soils could be determining the mass balance of the two representative parameters: nitrogen and phosphorus, for such purpose work with mean values, the final results are presented in tables and graphics, better explained in the next chapter.

Period	*Lasting days*	*Feed water composition (% v/v)*		*Main pollutants concentration (mg l^{-1})*		
		Grey water	*Leachate*	*TKN*	*COD*	*TP*
1	13	80%	20%	261.4	755.4	0.50
2	10	80%	20%	261.4	755.4	0.50
3	19	80%	20%	261.4	755.4	0.50
4	12	80%	20%	261.4	755.4	0.50
5	14	80%	20%	261.4	755.4	0.50
6	14	80%	20%	261.4	755.4	0.50
7	12	80%	20%	261.4	755.4	0.50

Table 3.11. Feeding water characteristics - phase 3.

3.5.1.4. Phase 4

Last experimental phase was performed in a bigger scale: eight 300 L tanks inside the greehouse were used (see scheme 3.5) four for each species: *Helianthus annus (H)* and *glycine max (G)*. The seedlings were germinated in the greenhouse of the department DAFNE, later on nine seedlings for each tank were transplanted.



Scheme 3.5. Reactors disposition - phase 4

Six tanks were irrigated with the leachate mixture (10-60% leachate and 90-40% tap water), and the other two with tap water as control. The characterization is described in table 3.12.

Parameters	*Leachate C(a)*	*Leachate C(b)*
pH	7.85	7.81
TS	2605	3738
VS	1010	1158
COD	607	1005
BOD	24	38
BOD/COD (ratio)	0.04	0.04
TOC	1002	1471
IC		
TKN	627	1015
+NH_4^+-N	588	1005
NO_3^--N	2.50	2.03
TP	7.60	2.90
PO_4^{3-}-P	<10	<10
Cl^-	594	886
SO_4^{2-}	<10	<10

Table 3.12. Leachate characterization - phase 4

Feeding quality, the irrigation with leachate dilution was periodically for a period of ten days submitted to maintaining a constant mixed of leachate concentrations. The irrigation volume was as needed as a result the quantity was 3.0 L/tank/day. Table 3.13 summarized the feeding characteristics through the different periods of the trial and emphasizes the progressive increase in concentrations of different parameters viewed as essential in the behaviour of phytoremediation during all the phase.

Leachate	*Period*	*Lasting days*	*Feed water composition (% v/v)*		*Main pollutants concentration (mg l^{-1})*		
			Tap water	*Leachate*	*TKN*	*COD*	*TP*
C (a)	1	7	90%	10%	65.2	69.3	0.77
	2	7	80%	20%	127.5	129.1	1.52
	3	7	70%	30%	189.9	188.8	2.28
	4	7	60%	40%	127.5	248.6	3.04
C (b)	5	7	70%	30%	306.5	308.2	0.88
	6	7	60%	40%	407.7	407.8	1.17

Table 3.13. Feeding water characteristics - phase 4.

Flowering occurred at the fourth week for *Helianthus annus* and at the sixth week for *Glycine max*. Chemical and physical analysis were performed in the output waters according to the research program, analyses were performed in duplicate for each tank. Similar analyses were performed for the ground substrate, at the beginning and at the end of the operating phase. With all data obtained, water, plants and soils could be determining the mass balance of the two representative parameters: nitrogen and phosphorus, for such purpose work with mean values, the final results are presented in tables and graphics, better explained in the third chapter.

3.5.2. Analytical methods

The efficiency of the experimentations was evaluated by measuring organic and inorganic parameters. The influent and effluent samples were performed following Italian analytic standards for water and wastewater samples (CNR-IRSA, 29/2003). pH was conventionally measured with a pHmeter, whereas a double step oven treatment (105°C for one day, 550°C for 4 hours) allows to define TS and VS content. COD was detected through the potassium dichromate oxidation method. Instead, BOD was evaluated by a 5 days BOD test in a Sapromat-E respirometer.

TOC was determined using a carbon analyzer by Shimadzu. TKN and ammonia were evaluated through a distillation-titration procedure, preceded by a digestion phase in the case of TKN.

Filtration of the sample with a 0,45 µm pore membrane was required to determine the dissolved components (nitrate, phosphate and sulphate ions) quantified by means of a UV-VIS spectrophotometer. The colorimetric method was used to detect also total phosphorus after samples digestion. Chloride and sulphide were measured by titration, whereas metals content was evaluated using an ICP-OES after samples digestion. In the following tables: 33(a,b and c); are describing the analytical method used for the different materials of the systems, namely: wastewater, plants and substrates.

PARAMETER	*Units*	*ANALYTICAL METHOD*
ORGANIC MATTER		
BOD5	mg/l O2	Respirometric
COD	mg/l O2	Potassium dichromate method
TOC	mg/l C	Carbon analyzer by Shimadzu
NUTRIENTS		
TKN	mg/l	Kjeldhal Metohd
NO3-	mg/l	Spectrophotometric analysis
Ptot	mg/l	Spectrophotometric analysis
PHYSICAL		
pH	pH unit	Potentiometric method
Total Solids (TS)	mg/l	Evaporation (105 °C)
Total Suspended Solids (SS)	mg/l	Filtration
Total Volatised Solid (VS)	mg/l	Evaporation (550 °C)
OTHERS		
Metals	mg/l	Spectrophotometry
Chlorides	mg/l	Mohr method

Table 3.14(a). Wastewater quality parameter and analytical methods employed

PARAMETER	*Units*	*ANALYTICAL METHOD*
ORGANIC MATTER		
TOC	mg/l C	Carbon analyzer by Shimadzu
NUTRIENTS		
TKN	mg/l	Kjeldhal Metohd
NO3-	mg/l	Spectrophotometric analysis
Ptot	mg/l	Spectrophotometric analysis
PHYSICAL		
Total Solids (TS)	mg/l	Evaporation (105 °C)
Total Volatised Solid (VS)	mg/l	Evaporation (550 °C)
OTHERS		
Metals	mg/l	Spectrophotometry

Table 3.14(b). Substrate quality parameter and analytical methods employed

PARAMETER	*Units*	*ANALYTICAL METHOD*
NUTRIENTS		
TKN	mg/l	Kjeldhal Metohd
NO3-	mg/l	Spectrophotometric analysis
Ptot	mg/l	Spectrophotometric analysis
OTHERS		
Content oils of seeds	%	UNE-EN 14214
Measures of the extracted plant	cm	
Weight of the plants	g	

Table 3.14(c). Plants quality parameter and analytical methods employed

- ✓ COD (Chemical Oxygen Demand)

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This parameter is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

A sample is refluxed in strongly sulphuric acid solution with a known excess of potassium dichromate (K2Cr2O7). After digestion, the remaining unreduced dichromate is titrated with Mohr salt (ferrous ammonium sulphate, Fe(NH4)2(SO4)2·6H2O) to determine the amount of oxidant consumed, and the oxidizable organic matter is calculated in terms of oxygen equivalent.

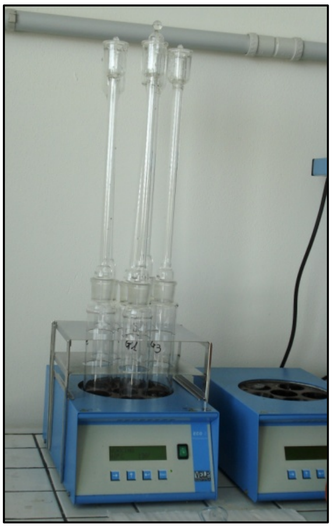

Figure 3.10. COD digestion

- ✓ BOD5 (Biochemical Oxygen Demand)

The method is based on the determination of dissolved oxygen in the sample, optionally diluted and inoculated, before and after incubation of 5 days in the dark and at a temperature of 20°C: the difference between the two determinations, expressed in mgO2/l, gives the value of BOD5.

If the sample requires dilution, it should be prepared the dilution water, containing the inoculum (slurry of sewage sediment), some salts adding as nutrients and a buffer solution at pH = 7,2.

Figure 3.11. BOD - Respirometer Sapromat

- ✓ TOC (Total Organic Carbon)

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The TOC is the result of the difference between the TC, total carbon, an the IC, inorganic carbon. Both these parameters are obtained oxidising the sample with air in a combustion tube in the presence of a catalyst: the carbon dioxide produced is quantified with an infrared detector. The difference in obtaining TC or IC is the working temperature (higher for TC) and the acid pre-treatment of the sample in the IC procedure, for transforming all the carbonate and bicarbonate in carbon dioxide.

The samples are analysed filtrated in case of presence of suspended solids.

- ✓ TKN (Total Kjeldal Nitrogen)

IRSA-CNR 29/2003 vol. 2 n. 5030

With this analysis it is possible to measure the sum of the organic nitrogen and the ammonia present in a sample. Measuring separately the ammonia and subtracting the amount from the TKN value it is possible to obtain the organic nitrogen.

A sample is refluxed in strongly sulphuric acid solution in the presence of a catalyst. At the end of the digestion all the nitrogen (organic and ammonia) is transformed in ammonium ion. All the ammonium ion is then changed in ammonia, increasing the pH with sodium hydroxide, and it is distilled in steam current. The stripped ammonia is collected in a boric acid solution and titrated with a standard sulphuric acid solution.

Figure 3.12. TKN for the crops

- ✓ Ammonia

IRSA-CNR 29/2003 vol. 2 n. 4030 C

The concentration of ammonia is estimated with the same procedure described for TKN, without the digestion step: the sample, after a pH changing with sodium hydroxide, is distilled in steam current and titrated with sulphuric acid.

Figure 3.13. Ammonia distillation

- ✓ Nitrates

IRSA-CNR 29/2003 vol. 2 n. 4040 A1

The evaluation of nitrates is carried through colorimetric determination with sodium salicylate.

Figure 3.14. Nitrates samples

- ✓ Total phosphorus

IRSA-CNR 29/2003 vol. 2 n. 4110 A2

With this analysis it is possible to measure the sum of the organic phosphorus and the phosphate present in a sample. For evaluating the organic phosphorus the sample must be digested with potassium persulfate to release all the phosphorus as orthophosphate. Then the evaluation of the phosphate ions is carried through colorimetric determination.

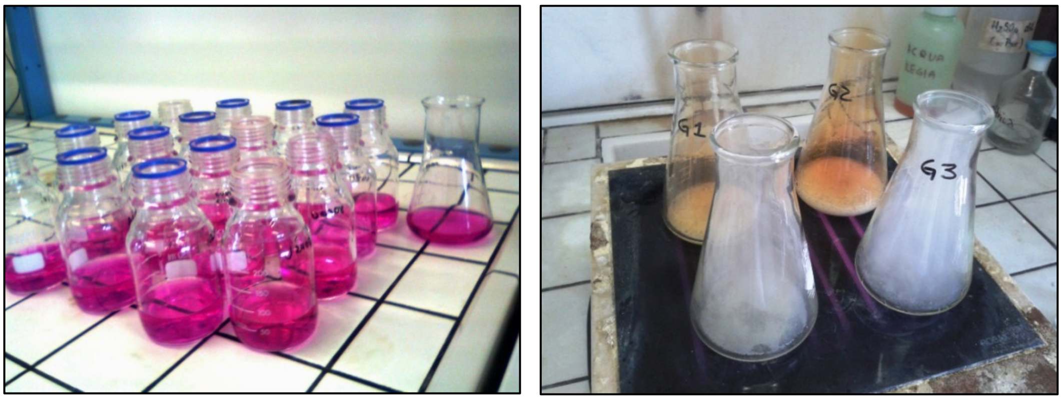


Figure 3.15. Phosphorus samples (liquid-solid)

- ✓ Chlorides

IRSA-CNR 29/2003 vol. 2 n. 4090 A1

The evaluation of the chlorides is made by titration with a silver nitrate solution so as to form silver chloride precipitate. The end of the titration is highlighted by the presence of a potassium chromate solution forming, with the chlorides of the sample, a silver chromate precipitate.

- ✓ Sulphates

IRSA-CNR 29/2003 vol. 2 n. 4140 B

The determination of the sulphates is carried through turbidimetric determination with barium chloride so as to form barium sulphate crystals of uniform size.

- ✓ pH

IRSA-CNR 29/2003 vol. 1 n. 2060

The pH is measured with a pH meter, after a calibration with two buffers at pH 4 and pH 7.

Figure 2.15.

- ✓ Solids

IRSA-CNR 64/84 vol. 2 n. 2 (modified for the liquid samples)

The total solids correspond to the solids that remain in a sample its evaporation in an oven at 105°C for 12 hours.

In the other hand the volatile solids correspond to the solids that remain in the dry sample after thermal treatment in a muffle at 550°C for 3 hours.

Figure 3.16. TS/VS capsule.

- ✓ Heavy metals

IRSA-CNR 29/2003 vol. 1 n. 3010 A (digestion)

IRSA-CNR 29/2003 vol. 1 n. 3020

The heavy metals are detected in an ICP-OES, after the digestion and filtration of the sample. The first operation, carried out in a strongly acid solution, allow the break of metal bonding with the organic matter; the second permit not to have particulate in the sample to be analyzed.

4 OUTCOMES & DISCUSSIONS

4.1. Phase 1

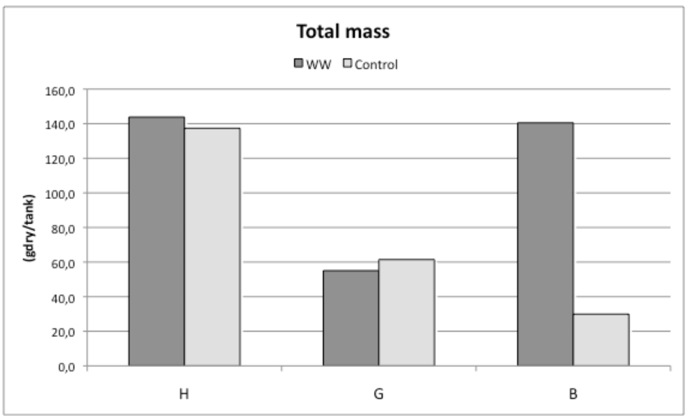
4.1.1. Vegetation growth

Once completed and life cycle of plants, these were extracted then be measured and weighed (see figure 4.1) - in wet and dry-differentiating the aerial part of the underground (roots) and also separating the seeds, the results of the total mass, root mass and root length are shown in the figures 4.2.(a,b,c). The evaluation of root development gives an idea of the possibility of exploring the ground and the potential phytoremediation capacity from an environmental point of view; this evaluation is enabled comparing the weight and length of the roots for each species with those of respective controls (see table 4.1).

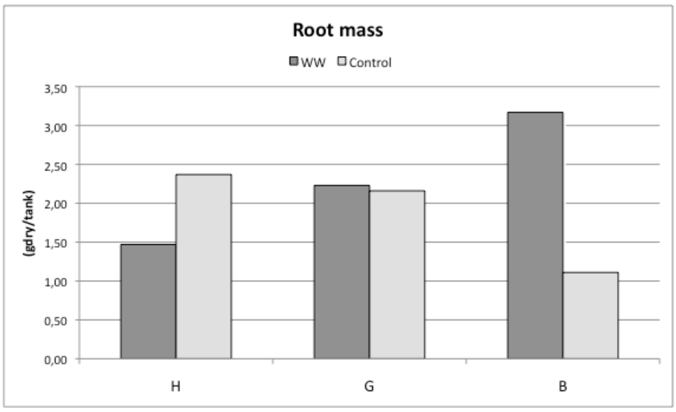
Figure 4.1. Species extracted in phase 1: H, G and B.

CROPS	MASS				LENGHT	
	Total		*Root*		*Main root*	
	g_{DRY}/*pot*	*%*	g_{DRY}/*pot*	*%*	*cm*	*%*
H	143,8	105%	1.47	62%	13.2	122%
H_control	137,4	-	2.37	-	10.8	-
G	55,1	90%	2.23	103%	22.3	60%
G_control	61,4	-	2.16	-	37.4	-
B	140,5	470%	3.17	286%	27.4	292%
B_control	29,9	-	1.11	-	9.4	-

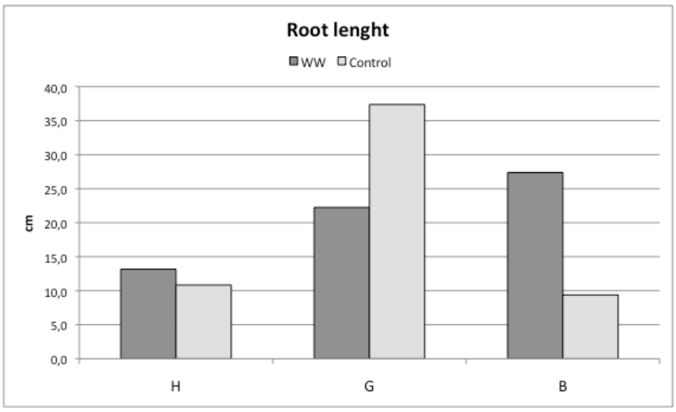
Table 4.1. Growth vegetation phase 1.



(H: Helihantus annus; G: Glycine max; B: Brassica napus).
Figure 4.2(a)Total biomass phase 1.



(H: Helihantus annus; G: Glycine max; B: Brassica napus).
Figure 4.2.(b) Root mass phase 1.



(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.2.(c) Root length phase 1.

In general, domestic wastewaters fed crops developed larger root biomass than corresponding controls, especially in the case of *brassica napus*. The shoot of *helianthus annus* took advantage to the fed wastewater in the first phase, with a less grew in a root mass in confront to the control one. *Glycine max* grew less than control plants, except in the root mass where denoted better response; but in general peak inhibitions was observed maybe due to a lack of nutrients at the beginning of plant growth period, not causing a deeper development in the root apparatus. *Brassica napus* responded definitely better at wastewater fed than it respective control, in terms of root mass and length and total biomass, totally opposite with a previous experiences in M.C. Lavagnolo et al, 2011.

4.1.2. Quality of oils seed cultivated

As a result of the seeds cultured have only two of the three species: *helianthus annus* and *glycine max*. To consider any feedstock (in this case seeds crops cultivation) as a biodiesel source, the oil percentage content and the oil yield are important parameters. In this line, we make the analysis correspondent at the two crops, taking 100 g for 1 g of the sample; like showed in table 18, we can verify the content of oils from the seed cultivated in the phase 1, according to the literature review and the limits of international standards, is located to the extent permitted, that is suitable for the production of biodiesel (see table 1.5; chapter 1), identifying the content of oil for the crops studied: 25-35 5 for H and 15-205 for G.

Oil seed and FAME (fatty acid methyl esters) content of the cultivated crops

Seed cultivated *Phase 1*	**Content of Oil** *(%)*	**FAME** *(%)*
H	35.87	34.29
G	16.69	18.82

(H = Helianthus annus; G = Glycine max.)
Table 4.2. Quality of oils seed - phase 1.

Even to controlled and verified the fatty acids compositions, we obtained this values of the principal component according to the profile given in literature, for the two species obtained (see table 19). The results confirmed that our values are in the permits limits (A.Karmakar et al, 2010)

Fatty acid compositions (wt.%) of seed oils		**H**	**G**
Lauric	C12:0	0.014	0.006
Myristic	C14:0	0.069	0.052
Palmitic	C16:0	1.829	3.058
Palmitoleic	C16:1	0.082	0.043
Stearic	C18:0	0.947	0.612
Oleic	C18:1	29.909	4.625
Linoleic	C18:2	0.933	9.121
Linolenic	C18:3	0.082	1.153
Arachidic	C20:0	0.106	0.065
Gadoleic	C20:1	0.077	0.041
Behenic	C22:0	0.002	0.008
Erucic	C22:1	0.002	0.000
Lignoceric	C24:0	0.222	0.037
Nervotic	C24:1	0.019	0.003

(H = Helianthus annus; G = Glycine max.)
Table 4.3. Fatty acids compositions - phase 1.

4.1.3. Removal efficiencies

Nitrogen, Phosphorus and COD were fundamental in furthering the understanding of the remediation effects of the three crops. Each graph features the different periods in order to improve the understanding of plant responses in terms of performance to variations remediation of contaminants introduced load. To better interpret the results and make a comparison with other findings present in literature, the values are expressed in international units.

The removal of nitrogen and organics, from wastewater, in such engineered ecosystem is very important, due to the following reasons:

- Uncontrolled discharge of nitrogen into natural water channels fosters eutrophication of lakes and rivers.
- Untreated organic materials often deplete dissolved oxygen (DO) concentration in open water channels, leading to the death of aquatic organisms (T.Saeed, G, Sun, 2012).

The performance disparity of phtytoremediation, in terms of nitrogen and organics removal could be attributed to the following reasons:

- Excessive presence of organics compounds in wastewater inhibits nitrification, as faster heterotrophic organic degradation depletes DO availability; and
- Lack of biodegradable organics often hinders classic denitrification metabolism (due to dependency on organic carbon) in wetland system.

4.1.3.1.COD removal

COD removal was above 70 %, through all the irrigation period and independently from the specific plant-substrate combination, see figure 4.3.

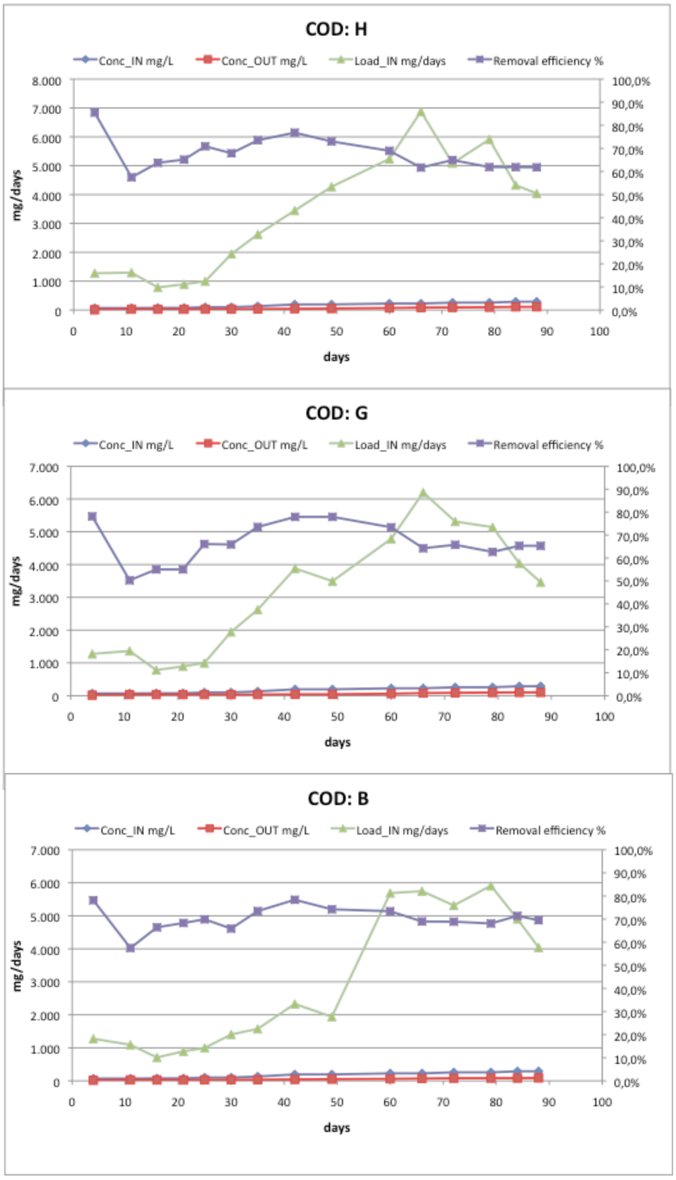


Figure 4.3. Phase 1: COD input and output concentration (mgO_2/L), COD input load ($mgO_2/days$) and COD η(%) removal efficiency during the experimental period. *(H:Helihantus annus; G: Glycine max; B: Brassica napus.)*

4.3.1.2.Nitrogen removal

Nitrogen is one of the principal pollutants in wastewater that can cause eutrophication, affect dissolved oxygen levels of receiving water, and may cause toxicity (depending on the nitrogen form) to the aquatic organism. Nitrogen exists in wastewater in both organic and inorganic form. Organic nitrogen can be represent in amido acids, urea, uric acids and purine, pyrimidines. The inorganic forms of nitrogen are ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), and dissolved elemental nitrogen or nitrogen gas (N_2). Gaseous nitrogen includes nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO_2), and free ammonia (NH_3). (T. Saeed, G. Sun, 2012).

The trend of nitrogen outlet concentrations reflects the nitrogen removal efficiency trend: values below 40 mgN/L have been detected for the total experimental phase, then the concentration rose up to 350 mgN/L. This kind of behaviour could be ascribed to the senescence of the plants.

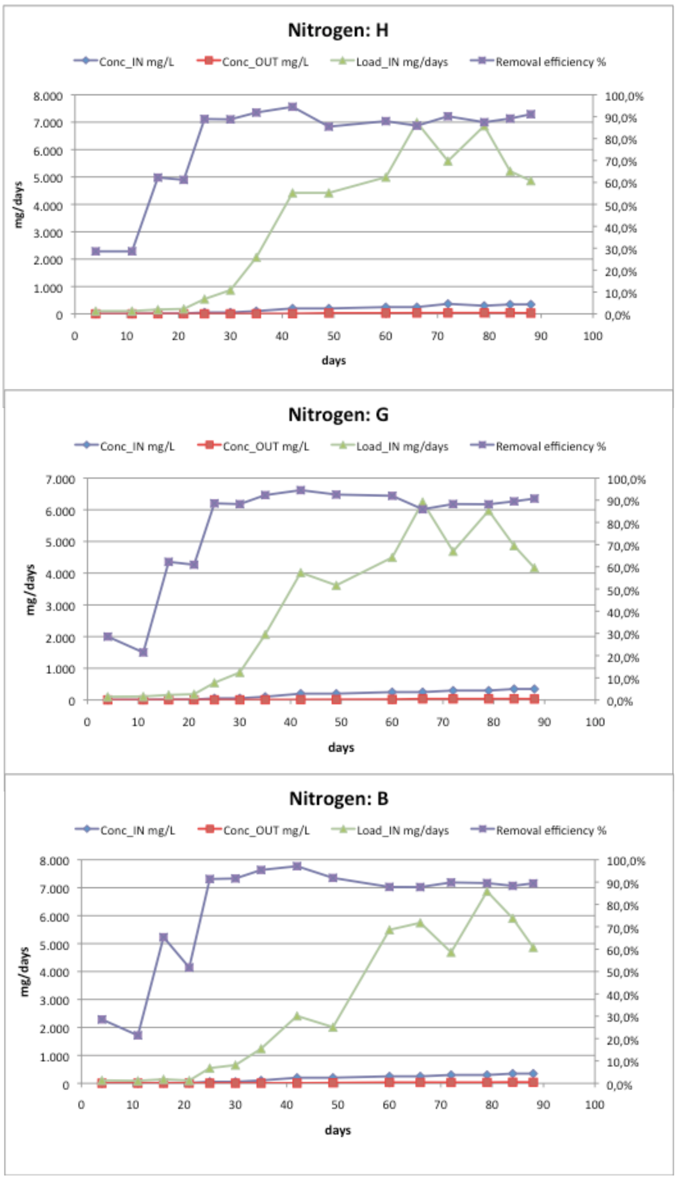


Figure 4.4. Phase 1: N input and output concentration (mgN/L), N input load (mgN/days) and N removal (%) removal efficiency during the experimental period. *(H: Helihantus annus; G: Glycine max; B: Brassica napus).*

4.1.3.3.Phosphorus removal

Removals efficiency in phosphorus rate were equal to approximately 30% for all the species, with outlet concentrations below the value of 1 mg/L, even below the 0.5 mg/l in compliance with the Italian discharge limit for surface water (D.lgs. 152/2006), indicating that this nutrient had been used by the plants. Phosphorus displayed excellent performances for the whole experimental period, for each phase; no depended on the input load, on the substrate for plants growth or on the specific vegetal essence.

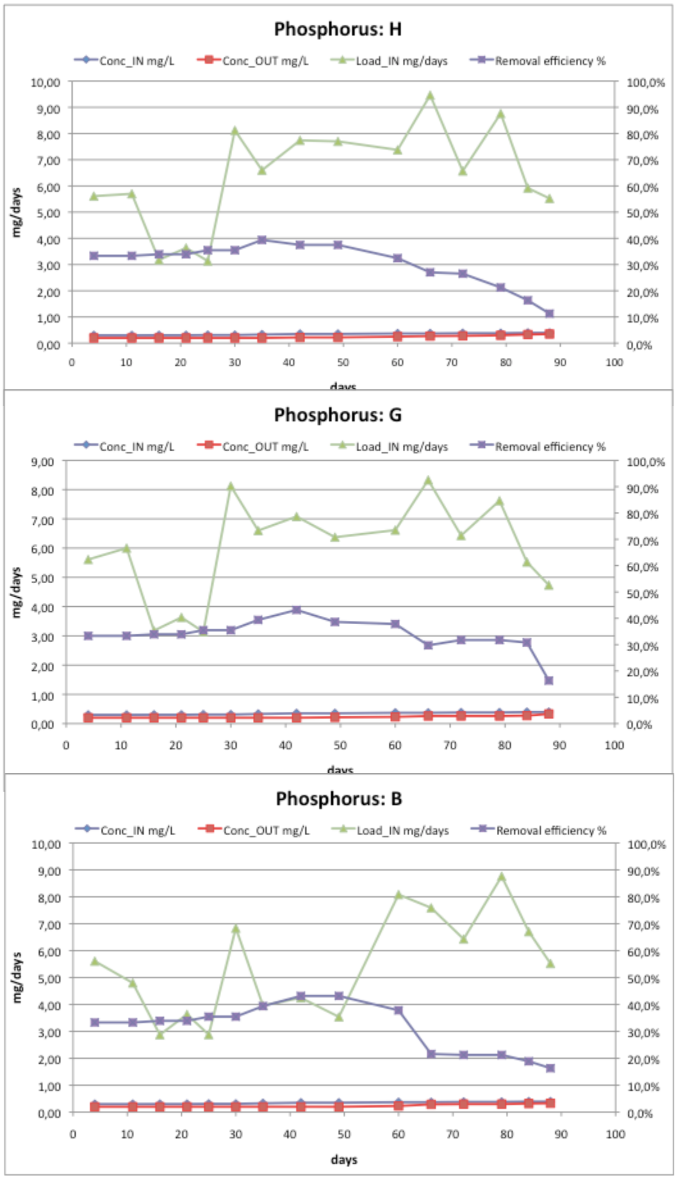


Figure 4.5. Phase 1: P input and output concentration (mgP/L), P input load (mgP/days) and P removal (%) removal efficiency during the experimental period. *(H: Helihantus annus; G: Glycine max; B: Brassica napus.)*

4.1.3.4.Another's chemical constituents

Table 4.4 reports the average input and output concentrations of other major wastewater constituents. TS and VS removal denoted some inefficiency, probably caused by the drag of solid material during drainage operations. Alkaline earth metals displayed approximately the same input and output values, meaning that the phytotreatment process did not affect them. Chlorides concentration in the influent water was occasionally higher than in the effluent water. The same can be noticed for copper, manganese, lead and zinc. Therefore, further investigations are required in order to understand chlorides and heavy metals distribution within the phytotreatment system.

PARAMETERS	WW	IN		OUT	
		CONCENTRATIONS (mg/l)			
		MIN-MAX	*MEAN VALUES*	*MIN-MAX*	*MEAN VALUES*
Cl-	*H*	43.7 – 119.5	75.70	49.6 – 243.9	113.1
	G			49.6 – 154.2	94.4
	B			46.1 – 168.4	84.9
SO42-	*H*	42.2 – 84.9	60.22	70.4 – 108.9	90.3
	G			63.2 – 145.9	90.0
	B			41.0 – 141.4	91.3
TS	*H*	448 - 1709	981	514 - 1702	975
	G			516 - 1790	1051
	B			407 - 1342	791
VS	*H*	281 - 984	594	257 - 1417	556
	G			345 - 1448	679
	B			292 - 655	402
		CONCENTRATIONS (µg/l)			
Cu	*H*	24 - 65	62	45 - 92	68.5
	G			10	10
	B			10 - 14	12
Fe	*H*	17 - 61	50	102 - 266	184
	G			61 - 282	260
	B			215 - 480	348

Table 4.4. Mean input _output concentrations - phase1.

4.1.4. Mass balance estimation

The mass balance of nitrogen and phosphorus was analysed in the distribution among the main system components: outlet waters, substrates and plants (energy crops). A higher content of Nitrogen and Phosphorus in plants subjected to phytoremediation is observed; and this finding confirmed that the capacity of the pyhtoremediation was not compromised, even contributing a higher rate of removal of pollutants. Both balances prove a large reduction of the N and P water content through the phytotreatment process. The system with agricultural soil displayed higher removal rates than the one with sand substrate. This confirms the complexity of the phytoremediation process, which consists in a combination of different phenomena, rather than in the isolated action of the plant (Jones et al., 2005).

To obtain the estimation of the general mass balance, we calculated with the equation for a chemical, written as follows:

$$M_{plant} = M_{IN} - M_{OUT} - M_{soil}$$

Where:

M_{plant} = mass of the chemical in the plant, (g/pot)
M_{IN} = mass of the chemical in the inflow, (g/pot)
M_{OUT}= mass of the chemical in the outflow, (g/pot)
M_{soil}= mass of the chemical in the soil, (g/pot)

These components of the chemical budget were estimated for each species according to the following expressions:

$$M_{plant} =(root\_mass*conc\_ root + aerial\_mass*conc\_aerial)*N°pots$$

Where:

root_mass= mass of the chemical in all the roots of the considered species, (g)
conc_ root = concentration of the chemical in the roots of the considered species, (g)
aerial_mass = mass of the chemical in all the aerial part of the considered species, (g)
conc_aerial = concentration of the chemical in the aerial part of the considered species, (g)
N°pots = number of pots occupied by the considered species

$$M_{IN}=V_{IN}*conc\_in* N°pots$$

Where:

V_{IN}= inflow volume fed water to the essences from time to time, (l)
Conc_in= concentration of the chemical in the inflow volume, (mg/l)

$$M_{OUT}=V_{OUT}*conc\_out*N°pots$$

Where:

V_{OUT}= outflow water volume from the essences pots, (l)
Conc_out= concentration of the chemical in the outflow volume, (mg/l)

$$M_{soil}=M\_tot*(conc\_soil\_f - conc\_soil\_i)*N°pots$$

Where:

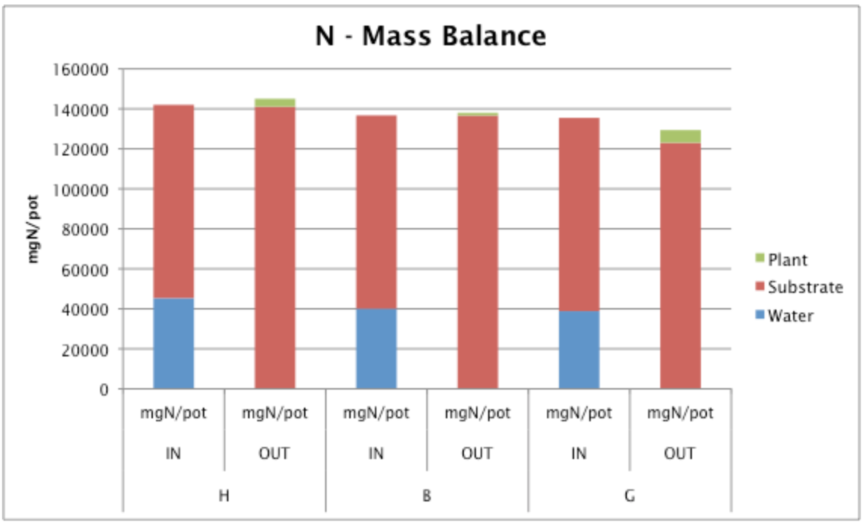
M_tot= total mass of the soil in the pots of the considered species, (kg)
Conc_soil_i= concentration of the chemical in the soil present at the beginning of the experimentation in the pots of the considered species, (mg/kg)
Conc_soil_f= concentration of the chemical in the soil present at the end of the experimentation in the pots of the considered species, (mg/kg).

4.1.4.1.Nitrogen mass balance

Nitrogen mass balance was obtained considering the total nitrogen input as the sum of all TKN and nitrate loads providing through feeding throughout the entire trial; by calculating the total output n mass as the sum of the residual nitrogen mass present in the wastewater subjected to remediation over the entire trial, with the N mass accumulated in the plants, in addition the n accumulated in the substrate. This final value has already been reported net of the amount of N present at the start of the phase (Table 4.5). Nitrogen is subsequently uptaken by plants, and a little part released in the drainage water. Plant uptake plays an important role in the enhancement of N removal (see figure 4.6), especially in treatment wetlands containing fast-growing plants, as the removal of N is facilitated by microbial activity. Nitrogen values in the substrate represented a substantial percentage of the total nitrogen, indicating how ammonia and nitric nitrogen input was transformed and used in the development of biomass (M.C. Lavagnolo et al, 2011).

	H		**B**		**G**	
	IN	*OUT*	*IN*	*OUT*	*IN*	*OUT*
Water	45383	205	40103	277	38854	228
Substrate	96626	140871	96626	136398	96626	122825
Plant		3967		1359		6340
Total	142009	145043	136729	138.033	135479	129393

H: Helihantus annus; G: Glycine max; B: Brassica napus.
Table 4.5. Nitrogen mass balance - phase 1. (Values expressed in mgN/tank).



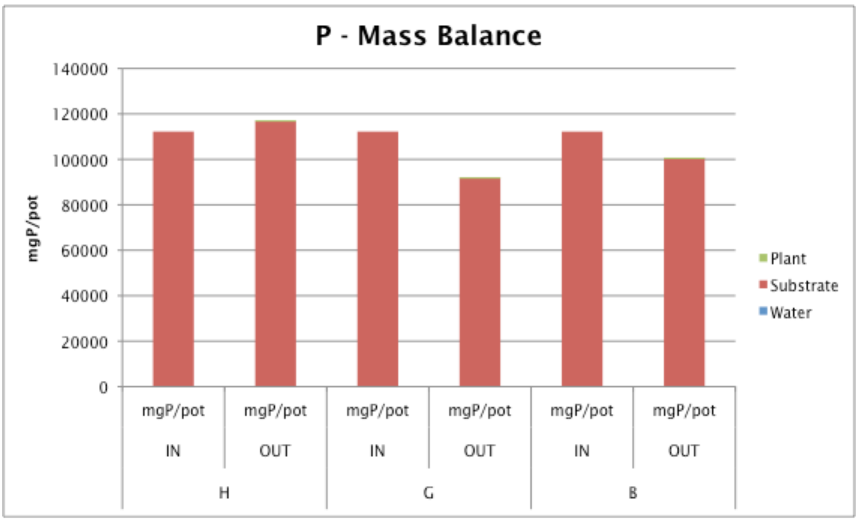
(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.6. Nitrogen mass balance phase 1.

4.1.4.2.Phosphorus mass balance

Phosphorus mass balance is far less complicated than nitrogen mass balance (see figure 4.7), incoming P simply partitions between the plant tissues and the soil matrix, with a very low release in the drainage water. The results are reported in table 4.6.

	H		G		B	
	IN	*OUT*	*IN*	*OUT*	*IN*	*OUT*
Water	95	17	89	8	81	18
Substrate	112138	116801	112138	92011	112138	100392
Plant		365.7		64.8		383.7
Total	112233	117184	112227	92083	112220	100793

(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Table 4.6. Phosphorus mass balance phase 1. (Values expressed in mgP/tank).



(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.7. Phosphorus mass balance phase 1.

4.2. Phase 2

4.2.1. Vegetation growth

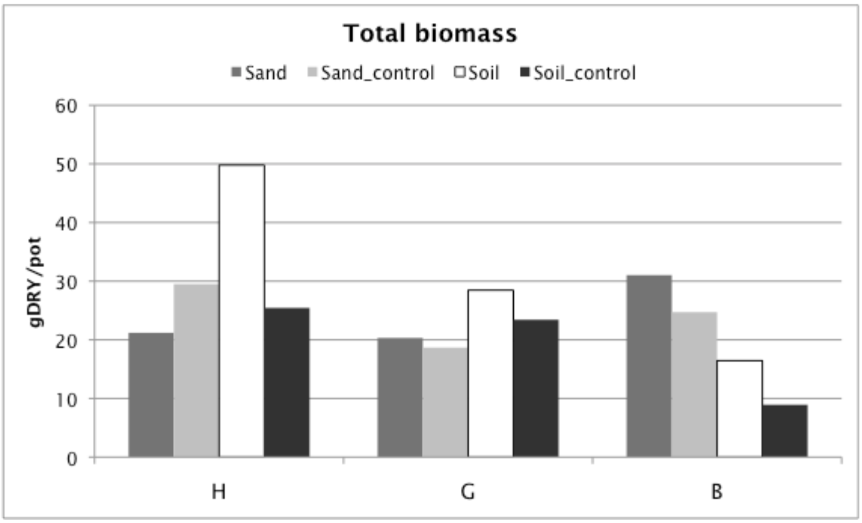
Once completed and life cycle of plants, these were extracted then be measured and weighed - in wet and dry- differentiating the aerial part of the underground (roots, figure 4.7) and also separating the seeds, Even if occasional signs of stress were detected during the irrigation period, namely leaves desiccation, foliage spotting and abnormal production of flowers and pods, leachate fed crops developed larger biomasses than correspondent controls (see Figure 4.8a). *Helianthus annus* and *Glycine max* performed better when associated to the soil substrate, while *Brassica napus* was favored by the sandy substrate. Peak inhibition emerged for *Helianthus annus* growing on sand. In this case, leachate fertilization effect was probably overcome by the inability of the substrate to buffer pollutants action. Root mass and root length were subsequently evaluated (see Table 4.7). As showed in Figure 4.8b and 4.8c, leachate sometimes promoted, other times inhibited the growth of the roots. Thus, feed water composition was not crucial for the development of plants radical system. Instead, the pot filling substrate played a major role. Thanks to its high porosity, sandy soil favored the expansion of *Glycine max* and *Brassica napus* radical system, while *Helianthus annus* preferred the soil substrate. *Glycine max* displayed the longest roots, as confirmed by Figure 4.8(c).

Figure 4.8. Root species extracted - phase 2. Examples: H, G and B.

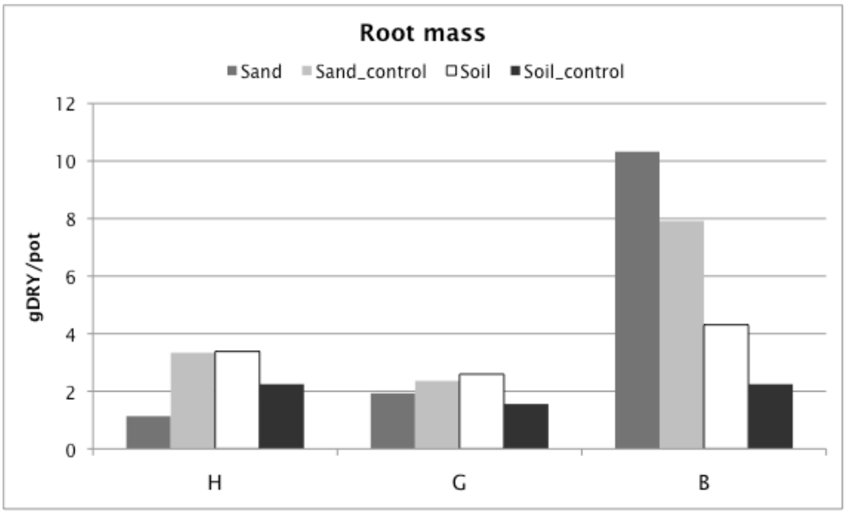
Crops		Total Mass		Root Mass		Main root Lenght	
		g_{DRY}/pot	*%*	*g_{DRY}/pot*	*%*	*cm*	*%*
H_sand	Leachate	21.2	72%	1.14	34%	40.0	94%
	Control	29.5	-	3.3	-	42.5	-
H_soil	Leachate	49.8	196%	3.4	150%	46.0	95%
	Control	25.4	-	2.2	-	48.5	-
G_sand	Leachate	20.3	109%	1.9	82%	66.0	102%
	Control	18.7	-	2.4	-	64.5	-
G_soil	Leachate	28.5	121%	2.6	166%	65.0	124%
	Control	23.4	-	1.6	-	52.5	-
B_sand	Leachate	31.0	126%	10.3	130%	51.0	91%
	Control	24.7	-	7.9	-	56.0	-
B_soil	Leachate	16.4	184%	4.3	192%	47.0	188%
	Control	8.3	-	2.2	-	25.0	-

Table 4.7. Growth vegetation - phase 2

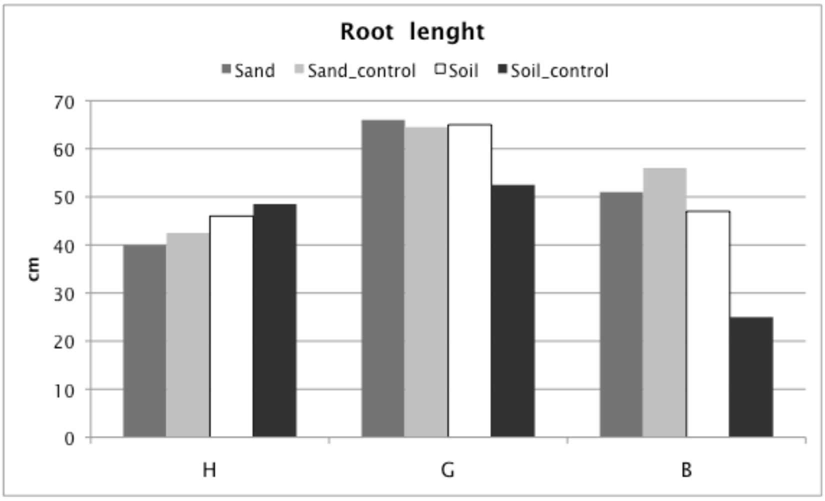
On the contrary, *Brassica napus* exhibited the largest root biomass (Figure 4.9b), which allowed it to reach very high pollutants removal efficiency. The evaluation of root development gives an idea of the possibility of exploring the ground and the potential phytoremediation capacity from an environmental point of view; this evaluation is enabled comparing the weight and length of the roots for each species with those of respective controls.



(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.9(a). Total biomass - phase 2.



(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.9(b). Root biomass - phase 2.



(H: Helihantus annus; G: Glycine max; B: Brassica napus.)
Figure 4.9(c). Root lenght - phase 2.

4.2.2. Quality of oils seed cultivated

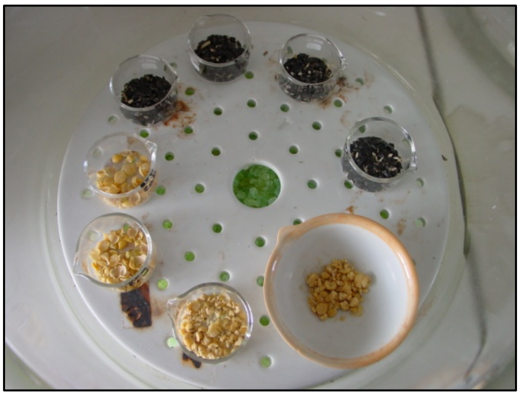


Figure 4.10. Seeds crops - phase 2

As a result of the seeds cultured have only two of the three species: *helianthus annus* and *glycine max*. To consider any feedstock (in this case seeds crops cultivation, figure 4.10) as a biodiesel source, the oil percentage content and the oil yield are important parameters. In this line, we make the analysis correspondent at the two seeds crops, taking 100 g for 1 g of the sample; like showed in table 4.8, we can verify the content of oils from the seed cultivated in the phase 1, according to the literature review and the limits of international standards, is located to the extent permitted, that is suitable for the production of biodiesel (table 1.3, A.E. Atabani et al 2012)

Oil seed and FAME (fatty acid methyl esters) content of the cultivated crops

Seed cultivation	Content of Oil *(%)*	FAME *(%)*
H_sand	43.03	41.14
H_soil	45.13	43.14
G_sand	17.03	16.28
G_soil	16.26	15.54

(H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate; G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).

Table 4.8. Quality of seed - phase 2.

Even to controlled and verified the fatty acids compositions, we obtained this values of the principal component according to the profile given in literature, for the two species obtained (see table 4.9) The results confirmed that our values are in the permits limits (table 1.5, chapter 1;A.Karmakar et al, 2010).

Fatty acid compositions (wt.%) of seed oils		*H_sand*	*H_soil*	*G_sand*	*G_soil*
Lauric	C12:0	0.009	0.007	0.011	0.010
Myristic	C14:0	0.063	0.065	0.043	0.040
Palmitic	C16:0	1.820	1.873	2.486	2.413
Palmitoleic	C16:1	0.070	0.078	0.037	0.035
Stearic	C18:0	0.775	0.675	0.464	0.462
Oleic	C18:1	36.285	37.900	2.912	2.903
Linoleic	C18:2	1.588	2.018	8.604	7.761
Linolenic	C18:3	0.110	0.084	1.592	1.778

Arachidic	C20:0	0.084	0.090	0.042	0.046
Gadoleic	C20:1	0.092	0.105	0.018	0.018
Behenic	C22:0	0.000	0.003	0.006	0.007
Erucic	C22:1	0.002	0.000	0.000	0.000
Lignoceric	C24:0	0.219	0.244	0.064	0.068
Nervotic	C24:1	0.019	0.003	0.001	0.001

(*H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate; G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).*

Table 4.9. Fatty acid composition - phase 2.

4.2.3. Removal efficiencies

Nitrogen, Phosphorus and COD were fundamental in furthering the understanding of the remediation effects of the three crops. Each graph features the different periods in order to improve the understanding of plant responses in terms of performance to variations remediation of contaminants introduced load. To better interpret the results and make a comparison with other findings present in literature, the values are expressed in international units.

The removal of nitrogen and organics, from wastewater, in such engineered ecosystem is very important, due to the following reasons:

- Uncontrolled discharge of nitrogen into natural water channels fosters eutrophication of lakes and rivers.
- Untreated organic materials often deplete dissolved oxygen (DO) concentration in open water channels, leading to the death of aquatic organisms (T.Saeed, G, Sun, 2012).

The performance disparity of phytoremediation, in terms of nitrogen and organics removal could be attributed to the following reasons:

- Excessive presence of organics compounds in wastewater inhibits nitrification, as faster heterotrophic organic degradation depletes DO availability; and
- Lack of biodegradable organics often hinders classic denitrification metabolism (due to dependency on organic carbon) in wetland system.

4.2.3.1.COD removal

COD outlet concentration kept below the Italian discharge limit of 120 mg/L (D.lgs. 152/2006) until the ninth experimental week. Subsequently, it increased up to 300 mg/L. This result suggests that a COD input of 3400 mgO2/m2·day (900 mgO2/pot·week, pot area = 0,038 m2) could be a valuable design parameter for larger scale treatment applications. Soil substrate filled pots (Figure 4.11) generally displayed higher removal efficiencies than sand filled pots.

Among the three experimental essences, rapeseed featured the best COD removal performances (Figure 4.11b and 4.11c). This contrasts the results obtained by Lavagnolo (Lavagnolo et al., 2011) in a pot trial assessing the ability of *Helianthus annus*, *Glycine max* and *Brassica napus*, to treat a mixture of yellow and grey waters. Under similar COD load conditions, *Glycine max* emerged as the most efficient COD removing essence, while *Brassica napus* performance was somehow inhibited by feed water composition.

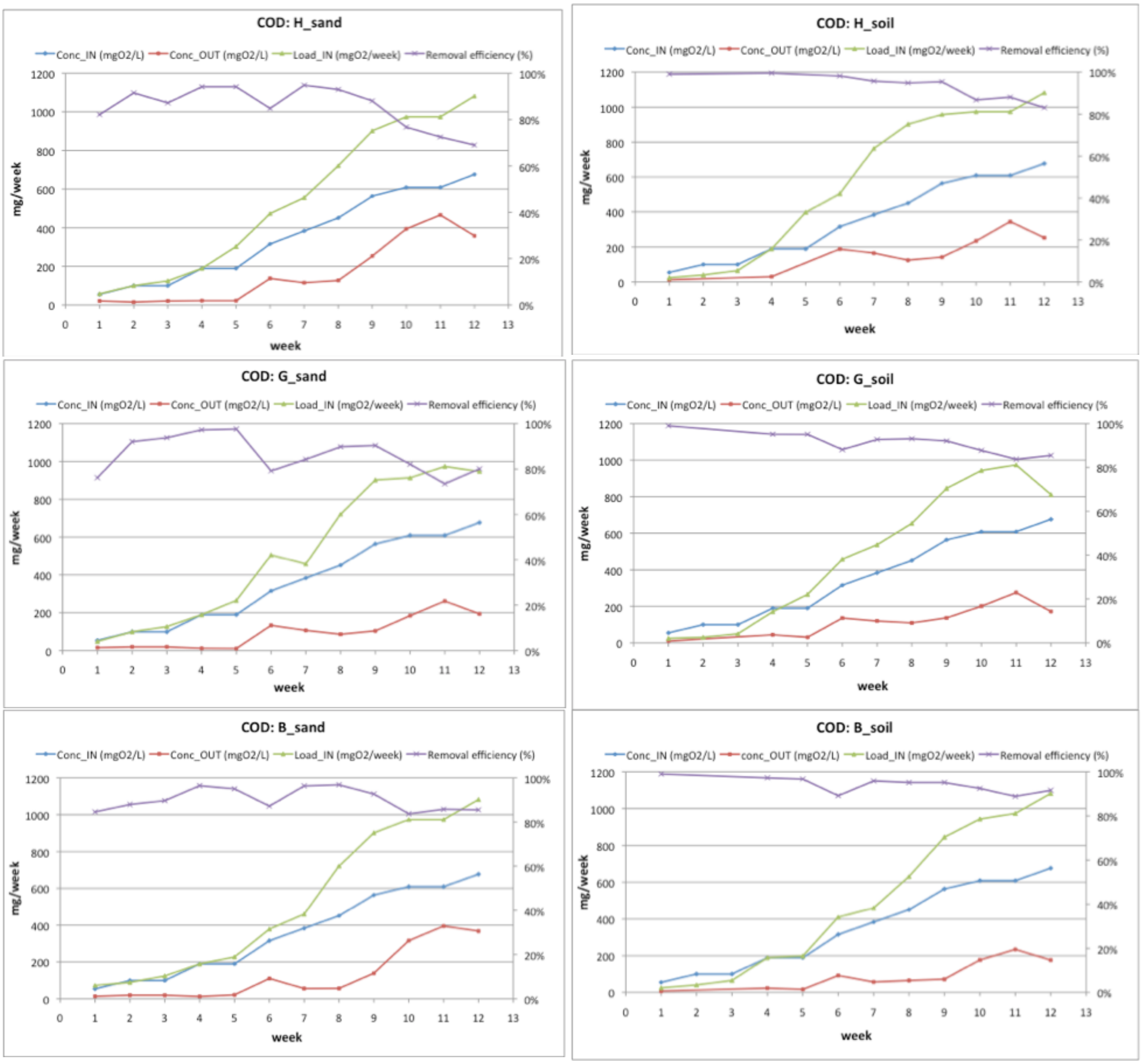


Figure 4.11. Phase 2: COD input and output concentration (mgO_2/L), COD input load (mgO_2/week) and COD η(%) removal efficiency during the experimental period. (*H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate;G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).*

4.2.3.2.Nitrogen removal

At the start of the trial, nitrogen input was set at 15 mgN/pot·week (55 mgN/m2·day). After three months of operations it achieved 600 mgN/pot·week (2200 mgN/m2·day), as illustrated in Figure 4.12. Nitrogen removal efficiency kept above 70% until the eighth experimental week. Afterwards, it dropped to very low percentage values. The drop was less marked in clayey soil filled pots with respect to sand filled pots. Rapeseed displayed once more higher removal efficiencies in comparison to the other vegetal essences. Further, the drop in nitrogen removal temporarily was equal to the drop in COD removal. Considering leachate characteristics listed in Table 4.9, this trial suggests that excellent performances could be achieved with a 20% v/v leachate concentration in the feed.

Nitrogen outlet concentration trend was aligned to nitrogen removal efficiency trend. Actually, outlet values below 100 mgN/L were maintained until the eight experimental week, when they rose up to 400 mgN/L. In a similar experience, Cheng and Chu (Cheng and Chu, 2011) demonstrated that nitrogen concentration in the effluent water stayed below 90 mgN/L with an input of 2800 mgN/m2·day. Such a different performance could be ascribed to a number of factors, namely the choice of the vegetal essences, the depth of the soil column or the schedule of nitrogen dosage through the experimental period.

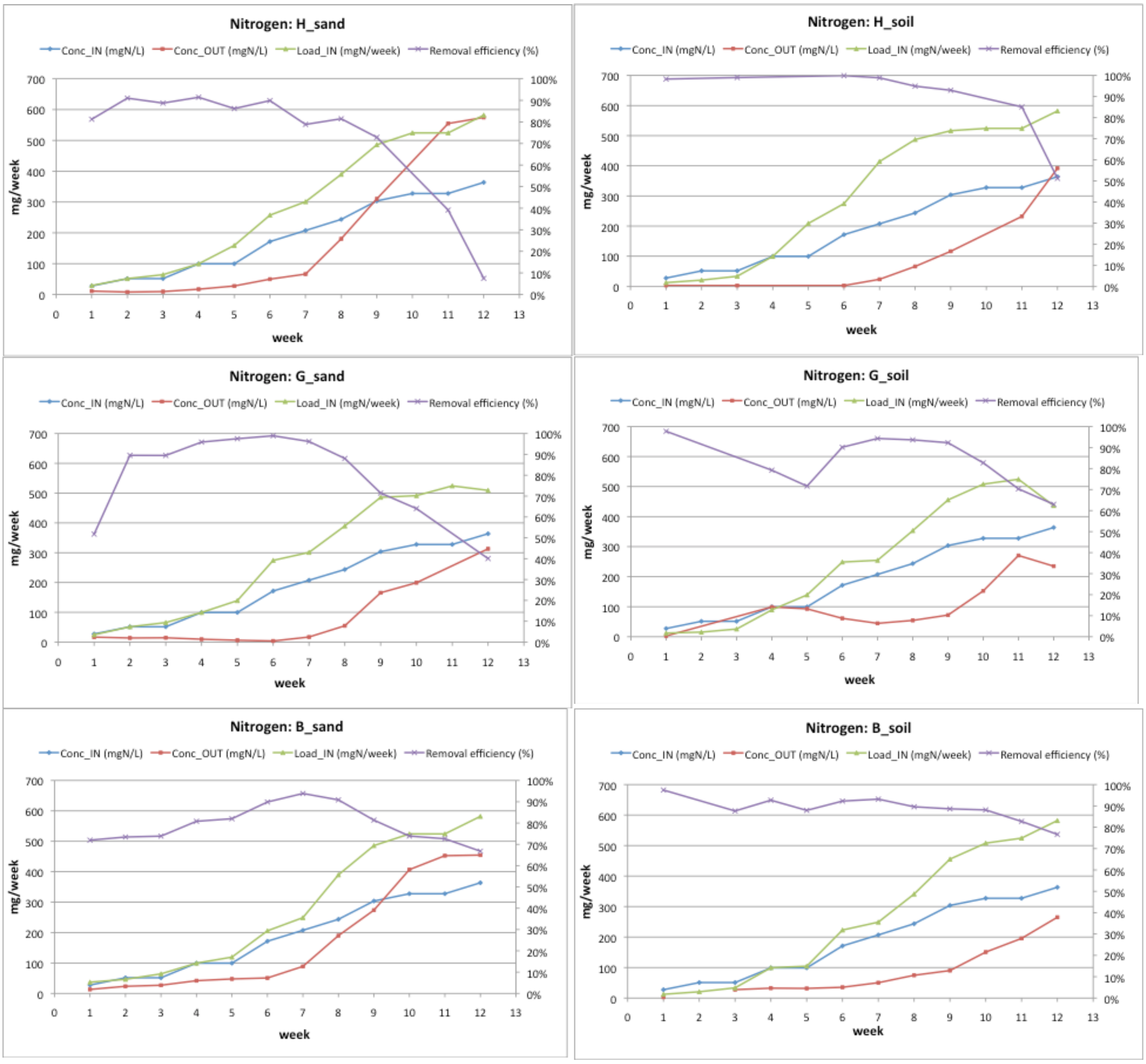


Figure 4.12. Phase 2: N input and output concentration (mgN/L), N input load (mgN/pot*week) and N removal (%) removal efficiency during the experimental period. *(H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate;G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).*

Nitrification rates:

Analyzing the composition of nitrogen input vs. output, it emerges that nitrogen enters the system in the form of ammonium (see leachate composition at table 3.7) but it gets out in the form of nitrate. This result ensures that enough oxygen is present in the system either to chemically or biologically oxidize occurring nitrogen, meanwhile transforming it in a more bioavailable form (Cheng and Chu, 2011; Tyrrel et al., 2001).

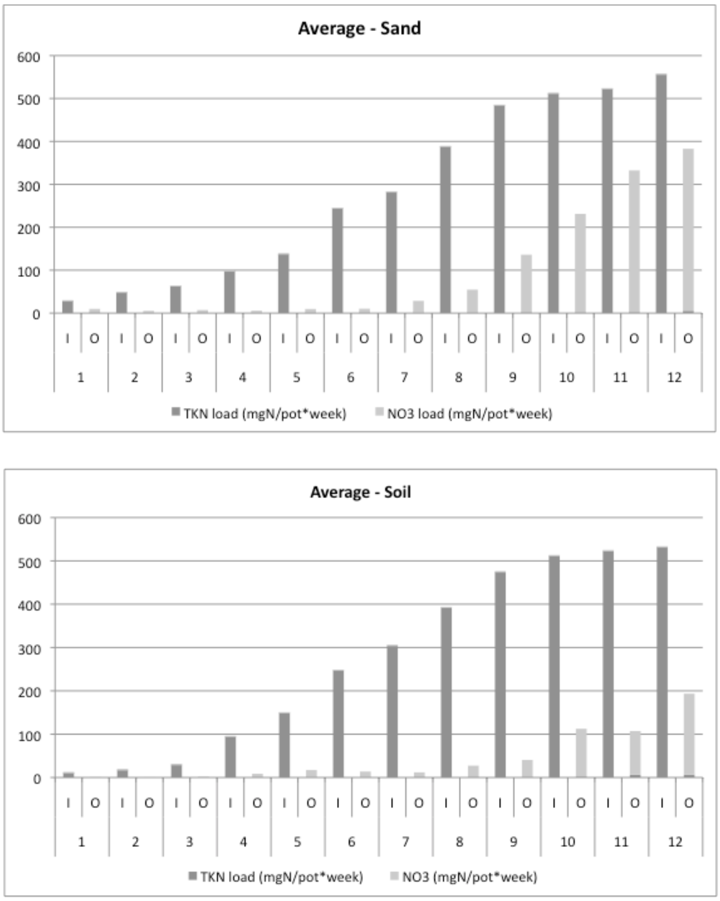


Figure 4.13. Comparison of input (I) and output (O) nitrogen load composition through the whole experimental phase 2. Values are obtained as an average among the essences growing on sand and the essences growing on clayey soil.

4.2.3.3.Phosphorus removal

Phosphorus removal displayed excellent performances for the whole experimental period (see Figure 4.14). No dependence on the input load, the substrate of growth or the specific vegetal essence was noticed. P outlet concentration kept always below the value of 1 mg/L, in compliance with the Italian discharge limit for surface water (D.lgs. 152/2006).

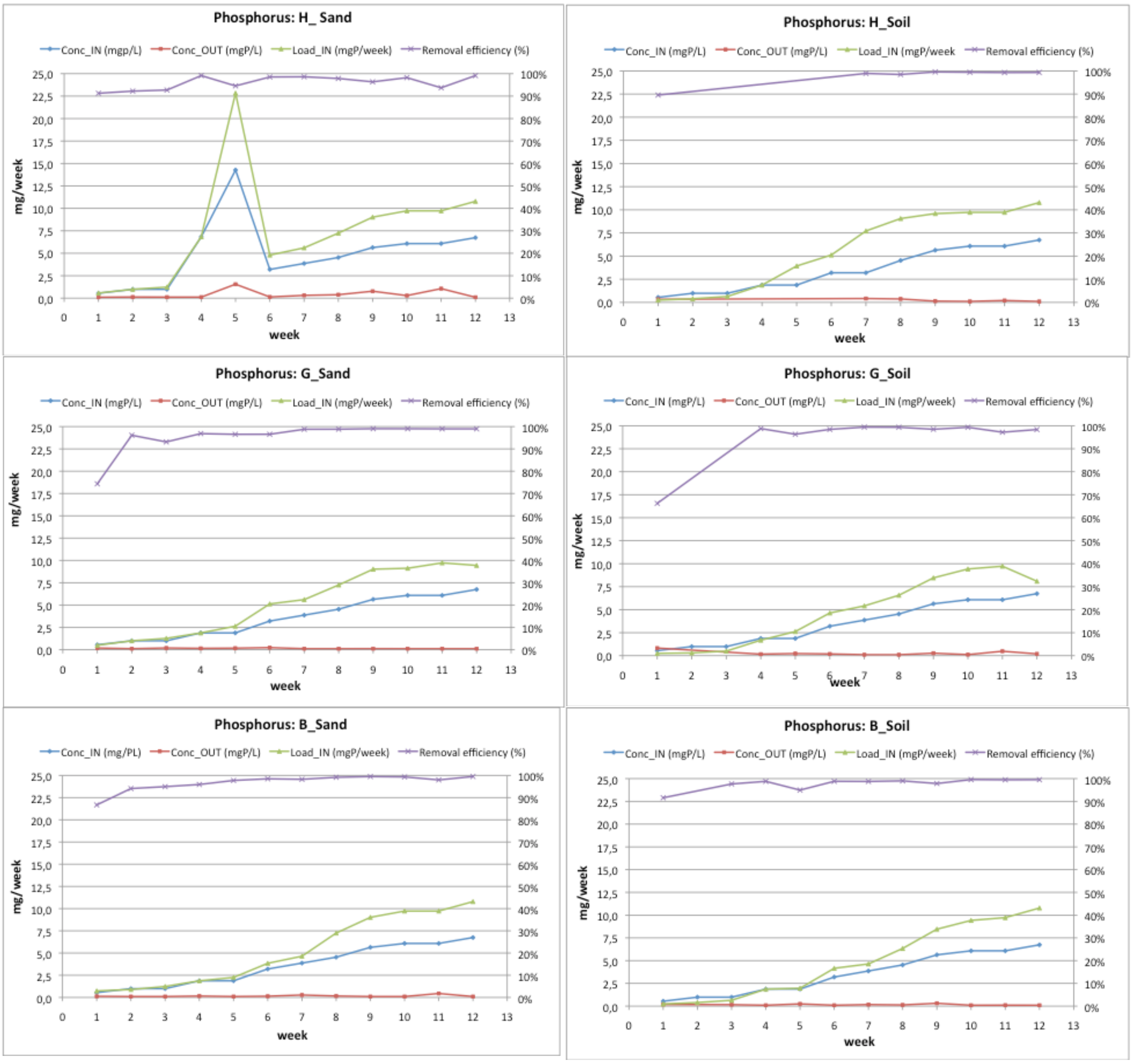


Figure 4.14. Phase 2: P input and output concentration (mgP/L), P input load (mgP/pot*week) and P removal (%) removal efficiency during the experimental period. (*H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate;G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).*

4.2.3.4.Another's chemical constituents

Table 4.10, reports the average input and output concentrations of other major leachate constituents. TS and VS removal denoted some inefficiency, probably caused by the drag of solid material during drainage operations. Alkaline earth metals displayed approximately the same input and output values, meaning that the phytotreatment process did not affect them. Chlorides concentration in the influent water was occasionally higher than in the effluent water. The same can be noticed for copper, manganese, lead and zinc. Therefore, further investigations are required in order to understand chlorides and heavy metals distribution within the phytotreatment system.

Parameters	IN	OUT					
		H_sand	G_sand	B_sand	H_soil	G_soil	B_soil
				mg/L			
TS	2278	2382	1778	2021	2993	2572	1920
VS	905	924	693	859	1117	1034	805
Cl^-	239	384	649	280	447	342	268
Ca	245	227	219	190	279	269	285
K	13.6	14.2	21.4	10.8	16.0	9.41	9.41
Mg	70.0	77.1	57.8	66.6	77.3	67.2	74.2
Na	271	308	197	277	365	244	236
				µg/L			
Cd	1.63	1.03	1.07	1.82	1.46	1.29	1.24
Cr	70.4	20.2	12.1	12.3	17.9	12.2	11.8
Cu	8.75	76.8	60.2	55.7	60.8	51.6	76.5
Fe	1093	837	925	405	178	1897	2251
Mn	27.9	38.1	49.5	23.1	32.0	68.2	73.5
Ni	23.5	42.6	30.8	39.7	46.0	28.9	30.8
Pb	4.90	22.5	16.7	19.3	29.9	30.0	13.3
Zn	27.9	59.2	47.4	33.0	40.8	36.9	57.6

Table 4.10. Mean input – output concentrations Phase 2.

4.2.4. Mass balance estimation

The mass balance of nitrogen and phosphorus was analysed in the distribution among the main system components: outlet waters, substrates and plants (energy crops). Nutrients mass balance was performed on leachate irrigated pots. The balance analyses nitrogen and phosphorus distribution among the main system components: water, substrate (either sand or clay) and plant (sunflower, soybean and rapeseed). Both balances prove a large reduction of the N and P water content through the phytotreatment process. The system with agricultural soil displayed higher removal rates than the one with sand substrate. This confirms the complexity of the phytoremediation process, which consists in a combination of different phenomena, rather than in the isolated action of the plant (Jones et al., 2005).

To obtain the estimation of the general mass balance, we calculated with the equation for a chemical, written as follows:

$$M_{plant} = M_{IN} - M_{OUT} - M_{soil}$$

Where:

M_{plant} = mass of the chemical in the plant, (g/pot)

M_{IN} = mass of the chemical in the inflow, (g/pot)

M_{OUT}= mass of the chemical in the outflow, (g/pot)

M_{soil}= mass of the chemical in the soil, (g/pot)

These components of the chemical budget were estimated for each species according to the following expressions:

$$M_{plant} =(root\_mass*conc\_ root + aerial\_mass*conc\_aerial)*N°pots$$

Where:

root_mass= mass of the chemical in all the roots of the considered species, (g)

conc_ root = concentration of the chemical in the roots of the considered species, (g)

aerial_mass = mass of the chemical in all the aerial part of the considered species, (g)

conc_aerial = concentration of the chemical in the aerial part of the considered species, (g)

N°pots = number of pots occupied by the considered species

$$M_{IN}=V_{IN}*conc\_in* N°pots$$

Where:

V_{IN}= inflow volume fed water to the essences from time to time, (l)

Conc_in= concentration of the chemical in the inflow volume, (mg/l)

$$M_{OUT}=V_{OUT}*conc\_out*N°pots$$

Where:

V_{OUT}= outflow water volume from the essences pots, (l)

Conc_out= concentration of the chemical in the outflow volume, (mg/l)

$$M_{soil}=M\_tot*(conc\_soil\_f - conc\_soil\_i)*N°pots$$

Where:

M_tot= total mass of the soil in the pots of the considered species, (kg)

Conc_soil_i= concentration of the chemical in the soil present at the beginning of the experimentation in the pots of the considered species, (mg/kg)

Conc_soil_f= concentration of the chemical in the soil present at the end of the experimentation in the pots of the considered species, (mg/kg)

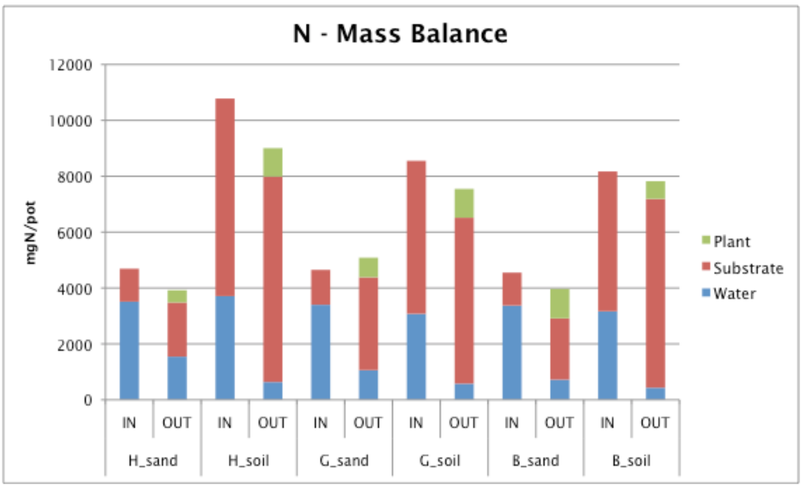
Both the balances prove a large reduction of the NP water content through the phytotreatment process. Higher removal rates were displayed by the plants growing on soil substrate. These observations support the results obtained in the previous paragraph, and confirm the effectiveness of leachate treatment via oily crops irrigation. The NP mass balance ensures a substantial accumulation of nutrients in the vegetal tissues of plants fed with landfill leachate. Indeed, Figure 4.13 shows a very high nitrogen uptake in comparison to a limited amount of accumulated phosphorus (Figure 4.14). These data may suggest an unbalanced nutrient availability for the leachate-irrigated crops, which is a common problem in many leachate phytotreatment applications (Vymazal, 2009).

4.2.4.1.Nitrogen mass balance estimation

Nitrogen mass balance highlights that nitrogen enters the system in the form of ammonium. A portion of it is adsorbed onto the soil matrix as organic nitrogen (see Table 3.3), while another portion is oxidized to nitrate. Nitrogen is subsequently uptaken by plants, or released in the drainage water. Nitrogen input exceeds 8% on average the correspondent output in all the trials. The results are expressed in table 4.11.

	H_sand		**H_soil**		**G_sand**		**G_soil**		**B_sand**		**B_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
Substrate	1180	1938	7070	7354	1249	3324	5476	5937	1180	2195	5005	6757
Plant		440		1017		702		1032		1053		633
Total	4692	3922	10781	9006	4650	5086	8552	7547	4552	3968	8171	7822

(H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate; G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).
Table 4.11. Nitrogen mass balance - phase 2. (Values expressed in mgN/pot).



(H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate;
G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).

Figure 4.15. Nitrogen mass balance - phase 2.

4.2.4.2.Phosphorus mass balance estimation

Phosphorus mass balance is far less complicated than nitrogen mass balance. Indeed, Figure 4.16 shows that incoming P simply partitions between the plant tissues and the soil matrix, with a very low release in the drainage water. Moreover, P conservative attitude is confirmed by the limited gap between the input and output term of the phosphorus balance (± 2% on average) values showed in table 4.12.

	H_sand		**H_soil**		**G_sand**		**G_soil**		**B_sand**		**B_soil**	
	IN	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*OUT*
Water	95.6	3.10	70.7	0.51	68.7	1.16	59.5	0.98	68.1	0.97	60.5	0.66
Substrate	1686	1776	4488	4602	1786	1857	3932	4001	1686	1664	3626	3768
Plant		45.3		77.4		43.0		58.5		39.0		20.4
Total	1782	1824	4559	4679	1854	1901	3991	4061	1754	1704	3687	3789

Table 4.12. Phosphorus mass balance phase 2. (Values expressed in mgP/pot). (*H_sand= Helianthus annus in sand substrate; H_soil= Helianthus annus in soil substrate;G_sand= Glycine max in sand substrate;G_soil= Glycine max in soil substrate).*

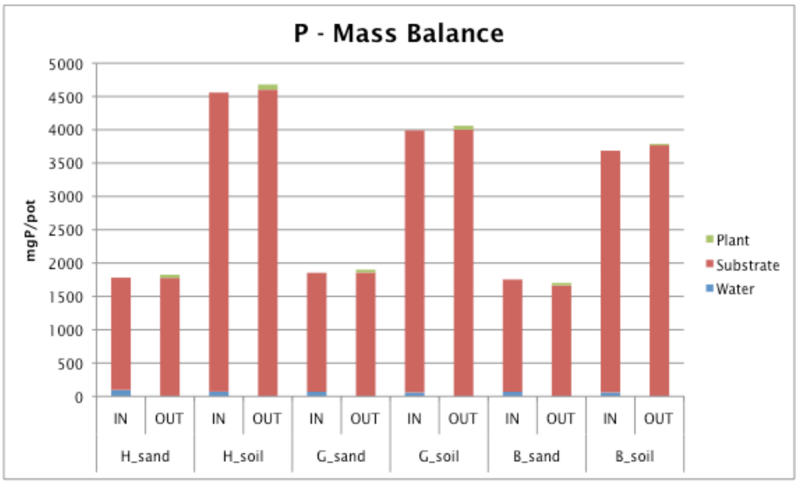


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Figure 4.16. Phosphorus mass balance phase 2.

4.3. Phase 3

4.3.1. Vegetation growth

Once completed and life cycle of plants, these were extracted then be measured and weighed (figure 4.17)- in wet and dry- differentiating the aerial part of the underground (roots) and also separating the seeds, Even if occasional signs of stress were detected during the irrigation period, namely leaves desiccation, foliage spotting and abnormal production of flowers and pods, leachate fed crops developed larger biomasses than correspondent controls (see Figure 4.18a), in both of the species, most evidenced for the *Helianthus annus*.

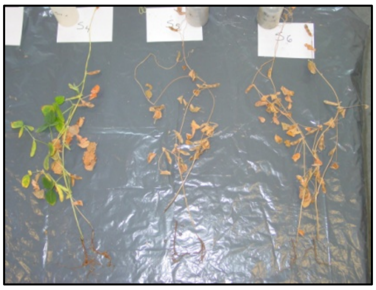
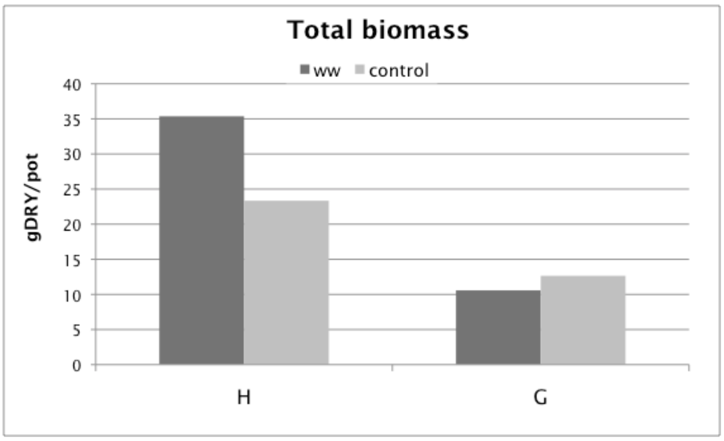


Figure 4.17. Species extracted - phase 3: H and G.

Root mass and root length were subsequently evaluated (see figures 4.18b and 4.18c) in this experimental phase, leachate promoted the growth of the roots in both species crops. The summarized values are reported in table 4.13.

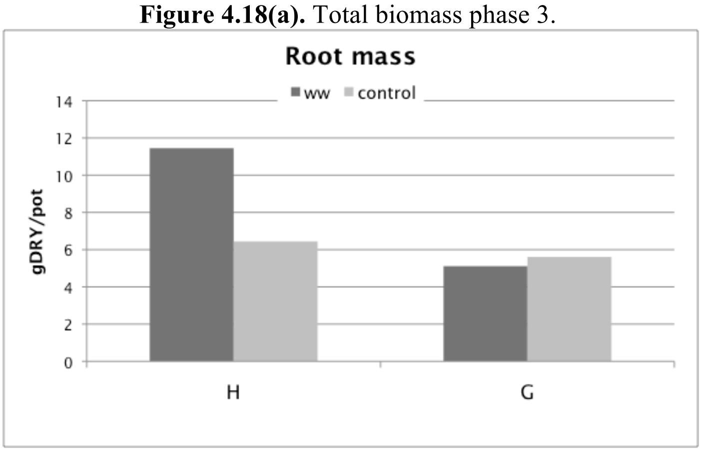
CROPS	MASS				LENGHT	
	Total		***Root***		***Main root***	
	g_{DRY}/pot	%	g_{DRY}/pot	%	cm	%
H	35.4	152%	11.45	178%	21.7	114%
H_control	23.3	-	6.44	-	19.0	-
G	10.6	84%	5.11	91%	17.8	92%
G_control	12.6	-	5.61	-	19.3	-

Table 4.13. Growth vegetation phase 3

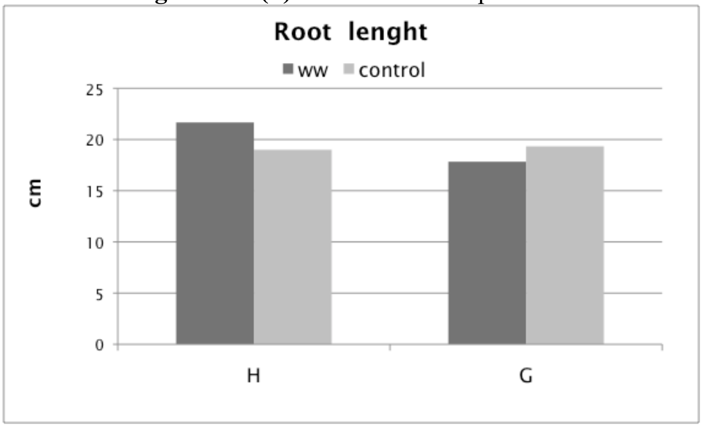


(H: Helihantus annus; G: Glycine max.)

Figure 4.18(a). Total biomass phase 3.



(H: Helihantus annus; G: Glycine max.)
Figure 4.18(b). Total root mass phase 3.



(H: Helihantus annus; G: Glycine max.)
Figure 4.18(c). Total root length phase 3.

4.3.2. Quality of oils seed cultivated

As a result of the seeds cultured have only two of the three species: *helianthus annus* and *glycine max*. To consider any feedstock (in this case seeds crops cultivation) as a biodiesel source, the oil percentage content and the oil yield are important parameters. In this line, we make the analysis correspondent at the two crops, taking 100 g for 1 g of the sample; like showed in table 4.14, we can verify the content of oils from the seed cultivated in the phase 1, according to the literature review and the limits of international standards, is located to the extent permitted, that is suitable for the production of biodiesel (table 2.7., A.E. Atabani et al 2012)

Oil seed and FAME (fatty acid methyl esters) content of the cultivated crops

Seed cultivated	**Content of Oil** *(%)*	**FAME** *(%)*
H	40.02	38.26
G	22.1	21.13

(*H: Helianthus annus ;G: Glycine max*).
Table 4.14. Quality of seed in phase 3.

Even to controlled and verified the fatty acids compositions, we obtained this values of the principal component according to the profile given in literature, for the two species obtained (see table 4.15). The results confirmed that our values are in the permits limits (A.Karmakar et al, 2010)

Fatty acid compositions (wt.%) of seed oils		**H**	**G**
Lauric	C12:0	0.007	0.004
Myristic	C14:0	0.076	0.060
Palmitic	C16:0	2.231	3.425
Palmitoleic	C16:1	0.102	0.042
Stearic	C18:0	0.921	0.770
Oleic	C18:1	33.152	5.069
Linoleic	C18:2	1.407	10.234
Linolenic	C18:3	0.058	1.247
Arachidic	C20:0	0.100	0.076

Gadoleic	C20:1	0.080	0.039
Behenic	C22:0	0.000	0.004
Erucic	C22:1	0.002	0.000
Lignoceric	C24:0	0.121	0.156
Nervotic	C24:1	0.002	0.002

(*H: Helianthus annus ;G: Glycine max).*

Table 4.15. Fatty acid compositions in phase 3.

4.3.3. Removal efficiencies

Nitrogen, Phosphorus and COD were fundamental in furthering the understanding of the remediation effects of the three crops. Each graph features the different periods in order to improve the understanding of plant responses in terms of performance to variations remediation of contaminants introduced load. To better interpret the results and make a comparison with other findings present in literature, the values are expressed in international units.

The removal of nitrogen and organics, from wastewater, in such engineered ecosystem is very important, due to the following reasons:

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The performance disparity of phytoremediation, in terms of nitrogen and organics removal could be attributed to the following reasons:

- Excessive presence of organics compounds in wastewater inhibits nitrification, as faster heterotrophic organic degradation depletes DO availability; and
- Lack of biodegradable organics often hinders classic denitrification metabolism (due to dependency on organic carbon) in wetland system.

4.3.3.1.COD removal

The COD concentration in the mixture of leachate used for irrigation of crops has remained constant throughout the experimental period (791 mgO2 / L), while the load has been increasing according to the needs of the plant (Table 1.10). For *Glycine max* plants has increased from about 1600 mgO2/pot in the first period (equal to 4210 mgO2/m2 · day), at 2500 mgO2/pot in recent times (equal to 6580 mgO2/m2 · day). The concentration of COD in water leaching is often higher than the limit of 120 mg/l, set by Italian law (Legislative Decree 152/2006) as regards the discharge into surface waters. Considering, however, the amount of COD in entry and exit, the removal efficiency by plants, is always higher than 80% for both species, exceeding in some periods even

98% in the waters of leaching vessels in which they are were grown *Helianthus annus* plants (see figure 4.19).

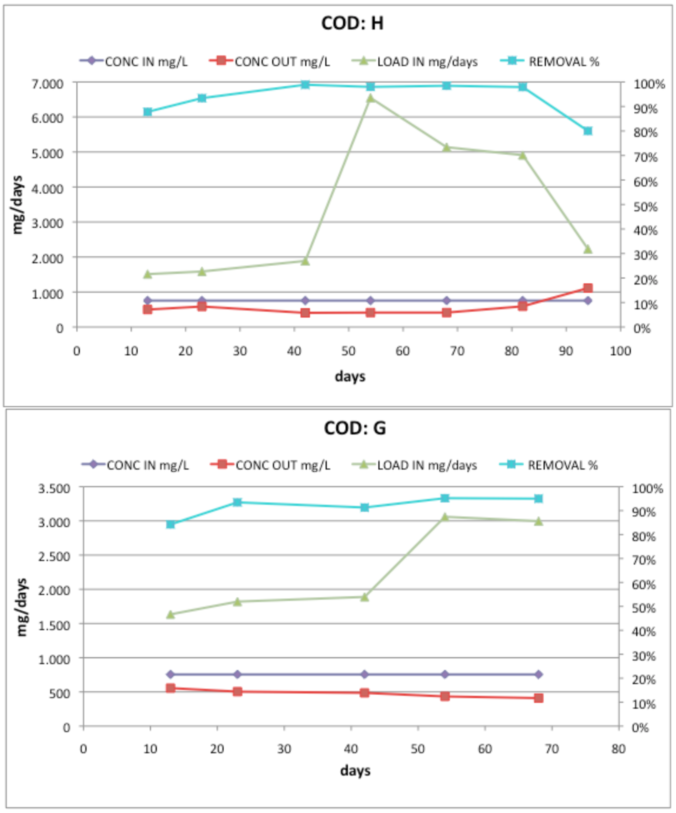


Figure 4.19. Phase 3: COD input and output concentration (mgO_2/L), COD input load ($mgO_2/days$) and COD η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

Surely the land has contributed as a filtering system in the removal of the organic load in solution, but comparing the two species, both characterized by a high percentage of removal, sunflower plants have been shown to have a better performance in the removal of COD from the mixture of irrigation to 20% of leachate.

4.3.3.2.Nitrogen removal

The concentration of nitrogen in the mixture of irrigation has remained stable throughout the experimental period (see figure 4.20). The load was different depending on the need demonstrated by plants, and is varied from a minimum of about 530 mgN / vessel for the first periods for soybean plants (corresponding to 1389 mgN/m2 · day) to a maximum of 2128 mgN / vessel (or 5600 mgN/m2 · day) in the fourth period for *Helianthus annus* plants. Even in this case, both cultures showed a high capacity for nitrogen removal from the irrigation solution, reaching even higher levels respects to the removal of COD; *Helianthus annus* plants were more efficient in the removal of nitrogen even above to 99 %, while the *Glycine max* has reached levels of removal above of 97%.

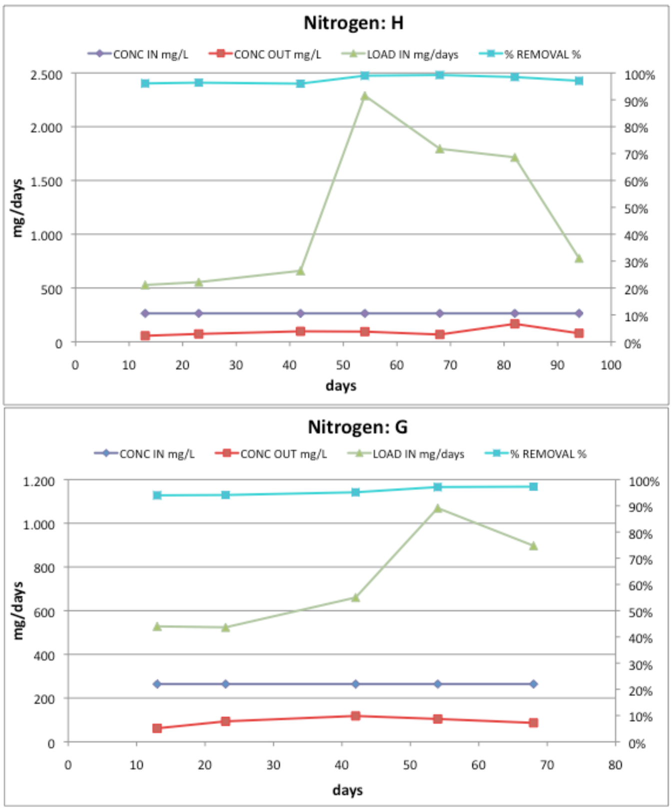
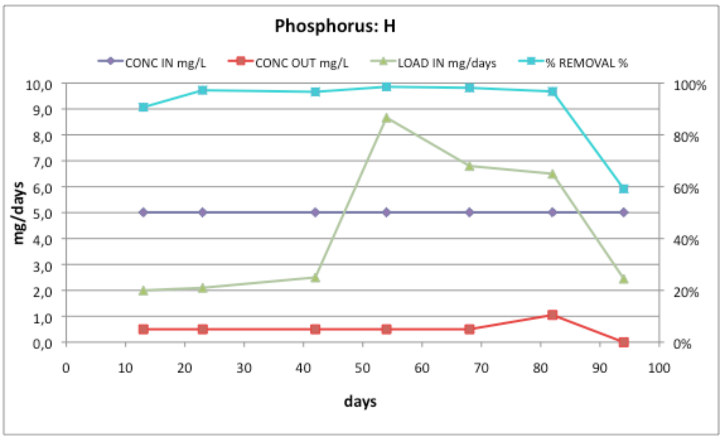


Figure 4.20. Phase 3: N input and output concentration (mgN/L), N input load (mgN/days) and N η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

4.3.3.3.Phosphorus removal

In the waters of leaching pots has not been found the presence of phosphorus (see figure 4.21), this is probably related to the strong reduction of total solids compared to the input load, as the phosphorus is conveyed in particular by the solid fraction, and then is likely to remain more closely linked to the substrate.



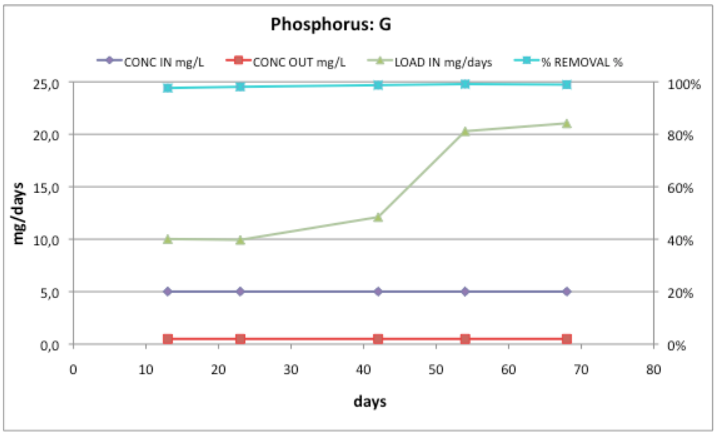


Figure 4.21. Phase 3: P input and output concentration (mgP/l), P input load (mgP/pot*period) and P η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

4.3.3.4.Another's chemical constituents

Table 4.16 reports the average input and output concentrations of other major leachate constituents. TS and VS removal denoted some inefficiency, probably caused by the drag of solid material during drainage operations. Alkaline earth metals displayed approximately the same input and output values, meaning that the phytotreatment process did not affect them. Chlorides concentration in the influent water was occasionally higher than in the effluent water (for glycine max).

Parameters	IN			OUT					
				H			G		
	Min	*Max*	*Average*	*Min*	*Max*	*Average*	*Min*	*Max*	*Average*
	mg/L			*mg/L*					
pH	8.16	8.20	8.17	6.93	7.72	7.22	6.89	7.25	7.07
NH3	10.9	238	200	25.9	43.4	31.7	14.8	36.6	26.9
Cl-	8.1	425.5	355.9	183.2	443.2	304.4	322.1	969.2	584.3
ST	296	1.858	1597	993	3166	1737	1517	2865	2462
VS	156	831.3	718.7	201.7	1170	611.1	477.5	1138	846.2

Table 4.16. Mean input_ output concentrations - phase 3.

The pH of the mixture was 8.17 approximately. In subsequent analysis of water leaching and soil testing the pH remained constant around neutrality (lower than that of the incoming solution), a situation favourable to the absorption of various nutrients, this is probably due to the addition of water to percolate, which in the period of stay in the ground has soured.

Chloride removal

In the previous phase (the second one) tested we could not complete the weekly analysis of chloride, due to the low amount of water we got from the packaging, but some values were demonstrated interest level removal of this parameter. In the present phase we have the possibility to obtain the graphics, demonstrating the ability of the phytotreatmen to remove the chloride (see figure 4.22), with values from 84% to 98% in the case of sunflowers and from 81% to 87% in the case of soybean.

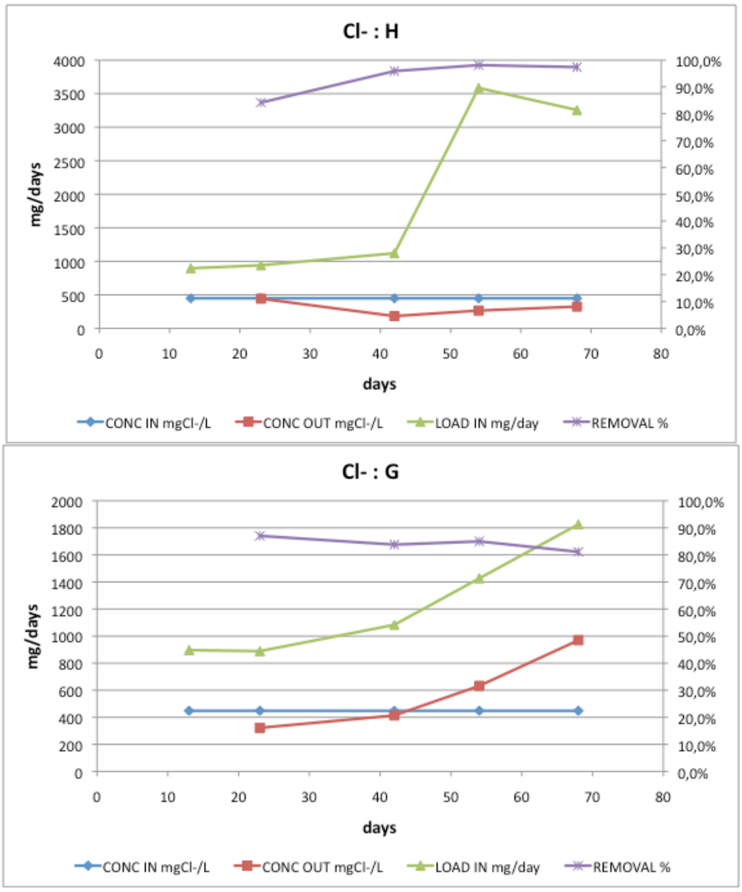


Figure 4.22. Phase 3: Cl- input and output concentration (mgCl-/L), P input load (mgP/days) and Cl- η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max)*.

4.3.4. Mass balance estimation

The mass balance of nitrogen and phosphorus was analysed in the distribution among the main system components: outlet waters, substrates and plants (energy crops). Nutrients mass balance was performed on leachate irrigated pots. The balance analyses nitrogen and phosphorus distribution among the main system components: water, substrate (either sand or clay) and plant (sunflower, soybean and rapeseed). Both balances prove a large reduction of the N and P water content through the phytotreatment process. The system with agricultural soil displayed higher removal rates than the one with sand substrate.

To obtain the estimation of the general mass balance, we calculated with the equation for a chemical, written as follows:

$$M_{plant} = M_{IN} - M_{OUT} - M_{soil}$$

Where:

M_{plant} = mass of the chemical in the plant, (g/pot)

M_{IN} = mass of the chemical in the inflow, (g/pot)

M_{OUT}= mass of the chemical in the outflow, (g/pot)

M_{soil}= mass of the chemical in the soil, (g/pot)

These components of the chemical budget were estimated for each species according to the following expressions:

$$M_{plant} =(root\_mass*conc\_ root + aerial\_mass*conc\_aerial)*N°pots$$

Where:

root_mass= mass of the chemical in all the roots of the considered species, (g)

conc_ root = concentration of the chemical in the roots of the considered species, (g)

aerial_mass = mass of the chemical in all the aerial part of the considered species, (g)

conc_aerial = concentration of the chemical in the aerial part of the considered species, (g)

N°pots = number of pots occupied by the considered species

$$M_{IN}=V_{IN}*conc\_in* N°pots$$

Where:

V_{IN}= inflow volume fed water to the essences from time to time, (l)

Conc_in= concentration of the chemical in the inflow volume, (mg/l)

$$M_{OUT}=V_{OUT}*conc\_out*N°pots$$

Where:

V_{OUT}= outflow water volume from the essences pots, (l)

Conc_out= concentration of the chemical in the outflow volume, (mg/l)

$$M_{soil}=M\_tot*(conc\_soil\_f - conc\_soil\_i)*N°pots$$

Where:

M_tot= total mass of the soil in the pots of the considered species, (kg)

Conc_soil_i= concentration of the chemical in the soil present at the beginning of the experimentation in the pots of the considered species, (mg/kg)

Conc_soil_f= concentration of the chemical in the soil present at the end of the experimentation in the pots of the considered species, (mg/kg)

Both the balances prove a large reduction of the NP water content through the phytotreatment process. Higher removal rates were displayed by the plants growing on soil substrate. These observations support the results obtained in the previous paragraph, and confirm the effectiveness of leachate treatment via oily crops irrigation. The NP mass balance ensures a substantial accumulation of nutrients in the vegetal tissues of plants fed with landfill leachate. Indeed, Figure 33 shows a very high nitrogen uptake in comparison to a limited amount of accumulated phosphorus (Figure 4.23). These data may suggest an unbalanced nutrient availability for the leachate-irrigated crops, which is a common problem in many leachate phytotreatment applications (Vymazal, 2009).

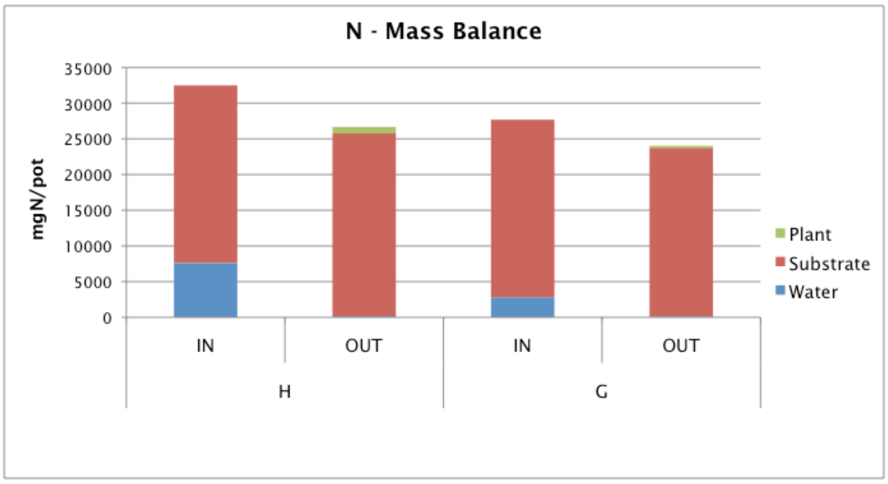
4.3.4.1.Nitrogen mass balance

At the end of the experimental analyzes of the soils used, have shown that the nitrogen content is decreased, due to the removal by plants and leaching water. In the pots in which were grown soybean plants were found 5937 mgN/pot, compared to the initial substrate, while in those with sunflower plants the quantity total nitrogen was 7354 mgN/pot, see table 4.17.

	H		***G***	
	IN	*OUT*	*IN*	*OUT*
Water	7626	154	2820	125
Substrate	24861	25594	24861	23610
Plant		909		290
Total	32487	26657	27680	24025

(Values expressed in mgP/pot) (*H: Helianthus annus;G: Glycine max)*

Table 4.17. Nitrogen mass balance phase 3



(H: Helianthus annus;G: Glycine max)
Figure 4.23. Nitrogen mass balance phase 3.

Based on mass balance analysis at HRT of 5 d, the crops plants were found to uptake about xx% of the TN input, while the xx% unaccounted values were postulated to be due to some microbial reactions, adsorption and volatilization.

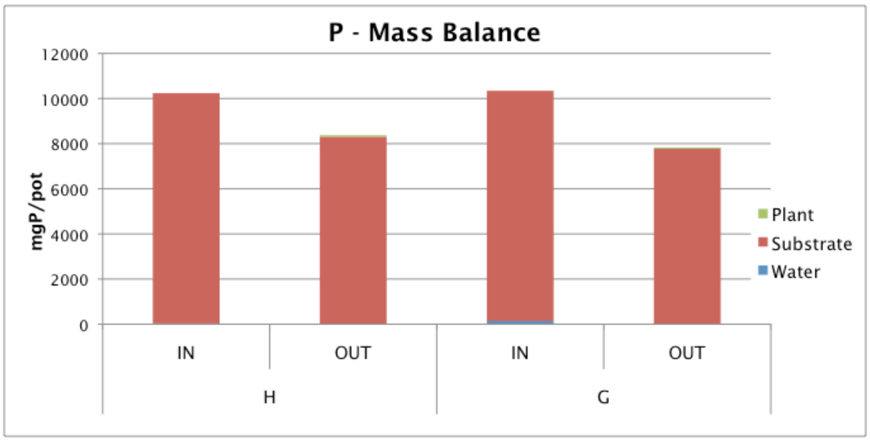
4.3.4.2.Phosphorus mass balance

Going instead to estimate the concentration of phosphorus in the initial substrate was 10203 mgP/pot and at the end of the experiment was 8308 MgP /pot, with a rise of 12% for the land in which were grown soybean plants and 7801 mgP /pot, 10% more, for those used in the sunflower plants. So we can consider that there was a high variation of the content of phosphorus and the contribution occurred through the mixture irrigation is more tied to the substrate (resulting in a small increase) like has occurred almost nothing in the presence water leaching of the plants have removed only a small part.

	H		G	
	IN	*OUT*	*IN*	*OUT*
Water	34.5	1.79	142.2	0.97
Substrate	10203	8308	10203	7801
Plant		64.7		22.7
Total	10238	8374	10346	7825

(Values expressed in mgP/pot) (*H: Helianthus annus;G: Glycine max*

Table 4.18. Phosphorus mass balance - phase 3.



(*H: Helianthus annus;G: Glycine max)*

Figure 4.24. Phosphorus mass balance - phase 3.

4.4. Phase 4

Because of time, the acceleration in the growth of plants (especially for those that were irrigated with leachate concentration) and other technical problems, it was decided not to continue with the experiment until the final stage of the same, that is, not obtained seeds at this stage, and the estimation of vegetation not was justified; one reason was that the mass unbalance it was predictable from the beginning; second we almost saw that the major part of the nutrients are release in the substrate.

4.4.1. Removal efficiencies

Nitrogen, Phosphorus and COD were fundamental in furthering the understanding of the remediation effects of the three crops. Each graph features the different periods in order to improve the understanding of plant responses in terms of performance to variations remediation of contaminants introduced load. To better interpret the results and make a comparison with other findings present in literature, the values are expressed in international units.

The removal of nitrogen and organics, from wastewater, in such engineered ecosystem is very important, due to the following reasons:

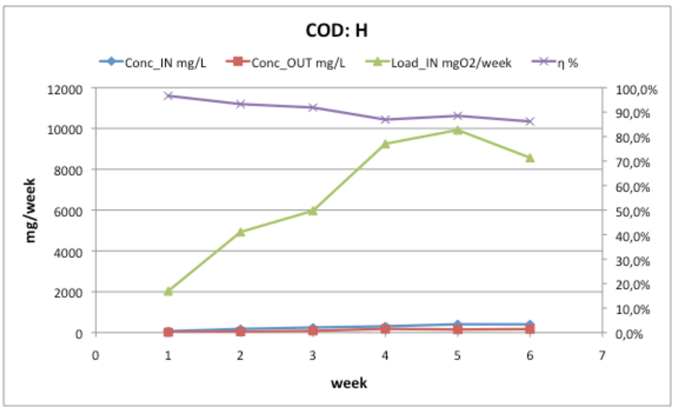
- Uncontrolled discharge of nitrogen into natural water channels fosters eutrophication of lakes and rivers.
- Untreated organic materials often deplete dissolved oxygen (DO) concentration in open water channels, leading to the death of aquatic organisms (T.Saeed, G, Sun, 2012).

The performance disparity of phytoremediation, in terms of nitrogen and organics removal could be attributed to the following reasons:

- Excessive presence of organics compounds in wastewater inhibits nitrification, as faster heterotrophic organic degradation depletes DO availability; and
- Lack of biodegradable organics often hinders classic denitrification metabolism (due to dependency on organic carbon) in wetland system.

4.4.1.1.COD removal

The amount of COD introduced weekly was gradually increased; the values in incoming and outgoing ones of the wastewater that has been collected from the bottom of the tanks weekly, after it is analyzed in the laboratory, reporting in the figure 4.22, who evidence the removal in the crops of *Helianthus annus* and *Glycine max*. We note that the percentages of removal (which were evaluated as regards the loads, "load") roam always above 85%.



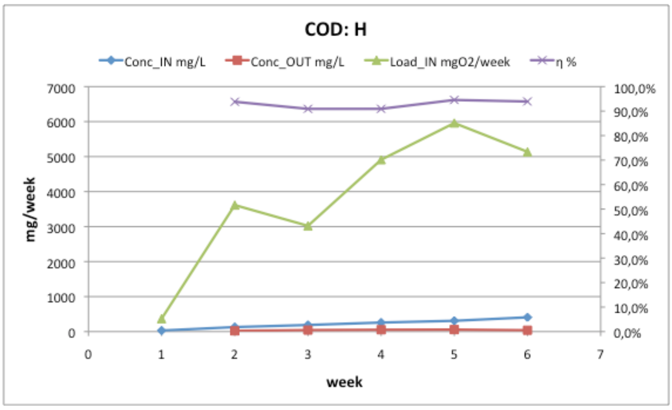


Figure 4.25. Phase 4: COD input and output concentration (mgO_2/l), COD input load (mgO_2/week) and COD η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

4.4.1.2.Nitrogen removal

We analyzed every week the release of nitrogen, by calculating the percentage removal of loads. In figure 4.22 are reported the efficiency of the nitrogen removal, considering above of 88% for *Helianthus annus* and about of 79% for *Glycine max*.

Figure 4.26. Phase 4: N input and output concentration (mgN/L), N input load (mgN/tank*week) and N η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

Nitrification rates:

Analyzing the composition of nitrogen input vs. output, it emerges that nitrogen enters the system in the form of ammonium (see leachate composition at table 3.7) but it gets out in the form of nitrate.

This result ensures that enough oxygen is present in the system either to chemically or biologically oxidize occurring nitrogen, meanwhile transforming it in a more bio-available form (Cheng and Chu, 2011; Tyrrel et al., 2001).

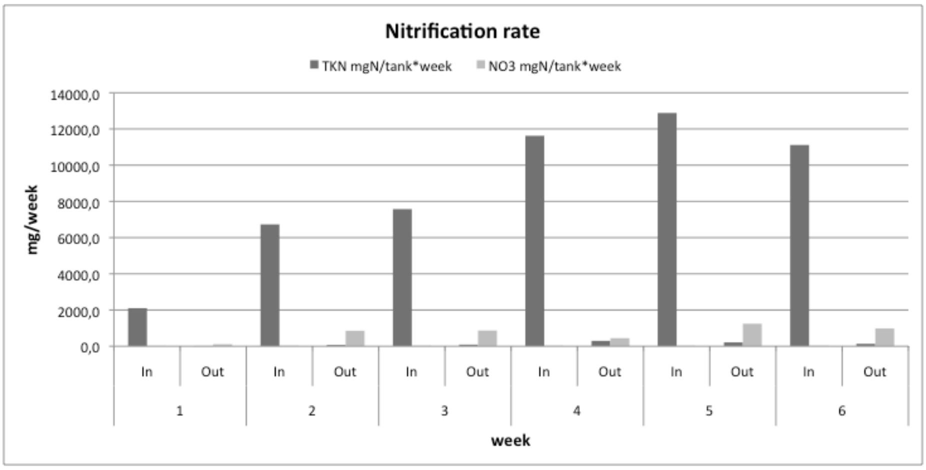


Figure 4.27. Comparison of input (I) and output (O) nitrogen load composition through the whole experimental phase 3. Values are obtained as an average among the essences growing on sand and the essences growing on clayey soil.

4.4.1.3.Phosphorus removal

Considered the loads of phosphorus outgoing from the following table we can see that the high percentage of removal was obtained every week consistently, considering above of 90% for both species: *Helianthus annus Glycine max*.

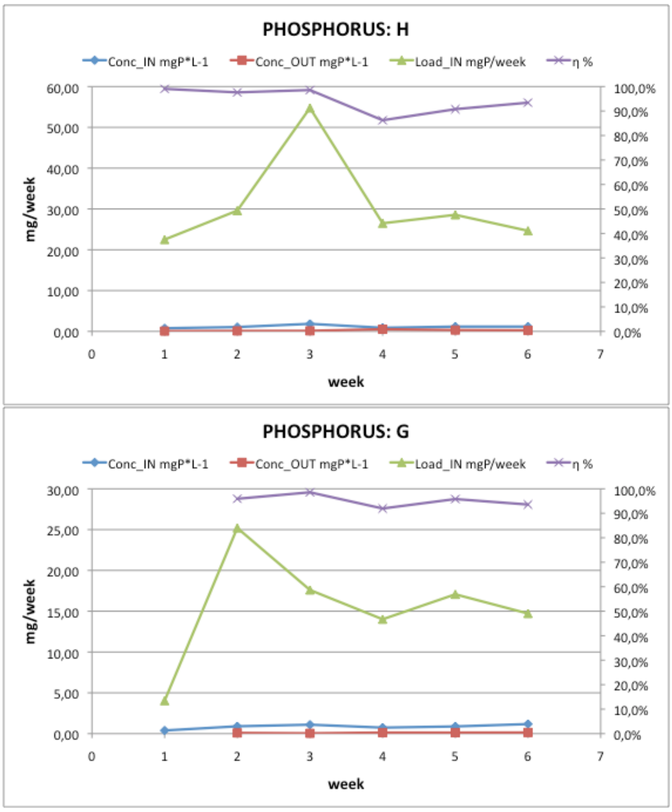


Figure 4.28. Phase 4: P input and output concentration (mgP/L), P input load (mgP/tank*week) and P η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max)*.

4.4.1.4.Chloride removal:

At the same way from phase 2, in this phase is specified the trend of the chloride during the experimental stage, giving as a result (see figure 4.25) values of removal: from 70% to 80% in the case of sunflower and from 72% to 88% in the case of soybeans

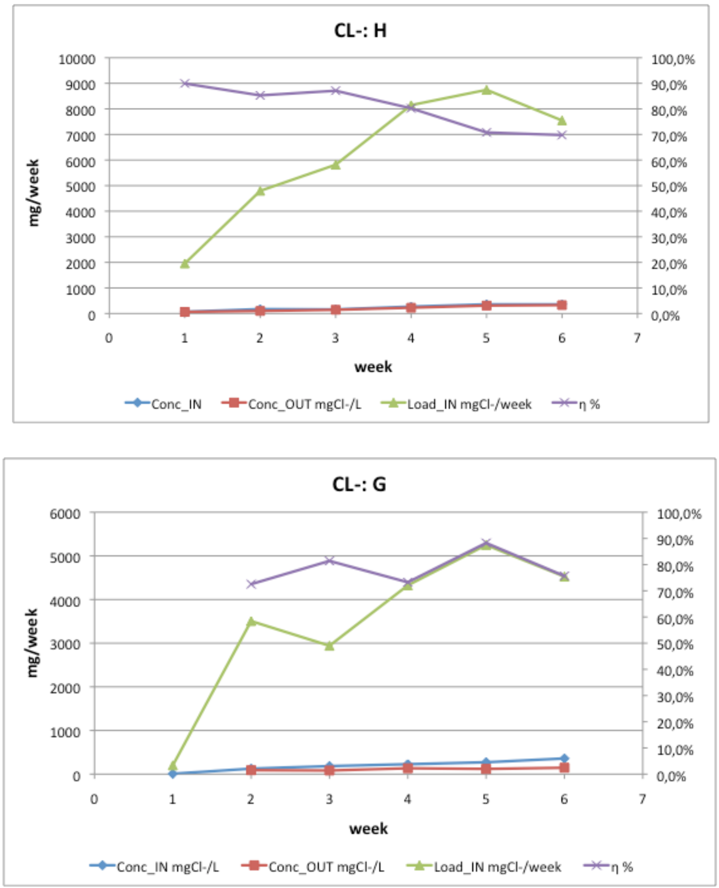


Figure 4.29. Phase 4: Cl- input and output concentration (mgCl-/L), N input load (mgCl-/tank*week) and Cl- η(%) removal efficiency during the experimental period. (*H: Helianthus annus; G: Glycine max).*

5 CONCLUSIONS

The treatment of municipal/domestic and industrial wastewater is a field of environmental and sanitary engineering characterized by many sustainable management, instead for the traditional practices; inspired by these, research developments particular interest providing promising opportunities. Accordingly, the wastewater treatment theory and processes concur largely with the theory and processes applied in solid waste management.

To limit the rising impacts of human society on the environment, sustainable management systems of waste must be made available. A management system may be made up of various phases and treatments. The common aim of all possible processes should be not only economical convenience but also environmental sustainability.

The present research work was carried out to tested several phytotreatment combinations, with the aim of identifying a sustainable system to be applied in full/pilot scale.

Energy crops;

The phytotreatment capacity of ***energy crops*** was through the depuration efficiencies and the plants growth, which at the end of their life cycle produced seeds, suited as biodiesel feedstock; thereby contributing towards increasing in the environmental sustainability of the waste management cycle. Accurate selection in base at the local conditions and the feasibility of plant cultivations, the energy crops were chosen.

In the firs phase: as general result of the different species treated with domestic wastewater (yellow and grey) grew better than those irrigated with tap water (controls); in terms of biomass developed and root length, the reduction of pollutant load and lastly, mass balance for N and P. The results obtained underline how controls for all species fed with tap water and nutrients. (Larger biomass than wastewater, inhibition in the growth of roots, etc.).

Leachate, for the next phases 2, 3 and 4: in general greater growth of plants irrigated with leachate treated than those with tap water (controls), that's mean, positive influence on the growth of plants and seed production.

The cost effectiveness in phytoremediation is achieved by recylcling, using energy saving biological processes, and by producing biomass, potentially biologically active compounds, and non-food products for energy production, green manure and building material. Additionally, is low-cost maintenance system (P. Schoder et al, 2007).

Wastewater treatment and mixing;

Grey water involves large quantity of volumes compatible with the phytoremediation process, low concentration of organic matter, nutrients N/P .

Good removal performances of the main pollutants (COD, N, P, metals) for the domestic wastewaters, especially mixture of GW and YW, higher efficiencies increasing the percentage of YW (3.5%), elicited an increase in the nitrogen concentration present in the feed, in turn resulting positive response of plants both in terms of removal efficiency and vegetation growth, as attested by the mass balance obtained for N and P. Evaluation of real water savings in the use of toilet flow separation (around 50%).

The research proved the capability of energy crops, irrigated with old landfill leachate, to ensure high pollutants removal rates in different operational conditions; energy crops positively affects the treatment of landfill leachate as there are lower TKN, NO3 and COD concentrations after every trial period. This is probably achieved through plant uptake and the effects of enhanced levels of oxygen and organic carbon in the rhizosphere on nitrification and denitrification. (Tyrrel et al, 2002).

The removal efficiency rates for N and P and COD remained high for all species throughout the trial. Some examples for best results:

COD [B_soil= 94.1 % phase2; H_soil= 93.4% phase2; G= 93.5 % phase3]

TN [B_soil=88.8 % phase2; H= 97.5 % phase3; G= 95.5 % phase3]

TP [B_soil= 98% phase2; H_soil= 98% phase2; G= 98.5% phase3]

The removal of nitrogen and organics and organics from wastewater by phytoremediation, though a inter-connected plants, media bulk water and biomass population, is very important, due to the uncontrolled discharge of nitrogen into natural water channels fosters eutrophication of lakes and rivers, and the untreated organic materials often deplete oxygen concentrations in open water channels, leading to the death of aquatic organisms; however, often exhibit poor nitrogen removal rates (Saeed and Sun, 2012).

A knowledge on the impact of environmental parameters (e.g. pH, oxygen, temperature etc.), and operating conditions (e.g. hydraulic and pollutant loading, detention time, influent feed mode, recirculation, organic carbon addition etc.) can improve the biodegradation routes, associated with nitrogen and organics removal in treatment wetlands; have significant impacts on nitrogen and organics removals in constructed wetland systems.

Ammonia nitrogen was removed efficiently in all variants. Like very high pollutants removal rates were ensured for both runs.

Phosphorus removal for all the experimentations, was not affected by the feeding leachate load, so that outlet P concentrations kept bellow 1 mg/L. At the contrary, COD and nitrogen removal displayed a certain dependence on feed water composition.

N and P mass balance confirmed the results obtained for water pollutants removals and plants nutrients accumulation, and highlight the crucial role played the substrate in the achievement of a high phytotreatment performance.

The results are much better than other types of treatment (septic tanks, Imhoff tanks) thickness used isolated settlements, with the use of a technology with very low demand and aesthetic value.

Substrate;

Sand was found to be a less suitable substrate for the growth even in the presence of leachate, except rapeseed (phase 2).

Agricultural soil showed better performance (key element for the removal).

Seeds;

The phytotreatment did not inhibit the production of seeds; rather the results obtained were higher than the regulars' values shown in others research (H= 25-35 %, G= 15-20; A. Karmakar, 2010).

% Oil content:

45.1 % for H_soil (phase 2), and 22,1 % for G (phase 3).

Future developments;

There are many benefits offered by plant-soil based forms from landfill leachate remediation; as a cost effective option (especially when spraying on large land areas adjacent to a landfill or on caped areas), technical equipment may require minimal supervision, being regarded as relatively uncomplicated, energy demands are low, and secondary products are not considered an issue (Jones et al, 2006).

For the hydraulic mass balance, is recommended to considered the most frequently used devices to estimate the evaporative demand in field conditions are: the Class A pan (CAP), the Piche

Atmometers (ATM), Andersson evaporimeters (ANE), etc.; all this devices are converted using adequate factors, into reference evapotranspiration values (ETo). Parallel, empirical equation have been developed that use climatic parameters to estimate ETo. The Penman-Monteith (PME) equation has been recently recommended as the sole method to determine ETo, although the FAO-Radiation (FRE) formula widely accepted as alternative. These methods give reasonable estimates of the crop water requirements, (advantages & disadvantages).

In this context, it is up till not clear whether greenhouse require more or less water per unit product than field culture, due to the absence of sufficient local measurements. Anyway, some publications specialized in this area compare the Etc values for irrigated crops using these methods with studies of water balance using lisymeters, there are used to monitor water, fertilizers, salts and other contaminants; lysimeters are particularly useful in transpiration and evapotranspiration studies as they provide measurements of water balance components. Design of lysimeters for crop studies has traditionally involved costly technologies that limit the scope and scale of their use. (A. Ben-Gal1 & U.Shani, 2002).

It is necessary to further develop to understanding of factors and processes that occur inside greenhouse, among which crop evapotranspiration (Etc), estimated from vegetative areas, is one of the main factors to consider in the calculation of water balance; in order to get adequate and opportune estimations of water demand for the irrigation system designs M. Casanova et al., 2009).

To finish, the main developments to this research is to field open treatment reutilizing derelict area (closed landfill, contaminated soil, etc.); not competing between land for food and for energy. In this way, during full/pilot scale projects the following will be verified:

- The accumulation of nutrients in the soil
- The fate of possible emerging pollutants

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ANNEXES

CHEMICAL BUDGET

Chemical budget Phase 1

		TKN											
		H				*G*				*B*			
Period	*Days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*
1	4	4,3	2,8	80,4	-	4,3	2,8	80,4	-	4,3	2,8	80,4	-
2	11	4,3	2,8	81,7	34,9%	4,3	2,8	86,0	34,9%	4,3	2,8	68,8	34,9%
3	16	14,1	2,8	147,6	80,1%	14,1	2,8	147,64	80,1%	14,1	2,8	133,6	80,1%
4	21	14,1	2,8	168,7	80,1%	14,1	2,8	168,7	80,1%	14,1	2,8	84,4	80,1%
5	25	53,1	2,8	531,0	80,1%	53,1	2,8	531,0	94,7%	53,1	2,8	531,0	94,7%
6	30	53,1	2,8	849,7	94,7%	53,1	2,8	849,66	94,7%	53,1	2,8	637,2	94,7%
7	35	101,9	4,8	2.038,1	95,3%	101,9	3,9	2.038,1	96,2%	101,9	2,8	1.222,9	97,3%
8	42	199,5	10	4.389,31	95,0%	199,5	6,0	3.990,3	97,0%	199,5	2,8	2.394,2	98,6%
9	49	199,5	24,6	4.389,3	87,7%	199,5	11,2	3.591,3	94,4%	199,5	12,8	1.995,1	93,6%
10	60	248,3	26	4.966,35	89,5%	248,3	12	4.469,72	94,0%	248,3	26,9	5.463,0	89,2%
11	66	248,3	29,7	6.952,9	88,0%	248,3	28,6	6.207,9	88,5%	248,3	27,0	5.711,3	89,1%
12	72	297,1	31,5	3.565,5	89,4%	297,1	28,0	2.674,1	88,7%	297,1	27,0	2.674,1	90,9%
13	79	297,1	31,5	6.833,8	89,4%	297,1	28,0	5.942,4	90,6%	297,1	28,0	6.833,8	90,6%
14	84	297,1	31,5	5.188,9	89,4%	297,1	29,1	4.842,9	90,2%	297,1	35,2	5.880,7	88,2%
15	88	345,9	30,8	4.842,9	91,1%	345,9	32,2	4.151,1	90,7%	345,9	36,7	4.842,9	89,4%

		N total											
		H				***G***				***B***			
Period	*Days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*η %*
1	4	5,6	4,0	104,7	28,6%	5,6	4,0	104,7	28,6%	5,6	4,0	104,7	28,6%
2	11	5,6	4,0	106,4	28,6%	5,6	4,4	112,0	21,4%	5,6	4,4	89,6	21,4%
3	16	15,4	5,8	160,4	62,3%	15,4	5,8	160,4	62,2%	15,4	5,3	145,2	65,4%
4	21	15,4	6,0	184,4	61,2%	15,4	6,0	184,4	60,9%	15,4	7,4	100,0	51,8%
5	25	54,4	6,0	544,3	89,0%	54,4	6,2	544,3	88,6%	54,4	4,7	544,3	91,4%
6	30	54,4	6,1	869,6	88,8%	54,4	6,4	869,6	88,2%	54,4	4,6	652,4	91,6%
7	35	103,3	8,4	2.065,0	91,9%	103,2	7,9	2.065,0	92,3%	103,2	4,7	1.239,0	95,4%
8	42	200,9	11,0	4.419,8	94,5%	200,8	11,0	4.018,0	94,5%	200,8	5,8	2.410,8	97,1%
9	49	200,9	29,2	4.419,8	85,5%	200,8	15,0	3.616,2	92,5%	200,8	16,3	2.009,0	91,9%
10	60	249,7	30,0	4.992,4	88,0%	249,6	20,0	4.495,1	92,0%	249,6	30,4	5.491,9	87,8%
11	66	249,7	35,2	6.993,0	85,9%	249,6	35,2	6.243,7	85,9%	249,6	30,5	5.744,1	87,8%
12	72	369,1	36,2	5.582,6	90,2%	298,4	35,0	4.687,0	88,3%	298,4	30,3	4.687,0	89,8%
13	79	298,6	37,4	6.867,0	87,5%	298,4	35,3	5.971,4	88,2%	298,4	31,2	6.867,0	89,5%
14	84	347,4	37,6	5.210,6	89,2%	347,2	36,5	4.863,3	89,5%	347,2	40,5	5.905,4	88,3%
15	88	347,4	30,8	4.863,3	91,1%	347,2	32,2	4.168,5	90,7%	347,2	36,7	4.863,3	89,4%

Ptotal

		H				G				B			
Period	Days	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %
1	4	0,30	0,20	5,61	33,3%	0,30	0,20	5,61	33,3%	0,30	0,20	5,61	33,3%
2	11	0,30	0,20	5,70	33,3%	0,30	0,20	6,00	33,3%	0,30	0,20	4,80	33,3%
3	16	0,30	0,20	3,18	33,9%	0,30	0,20	3,18	33,9%	0,30	0,20	2,88	33,9%
4	21	0,30	0,20	3,63	33,9%	0,30	0,20	3,63	33,9%	0,30	0,20	3,63	33,9%
5	25	0,31	0,20	3,14	35,5%	0,31	0,20	3,14	35,5%	0,31	0,20	2,88	35,5%
6	30	0,31	0,20	8,12	35,5%	0,31	0,20	8,12	35,5%	0,31	0,20	6,84	35,5%
7	35	0,33	0,20	6,60	39,4%	0,33	0,20	6,60	39,4%	0,33	0,20	3,96	39,4%
8	42	0,35	0,22	7,74	37,5%	0,35	0,20	7,08	43,2%	0,35	0,20	4,25	43,2%
9	49	0,35	0,22	7,70	37,5%	0,35	0,22	6,37	38,6%	0,35	0,20	3,54	43,2%
10	60	0,37	0,25	7,38	32,4%	0,37	0,23	6,62	37,8%	0,37	0,23	8,09	37,8%
11	66	0,37	0,27	9,46	27,0%	0,37	0,26	8,33	29,7%	0,37	0,29	7,59	21,6%
12	72	0,38	0,28	6,57	26,5%	0,38	0,26	6,43	31,8%	0,38	0,30	6,43	21,3%
13	79	0,38	0,30	8,76	21,3%	0,38	0,26	7,62	31,8%	0,38	0,30	8,76	21,3%
14	84	0,39	0,33	5,92	16,3%	0,39	0,27	5,52	30,8%	0,39	0,32	6,71	18,9%
15	88	0,39	0,35	5,52	11,3%	0,39	0,33	4,73	16,3%	0,39	0,33	5,52	16,3%

COD

		H				G				B			
Period	Days	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	η %
1	4	68,2	9,9	1.275,3	85,5%	68,2	14,9	1.275,3	78,2%	68,2	14,9	1.275,3	78,2%
2	11	68,2	29,0	1.295,8	57,5%	68,2	33,9	1.364,0	50,3%	68,2	29,0	1.091,2	57,5%
3	16	74,5	27	782,2	63,8%	74,5	33,5	782,2	55,0%	74,5	25,0	707,7	66,4%
4	21	74,5	26,0	893,9	65,2%	74,5	33,5	893,9	55,0%	74,5	23,6	893,9	68,3%
5	25	99,7	29,0	996,7	70,9%	99,7	33,8	996,7	66,1%	99,7	30,0	996,7	69,9%
6	30	99,7	32,0	1.943,3	67,9%	99,7	34,0	1.943,3	65,9%	99,7	34,0	1.405,2	65,9%
7	35	131,1	34,7	2.623,0	73,5%	131,1	34,7	2.623,0	73,5%	131,1	34,8	1.573,8	73,5%
8	42	194,1	45	3.445,0	76,8%	194,1	42,8	3.881,9	77,9%	194,1	42,0	2.329,2	78,4%
9	49	194,1	52,4	4.270,1	73,0%	194,1	42,8	3.493,7	77,9%	194,1	50,0	1.941,0	74,2%
10	60	225,6	70	5.236,3	69,0%	225,6	60,0	4.785,2	73,4%	225,6	60,0	5.687,5	73,4%
11	66	225,6	86,4	6.875,3	61,7%	225,6	80,6	6.198,6	64,3%	225,6	70,0	5.747,5	69,0%
12	72	257,0	90,1	5.085,0	64,9%	257,0	88,0	5.313,0	65,8%	257,0	80,0	5.313,0	68,9%
13	79	257,0	97,9	5.912,0	61,9%	257,0	96,0	5.140,9	62,7%	257,0	82,0	5.912,0	68,1%
14	84	288,5	110,0	4.327,8	61,9%	288,5	100,0	4.039,3	65,3%	288,5	82,5	4.904,8	71,4%
15	88	288,5	110,2	4.039,3	61,8%	288,5	100,1	3.462,2	65,3%	288,5	88,0	4.039,3	69,5%

TS

		H			G			B		
Period	Days	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days	Conc_IN mg/L	Conc_OUT mg/L	Load_IN mg/days
1	4	448	514	8.385	448	516	8.385	448	494	8.385
2	11	448	592	8.520	448	620	8.968	448	407	7.174
3	16	484	580	4.022	484	620	4.022	484	473	3.639
4	21	484	524	5.205	484	622	5.205	484	564	5.205
5	25	629	610	6.285	629	626	6.285	629	570	6.285
6	30	629	625	11.737	629	629	11.737	629	866	14.836
7	35	809	647	16.173	809	810	16.173	809	623	9.704
8	42	1.169	750	25.717	1.169	925	23.379	1.169	432	14.027
9	49	1.169	951	25.717	1.169	1.103	21.041	1.169	988	11.689
10	60	1.349	1.214	26.627	1.349	1.246	23.929	1.349	1.275	29.325
11	66	1.349	1.782	33.847	1.349	1.790	29.800	1.349	1.116	27.101
12	72	1.529	1.542	18.350	1.529	1.786	13.763	1.529	1.002	13.763
13	79	1.529	1.611	35.065	1.529	1.796	30.478	1.529	924	35.065
14	84	1.709	1.702	25.640	1.709	1.621	23.931	1.709	1.342	29.059
15	88	1.709	n.d.	23.931	1.709	n.d.	20.512	1.709	n.d.	23.931

		VS								
		H			G			B		
Period	*Days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*
1	4	260	341	4.868	260	345	4.868	260	299	4.868
2	11	260	373	4.946	260	370	5.206	260	332	4.165
3	16	281	296	1.554	281	362	1.554	281	292	1.406
4	21	281	257	2.574	281	360	2.574	281	365	2.574
5	25	364	302	2.532	364	356	2.532	364	345	2.532
6	30	364	337	7.358	364	355	7.358	364	295	5.288
7	35	467	370	9.344	467	430	9.343	467	381	5.606
8	42	674	410	14.828	674	531	13.480	674	415	8.088
9	49	674	460	14.828	674	605	12.132	674	436	6.740
10	60	777	586	15.309	777	820	13.754	777	560	16.864
11	66	777	892	20.071	777	1.186	17.739	777	655	16.184
12	72	881	761	10.570	881	1.200	7.928	881	580	7.928
13	79	881	1.109	20.158	881	1.269	17.515	881	348	20.158
14	84	984	1.288	14.764	984	1.316	13.780	984	326	16.733
15	88	984	1.417	13.780	984	1.448	11.812	984	359	13.780

		Cl-								
		H			G			B		
Period	*Days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*
1	4	43,7	49,6	817,2	43,7	49,6	817,2	43,7	56,7	817,2
2	11	43,7	56,7	830,3	43,7	63,8	874,0	43,7	55,4	699,2
3	16	45,9	52,0	446,7	45,9	65,0	446,7	45,9	36,5	404,1
4	21	45,9	41,3	530,4	45,9	66,0	530,4	45,9	37,5	530,4
5	25	54,5	44,0	545,2	54,5	68,0	545,2	54,5	46,1	545,2
6	30	54,5	49,0	1.344,3	54,5	70,9	1.344,3	54,5	46,1	1.004,9
7	35	65,3	53,2	1.845,8	65,3	89,8	1.845,8	65,3	81,5	1.107,5
8	42	87,0	76,0	3.179,6	87,0	99,0	2.794,7	87,0	90,0	1.676,8
9	49	87,0	140,0	4.234,6	87,0	109,9	3.464,6	87,0	91,0	1.924,8
10	60	97,8	160,0	4.267,9	97,8	110,0	3.809,7	97,8	100,0	4.726,1
11	66	97,8	206,6	6.047,9	97,8	123,4	5.360,6	97,8	109,9	4.902,4
12	72	108,6	198,6	1.303,5	108,6	122,0	977,7	108,6	115,0	977,7
13	79	108,6	212,5	2.003,9	108,6	129,4	1.678,1	108,6	155,0	2.003,9
14	84	119,5	243,9	1.791,8	119,5	154,2	1.672,3	119,5	168,4	2.030,7
15	88	119,5	n.d.	4.232,8	119,5	n.d.	3.628,1	119,5	n.d.	4.232,8

		SO_4^{2-}								
		H			G			B		
Period	*Days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*	*Conc_IN mg/L*	*Conc_OUT mg/L*	*Load_IN mg/days*
1	4	42,2	n.d.	789,1	42,2	n.d.	789,1	42,2	n.d.	789,1
2	11	42,2	70,4	801,8	42,2	78,2	844,0	42,2	71,0	675,2
3	16	43,4	74,0	501,3	43,4	72,0	455,9	43,4	41,0	453,5
4	21	43,4	85,2	547,0	43,4	68,0	521,0	43,4	58,1	547,0
5	25	48,3	82,0	482,9	48,3	65,0	434,2	48,3	92,0	482,9
6	30	48,3	92,0	870,0	48,3	63,2	694,7	48,3	85,8	637,9
7	35	54,4	108,9	1.087,8	54,4	83,8	1.331,5	54,4	84,9	652,7
8	42	66,6	103,0	1.464,7	66,6	92,0	1.331,5	66,6	85,0	798,9
9	49	66,6	100,5	1.464,7	66,6	103,3	1.198,4	66,6	85,2	665,8
10	60	72,7	95,0	1.754,2	72,7	92,0	1.608,8	72,7	95,0	1.899,5
11	66	72,7	85,4	1.985,3	72,7	88,1	1.767,3	72,7	110,6	1.621,9
12	72	78,8	86,7	945,2	78,8	92,0	708,9	78,8	115,0	708,9
13	79	78,8	83,8	1.811,6	78,8	126,5	1.575,3	78,8	122,3	1.811,6
14	84	84,9	106,4	1.272,9	84,9	145,9	1.188,0	84,9	141,4	1.442,6
15	88	84,9	n.d.	1.188,0	84,9	n.d.	1.018,3	84,9	n.d.	1.188,0

Chemical budget Phase 2

TKN

	H_SAND				***G_SAND***				***B_SAND***			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	2,80	n.d.	2,80	-	2,80	n.d.	2,80	-	2,80	n.d.	2,80	-
1	26,8	2,80	28,3	95,1%	26,8	2,80	24,1	91,7%	26,8	2,80	36,2	93,9%
2	50,8	2,80	50,8	96,8%	50,8	2,80	50,8	97,9%	50,8	2,80	45,8	96,8%
3	50,8	2,80	63,6	96,6%	50,8	2,80	64,8	97,9%	50,8	2,80	63,6	97,2%
4	98,9	2,80	98,9	98,6%	98,9	2,80	98,9	98,8%	98,9	2,80	98,9	98,6%
5	98,9	2,80	158	98,6%	98,9	2,80	138	98,9%	98,9	2,80	119	98,8%
6	171	3,36	256	99,3%	171	3,08	274	99,1%	171	2,80	205	99,4%
7	207	3,64	300	99,7%	207	2,80	300	99,4%	207	2,80	248	99,7%
8	243	3,64	389	99,6%	243	3,08	389	99,3%	243	2,80	389	99,7%
9	303	4,34	485	99,6%	303	3,08	485	99,5%	303	3,36	485	99,7%
10	327	2,80	523	99,7%	327	2,80	491	99,5%	327	3,36	523	99,7%
11	327	2,80	523	100%	327	2,80	523	99,5%	327	6,44	523	99,6%
12	363	9,24	581	98,5%	363	4,20	508	99,2%	363	8,40	581	99,4%

TKN

	H_SOIL				***G_SOIL***				***B_SOIL***			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	2,80	n.d.	0,84	-	2,80	n.d.	0,84	-	2,80	n.d.	0,84	-
1	26,8	2,80	12,1	98,3%	26,8	2,80	12,1	97,7%	26,8	2,80	12,1	97,4%
2	50,8	n.d.	20,3	-	50,8	n.d.	15,3	-	50,8	n.d.	20,3	-
3	50,8	n.d.	33,1	-	50,8	n.d.	25,4	-	50,8	2,80	33,1	98,7%
4	98,9	n.d.	98,9	-	98,9	2,80	89,0	99,4%	98,9	2,80	98,9	99,4%
5	98,9	n.d.	208	-	98,9	2,80	138	99,1%	98,9	2,80	104	98,9%
6	171	n.d.	274	-	171	3,08	248	99,5%	171	2,80	222	99,4%
7	207	10,1	413	99,5%	207	3,36	254	99,6%	207	2,80	248	99,6%
8	243	3,64	486	99,7%	243	3,36	352	99,6%	243	2,80	340	99,6%
9	303	2,80	516	99,8%	303	5,04	455	99,5%	303	4,20	455	99,5%
10	327	2,80	523	99,7%	327	6,45	507	99,3%	327	3,92	507	99,7%
11	327	17,08	523	98,9%	327	22,4	523	97,5%	327	7,84	523	99,3%
12	363	16,52	581	97,9%	363	9,24	436	98,5%	363	3,36	581	99,7%

N total

	H_SAND				***G_SAND***				***B_SAND***			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	44,8	6,46	44,8	89,7%	44,8	11,5	44,8	84,3%	44,8	7,3	44,8	90,3%
1	27,4	10,8	28,9	81,2%	27,4	16,7	24,7	51,8%	27,4	13,2	37,0	72,0%
2	51,4	7,86	51,4	91,0%	51,4	13,9	51,4	89,5%	51,4	23,4	46,3	73,4%
3	51,4	9,34	64,3	88,7%	51,4	14,5	65,6	89,5%	51,4	26,9	64,3	73,9%
4	99,5	17,1	99,5	91,4%	99,5	9,6	99,5	95,9%	99,5	41,8	99,5	80,8%
5	99,5	27,5	159	86,2%	99,5	6,2	139	97,5%	99,5	47,6	119	82,0%
6	172	49,6	257	89,9%	172	3,7	274	98,9%	172	50,7	206	89,9%
7	208	66,2	301	78,8%	208	17,1	301	96,2%	208	88,8	249	93,9%
8	244	181	390	81,5%	244	54,8	390	88,0%	244	190	390	90,9%
9	304	310	486	72,9%	304	166	486	71,5%	304	274	486	81,4%
10	328	593	524	35,0%	328	199	492	64,0%	328	407	524	73,9%
11	328	555	524	39,2%	328	344	524	35,3%	328	453	524	72,6%
12	364	574	582	7,5%	364	313	509	40,1%	364	455	582	66,8%

	N total											
	H_SOIL				***G_SAND***				***B_SOIL***			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	44,8	113	13,4	-	44,8	108	13,4	-	44,8	n.d.	13,4	-
1	27,4	2,80	12,3	98,3%	27,4	2,80	12,3	97,7%	27,4	2,80	12,3	97,4%
2	51,4	n.d.	20,6	-	51,4	n.d.	15,4	-	50,8	n.d.	20,6	-
3	51,4	n.d.	33,4	-	51,4	n.d.	25,7	-	50,8	27,5	33,4	87,6%
4	99,5	n.d.	99,5	-	99,5	99,2	89,5	79,2%	98,9	32,3	99,5	92,7%
5	99,5	n.d.	209	-	99,5	92,9	139	71,6%	98,9	31,6	104	87,9%
6	172	2,54	274	99,9%	172	61,3	249	90,1%	171	35,4	223	92,3%
7	208	23,3	414	98,9%	208	44,7	255	94,3%	207	50,3	249	93,2%
8	244	66,0	487	94,9%	244	54,4	353	93,6%	244	74,7	341	89,6%
9	304	116	517	93,0%	304	72,2	456	92,3%	304	90,1	456	88,6%
10	328	345	524	63,8%	328	153	508	82,7%	327	151	508	88,1%
11	328	232	524	85,1%	328	271	524	70,3%	327	195,6	524	82,7%
12	364	392	582	51,2%	364	235	436	63,0%	363	265	582	76,7%

	Ptotal											
	H_SAND				**G_SAND**				**B_SAND**			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	6,20	0,10	6,20	98,9%	6,20	0,10	6,20	99,0%	6,20	0,10	6,20	99,0%
1	0,54	0,10	0,57	91,3%	0,54	0,18	0,49	74,3%	0,54	0,12	0,73	86,7%
2	0,99	0,13	0,99	92,2%	0,99	0,10	0,99	96,1%	0,99	0,10	0,89	94,1%
3	0,99	0,12	1,23	92,7%	0,99	0,18	1,26	93,1%	0,99	0,10	1,23	94,9%
4	6,83	0,12	6,83	99,1%	1,87	0,14	1,87	96,9%	1,87	0,15	1,87	95,9%
5	14,3	1,55	22,8	94,6%	1,87	0,16	2,62	96,5%	1,87	0,10	2,25	97,7%
6	3,20	0,13	4,80	98,5%	3,20	0,22	5,12	96,6%	3,20	0,14	3,84	98,5%
7	3,86	0,31	5,60	98,6%	3,86	0,10	5,60	98,8%	3,86	0,27	4,64	98,2%
8	4,53	0,39	7,24	97,9%	4,53	0,10	7,24	98,8%	4,53	0,16	7,24	99,1%
9	5,64	0,77	9,02	96,4%	5,64	0,10	9,02	99,0%	5,64	0,10	9,02	99,5%
10	6,08	0,29	9,72	98,2%	6,08	0,10	9,12	99,0%	6,08	0,10	9,72	99,3%
11	6,08	1,06	9,72	93,7%	6,08	0,10	9,72	99,0%	6,08	0,44	9,72	98,0%
12	6,74	0,10	10,8	99,1%	6,74	0,10	9,44	99,0%	6,74	0,10	10,79	99,5%

	Ptotal											
	H_SOIL				***G_SOIL***				***B_SOIL***			
WEEK	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*	*CONC IN mg/L*	*CONC OUT mg/L*	*LOAD IN mg/week*	*REMOVAL %*
0	6,20	0,10	1,86	99,2%	6,20	0,10	1,86	99,1%	6,20	0,10	1,86	99,9%
1	0,54	0,34	0,24	89,6%	0,54	0,83	0,24	66,2%	0,54	0,18	0,24	91,6%
2	0,99	n.d.	0,39	-	0,99	n.d.	0,30	-	0,99	n.d.	0,39	-
3	0,99	n.d.	0,64	-	0,99	n.d.	0,49	-	0,99	0,16	0,64	97,7%
4	1,87	n.d.	1,87	-	1,87	0,16	1,68	98,8%	1,87	0,10	1,87	98,8%
5	1,87	n.d.	3,93	-	1,87	0,23	2,62	96,3%	1,87	0,25	1,96	95,0%
6	3,20	n.d.	5,12	-	3,20	0,19	4,64	98,4%	3,20	0,10	4,16	98,8%
7	3,20	0,42	7,72	98,9%	3,86	0,10	5,41	99,4%	3,86	0,17	4,64	98,8%
8	4,53	0,37	9,06	98,5%	4,53	0,10	6,57	99,3%	4,53	0,13	6,34	99,0%
9	5,64	0,14	9,6	99,6%	5,64	0,27	8,45	98,5%	5,64	0,31	8,45	97,9%
10	6,08	0,11	9,72	99,4%	6,08	0,11	9,42	99,3%	6,08	0,10	9,42	99,6%
11	6,08	0,21	9,72	99,3%	6,08	0,48	9,72	97,2%	6,08	0,11	9,72	99,5%
12	6,74	0,10	10,8	99,3%	6,74	0,19	8,09	98,3%	6,74	0,10	10,8	99,5%

COD

WEEK	H_SAND				G_SAND				B_SAND			
	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %
0	9,60	n.d.	9,60	-	9,60	n.d.	9,60	-	9,60	n.d.	9,60	-
1	54,5	20,5	57,5	82,2%	54,5	16,4	49,0	76,2%	54,5	14,4	73,6	84,6%
2	99,4	14,4	99,4	91,5%	99,4	20,5	99,4	92,0%	99,4	20,5	89,5	88,0%
3	99,4	20,5	124	87,2%	99,4	20,5	127	93,7%	99,4	20,5	124	89,7%
4	189	22,1	189	94,2%	189	12,5	189	97,2%	189	13,5	189	96,4%
5	189	22,1	303	94,2%	189	11,5	265	97,6%	189	22,1	227	95,0%
6	316	137	473	84,8%	316	134	505	79,2%	316	111	379	87,2%
7	383	115	556	94,8%	383	107	458	84,3%	383	56,2	460	96,3%
8	451	127	721	93,0%	451	86,8	721	89,8%	451	57,1	721	96,8%
9	564	253	902	88,1%	564	104	902	90,3%	564	139	902	92,7%
10	609	394	974	76,7%	609	184	913	82,1%	609	316	974	83,8%
11	609	466	974	72,5%	609	262	974	73,5%	609	395	974	85,8%
12	676	358	1082	69,0%	676	194	947	80,1%	676	368	1082	85,5%

COD

WEEK	H_SOIL				G_SOIL				B_SOIL			
	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	REMOVAL %
0	9,60	n.d.	2,88	-	9,60	n.d.	2,88	-	9,60	n.d.	2,88	-
1	54,5	n.d.	24,5	-	54,5	n.d.	24,5	-	54,5	n.d.	24,5	-
2	99,4	n.d.	39,8	-	99,4	n.d.	29,8	-	99,4	n.d.	39,8	-
3	99,4	n.d.	64,6	-	99,4	n.d.	49,7	-	99,4	n.d.	64,6	-
4	189	n.d.	189	-	189	43,9	170	95,2%	189	23,1	189	97,3%
5	189	n.d.	397	-	189	30,4	265	95,1%	189	16,3	199	96,7%
6	316	188	505	98,1%	316	136	458	88,2%	316	91,1	410	89,2%
7	383	165	764	95,7%	383	120	537	92,8%	383	56,5	460	95,9%
8	451	124	902	94,8%	451	109	654	93,1%	451	64,5	631	95,1%
9	564	142	958	95,4%	564	137	845	92,1%	564	71,4	845	95,1%
10	609	234	974	86,8%	609	201	944	87,8%	609	177	944	92,5%
11	609	345	974	88,1%	609	275	974	83,8%	609	235	974	88,9%
12	676	253	1082	83,1%	676	172	812	85,5%	676	177	1082	91,6%

TS

WEEK	H_SAND			G_SAND			B_SAND		
	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week
0	296	n.d.	296	296	n.d.	296	296	n.d.	296
1	416	343	439	416,4	542	375	416,4	360	562
2	537	528	537	536,8	503	537	536,8	506	483
3	537	617	671	536,8	850	684	536,8	554	671
4	778	690	778	778	784	778	778	624	778
5	778	833	1244	778	789	1089	778	732	933
6	1139	1235	1708	1139	988	1822	1139	959	1366
7	1319	1651	1913	1319	1296	1913	1319	1176	1583
8	1500	2241	2400	1500	1763	2400	1500	1544	2400
9	1801	3044	2881	1801	2281	2881	1801	2686	2881
10	1921	5530	3074	1921	3590	2882	1921	4082	3074
11	1921	6204,00	3074	1921	3930,00	3074	1921	5150,00	3074
12	2102	5668	3363	2102	4021	2942	2102	5876	3363

TS

WEEK	H_SAND			G_SAND			B_SAND		
	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/week
0	296	n.d.	296	296	n.d.	296	296	n.d.	296
1	416	343	439	416,4	542	375	416,4	360	562
2	537	528	537	536,8	503	537	536,8	506	483
3	537	617	671	536,8	850	684	536,8	554	671
4	778	690	778	778	784	778	778	624	778
5	778	833	1244	778	789	1089	778	732	933
6	1139	1235	1708	1139	988	1822	1139	959	1366
7	1319	1651	1913	1319	1296	1913	1319	1176	1583
8	1500	2241	2400	1500	1763	2400	1500	1544	2400
9	1801	3044	2881	1801	2281	2881	1801	2686	2881
10	1921	5530	3074	1921	3590	2882	1921	4082	3074
11	1921	6204,00	3074	1921	3930,00	3074	1921	5150,00	3074
12	2102	5668	3363	2102	4021	2942	2102	5876	3363

TS

WEEK	H_SOIL CONC IN mg/L	H_SOIL CONC OUT mg/L	H_SOIL LOAD IN mg/week	G_SOIL CONC IN mg/L	G_SOIL CONC OUT mg/L	G_SOIL LOAD IN mg/week	B_SOIL CONC IN mg/L	B_SOIL CONC OUT mg/L	B_SOIL LOAD IN mg/week
0	296	n.d.	88,8	296	n.d.	88,8	296	n.d.	88,8
1	416	n.d.	187	416	n.d.	187	416	n.d.	187
2	537	n.d.	215	537	n.d.	161	537	n.d.	215
3	537	n.d.	349	537	n.d.	268	537	n.d.	349
4	778	n.d.	778	778	1661	700	778	539	778
5	778	n.d.	1633	778	1860	1089	778	807	816
6	1139	n.d.	1822	1139	2046	1651	1139	1251	1480
7	1319	1772	2662	1319	2063	1847	1319	1238	1583
8	1500	1991	3000	1500	2603	2175	1500	1691	2100
9	1801	2659	3091	1801	2686	2701	1801	2032	2701
10	1921	3418	3074	1921	3164	2978	1921	2788	2978
11	1921	4154	3074	1921	3317	3074	1921	3166	3074
12	2102	3965	3363	2102	3748	2522	2102	3766	3363

VS

WEEK	H_SAND CONC IN mg/L	H_SAND CONC OUT mg/L	H_SAND LOAD IN mg/week	G_SAND CONC IN mg/L	G_SAND CONC OUT mg/L	G_SAND LOAD IN mg/week	B_SAND CONC IN mg/L	B_SAND CONC OUT mg/L	B_SAND LOAD IN mg/week
0	156	n.d.	156	156	n.d.	156	156	n.d.	156
1	184	91	194	183,8	160	165	184	106	248
2	212	152	212	211,7	170	212	212	211	190
3	212	287	265	211,7	289	270	212	292	265
4	267	300	267	267	323	267	267	330	267
5	267	371	428	267	332	374	267	376	321
6	351	559	526	351	358	561	351	495	421
7	393	670	569	393	396	569	393	586	471
8	434	784	695	434	619	695	434	811	695
9	504	1284	806	504	800	806	504	1281	806
10	532	2150	957	532	1402	798	532	1655	851
11	532	2242	851	532	1683	851	532	1931	851
12	573	2192	918	573	1778	803	573	2234	916

VS

WEEK	H_SOIL CONC IN mg/L	H_SOIL CONC OUT mg/L	H_SOIL LOAD IN mg/week	G_SOIL CONC IN mg/L	G_SOIL CONC OUT mg/L	G_SOIL LOAD IN mg/week	B_SOIL CONC IN mg/L	B_SOIL CONC OUT mg/L	B_SOIL LOAD IN mg/week
0	156	n.d.	46,8	156	n.d.	46,8	156	n.d.	46,8
1	184	n.d.	83	184	n.d.	83	184	n.d.	83
2	212	n.d.	85	212	n.d.	63	212	n.d.	85
3	212	n.d.	138	212	n.d.	106	212	n.d.	138
4	267	n.d.	267	267	935	241	267	308	212
5	267	n.d.	561	267	878	374	267	397	281
6	351	n.d.	561	351	756	509	351	464	456
7	393	756	799	393	806	550	393	581	471
8	434	635	869	434	911	630	434	708	608
9	504	1078	872	504	990	756	504	913	756
10	532	1338	851	532	1144	824	532	1078	824
11	532	1315	851	532	1442	851	532	1327	851
12	573	1580	918	573	1442	688	573	1468	918

Chemical budget Phase 3

TKN

Days	H CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %
0	2,80	n.d	9,80	-	2,80	n.d.	9,8	-
13	261,4	55,1	523	96,1%	261,4	51,10	523	95,3%
23	261,4	60,4	549	96,9%	261,4	61,18	518	96,5%
42	261,4	83,7	653	96,2%	261,4	73,08	653	96,2%
54	261,4	93,8	2265	99,0%	261	59,36	1059	98,0%
68	261,4	66,85	1777	99,2%	261	57,40	887	97,3%
82	261,4	29,8	1699,0	99,7%				-
94	261,4	33	771,07	-				-

N total

	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %
0	3,37	n.d	11,80	-	3,37		11,80	-
13	264,07	55,6	528,14	96,1%	264,07	61,6	528,14	94,0%
23	264,07	73,0	554,55	96,4%	264,07	93,5	523,74	94,1%
42	264,07	97,2	660,18	96,0%	264,07	118,2	660,18	95,1%
54	264,07	94,4	2288,62	99,0%	264,07	104,5	1069,49	97,1%
68	264,07	67,4	1794,52	99,2%	264,07	86,4	897,08	97,3%
82	264,07	166,6	1715,12	98,5%	n.d.			-
94	264,07	78,7	776,32	97,1%	n.d.			-

Ptotal

	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %
0	0,10	n.d	3,50	-	0,10	n.d.	0,35	-
13	5,01	0,50	2,00	90,7%	5,01	0,50	10,02	97,6%
23	5,01	0,50	2,10	97,2%	5,01	0,50	9,94	98,1%
42	5,01	0,50	2,50	96,6%	5,01	0,50	12,11	98,7%
54	5,01	0,50	8,67	98,6%	5,01	0,50	20,29	99,2%
68	5,01	0,50	6,80	98,2%	5,01	0,50	21,05	98,9%
82	5,01	1,05	6,50	96,7%				-
94	5,01	0,00	2,45	59,2%				-

COD

	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/days	REMOVAL %
0	9,6	n.d	34	-	9,6	n.d	33,6	-
13	755,4	499,3	1.511	87,8%	755,4	555,4	1.634	84,2%
23	755,4	586,3	1.586	93,4%	755,4	504,2	1.820	93,4%
42	755,4	405,0	1.889	98,9%	755,4	486,0	1.889	91,3%
54	755,4	413,0	6.547	98,0%	755,4	433,8	3.059	95,2%
68	755,4	413,0	5.137	98,5%	755,4	409,8	2.997	95,0%
82	755,4	591,8	4.910	97,9%				-
94	755,4	1.113,0	2.229	80,0%				-

	TS							
	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %
0	296,0	n.d.	1.036,0	-	296,0	n.d.	1.036,0	-
13	1.767	2.483,7	3.534,0	72,4%	1.767	2.751,0	3.534,0	62,8%
23	1.767	3.165,7	3.651,8	75,3%	1.767	2.864,7	3.504,5	80,8%
42	1.767	1.023,3	4.123,0	93,8%	1.767	1.517,5	4.050,4	81,3%
54	1.767	993,3	12.015,6	97,9%	1.767	2.656,3	5.624,9	84,0%
68	1.767	1.016,7	11.249,9	97,7%	1.767	2.522,9	4.653,1	89,8%

	VS							
	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %
0	156,0	n.d.	234,0	-	156,0	n.d.	546,0	-
13	692,5	1.075,3	1.385,0	69,8%	692,5	1.138,5	1.385,0	60,3%
23	692,5	1.170,3	1.454,3	76,8%	692,5	1.116,8	1.373,5	82,3%
42	692,5	278,3	1.731,3	93,3%	692,5	477,5	1.673,5	85,8%
54	692,5	201,7	5.540,0	99,1%	692,5	725,8	2.204,5	89,0%
68	692,5	330,0	4.408,9	98,4%	692,5	772,5	1.823,6	90,5%

	Cl-							
	H				G			
Days	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %	CONC IN mg/L	CONC OUT mg/L	LOAD IN mg/day	REMOVAL %
0	8,05	n.d.	28,18	-	8,05	n.d.	28,18	-
13	448,17	n.d.	896,34	100,0%	448,17	n.d.	896,34	100,0%
23	448,17	443,20	941,16	84,2%	448,17	322,07	888,87	87,0%
42	448,17	183,20	1120,43	95,9%	448,17	413,65	1083,08	83,8%
54	448,17	266,00	3585,38	98,1%	448,17	632,33	1426,68	85,0%
68	448,17	325,02	3256,19	97,4%	448,17	969,16	1825,64	81,1%

Chemical budget Phase 4

	TKN							
	H				G			
Week	Conc_in mg/L	Conc_out mg/L	Load_IN mg/week	η %	Conc_in mg/L	Conc_out mg/L	Load_IN mg/week	η %
1	65,2	5,90	1.912	98,9%	34,0	n.d.	381,85	-
2	179,5	4,21	4.943	97,9%	127,5	3,2	3.571	99,0%
3	252,3	27,20	6.055	97,9%	189,9	6,93	3.039	98,3%
4	306,5	82,30	9.194	94,4%	255,9	8,40	4.861	98,3%
5	407,7	40,70	9.920	96,6%	306,5	13,80	5.925	98,5%
6	407,7	14,84	8.561	98,9%	407,7	26,55	5.108	96,6%

	N total							
	H				G			
WEEK	Conc_IN mgN/L	Conc_OUT mgN/L	Load_IN mg/week	η %	Conc_IN mgN/L	Conc_OUT mgN/L	Load_IN mg/week	η %
1	65,9	85,35	3836,8	99,4%	34,4	n.d.	395,4	-
2	180,4	89,18	9908,9	88,9%	128,2	97,5	3593,5	71,3%
3	253,6	134,17	12127,0	89,4%	191,0	167,0	3056,2	60,4%
4	307,5	161,92	18405,8	95,8%	256,8	43,5	4879,4	92,9%
5	408,8	256,92	19859,3	94,2%	307,4	164,6	5943,7	85,0%
6	408,8	255,30	17138,9	96,2%	408,8	96,2	5124,0	85,9%

Ptotal

WEEK	H Conc_IN mgP/L	H Conc_OUT mgP*L-1	H Load_IN mg/week	H η %	G Conc_IN mgP/L	G Conc_OUT mgP/L	G Load_IN mg/week	G η %
1	0,77	0,07	22,50	99,1%	0,39	n.d.	4,04	-
2	1,07	0,11	29,60	97,6%	0,90	0,09	25,20	95,9%
3	1,85	0,13	54,75	98,6%	1,10	0,04	17,60	98,6%
4	0,88	0,53	26,49	86,2%	0,74	0,13	14,01	91,9%
5	1,17	0,32	28,57	90,8%	0,88	0,12	17,07	95,8%
6	1,17	0,26	24,65	93,4%	1,17	0,14	14,72	93,6%

COD

WEEK	H Conc_IN mg/L	H Conc_OUT mg/L	H Load_IN mg/week	H η %	G Conc_IN mg/L	G Conc_OUT mg/L	G Load_IN mg/week	G η %
1	69,35	19,81	2034,18	96,6%	29,52	n.d.	367,49	-
2	178,9	46,9	4929,1	93,3%	129,1	21,0	3614,6	93,8%
3	248,6	86,0	5966,1	91,9%	188,8	43,0	3021,5	90,9%
4	308,2	182,8	9247	86,9%	258,5	52,5	4911	90,9%
5	408	150,0	9922	88,5%	308	56,7	5959	94,5%
6	408	173	8563	86,2%	408	39	5137	93,9%

TS

WEEK	H Conc_IN mg/L	H Conc_OUT mg/L	H Load_IN mg/week	G Conc_IN mg/L	G Conc_OUT mg/L	G Load_IN mg/week
1	527	906	15456	296	n.d	8555
2	950	1807	26298	758	1868	21218
3	736	2368	29270	989	2324	15819
4	1329	2295	39858	1157	2013	21974
5	1673	3274	40705	1329	2195	25686
6	1673	4370	35129	1673	2777	22143

VS

VS

WEEK	H Conc_IN mg/L	H Conc_OUT mg/L	H Load_IN mg/week	G Conc_IN mg/L	G Conc_OUT mg/L	G Load_IN mg/week
1	182,7	417	7081	156,0	n.d.	4327
2	398,0	738	11029	326,8	738	9150
3	331,9	1245	11942	440,7	1088	6595
4	456,6	1062	13698	406,5	1021	7724
5	556,8	1724	13549	456,6	976	8828
6	556,8	1342	11693	556,8	1425	7610

Cl-

WEEK	H Conc_IN mgCl-/L	H Conc_OUT mgCl-/L	H Load_IN mg/week	H η %	G Conc_IN mgCl-/L	G Conc_OUT mgCl-/L	G Load_IN mg/week	G η %
1	66,64	63,80	1955	89,9%	8,05	n.d.	201	-
2	174,04	99,30	4795	85,3%	125,22	89,83	3506	72,5%
3	161,89	146,57	5817	87,1%	183,81	82,73	2941	81,4%
4	271,56	226,93	8147	80,2%	227,64	134,77	4325	73,3%
5	359,39	310,70	8745	70,8%	271,56	119,63	5250	88,3%
6	359,39	328,10	7547	69,8%	359,39	146,30	4526	75,7%

WATER BUDGET

Water budget Phase 1

	H		***G***		***B***	
Period	*Vin*	*Vout*	*Vin*	*Vout*	*Vin*	*Vout*
			(L)			
1	18,70		18,70		18,70	
2	19,00	1,87	20,00	1,44	16,00	6,50
3	10,50	0,00	10,50	0,00	9,50	2,81
4	12,00	2,04	12,00	0,00	12,00	5,76
5	10,00		10,00		10,00	4,70
6	16,00		16,00	1,55	12,00	5,10
7	20,00	4,06	20,00	6,30	12,00	5,02
8	22,00		20,00		12,00	
9	22,00	3,20	18,00	2,83	10,00	0,33
10	20,00		18,00		22,00	
11	28,00	1,50	25,00	1,74	23,00	1,85
12	12,00	1,40	9,00		9,00	
13	23,00	0,55	20,00	2,45	23,00	1,50
14	15,00	1,73	14,00	3,78	17,00	2,00
15	14,00	1,70	12,00	4,53	14,00	2,43
Total	***262,20***	***18,04***	***243,20***	***24,61***	***220,20***	***37,99***

Water budget Phase 2

	H_SAND			***H_SOIL***			***G_SAND***			***G_SOIL***			***B_SAND***			***B_SOIL***		
Week	*Vin*	*Vout*	*Vev*	*V in*	*V out*	*Vev*	*V in*	*V out*	*Vev*	*V in*	*V out*	*Vev*	*V in*	*V out*	*Vev*	*V in*	*V out*	*Vev*
										(L)								
0	1,00	0,71	0,00	0,30	0,15	0,00	1,00	0,61	0,00	0,30	0,16	0,00	1,00	0,60	0,00	0,30	0,03	0,00
1	1,06	0,50	0,12	0,45	0,08	0,05	0,90	0,71	0,10	0,45	0,10	0,05	1,35	0,79	0,15	0,45	0,11	0,05
2	1,00	0,59	0,09	0,40	0,00	0,04	1,00	0,39	0,11	0,30	0,00	0,03	0,90	0,53	0,08	0,40	0,00	0,04
3	1,25	0,78	0,07	0,65	0,00	0,03	1,28	0,48	0,07	0,50	0,00	0,03	1,25	0,63	0,07	0,65	0,15	0,03
4	1,00	0,50	0,04	1,00	0,00	0,04	1,00	0,43	0,04	0,90	0,19	0,04	1,00	0,50	0,04	1,00	0,23	0,04
5	1,60	0,80	0,10	2,30	0,00	0,14	1,40	0,56	0,08	1,40	0,43	0,08	1,20	0,51	0,07	1,05	0,40	0,06
6	1,50	0,53	0,03	2,00	0,05	0,04	1,60	0,79	0,04	1,45	0,40	0,03	1,20	0,44	0,03	1,30	0,49	0,03
7	1,45	0,25	0,09	3,30	0,20	0,20	1,45	0,68	0,09	1,40	0,33	0,08	1,20	0,30	0,07	1,20	0,34	0,07
8	1,60	0,40	0,10	2,90	0,38	0,17	1,60	0,85	0,10	1,45	0,41	0,09	1,70	0,40	0,10	1,40	0,48	0,08
9	1,60	0,43	0,07	2,30	0,31	0,10	1,60	0,84	0,07	1,60	0,49	0,07	1,80	0,48	0,08	1,50	0,58	0,07
10	1,80	0,58	0,08	2,80	0,55	0,13	1,70	0,89	0,08	1,70	0,58	0,08	2,00	0,50	0,09	1,75	0,40	0,08
11	1,60	0,58	0,10	1,60	0,34	0,10	1,60	0,99	0,10	1,60	0,58	0,10	1,60	0,35	0,10	1,60	0,46	0,10
12	1,60	0,94	0,07	2,10	0,73	0,09	1,40	0,98	0,06	1,20	0,69	0,05	1,60	0,43	0,07	1,60	0,51	0,07
TOTAL	**18,06**	**7,56**	**0,95**	**22,10**	**2,78**	**1,14**	**17,53**	**9,18**	**0,93**	**14,25**	**4,34**	**0,73**	**17,80**	**6,44**	**0,95**	**14,20**	**4,17**	**0,72**

Water budget Phase 3

	H		**G**	
Period	*V in*	*V out*	*V in*	*V out*
		(L)		
0	3,50		3,50	
1	*2,00*	0,37	2,00	0,47
2	2,10	0,29	1,98	0,235
3	2,50	0,17	2,50	0,37
4	8,67	0,25	4,05	0,35
5	6,80	0,20	4,20	0,38
6	6,50	0,17		
7	2,45	0,50		
Total	***34,52***	***1,94***	***18,23***	***1,80***

Water budget Phase 4

	H			Hc			G			Gc		
	Vin	*Vout*	*Vev*	*Vin*	*Vout*	*Vev*	*Vin*	*Vout*	*Vev*	*Vin*	*Vout*	*Vev*
Week						(L)						
0	24,00	0,00	0,00	24,00	0,00	0,00	24,00	0,00	0,00	24,00	0,00	0,00
1	29,33	2,22	0,70	25,00	2,50	0,56	27,17	2,36	0,42	25,00	1,50	0,56
2	28,00	7,24	0,63	28,00	12,53	0,63	28,00	9,89	0,75	28,00	12,63	0,63
3	24,00	5,42	1,26	24,00	5,52	1,26	24,00	5,47	1,51	16,00	8,70	0,84
4	30,00	7,40	1,58	30,00	9,70	1,58	30,00	8,55	2,05	19,00	8,80	1,00
5	24,33	7,34	1,42	25,00	9,60	1,69	24,67	8,47	2,37	20,00	8,25	1,35
6	14,00	8,96	0,95	17	9,4	1,156	15,50	9,18	2,37	17	9,1	1,15
Total	**173,7**	**38,6**	**6,5**	**173,0**	**49,3**	**6,9**	**173,3**	**43,9**	**6,5**	**149,0**	**49,0**	**5,5**