UNIVERSITY OF NOVA GORICA
GRADUATE SCHOOL

BLACKWATER TREATMENT AT TOURIST FACILITIES

DISSERTATION

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Every little new step we do forward

with good and constructive intentions is important,

very important.

Because everything is connected after all.

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ABBREVIATIONS

BA	Total Bacteria
BF	Biofilter
BOD	Biochemical Oxygen Demand
BOD_5	Biochemical Oxygen Demand over a 5-day period
BW	Blackwater
C/N	Carbon-Nitrogen ratios
CO	Coliforms
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DM	Dry Matter
EC	Electrical Conductivity
EN	Enterobacteria
GW	Greywater
FW	Food waste
KTOT	Total Potassium
LOI	Loss Of Ignition
MPN	Most Probable Number
MTF	Multiple Tube Fermentation
NECCO	Non-*E. coli* Coliforms
NECEN	Non-*E. coli* Enterobacteria
NECNENBA	Non-*E. coli* Non Enteric Bacteria
NH_4^+-N	Ammonium nitrogen
NO_2^--N	Nitrite nitrogen
NO_3^--N	Nitrate nitrogen
OCE	BW, oat and bran compost mixture
OCB	BW, bark, oat and bran compost mixture
OCP	BW, peat, oat and bran compost mixture
PCR	Polymerase Chain Reaction
PLC	Programmable Logic Controller
TP	Total phosphorus
PF	Peat Filters
rRNA	Ribosomal RNA
OCS	BWS, sawdust, oat and bran compost mixture
S	Sulphur
SBR	Sludge Batch Reactor
TC	Total Carbon
TOC	Total Organic Carbon
TS	Total solids
TSS	Total Suspended Solids
TW	Treatment Wetland
VS	Volatile Solids
Y&M	Yeast and Moulds

1. INTRODUCTION

1.1 Sustainability and recycling of nutrients

Sustainable development was defined by the Brundtland commission as: “Development that meets the needs of the present without compromising the ability of future generations to meet their own needs” (WCED, 1987). As a response to this work, sustainability has become important in all aspects of society including sanitary engineering. In the book “Ecological Engineering” (Mitsch and Jørgensen, 1989), a holistic perspective on engineering is presented where not only technical aspects, but also the interactions between technology, nature and society become important in engineering processes and design. In a sustainability evaluation of sanitary systems both energy aspects and recycling are very important (Guterstam, 1991). Werner et al., (2004) introduces sanitation to the systematic closure of local material flow cycles and point to this as a main principle of integrated eco-systems, water oriented and natural resources management. The linear attitude regarding nutrient flow needs to be changed towards a sustainable one, advancing to a “recycling society”.

1.1.1 Macronutrients

Macronutrients (carbon (C), nitrogen (N), phosphorus (P), sulphur (S), potassium (K), calcium (Ca), magnesium (Mg)) and trace elements are necessary for well-functioning of living organisms and all biological systems. N and P and K are the most common macronutrients in mineral fertilizers. Nowadays, a massive flow of nutrients, in the form of food waste comes from rural and urban areas. If we consider the flow of nutrients and trace elements in agriculture (e.g. farms), they are circulated mostly on the farms. Some of these elements are transported as food from the farms to the urban communities where some of them are consumed and some are disposed as waste. In urban or rural areas, these elements in household waste have the same fate. The linear flows of nutrients which do not often appear in nature are producing a deficit. This deficit calls for compensation of these elements. Nowadays this compensation is mainly done by using mineral fertilizer containing P, from rapidly decreasing sources (Vinnerås, 2001). Production of mineral fertilizer is also energy demanding and production of 1kg mineral N fertilizer requires 38 MJ or 10.5kWh of energy (Johansson, 2001).

The N compounds are of pronounced interest, due to the fact that they can play an important role in plant growth (Paredes at el., 2007). On the other hand, removal of N compounds is required in most of European countries in order to reduce the input to water bodies or the sea because this element can trigger eutrophication, in waterways causing negative effects on invertebrate and verterbrate species, including humans (Heeb, 2007). The removal of N can be done by physicochemical and biological processes. The biological processes were adopted in the favour of physicochemical processes, because they are more efficient and less expensive (Ahn, 2006). The common biological process for N removal is by nitrification followed by subsequent denitrification. In the last few years new and cost-effective processes of N removal have been developed such as: partial nitritation, nitrifier denitrification, anaerobic ammonium oxidation (Annamox), single reactor systems for high ammonification removal over nitrate (SHARON), completely autotrophic N removal over nitrite (CANON), oxygen limited autotrophic nitrification-denitrification (OLAND), and nitrification-denitrification via nitrite accumulation (Ruiz et al., 2006). An upcoming method that is receiving increasing interest is struvite precipitation where magnesium-ammonium-phosphate (often termed MAP) is precipitated as the mineral struvite (Ban and Dave, 2004; Baur et al., 2009).

Ammonium (NH_4^+) in natural water environments when exceeding the concentration of 0.2 mg/l is toxic to aquatic species and causes eutrophication (Kadlec and Knight, 1996; Tchobanoglous et al., 2003).

Between the N species (e.g. NH_4^+, NO_3^-, NO_2^-) very complex interrelationships exist as well as different transformation mechanisms and a number of environmental factors influence nitrification such as temperature, pH, retention time, DO, etc. (Paredes et al., 2007). Figure 1 shows the redox cycle for N, and different N-species depending on pH, temperature, DO and free ammonia (Brock et al., 1994; Paredes et al, 2007).

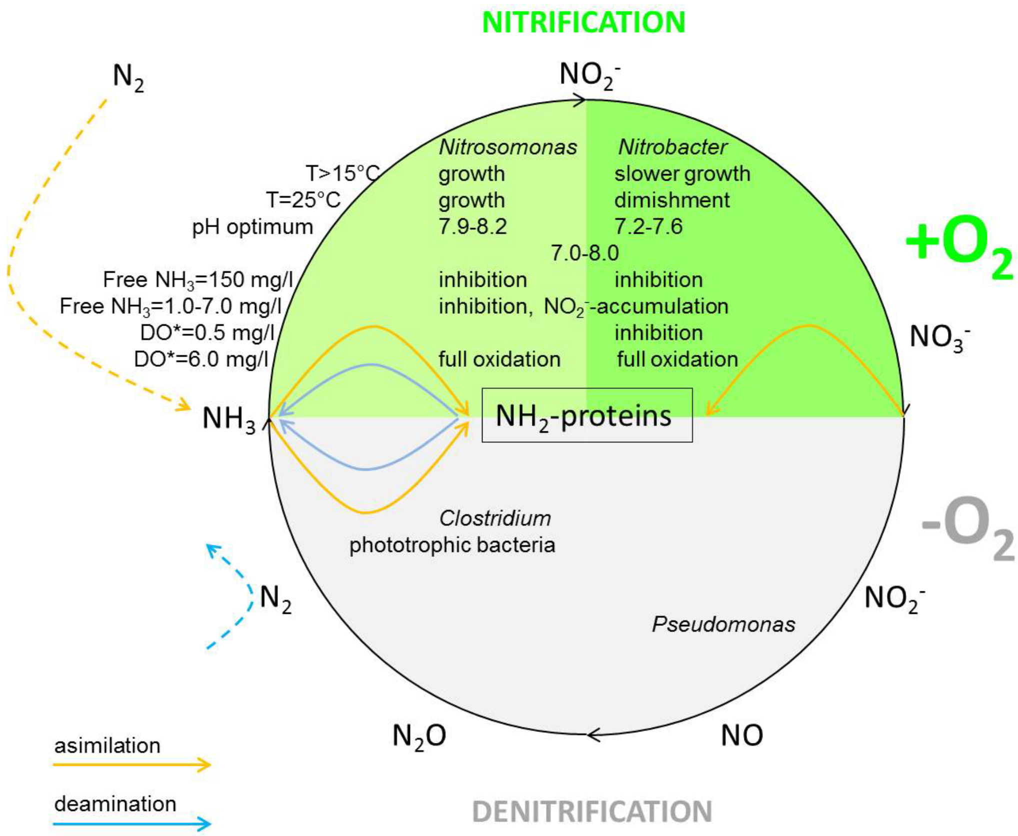


Figure 1*: Redox cycle for nitrogen. Effect of temperature, pH, free ammonia (NH_3) and dissolved oxygen (DO) on nitrification in wastewater. DO*- in suspended growth of biomass. Compiled from Brock et al., 1994 (nitrogen cycle and main bacterial representatives) and Parades et al., 2007.*

Phosphorus is an important component of all living cells, thus an essential nutrient to all living organisms, and particularly essential in food production for humans (Voet and Voet, 1995). P never occurs in pure form but is always bonded with other elements forming compounds, such as phosphate rock. The extraction of phosphorus is a nature-damaging process (strip-mining is most common method) having as result toxic by-products (arsenic, cadmium etc.). Because P is highly bondable, bonding with soil molecules renders most P unavailable for plant uptake until the soil is saturated with P; these results in a high demand on mineral P-fertilisers to increase the yields of arable land. On the other hand excess P is a problem in effluents from wastewater treatment plants because it contributes to eutrophication and is probably the main controlling element for eutrophication in freshwater (Ecosanres, 2005).

Recycling of P from wastewater is currently very limited (Corcoran et al., 2010). Ways of incorporating P into the sewage sludge exist, but the reuse of the latter is often restricted due to contamination of the sludge with pollutants as heavy metals and organic micropollutants. The plant availability of chemically precipitated phosphorus in sewage sludge is also very low when iron or aluminium salts are used as coagulants in P-removal processes (Krogstad et al., 2005).

Of the macronutrients N, P and K, P is the only element where the main sources are facing depletion. Studies show that at current rates of extraction, global commercial phosphate reserves will be depleted in 50-100 years, and the peak in phosphorus production is predicted to be shortly after 2030 (Cordell et al., 2009). The increase in world population and demand for food calls for a continuously increasing demand for phosphate fertilisers. Due to the fact that there are a small number of countries with minable P-resources, future conflicts regarding phosphate resources are likely to appear (Rosemarin, 2004). In order to avoid a future food-related crisis, phosphorous scarcity must be mitigated (Cordell et al., 2009).

1.1.2 Human excreta as a resource and pollution

During food digestion only a small proportion of the metabolized nutrients is taken up by the body, the rest is excreted, and end up in the wastewater. In this way they are often lost and cause adverse environmental impacts such as eutrophication (Corcoran et al., 2010).

In theory, the annual amount of nutrients in excreta of one person corresponds to the amount of fertiliser needed to produce 250 kg of cereal which is also the amount of cereal that one person needs to consume in one year (Wolgast, 1993). However, there are losses from the agricultural fields and in the processing of food so additional sources are needed. In the future human excreta together with animal manure will become the two most important sources of P-fertilizer (Vinnerås et al., 2006).

In industrialized countries the daily water use ranges from 150 to 250 litres per capita. The volume of our excreta is roughly 1.5 litres/capita/day (Todt et al., 2010). Thus our excreta (urine and faeces) constitute less than 1% of the wastewater volume. This 1% excreta contributes the majority of pathogens in wastewater (WHO, 2006) as well as about 90% of the N, 80% of the P and roughly 60% of COD (Meinzinger and Oldenburg, 2009). A logical consequence of this distribution of nutrients between the wastewater fractions would be to avoid mixing of our excreta with a lot of water and collect the excreta separate from the rest of the wastewater (Jenssen et al., 2009).

Release of untreated human excreta (often termed blackwater) into water, as is the common practice today, causes eutrophication of surface water and contamination of underground water bodies, leading to spreading and propagation of pathogens, causing outbreaks of diseases, as hepatitis (Grove et al., 2006) and cholera (Smith and Perdek, 2003; Gerba and Smith, 2005). The fraction of wastewater, represented by human excreta, should therefore, ideally, be removed from the wastewater before discharge into the aquatic environment.

1.2 Treatment options for blackwater

Over the last couple of decades several solutions for treating the BW fractions separate from the greywater (GW, wastewater from kitchen and shower) have been developed (Jenssen and Skjelhaugen, 1994; Werner et al 2004; Jenssen et al., 2004). Treatment of each fraction is efficient because it can be adapted to its specific composition (Vinnerås, 2001). Human excrements can be divided in liquid and solid fraction. The liquid fraction represents urine and flush water, and solid fraction is faeces. Faeces are also often called brown water, urine – yellow water, and together with GW represent the new colours of sanitation. Toilet wastes (urine, faces and toilet paper) which are collected and kept together are called blackwater (BW). The technology used for BW treatment depends on the type of toilet used (Jenssen et al., 2004). Common for all toilet types used in source separating systems is that they use very little or no water, so substantial water savings can be obtained (Jenssen et al., 2004).

Urine is the most nutrient rich fraction of the wastewater (Figure 2) thus, toilets that separate urine from faeces and facilitate urine collection have been developed.

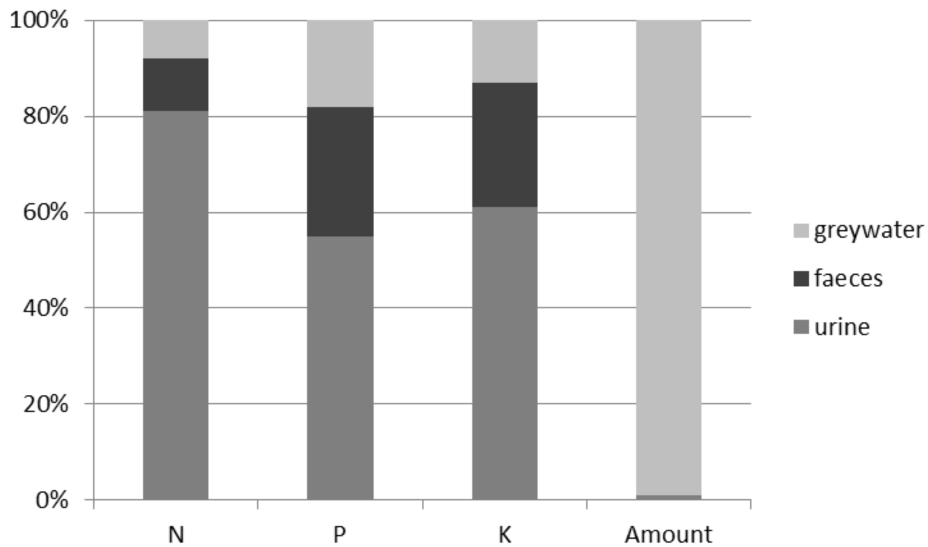


Figure 2*: Percentage content of nutrients in domestic wastewater and amounts of produced wastewater fractions (Jönsson et al., 1999).*

Urine is sterile if collected from healthy humans, but some faecal contamination is unavoidable in urine diverting toilets (Vinnerås, 2001), thus urine does not need sophisticated treatment to reduce the bacteria concentration to acceptable levels. Six months storage before use in agriculture is the requirement from WHO (2006). During storage urea ($(NH_2)_2CO$) is converted to ammonia (NH_3). This reaction increases the pH up to about 9. The increase in pH and the fact that NH_3 is toxic to many bacteria are the main reasons for reduction in the pathogen levels (Camargo and Alonso, 2006). Recycling of urine does not call for sophisticated treatment (WHO, 2006). In the urine diverting toilets the faecal fraction can be collected dry or flushed down with water, usually 4 litres.

Composting or dry toilets collect urine and faeces without additional use of water (WHO, 2006; Morgan, 2007). Such toilets may be equipped with urine diversion or they process both urine and faeces. Composting toilets are used in cabins or remote/rural areas without connection to the sewerage treatment works. Modern compost toilets have the advantage of high biodegradation rates, zero discharge of water and the sanitized compost that can be used as fertilizer in the agriculture. The disadvantages of these systems are more maintenance for the user compared to a water toilet, loss of N during processing of the compost if urine diversion is not practiced, and odour if the ventilation system is not properly designed. However, international research shows that dry sanitation may give an equal or higher reduction of pathogens than a water toilet and a high reduction in risk of exposure (Stenstrøm, 2002). The main reasons for limited use of composting toilets are probably the user friendliness and the fact that they are different from the traditional water toilet. It is also very difficult to connect several compost toilets in a building to one central processing unit (Lopez Zavala et al., 2005). To sanitize the residues from a traditional composting toilet 6-24 months is needed depending on temperature (WHO, 2006).

Vacuum toilets that collect BW are very similar to traditional water toilets, but they use only 0.5-1.5 litres per flush. They offer more traditional comfort than compost toilets, as well as low water use (Jenssen et al., 2004). When using toilets that use a small amount of water for flushing, such as vacuum toilets, the concentration of nitrogen, phosphorus and potassium increase in BW compared to BW from the toilets with a classic flush system, while the hydraulic load of BW can be significantly reduced using the low flush options. BW from vacuum toilets has also specific characteristics in terms of size and distribution of particles (Couillard, 1994, Lens et al., 1994, Gajurel et al., 2003). However, various aerobic and anaerobic systems for BW treatment exist for source-separating toilets (Skjelhaugen, 1999; Vinnerås, 2002).

Without taking into account the complexity of the system (energy), anaerobic treatment systems for BW treatment often have advantages over aerobic ones (van Voorthuizen et al., 2008). In the anaerobic BW decomposition methane is produced (biogas) with less sludge. Anaerobic treatment of BW performs best at relatively high temperatures (30-40°C) being the largest weakness of these systems (Wendland, 2008). Another method of treating BW is a combined anaerobic membrane bioreactor (MBR) with a sludge blanket reactor and an aerobic MBR (van Voorthuizen et al., 2008). It is important to stress that chemical oxygen demand (COD) in the effluent of this treatment systems is still relatively high and may inhibit physical and chemical processes required for decomposition of organic compounds, indicating that such systems are struggling with the high COD concentrations. MBR systems are usually too expensive and too sophisticated for use (Masi et al., 2010).

1.2.1 Use of natural organic filters

Small scale decentralized activated sludge treatment plants or similar package treatment systems are difficult to control in terms of sludge bulking and peak loading events (Johannessen, 2012). This can result in significant TSS loads in the effluent and an appropriate post-treatment is needed (Kujawa-Roeleveld and Zeeman, 2006). Organic filter systems are a promising pre-treatment method for reduction of TSS and nutrients in BW (Gajurel et al., 2003; Taylor et al., 2004). Organic filter media such as bark, sawdust and especially peat, have shown good filtration and adsorption effects for particles and nutrients (Lens et al., 1994; Kõiv et al., 2009). Peat is a light-weight media, easy to use and provides a large surface area with high capacity to retain different types of organic pollutants (Novoselova and Sirotkina, 2008). Particle retention capacity and TSS removal rates of more than 90% have been reported for peat filters (Corley et al., 2006). Other media such as bark has also been reported to have high particle retention (Lens et al., 1994). In opposite to the challenge of removing dissolved nutrient fraction, particle-bound nutrients can in principle be relatively easy removed with simple physical treatment methods such as sedimentation and filtration and the filter residue can be processed further into a hygienized dried sludge or compost product (Koohestanian et al., 2008). In using low flush toilet system such as vacuum toilets the concentration of N, P, and K increases compared to classic flush toilets while the hydraulic load of the BW stream can be substantially reduced. This can counteract some hydraulic shortcomings that have been reported when using organic filters (Couillard, 1994; Lens et al., 1994; Gajurel et al., 2003). However, hydraulic conductivity and structural stability of the filter media are crucial issues when using organic filters (Couillard, 1994). Many studies have been conducted on the application of organic filters in the treatment of domestic wastewater. However, due to limitations in the hydraulic capacity such filters have not gained widespread use so far. A proper selection and assessment of available filter media is therefore a key factor in the development of organic filter systems (Couillard, 1994; Lens et al., 1994; Gajurel et al., 2003).

One of the main challenges in BW treatment is the high concentrations of TSS, COD, and nutrients. Many of the available high tech-solutions are difficult to apply for BW treatment because the majority of them were developed for relatively low concentration municipal wastewater (Winker et al., 2009).

1.2.2 Composting

Composting of BW is a suitable way to sanitize the material, to recycle nutrients, to eliminate odours and to reduce disposal areas. Due to its rich content in nutrients and organic matter, BW compost can improve the soil structure and raise the tolerance of plants to salts (Smith et al., 2001; Engel et al., 2001). Adding compost to soil also improve the plants tolerance to water stress as well as improve the utilization of added nutrients (Baumeyer, 2003). Several studies have been performed regarding composting of source separated faeces and mixtures of urine and faeces. Niwagaba et al. (2009a, b) found that faeces separated from urine, and collected with ash, were sanitized when the temperature was kept at persistently 50°C for two weeks and the entire material was turned at least four times. A mixture of BW compost with sawdust was used as a soil conditioner for *Jatropha curcas* (purging nut) (Triastuti et al., 2009) with good results. Kim et al. (2010) found out that the aeration rate was an important parameter affecting nitrogen dynamics during composting of BW solids (solid part of the BW that remains after separation from liquid) mixed with sawdust, by controlling the ammonification, the ammonia emission and the nitrification processes. The authors also reported that at high aeration rate (500, 750 and 1100 l/h) a considerable amount of nitrogen, emitted in form of ammonia, is lost (75-94%). Among the disadvantages of composting are possible loss of nutrients, nitrogen especially, which could become a biohazard for staff at the composting plants (Hotta et al., 2004).

There are several crucial steps which have to be taken into consideration prior to composting: (1) mixture composition and texture to ensure equal distribution of particles, (2) sufficient moisture to support biochemical reactions, (3) air exchange to prevent anaerobic conditions, (4) and proper setup to ensure fast thermophilic composting and sanitation of material (Hotta et. al., 2004; Himanen et al., 2011).

1.2.3 Blackwater treatment by separation

BW collected by vacuum of extremely low flush gravity toilets contains a mixture of urine, faeces and toilet paper. BW can be treated both aerobically and anaerobically as mentioned above (section 1.2).

An efficient method of treatment, under the umbrella of the principle of "closing the loop" is separation of BW. Source separation of wastewater is a new approach to wastewater handling (Jenssen et al., 2009; Jenssen et al., 2010; Bulc et al., 2010). The separate treatment of waste fractions facilitates recovery and reuse of nutrients, water and organic material, and also offers ecological and economic benefits for the human society (Jenssen et al., 2004). Sanitized and stabilized end products can be used in agriculture as fertilizers (Heinonen-Tanski and van Wijk-Sijbesma, 2004).

1.3 Separation of wastewater at touristic and sensitive remote areas

Untreated wastewater discharged into aquatic environment, especially in more sensitive aquatic environment and sensitive areas can have different levels of impact. There is a large need for improved sanitary systems to meet the growing requirements in sensitive areas without sewage systems. To this date the market lacks compact small-scale treatment systems utilizing separate units to process BW and GW, and most current wastewater treatment systems treat a mixture of BW and GW in the same unit. A large number of such systems already exist on the market (Johannesen, 2012). Some of these systems require chemicals to operate properly and with few exceptions the energy consumption needed to run the system is high, and they are not designed to operate on solar power. Other disadvantages of the existing compact package systems are unstable purification performance and poor pathogen removal (Johannesen et al., 2007; Yri et al., 2007). Interestingly, Anastasi et al. (2010) demonstrated that some strains of uropathogenic *Escherichia coli* survived treatment stages of sewage treatment plants. Later it was shown that in waters near to the sewage treatment plants a high percentage of environmental *E. coli* was carrying virulence genes (Anastasi et al., 2012). This demonstrates the need for treatment of BW before discharge into aquatic environment, especially in more sensitive aquatic environment and sensitive areas. Microbial presence and organic load are among the key parameters that must be taken into account before releasing treated wastewater into the environment (Khopkar, 2004). Efficient monitoring is often expensive, time consuming and not easily executed, and demands training of laboratory staff (Khopkar, 2004). Currently there are a few commercially available biological kits which require little equipment, are easy to use, and the results of which are easy to read, thereby simplifying the procedure (e.g. 3M™, Petrifilm™ E. coli/Coliform Count, Bio-Rad RAPID'E. coli2, Colichrom-NPS). One of the most important parameters to monitor is coliform bacteria and *E. coli*.

Most of these systems are therefore not able to meet the strict discharge requirements needed in vulnerable areas without expensive and sometimes large polishing and/or disinfection units. In the EU Directive for urban wastewater (91/271/EEC) the biohazard issue is not properly emphasized, especially concerning the risk for pathogen transmission to humans. That is why parameters concerning human health need to receive more focus in relation to effluent quality.

This thesis tests components and processes of a treatment system (Figure 3 and 4) in Salina Nature Park in Slovenia that close the material flows through source separation.

2. HYPOTHESIS

The specific hypotheses of this thesis are listed below and the layout of the pilot scale system at Sečovlje Salina Nature Park is illustrated in Figure 3:

- Organic filters composed of peat or peat mixed with sawdust, peat mixed with bark, or bark remove more than 50% particulate matter measured as total suspended solids (TSS) when filtering blackwater (BW).

- Efficient removal of TSS and nutrients in the BW is obtained by separation in organic filters and recirculation in a biofilter.

- Adding peat, sawdust, bark, cereal bran, oat husks to BW is an important factor for enhancing both the BW composting and sanitizing.

- The novel source separation system tested in this work yields a pathogen free and nutrient rich end-product suitable for agricultural purposes.

- Commercially available agar plates, RIDA®COUNT plates, can be used for monitoring of microbial indicator groups (e.g. *E .coli* and Enterobacteriaceae) in concentrated wastewater as BW.

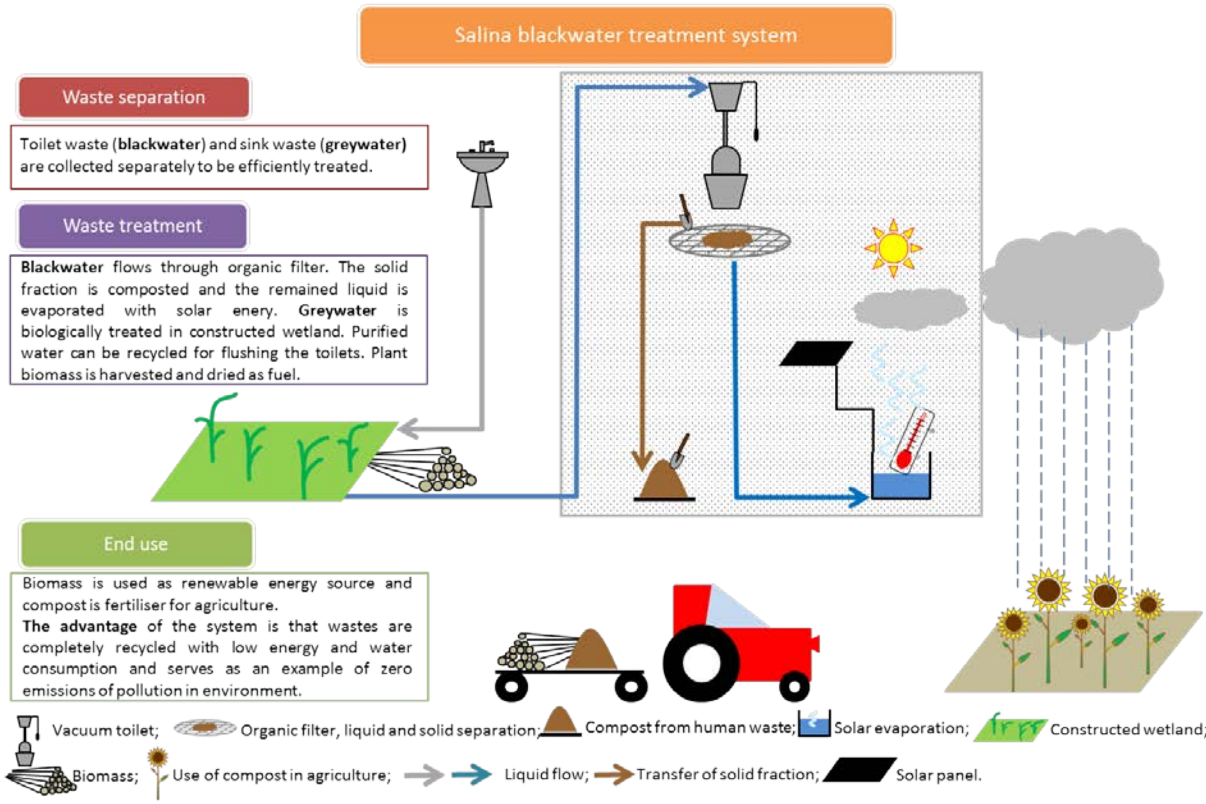


Figure 3: *Conceptual framework of the study. A grey frame indicates modules that were studied in detail.*

2.1 Structure of thesis

The thesis comprises of three interconnected parts: blackwater separation and liquid fraction treatment; blackwater solid fraction treatment and a section dedicated to bacteriological monitoring. The thesis starts with an introductory chapter giving a background about sustainability, nutrients, especially N and P, blackwater and its treatment options, followed by a list of hypotheses (Chapter 2). Forward, in the methodology chapter (Chapter 3), are presented in detail experimental setups and analytical methods in the following order: laboratory scale experiment and subsequently their application on pilot scale. Following the methodology chapter is a results and discussion chapter (Chapter 4) where the results from each experiment respecting the order given in the methodology are presented and discussed, as well as future application, advantages and disadvantages of the presented processes in the frame of this system. Overall conclusions and a summary of the PhD project are given in a Chapter 5 and 6, respectively.

3. MATERIAL AND METHODS

3.1 Pilot scale experimental setting

In the study two sets of experiments were conducted, under laboratory conditions and on a field as pilot scale experiments. Generally, before the pilot scale experiments, research objectives (chapter 1.5) were addressed first on a laboratory level. These results gave a frame to optimize the pilot level experiment in Salina Nature Park.

The system used in this study was located in a sensitive Mediterranean coastal area in northern part of Adriatic Sea in Slovenia - Sečovlje Salina Nature Park (45°29′32.12″ N; 13°36 ′32.94″ E). Efficiency of pollutants removal, recovery of nutrients and water saving from sanitary wastewater on a pilot scale was tested because of special requirements of the place: (1) no emissions of pollution allowed, especially because of traditionally harvesting of salt from the sea, (2) strong seasonal fluctuation in number of guests and (3) wastewater load which is typical for tourist infrastructures.

Data from Slovenia indicate peaks in popular vacation periods that can result in more than fivefold number of guests than the annual average. Summer peaks of approximately 15000 visitors compared with annual average of 35000, and the fact that the facility is situated at the sea side in a protected area indicates a strong need of water treatment and nutrients recycling. Preliminary studies on GW using hybrid treatment wetlands (TW) were also performed at pilot scale in Salina Nature Park, but BW treatment and recycling was the main focus of the research presented here.

The system design was based on separate treatment of BW and GW (Figure 3). The prototype was designed based on the laboratory-scale experiments of BW filtration with different organic filter media (described in chapter 2.2) and based on tourist visits from Salina Nature Park. High peaks of BW were expected in spring and autumn, while low peaks were expected in winter.

The system consisted of different treatment modules:

- Blackwater module was composed by peat filters (PF). The BW module was treating the BW from vacuum toilets. It consisted of two 80 l containers filled with commercially available organic media (peat) where separation of solid and liquid fraction took place. The PF is based on the same principles than so called rottebehälter developed in Germany (Peter-Fröhlich et al., 2007). It is a filter using an organic media for supporting particle, nutrient retention and decomposition. At low loading rates both processes can take place at the same time.
- Biofilter module (BF) – after separation of liquid from solid in BW module, the liquid fraction passed over one 80 l container filled with expanded clay and recirculates for two hours per day.
- Evaporation module – after the liquid circulation terminates in BF, the liquid fraction flowed to the evaporation module where was heated up (>60°C) and was finally evaporated in the environment. The evaporation module had five solar panels (vacuum collectors), a solar evaporation tank where the water was heated up and a cascade dryer which supported the evaporation process.
- Compost module consisted of compost reactors. BW solids (solid fraction of BW which resulted after separation of the liquid fraction) in two 80 l containers from the BW module were composted to obtain a pathogen free and nutrient rich end-product for agricultural purposes.
- Greywater module was a treatment wetland which treated the GW from sinks in a hybrid treatment wetland (horizontal and vertical). The horizontal treatment wetland was 20 m^2 (8 m long, 2.5 m width, depth 0.5 m) and the vertical treatment wetland was 9 m^2 (3 m long, 3 m width and 0.5 m deep). The hydraulic load was of 0.5 cm d^{-1} and continuous. The hybrid treatment wetland was filled with sand media of 2-4 and 8-16 mm. Microbial biofilm in association with roots of common reeds (*Phragmites australis*) stabilized and enhanced the treatment process of GW. The treatment efficiency of GW expressed as reduction of TSS, NH_4^+ and BOD_5 was 46%, 74% and 89%, respectively (Bulc et al., 2011).

The containers used for BW separation, recirculation and compost were adapted waste containers (80 l capacity, conic shape, inner dimensions of 35 cm x 34 cm at the top, 32 cm x 32 cm at the bottom, back side height 72 cm and front side 83 cm).

Samples were taken weekly at selected points (Figure 4) from the beginning of March 2011 till the end of September 2011. Number of flushes was counted weekly by programmable logic controller system (PLC). At BW module sampling was performed in two replicates at the inflow and at the outflow of PF and in outflow at BF. At compost module sampling was performed in two replicates. The evaporation tank represented the sampling point of the evaporation module (Figure 4). The physicochemical and microbial parameters and methods are described in detail at each experiment individually.

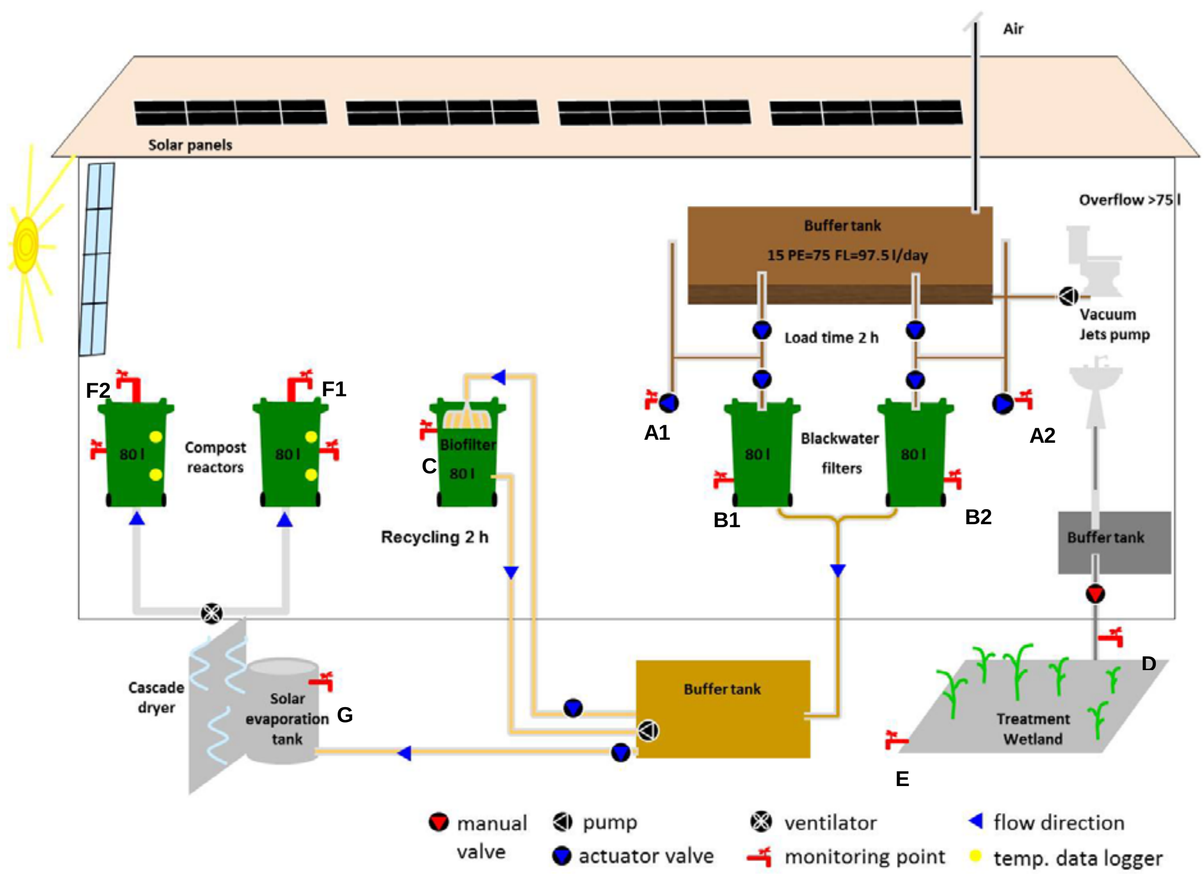


Figure 4: *Concept of the prototype with a source separating sanitation system at Sečovlje Salina Nature Park in Slovenia and sampling points: A1, A2- inflow into peat filters; B1, B2- outflow from peat filters; C- biofilter; D-inflow to treatment wetland; E- outflow from the treatment wetland; F1, F2- compost reactors; G- solar evaporation tank.*

3.2 Blackwater separation with organic filters and liquid fraction treatment

3.2.1 Organic filters – a laboratory scale experiment

Experimental design

Two laboratory scale experiments were performed using four types of organic filters and two types of wastewaters.

First experiment was done for assessing particulate matter retaining capacity of four locally available organic filter media: ground bark of conifers (pH 5, particle size of 0-40 mm, degradation rate H1-H2), pure peat (pH 3-4, particle size of 0-20 mm, degradation rate H2-H5), peat-ground bark mixture (50% of pure peat and 50% of ground bark of conifers) and peat-sawdust mixture (80% of pure peat and 20% of wood shaving mixture, pH 3-4, particle size of 0-20 mm) for sequencing batch reactor (SBR) sludge mixed with raw municipal sewage (for simulating potential storm loads typical for remote tourist facilities).

The second experiment was conducted in collaboration with colleagues from Life Science University, Aas, Norway, to assess particulate matter retention capacity for settled BW on peat-sawdust mixture, the most efficient filter-material in the first experiment. The results from this experiment were compared with peat/sawdust mixture from the experiment with SBR mixture. The experiment with settled BW used the same experimental set-up and analysis methods as in the experiment with the SBR mixture (Figure 5). The settled BW has been taken from a student dormitory. Before the settling tank the BW has passed a macerator pump (Jets™ vacuum sewer system). To assess the effect of filter depth, two columns have been filled with 30 cm and two columns with 15 cm of filter media. Due to a small number of samples (n<24) and particular slight deviation from normality a Wilcoxon signed rank tests (Wilcoxon, 1945) have been chosen for comparison of samples.

Four columns each having a cross sectional area of 0.0314 m^2 were used. Three were filled with selected organic filter media while one was used as a control (Figure 5). The columns were made of Plexiglas® and equipped with 1000 µm woven textile nets (Sefar PETEX® 07-1000/45) at the bottom.

The SBR effluent was pumped from the SBR to the distribution system which consists of high density polyethylene pipes or verticals (PEHD) with an inner diameter of 26 mm. The four columns were 1.58 m long with a control valve (nominal diameter of 25 mm) on the bottom and an overflow on the top site of the pipe. The filter systems were dosed periodically controlled by a PLC, Moeller Easy 820-DC-RC Controlunit, Germany (Table 1). When the SBR effluent was pumped into the system the valves were closed. The SBR effluent was pumped into the system for about 2 minutes and 40 seconds. When all the columns were full the surplus water overflowed back into the SBR. The air release valve enabled the pipes to empty completely. After the pump stopped the actuators started to open the valves and emptying the verticals. Opening and closing the valves took 2 minutes (1 minute to open and 1 to close). Each column received 22.5 l of wastewater per day divided into 3 intervals of 10, 5, and 10 loadings (simulating toilet flushes), with a total hydraulic loading rate of 71 cm per day. The amount of each loading was 0.8 l that corresponds to an average toilet flush of the vacuum toilets (Jets™). This loading regime has been selected in terms of simulating diurnal patterns in a household wastewater stream. Below the columns the filtrate was collected in 25 l containers prior to analysis.

Table 1*: Pumping intervals, dozing and number of flushes in laboratory experiment with organic filters.*

Interval	Time	Number of flushes	Interval between flushes (min)
1	8:00-9:00 am	10	5
2	11:00-12:00 am	5	12
3	14:00-15:00 pm	10	5

Monitoring and analysis methods

Degradation rate (H) of organic filter media was analysed according to VON POST (DIN 11540). Considering H1-H4 poorly decomposed media, H5-H6 medium decomposed media and H7-H10 strongly decomposed media, the organic filter media had following characteristics: pure peat had pH of 3-4, particle size 0-20 mm and degradation rate H2-H5; pure bark had pH of 5, particle size 0-40 mm and degradation rate H1-H2; part and sawdust mixture had pH 3-4, particle size 0-20 mm and degradation rate H2-H5.

The SBR mixture was filtered through 30 cm thick organic filters composed from above mentioned filter media. For each filter media a separate experiment was conducted.

A composite sample of 25 flushes from the inflow and outflow of the filter columns were taken daily for measuring TSS (SIST ISO 11923). A randomly selected number of samples have, in addition, been analysed for particle size distribution using woven textile nets (Sefar PETEX® 07-1000/45; 07-100/32; 07-10/2) in size of 10, 100 and 1000 µm. At the end of each trial the accumulation of mineral material was assessed with loss of ignition (LOI) (ASTM D7348-08) for all filter media. The trials in each set were stopped when clogging occurred after 10 to 14 days.

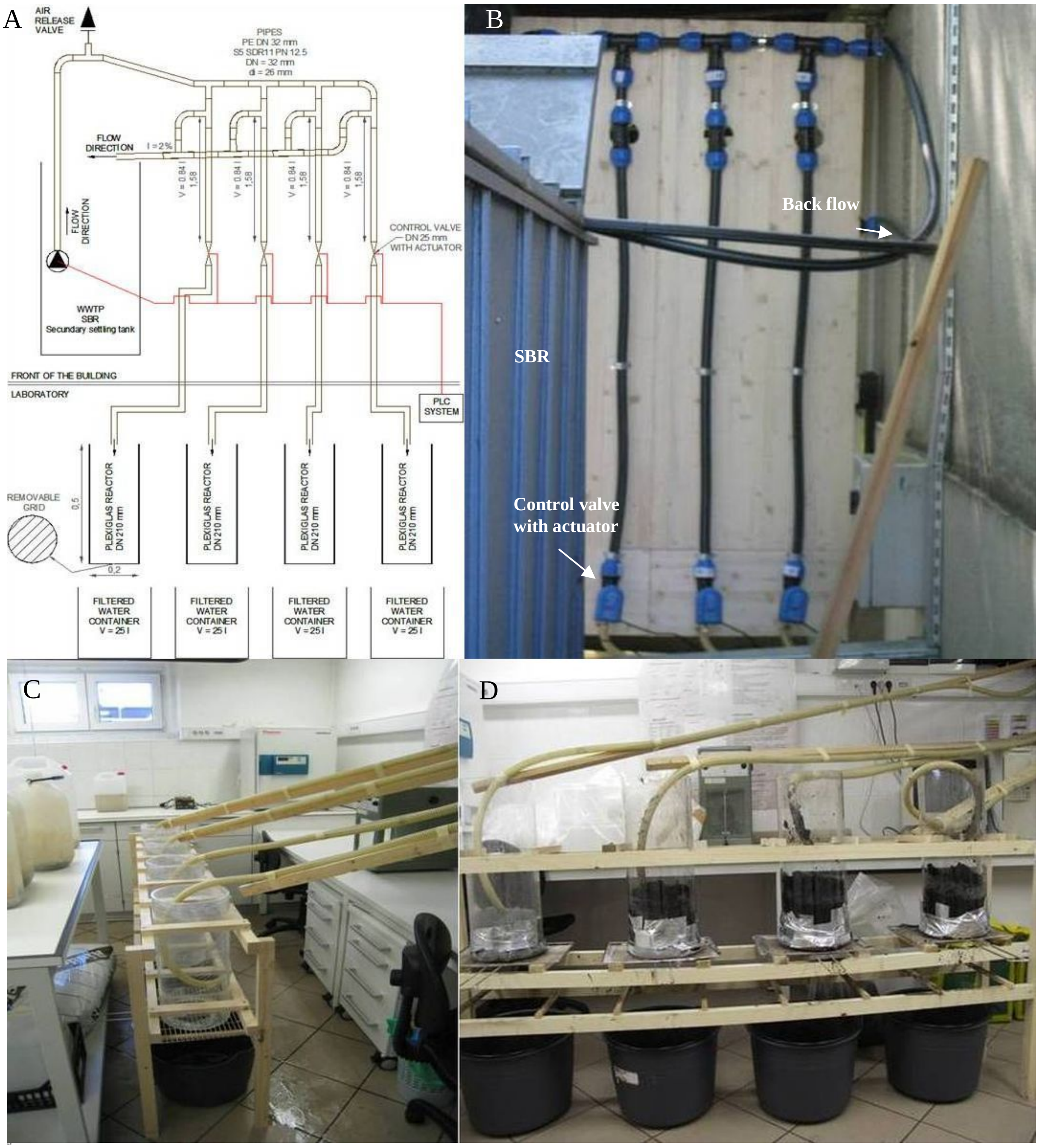


***Figure 5**: Secondary settling tank (SBR) with system of pipes with valves (A, B) and experimental columns loaded with SBR mixture (C, D).*

3.2.2 Organic filters and biofilter – a pilot scale experiment

Experimental design

The BW was pumped with a vacuum pump from 2 vacuum toilets into a 400 l storage tank (buffer tank). The BW in the buffer tank was permanently mixed with a small submerged pump. The designed daily load was 76 flushes (38 flushes/bin) or 97.5 l/day or 2.25 kg BOD_5/day. These calculations were based on the assumption that one person produced 1.3 l of BW (0.8 l of tap water and 0.3 l of faeces and urine) and 50 % of 60 g BOD_5/day due to urine dilution. Based on the laboratory-scale experiments (see chapter 2.2) and assumption that sludge will contain on average 1% TSS (10g TSS/l) and taking into account that 50 % of the load will be urine the necessary filter area for foreseen hydraulic and pollution load was calculated to be 0.2 m^2 (approximately 0.1 m^2 per bin; 0.102 m^2 at the bottom and 0.119 m^2 at the top). Therefore two filters (adapted waste containers) were constructed and installed for filtration purposes. They were filled with 15 cm layer of peat (chapter 2.2 for description) and placed in two sludge dewatering bags (Figure 6).

A B

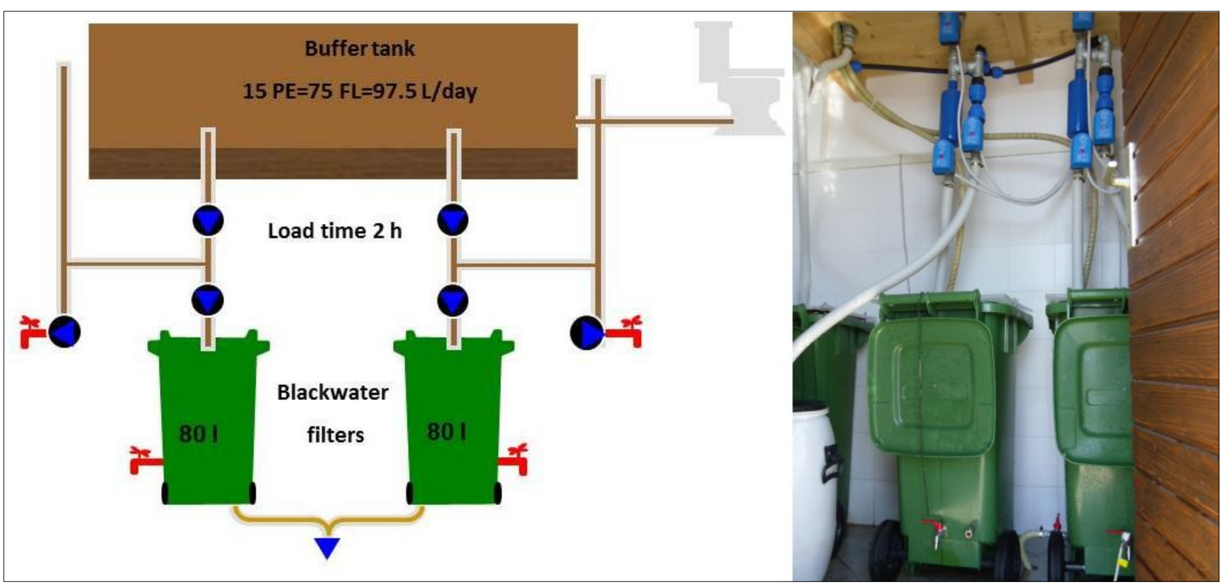


***Figure 6**: Buffer tank and peat filters (A- sketch; B- in situ installation) at pilot scale in Salina Nature Park.*

BW liquid fraction was flowing from the PF separation unite into a buffer tank of 200 l placed in a special constructed shaft (Figure 7).

A B C

***Figure 7**: Buffer tanks for blackwater in constructed shaft before (A, B) and after operation (B) outside of the building of the sanitary system in Salina Nature Park.*

From here the liquid fraction was pumped into the BF (Figure 4 and 8) filled with expanded clay (approx. 70 l) with particle size of 4-8 mm to 8-16 mm, Melk, Germany. In BF the BW liquid fraction was recirculated within expanded clay for at least 2 hours interval daily (11am-13pm). The regime of the pump was changed and optimized regarding performance efficiency. The pump operated in a continuous mode, although it could be interrupted if there was not enough water due to small number or no visitors in the Park. After the treatment cycle, the BW liquid fraction (at 5am) flowed into the solar module to be disinfected and evaporated.

A B

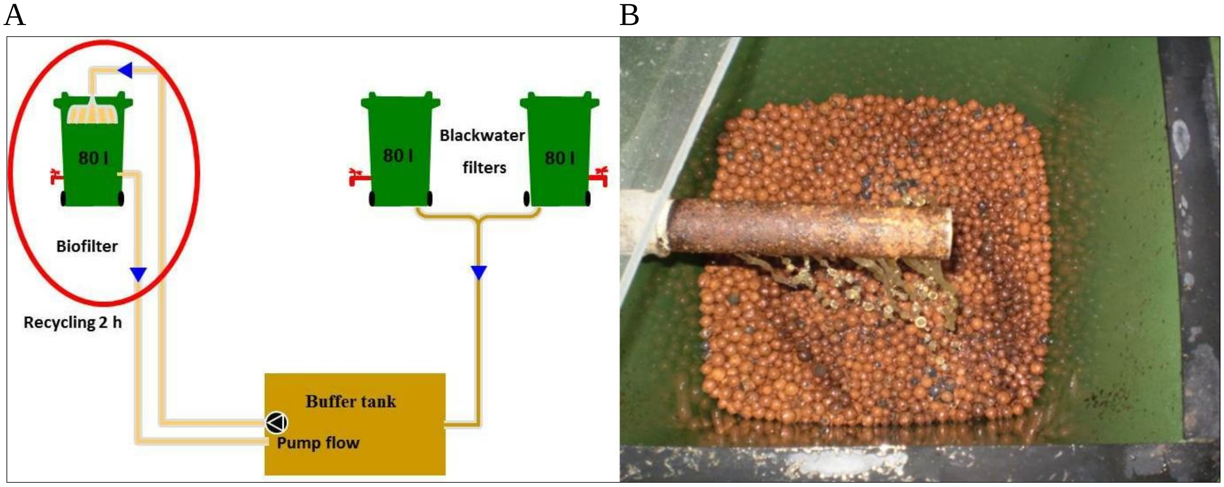


***Figure 8**: Biofilter (A- sketch; B- in situ installation) at pilot scale in Salina Nature Park.*

The evaporation module (Figure 9) comprised the following components:

- Solar panels (five vacuum collectors, 3.12 m^2 per collector), empty weight 63 kg, stagnation temperature 192 °C. The vacuum collectors were mounted to the roof of the sanitary facility;
- Solar evaporation tank with a 140 l volume, height of 1500 mm, outer diameter 820 mm, insulation – hermetically welded with outer shell. The tank was installed in front of the sanitary facility;
- Cascade dryer, 254 mm length, 5 layers, fleece layer dimension of 1000 mm. The cascade dryer was set near the tank in front of the sanitary facility.

The solar panels give energy to heat up the treated BW in the solar evaporation tank to the temperature of 62°C for at least 30 min what is enough for pasteurization (Safapour and Metcalf, 1999). In this first step, pathogenic microbes are reduced, thereby disinfecting the water (Safapour and Metcalf, 1999) for safe release into the environment. In a second step, to increase the evaporation that was already taking place in the tank, the water was pumped into a cascade dryer. The cascade dryer was connected to the vacuum collectors by a heat exchanger. The heat exchanger was located behind the solar evaporation tank and transformed the unused energy to hot air. The air was provided to the cascade dryer and supported the evaporation process. The evaporation process within the cascade dryer was also intensified by five layers of fleece which increased surface that serves evaporation. The air carrying the vapour passed through a condenser, further on through an air filter to be finally led off to the environment. In this way the disinfected liquid fraction of BW was efficiently volatized to reach zero effluent goal.

With the favourable climatic conditions in Slovenia with a solar irradiance of 350-450 W/m^2 and average daily summer temperature of 18°C it is aimed to evaporate all wastewater reaching the evaporation module.

A B

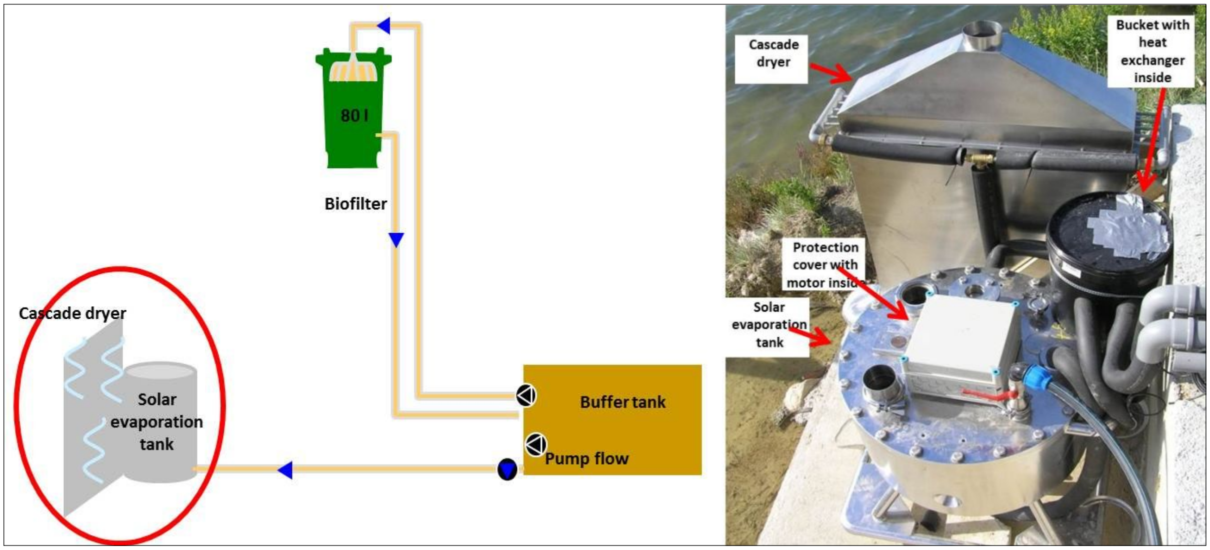


***Figure 9**: Evaporation module (A- sketch; B- in situ installation) at pilot scale in Salina Nature Park.*

Monitoring and analysis methods

Samples were taken weekly for a period of 7 months. Number of flushes was counted weekly by PLC. The organic filters were sampled in two replicates at the inflow and at the outflow and the BF in one replicate. The evaporation module was sampled only for microbial parameters.

In both PE and BF, in the BW liquid electrical conductivity (EC), dissolved oxygen (DO), temperature (T°C) and pH were measured on site with transportable multimeters. The samples were stored in a cooler bag and transported to laboratory for analyses. In each sample total suspended solids (TSS), ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), ortho-phosphate (PO_4^--P), total phosphorous (TP), chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were analysed according to standards described in Table 2. At the end of experiment, LOI was analysed in three different layers in the organic filters.

Table 2: *Environmental parameters measured at the pilot experiment in Salina Nature Park with reference to international (ISO), European (EN) or German (DIN) standardized determination method. The properties in bold were measured during field sampling.*

Parameter	Unit	Details	Method
Electrical conductivity (EC)	µS/cm	Multimeter HACH HQ40d	Electrometric
Dissolved oxygen (DO)	mg/l	Multimeter HACH HQ40d	Electrometric
Temperature	°C	Multimeter HACH HQ40d	DIN 38404-C4
pH		Multimeter HACH HQ40d	ISO 10523
Total suspended solids (TSS)	mg/l	Analytical scale Mettler	SIST ISO 11923
Ammonium (NH_4^+-N)	mg/l	Spectrophotometer HACH DR 2800	ISO 7150-1,2
Nitrite (NO_2^--N)	mg/l	Spectrophotometer HACH DR 2800	SIST EN 26777
Nitrate (NO_3^--N)	mg/l	Spectrophotometer HACH DR 2800	SIST ISO 7890-1
Orthoposphate (PO_4^--P)	mg/l	Spectrophotometer HACH DR 2800	ISO 6878–1
Phosphorus total (TP)	mg/l	Spectrophotometer HACH DR 2800	ISO 6878–1
Chemical oxygen demand (COD)	mg/l	Spectrophotometer HACH DR 2000	ISO 6060
Biochemical oxygen demand (BOD_5)	mg/l	Manometer WTW Oxi top	SIST EN 1899-2

Liquid and solid BW samples for microbial analyses were taken during operation of the system.

To evaluate the efficiency of microbial reduction in the liquid BW fraction from the solar evaporation tank and biofilter total bacterial count, total coliforms, faecal enterococci, staphylococci and *E. coli* were screened and evaluated using direct count on agar plates (CFU/ml). In the case of *E. coli*, after cultivation on DEV ENDO agar (Table 3) the fluorescent colonies surrounded by red precipitation zones were presumptive *E. coli*, which were later confirmed/refused by a biochemical test API 10 S (BioMerieux). Total coliforms and faecal enterococci concentration was not determined using the membrane filtration technique as is usual procedure in ISO Standards, but adapted to direct count plate (Table 3). Prior media inoculation, samples were serially diluted in physiological solution (0.9% NaCl) as follows: total bacterial count from 10^{-3} to 10^{-5}, from 10^{-1} to 10^{-4} for total coliforms, from 10^{-3} to 10^{-5} for faecal enterococci, up to 10^{-2} for staphylococci and *E. coli* (Table 12). Next step was mixing of diluted samples with a vortex, and application of appropriate dilutions in triplicates on the media.

An additional evaluation of microbial reduction was done in liquid fraction from PF, BF, solar evaporation tank, greywater from treatment wetland, and in solid fraction from the compost module using RIDA®COUNT plates. RIDA®COUNT cultivation media were selected because they were easy to handle in conditions with limited laboratory equipment, and as so they were suitable for remote (tourist) facilities. RIDA®COUNT plates and their comparison with agar plating are described in detail, in a separate chapter (3.4).

***Table 3**: Microbial parameters analysed and methods used at pilot scale experiment in Salina Nature Park.*

Microbial group	Growth media	Enumeration method, Unit	Method/Description
Solar evaporation tank, biofilter			
Total bacterial count	Yeast extract agar	Direct count plate,CFU/ml	ISO 6222 1999
Total coliforms	VRB agar	Direct count plate,CFU/ml	ISO 9308-1 2000 modified for agar plating
Faecal enteroccoci	Slanetz-Bartley agar	Direct count plate,CFU/ml	ISO 7899-2 2000 modified for agar plating
Staphylococci	Mannitol Salt Phenol-red Agar	Direct count plate,CFU/ml	Method complies with the recommendations of the harmonised Method in the Ph.Eur. 5.6 and the USP 29 (Merk Microb. Manual)
Escherichia coli	DEV ENDO agar	Direct count plate,CFU/ml Biochemical tests API 10 S (BioMerieux)	
Peat filters, biofilter, solar evaporation tank, treatment wetland, compost module			
Total bacterial count	RIDA®COUNT Total Aerobic Count	Direct count plate, CFU/ml CFU/g	RIDA®COUNT Total granted AOAC Performance Tested Method status, license No. 011001 (http://www.r-biopharm.com)
E. coli/Coliforms	RIDA®COUNT *E. coli*/Coliform	Direct count plate, CFU/ml CFU/g	RIDA®COUNT E. coli/Coliform granted AOAC Performance Tested Method status, license number 070901 (http://www.r-biopharm.com)
Enterobacteriaceae	RIDA®COUNT Enterobacteriaceae	Direct count plate,CFU/ml CFU/g	System certified ISO 9001, ISO 13485
Yeasts and Molds	RIDA®COUNT Yeast&Mold Rapid	Direct count plate,CFU/ml CFU/g	Satisfactory growth on RIDA®COUNT Yeast&Mold Rapid: *Aspergillus niger* IFO4091, *Candida tropicalis* IFO0589, *Pichia anomala* IFO0142, *Rhodotorula glutinis* IFO0389 (http://www.r-biopharm.com)

3.3 Blackwater solid fraction treatment

3.3.1 Composting of blackwater solids – a laboratory scale experiment

Experimental design

A bench scale compost experiment was performed with mixtures of BW solids (solid fraction which remained after BW separation) and different bulk materials, namely bark (B), sawdust (S), peat (P), oat husks (O), shrimp waste (SW), and finally cereal bran (C) (Table 4). BW was collected from a student dormitory with 48 students served by vacuum toilets. The vacuum toilet and transport system (Jets^{TM}) contained a macerator and a dewatering unit rendering macerated BW with a dry matter content of approximately 14% (measured by drying overnight at 105°C) prior to storage at 4°C. The bulk materials were commercially available materials.

Table 4*: Details on bulk and filter materials used for composting and blackwater separation in laboratory and at pilot scale experiment in Salina Nature Park.*

Material	Use	pH	Particle size	Composition	Producer
Peat	Filter material, bulk material	5.5-6.5	Various	Sphagnum peat	Klasmann-Deillmann GmbH, Geeste, Germany
Bark	Bulk material	5	0-40 mm	Pine bark	Terrasan Hungaria Kft, H-2046, Törökbalint, Hungary
Bran	Bulk material	Not labelled	Not labelled	Wheat fodder, crude fiber (9.8%), moisture 14%	Mlinotest, Ajdovščina, Slovenia
Husks	Bulk material	Not labelled	Not labelled	Dry oat husks	Grain mill, Norway
Sawdust	Bulk material	Not labeled	Not labeled	Not labeled	Local wood industry

The characteristics of the separate substrates used in the composting experiment are summarized in Table 4 and 5.

***Table 5**: Characteristics of the bulk materials used in composting laboratory experiment (B- bark, C- wheat bran, O- oat husks, P- peat, S- sawdust, CON- control).*

Substrate	TS (%)	VS (% dry weight)	Composition
B	14.3	92.3	Pine bark
C	87.6	93.7	Wheat fodder, crude fodder (9.8%)
O	68.4	95.7	Dry oat husks
P	64.3	97.0	Peat
S	92.9	99.8	Sawdust
CON	14.3	92.3	Toilet waste

The bench scale compost reactors (see Figure 10) were designed to stimulate the process of composting in a self-heating mode, without stirring, using various combinations of the tested bulk materials. The different mixtures and amounts of each substrate are shown in Table 6. In order to decrease the water content of the BW compost it was necessary to establish a proper ratio of the bulking. The ratio depended on total solids and moisture of each component.

***Table 6**: Ratios of bulking materials and blackwater used for composting laboratory experiment (B- bark, BW- black water, C- cereal bran, O- oat husks, P- peat, S- sawdust, SW- shrimp waste).*

Mixture	Ratio (kg/reactor)	No. of reactors
OCE	1 BW+0.05 O+0.05 C	2
OCB	1 BW+0.025 B+0.05 O+0.05 C	3
OCP	1 BW+0.025 P+0.05 O+0.05 C	3
OCS	1 BW+0.025 S+0.05 O+0.05 C	3
PSW	0.60 BW+0.030 P+0.030 SW	2
CON	1 BW	2

15 vertical cylindrical polyethylene reactors with a volume of 2 l were designed for the second part of the experiments (height 32 cm, diameter 10 cm). To minimize the heat losses, the reactors were insulated in thick Styrofoam boxes (height 55 cm, width 60 cm) made of 11 layers of Styrofoam (thickness 5 cm). The reactors were air tight. A perforated PVC sieve (2 mm) was installed at the bottom of each reactor. Aeration of the reactors was supplied with up-flow aeration from the bottom of the reactors with pressure pumps (Trixie Ap120, 120L/h, Australia), controlled by a time switch (24 Hour - In Time Switch, UK). Each reactor had its own pump in order to avoid differences of air diffusion due to the different texture of bulking

material. During the experiment the air pump operated every 3rd hour for 30 min. The temperature was measured continuously with temperature sensors buried in the middle layer of the composite materials at 12 cm depth. The ambient temperature was also monitored. A data logger (Delta T Logger DL2e, software Ls2Win PC) was used to register temperature data continuously with a sampling rate of 4 hours. A schematic diagram of the reactor setup can be seen in Figure 10.

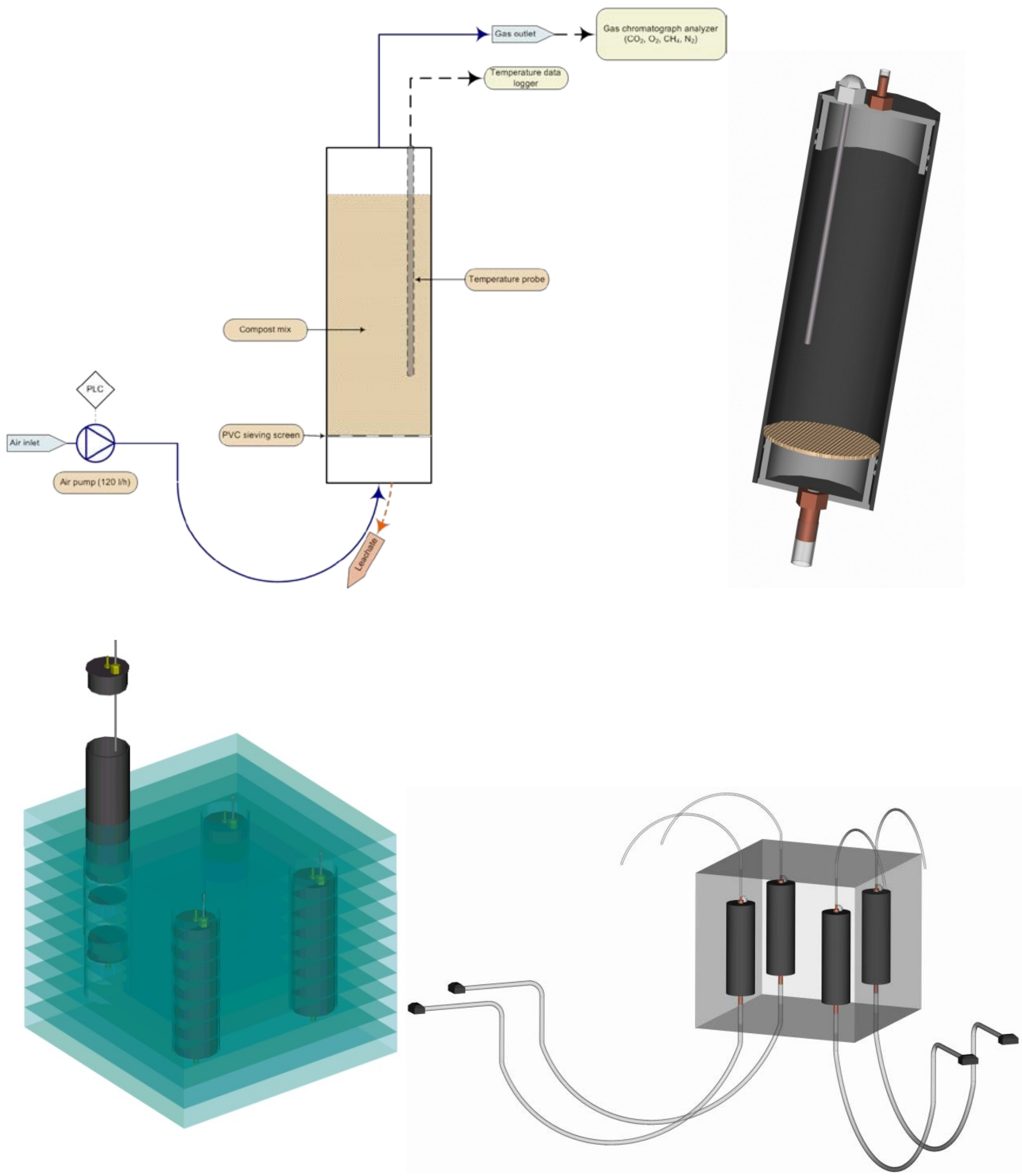


***Figure 10**: Experimental design of the bench scale composting reactors.*

The mixtures OCS, OCB and OCP were loaded in reactors in a quantity of 1.060 kg from 1.125 kg of the total quantity of mixtures prepared initially, due to available space in the reactors. The rest of the reactors were loaded with 1.100 kg of OCE mixture, 0.660 kg of PSW. The control reactors were loaded with 1.000 kg of BWS (Table 7). Mixtures OCS, OCB and OCP were tested in triplicates and mixtures OCE and PSW and the control (CON) in duplicates. The experiment lasted for 62 days. At the beginning of the experiment 1.000 kg of each mixture was shared in 3 parts for analysis: one part for immediate microbiological analysis, another part for drying and the third part was frozen at -20°C for chemical analysis. The same procedure was done at the end of experiment but with the whole composted material. During the process, the compost mass in reactors was not mixed, but after 29 days of composting water was added due to drying of the composting material.

Monitoring and analysis methods

The temperature and gas were the parameters which monitored the composting process over the whole experimental period. The temperature and gas concentration was measured in the free space between compost mass and reactor's lid. The gas was measured daily in the last 20 minutes before next pumping interval. CO_2, O_2 and CH_4 were analysed using an infrared gas analyser (GA2000 Plus, Geotechnical Instruments, UK). Gas was pumped out by the gas analyser through silicone tubes installed in the lid of each composting reactor. This experiment included a sub-experiment which included measurements of the aeration rate and temperature raise. The aeration rate was established as result of 5 days trials which consisted of different duration of aeration rates, time cycles and gases release measurements. As result, during the experiment the air pump operated every 3rd hour for 30 min. Dry matter (DM) at 105°C for 24 h and ash content (505°C, 24 h) of compost samples were measured and calculated according to CEN13039 (European Committee for Standardization, 1999c) at beginning and at the end of experiment. Total and volatile solids (TS and VS, respectively) were calculated at the beginning and at the end of experiment according to Standard Methods (APHA, 2005). The organic matter degradation was calculated at the end of experiment based on ash measurements (Rekha et al., 2005).

Other chemical factors analysed at the beginning and at the end of the experiments were EC, pH, total nitrogen (TN), total carbon (TC), ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N), total phosphorus (TP) and total potassium (KTOT) according to Standard Methods (APHA, 2005).

The analysed microbial parameters were faecal streptococci and *E. coli*. For enumeration of *E. coli* the multiple tube fermentation (MTF) technique was used according to Eaton et al. (2005). Triplicate experiments were performed and most probable number (MPN) values were calculated from the number of positive tubes as described in Blodgett (2010). Triplicate analyses for faecal streptococci were determined by direct plate count. Before inoculation into the different growth media 10-fold dilution series of the samples were prepared with 0.9% NaCl by manual shaking and with a mini shaker at medium speed. The different growth media and incubation conditions are shown in Table 7.

Table 7*: Growth media and enumeration methods for E. coli and enterococci used in composting laboratory experiment.*

Microbial group	Growth media	Enumeration method	Incubation temperature (°C)	Incubation time (h)
E. coli	Tryptone water + Ehlrich reagent (indole test)	MTF method	44	24 ±2 h
Enterococci	Slanetz-Bartley agar	Direct plate count	37	44 ± 4 h

3.3.2 Composting of blackwater solids – a pilot scale experiment

Experimental design

The system is described in details in chapter 2.1. In short, the BW was collected in a fiberglass tank using low flush (0.8 l per flush) vacuum toilets (BW source) with a macerator (Jets^{TM} system). The first treatment module was a light sphagnum PF (see Table 4 for description) where separation of solid and liquid fraction takes place (Figure 11A). The PF were HDPE plastic containers and had 80 l capacity, conical shape, inner dimensions of 35 cm x 34 cm at the top, 32 cm x 32 cm at the bottom, back side height 72 cm and front side 83 cm. The two PF were mixed after separation and collection of BW solids with commercially available wheat bran and pine bark chips (particle size 0-40 mm): BW- 21 kg, peat 2 kg, bark 1.5 kg and bran 5.5 kg as bulking material per compost reactor. The PF were converted in two compost reactors (CR1 and CR2, Figure 6B, 11A, B) by insulation with Styrofoam (6 cm thickness) and air tight. Additionally, the lid had an exhaust tube for gases and in the bottom a sieve for liquid separation. The up flow aeration was supplied with aquarium air pressure pumps (Sera 110 plus, Spain) controlled by a time switch (24 Hour - In Time Switch, UK). Compost was aerated with the air inflow of 110 l/h within 2 hours interval what gives 55 reactor volumes of air per day (Figure 11B).

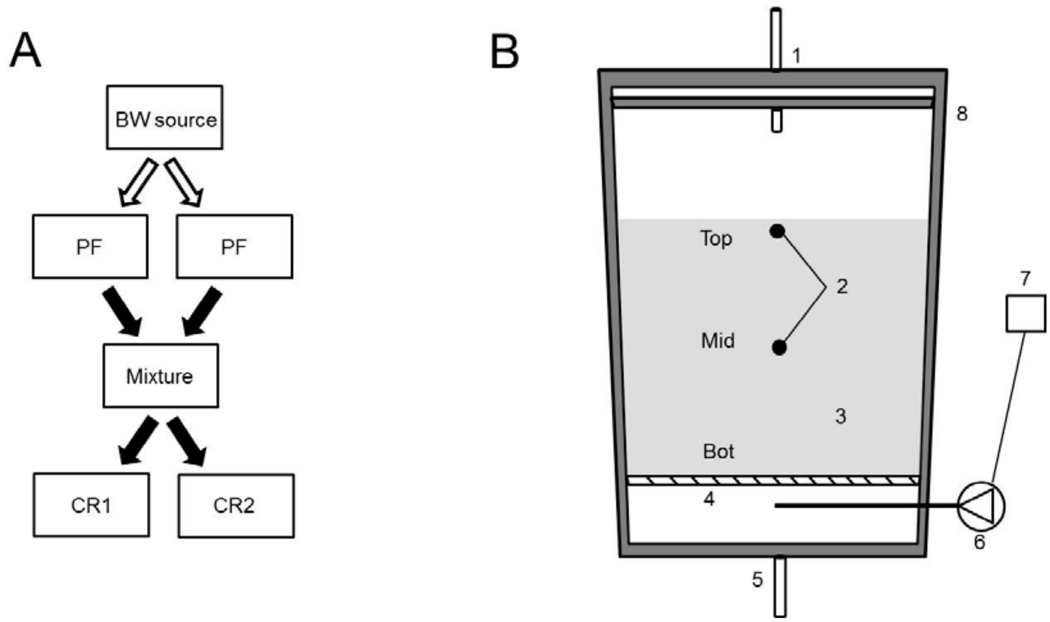


***Figure 11**: Compost experimental design at pilot scale in Salina Nature Park. A: BW- blackwater; PF- peat filters; CR1, CR2- compost reactors; empty arrow-liquid flow; filled arrow-solid flow; B: 1-gas release; 2-temperature data loggers; 3-compost mix; 4-sieve; 5- leachate; 6-air inlet and air pump; 7-time switch; 8-insulation. Designated sample points: Top- top layer; Cor- core layer; Bot- bottom layer.*

Monitoring and analysis methods

The pilot scale experiment lasted 19 weeks in the two independent compost reactors with the same material and conditions. The samples were collected in 1, 2, 3, 5 and 19 weeks after the compost process started. Samples were taken from the top (Top- corresponding to 1/10 depth: 0-5 cm), middle (Mid- 4/10 depth: 20-30 cm) and bottom (Bot- 9/10 depth: 50-60 cm) layers of the compost pile from both reactors. A total number of 30 samples were collected and frozen at -20 °C. Prior to analyses the samples were thawed at room temperature.

Temperature was monitored in the compost during the entire process. Data loggers (Thermometer data logger 22L, Repa System Solutions, software ThermoTrack PC, temperature rage -40°C/+85°C, sampling rate 1h) were placed 2 cm below the surface, and in the middle layer 20 cm deep of the compost pile. The pH and electrical conductivity (EC) were analysed according to Standard Methods (APHA, 2005).

Prior to the analysis of nutrients, the samples were dried at 30°C for 24 h and macerated. Total carbon (TC), total nitrogen (TN) and sulphur (S) were measured with Vario Max elemental analyser using a TCD detector after combustion at 950 to 1200°C (VarioMax CN, Elementar Analysensysteme GmbH). Total phosphorus (TP) and total potassium (TK) were measured according to SIST EN 13650.

The concentration of water-soluble ammonium and nitrate in compost-distilled water extract (5 g of compost in 200 ml of distilled water) was determined using test strips (Reflectoquant ammonium test and nitrate test; Merck KGaA, Darmstadt, Germany); detection limit for NH_4^+ was 0.2-7.0 mg/l, and NO_3^- 3-90 mg/l.

Dry matter (DM) and ash (ASH) were measured and calculated according to Standard Methods (APHA, 2005). The organic matter degradation was calculated based on ash measurements.

The toxicity of BW compost to plants was tested with a 36 h germination assay using seeds of garden cress (*Lepidium sativum*), which is a quick method for evaluation of compost toxicity (Himanen et al., 2011). A total number of 30 BW compost samples were collected over the entire composting process (19 weeks) from top, middle and bottom layers and stored at -20°C. Each sample was processed in triplicates.

In order to provide an integrative interpretation, the root elongation and seed germination of *Lepidium sativum* were combined in an Index of Germination (IG) (Gariglio et al., 2002).

Top, middle and bottom layers were also screened for human parasites (helminths and protozoans). Parasites were concentrated from the faecal material using the SAF-concentration method (sodium acetate-acetic acid-formalin), however, ethanol was substituted for formalin to allow also DNA isolation. 1 g of each sample was fixed in 5 ml SAF and examined by light and phase contrast microscopy (Nikon Eclipse E800). In the next step DNA was isolated from the concentrated samples using the QIAamp DNA Stool Mini Kit (Qiagen, Vienna, Austria) and strictly following the manufacturer's instructions. All samples were investigated by an *Entamoeba*-specific realtime PCR that detects and synchronously differentiates between *Entamoeba histolytica* and *E. dispar* (Blessmann et al., 2002). The PCRs were run in 20 µl reactions on a ROCHE LightCycler (Roche, Vienna, Austria) using 6 capillaries per sample, including for both, *E. histolytica* and *E. dispar,* a negative and a positive control, and always running first the *E. dispar* amplification. Reaction conditions were 5 min. 95°C, followed by 50 cycles of 58°C/10 seconds and 72°C/20 seconds.

During the composting, samples were taken for microbiological analyses to observe total number of cultivable bacteria and reduction of bacterial pathogens. Four different bacterial indicator groups based on RIDA®COUNT test plates (R-Biopharm, 2012) were used: total counts of heterotrophic aerobic bacteria (RIDA®COUNT Total Aerobic Count), *E .coli* and total coliforms (RIDA®COUNT E. coli/Coliform) and Enterobacteriaceae (RIDA®COUNT Enterobacteriaceae). All samples were aseptically applied on the RIDA®COUNT plates, when possible in the field, and incubated at 35°C for 24 h for bacteria (R-Biopharm, 2012). After the incubation period, microbial colonies were enumerated and expressed as Colony-Forming Units (CFU) per gram of BW compost (for details see Oarga et al., 2012).

One way ANOVA (Zar, 1984) was used to study composting rates and effects of sampling positions in the two independent compost reactors. The test was performed for each sampling date representing 1, 2, 3, 5 and 19 weeks of composting process.

Pearson's correlation analysis was used to correlate the data on phytotoxicity (GI) and physical and chemical parameters.

3.4 Bacteriological monitoring

Experimental design

The samples were collected from the system described in chapter 2.1, Figure 4. Characteristics are summarized in the Table 8. The BW was collected using low flush vacuum toilets (sample BW1). The treatment module was composed of a peat filter (sample BW2) where separation of solid and liquid fraction takes place. The liquid fraction passes over the BF filled with expanded clay (sample BW3) and recirculates for two hours per day to achieve a higher microbial mineralization of organic matter. After the liquid circulation terminates, the liquid fraction (sample BW4) flowed to the evaporation module where it was heated up (>60°C) to achieve disinfection, and was finally evaporated in the environment. The PF which contain BW solids were composted with bran and bark in a mass ratio of 3:1 (see chapter 2.4 for characteristics). A sample designated as SW1 represented the initial mixture of solid waste before composting and sample SW2 after 40 days of composting. In the GW hybrid treatment wetland (Kadlec and Wallace 2009; Bulc, 2006) the GW (GW1) was treated in a horizontal flow treatment wetland (sample GW2) followed by a vertical flow treatment wetland – (sample GW3). In a treatment wetland, biofilms in association with plant roots, in our case common reeds, stabilize and enhance the treatment process (Brix, 1997). Samples were taken on August and September 2011 when the highest number of tourist visited the park, which resulted in a high load of black and GW. In this period a total of 2192 flushes from vacuum toilets were recorded, with an average of 1.3 l per flush giving the total input of 2849.6 l of BW. Compost samples were taken at the beginning (September 2011) and after 40 days of composting process (Table 8).

Table 8*: Summary of treatment systems and samples characteristics (BW- blackwater; GW- greywater; SW- solid wastes; MW- municipal wastewater; HFTW- horizontal flow treatment wetland; MW- municipal wastewater; PE- population unit; VFTW- vertical flow treatment wetland).*

System/PU	Sample	Type	Description
Decentralized source separation/100	BW1	Liquid	BW input from toilets in PE
	BW2	Liquid	BW output from PE
	BW3	Liquid	BW from biofilter after 2 h of recirculation
	BW4	Liquid	BW from evaporation module
	GW1	Liquid	GW input in HFTW from sinks
	GW2	Liquid	GW output from HFTW
	GW3	Liquid	GW output from VFTW
	SW1	Solid	Mixture of solid waste prior composting
	SW2	Solid	Compost after 40 days
Centralized municipal/15000	MW1	Liquid	MW input from the city
	MW2	Liquid	MW output after biological treatment processes

At each sampling campaign approximately 0.5 l of material was aseptically taken. Samples were transferred in a cool box to the laboratory and subdivided to execute microbial and chemical analyses. Samples were processed in triplicates (Figure 12).

As an example of another type of organically polluted water, samples from the municipal wastewater treatment plant from Postojna, Slovenia, were introduced into the study. The plant in Postojna treats municipal waters (sample MW1) based on mechanical separation and biological oxidation (sample MW2 after the treatment) for 15000 population units (Kovod, 2011) (Figure 12).

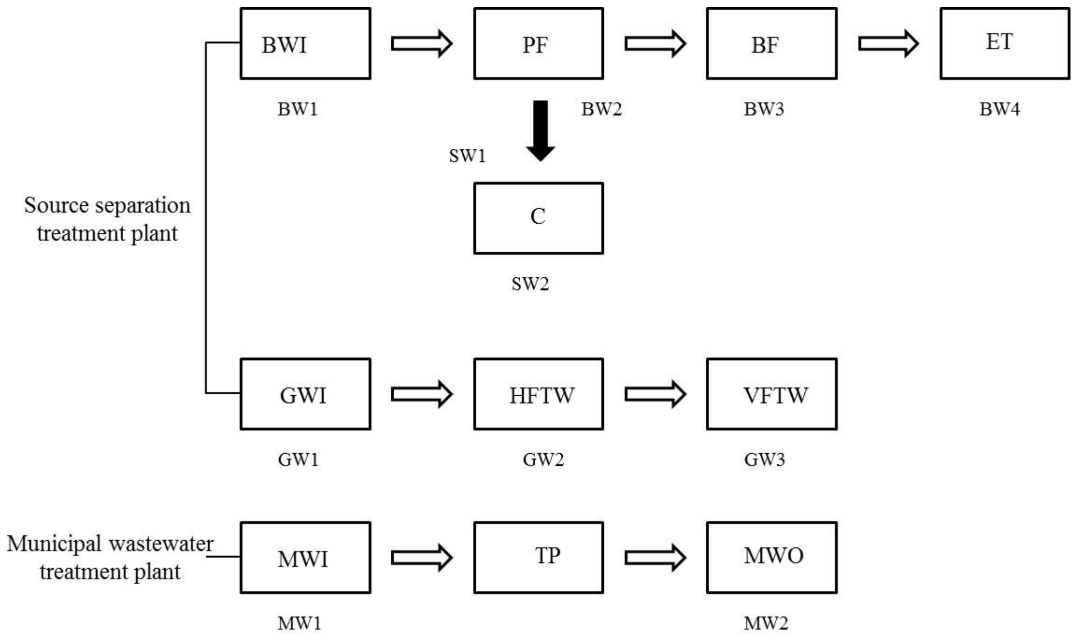


Figure 12*: Experimental design of wastewater treatment plants with indicated sampling points: BWI- blackwater input (sample BW1); PF- peat filter (sample BW2); BF- biofilter (sample BW3); ET- evaporation module (BW4); C- compost (samples SW1 and SW2); GWI- greywater input (sample GW1); HFTW- horizontal flow treatment wetland (sample GW2); VFTW- vertical flow treatment wetland (sample GW3); MWI- municipal wastewater input (sample MW1); TP- treatment pool; MWO- municipal wastewater output (sample MW2); blank arrow-liquid flow; black arrow-solid flow.*

Monitoring and analysis methods

RIDA®COUNT plates are medium sheets, coated with dry culture media which are activated when liquid samples (1 ml) are applied (Figure 13). Different plates were used to enumerate microorganisms from liquid and solid samples: total counts of heterotrophic aerobic bacteria (RIDA®COUNT Total Aerobic Count), *E. coli* and total coliforms (RIDA®COUNT *E. coli*/Coliform), enterobacteria (RIDA®COUNT Enterobacteriaceae), and cultivable yeasts and moulds (RIDA®COUNT Yeast&Mold Rapid). On RIDA®COUNT test plates, microorganisms form colonies that become coloured after the enzymatic reaction with the substratum in the media. We combined several different RIDA®COUNT test kits for the same sample by simultaneous use of different selective growth media allow us to extract more information on the presence of specific microbial groups. For example, the RIDA®COUNT *E.*

coli/coliforms kit gives the number of *E .coli* (violet colonies) and non- *E. coli* coliforms (blue colonies), while the RIDA®COUNT Enterobacteriaceae kit gives the total number of enterobacteria: *Cedecea*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Morganella*, *Proteus*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia* (R-Biopharm, 2012).

All samples were aseptically applied on the RIDA®COUNT plates, when possible in the field, and incubated at 35°C for 24 h for bacteria and at 25°C for 48 h for yeasts and moulds (R-Biopharm, 2012). After the incubation period, microbial colonies were enumerated and expressed as colony-forming units (CFU) per gram for solid material or CFU/ml for liquid samples. The solid samples were resuspended in physiological solution (0.9% NaCl) and appropriate serial dilution was applied on RIDA®COUNT plates. Physiological solution was used for all dilutions as it was recommended by the producer (R-Biopharm, 2012). On RIDA®COUNT media, the dilutions from 10^{-4} to 10^{-7} were applied for total bacterial count, from 10^{-1} to 10^{-5} for *E. coli*/coliforms and Enterobacteriaceae, and from 10^{-1} to 10^{-4} for yeast and moulds (Table 19).

Figure 13*: RIDA®COUNT plates, example of samples of wastewater from Postojna wastewater treatment plant, after 24 h of incubation at 35°C: left- RIDA®COUNT Total Aerobic Count; middle-RIDA®COUNT E. coli/Coliform; right- RIDA®COUNT Enterobacteriaceae.*

The comparative method for RIDA®COUNT Total (% against Petrifilm AC plate) ranges between 94-110% and is considered satisfactory (Quality Management System certified ISO

9001, ISO 13485, R-Biopharm, 2012). In this study, to check the repeatability and efficiency of RIDA®COUNT plates, nutrient agar (Sigma, USA) was tested in triplicates against RIDA®COUNT Total for liquid and solid BW samples. Nutrient agar is widely used to isolate nutrient non-demanding bacteria (Tanner, 2007). The comparison was based on sample streak and pour plating on nutrient agar plates, and application of liquid sample on a dry RIDA®COUNT medium sheet.

Simultaneously with sampling for cultivable microorganisms, physical parameters of the samples were measured at the site (specific electrical conductivity-SEC, pH, temperature-T, redox potential-ORP, and dissolved oxygen-DO) by Multimeter HACH HQ40d, USA. In the laboratory the following parameters were measured: total suspended solids-TSS (Analytical balance Mettler, USA), NH_4^+-N, NO_2^--N, NO_3^--N, orthophosphate (o-P), total phosphorus (TP), COD (Spectrophotometer HACH DR 2800, USA), BOD_5 (Manometer WTW OxiTop, USA), after Standard Methods (APHA, 2005).

The extracted data of defined cultivable microbial groups (*E. coli*, non- *E. coli* enterobacteria, non- *E. coli* non enteric bacteria, yeasts and moulds) and their abundance were correlated with environmental variables. Relative standard deviation (RSD) was calculated to evaluate the repeatability of total bacterial counts on RIDA®COUNT Total Aerobic Count compared to the number of cultivable bacteria on the nutrient agar plates.

4. RESULTS AND DISCUSSIONS

4.1 Blackwater separation with organic filters and liquid fraction treatment

4.1.1 Organic filters – laboratory experiment

Characteristics of the organic filters

In this experiment the suitability and the particle retention efficiency of selected organic filters for BW separation was investigated. The objective of the experiment was to test filters as a solid separation unit for BW from low-flush toilets prior to downstream treatment steps in the sanitary system at Salina Nature Park and their possible applications as polishing unit for package treatment plants in general. The selected organic filters were peat, bark, and mixtures from peat/bark and peat/sawdust. Solutions to increase the hydraulic capacity and clogging avoidance are also briefly discussed.

In the experiment a mixture of raw sewage and secondary sludge (SBR mixture) taken from a sequential batch reactor (SBR) package treatment plant was used. A subsequent common experiment using the same experimental setting was performed with settled BW and peat/sawdust mixture (Todt et al., submitted). The TSS from SBR mixture and settled BW were in a comparable range: 90-845 mg/l and 403-1590 mg/l, respectively.

Performance of the organic filters

The results (Figure 14A) with SBR mixture indicate that organic filter media reduced TSS from 60% to 80%. The highest performance of 81% has been reached with the peat/sawdust mixture. The average reduction using peat and peat/bark mixture were 75% and 76%, respectively, while the reduction using pure bark was lower (58%). These results confirmed the findings of Lens et al. (1994) who were testing peat, bark and a mixture of peat/bark and obtained the best performance in the peat/bark mixture followed by peat and bark. Despite the relatively small differences between the four tested media, both mixed media showed better results for TSS reduction than single media filters (Figure 14A).

A B

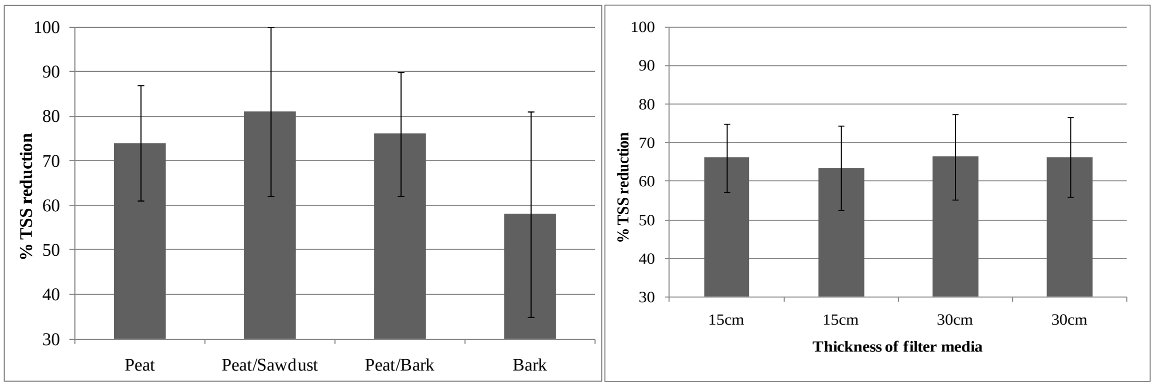


Figure 14*: A- Total suspended solids (TSS) reduction with standard deviations of SBR mixture filtered using four different types of organic filter media, (n=24) and B- two different filter thicknesses (n=11).*

Higher tortuosity could be an explanation for better results with the media mixtures. Studies in unsaturated natural peat soil systems showed that a more heterogeneous particle distribution promotes more tortuous flow pathways and longer retention times (Rezanezhad et al., 2010). These results also indicate that peat, which in some places is not always available, can be replaced with the more environmentally friendly and cheaper material sawdust. An even further reduction might be possible in increasing the sawdust ratio in the mixture. However more experiments are needed for testing different peat-sawdust mixtures or even pure sawdust.

The peat/sawdust mixture was selected for a further assessment using a similar column set-up, different filter media thickness and settled BW by Todt et al. (submitted), and compared with peat/sawdust mixture from the experiment with SBR mixture. To assess the effect of filter depth, two columns have been filled with 30 cm and two columns with 15 cm of peat/sawdust mixture. The TSS in settled BW varied in a range of 403-1590 mg/l, which is significantly higher than the SBR mixture (range of 90-845 mg/l). The effluent from the settled BW the average concentration was with an average of 720 mg/l, compared to a normal TSS range of 250-600 mg/l in conventional wastewater (Henze and Comeau, 2008). TSS varied in the settled BW, and a majority of the samples where in a range between 600 and 800 mg/l TSS –

inflow/outflow (Todt et al., submitted). A substantial amount (45-87%) of the TSS in the BW samples refers to small particulate material (<10 µm). The two types of wastewaters that have been applied to the peat filters therefore differed substantially in terms of both particle content and particle size distribution.

Thickness of the filter media had no significant impact on TSS reduction (Figure 14). Filter performance showed 65% reduction in average (Figure 14B), which was lower than the 81% reached with peat/sawdust in the first experiment with four types of mixtures (Figure 14A). A Wilcoxon test showed that the difference in TSS reduction using 15 cm and 30 cm thick layers of peat-sawdust mixture loaded with settled BW was not statistically significant ($p<0.1$). This indicates that most of the filtration process took place in the uppermost part of the filter.

LOI was significantly lower in the upper 5 cm of peat/sawdust mixture than in the lower layers (Figure 15A). This indicates that more mineral matter is retained on the upper layer of this filter media. A tendency towards top layer accumulation has also been indicated by two of the four tested media in the experiment with SBR mixture (Figure 15B) and in a study performed by Taylor et al. (2004). Only the bark filter showed a lower LOI in the bottom layer (Figure 15B). Compared to the other filter media, the larger pore sizes in the bark filter can be the reason for accumulation of BW particles in the bottom layers. In the experiment with settled BW, analysis of LOI (Figure 15B) showed that most of the particles were retained in the upper 7 cm of the filter media. The larger range of pore sizes of peat-sawdust as in the case of bark is one possible explanation for the deeper penetration of particles. TSS retention in the deeper filter layers on the other hand was lower, and indicates that a filter media thickness of more than 15 cm have little additional effect on the filter performance regarding removal of TSS.

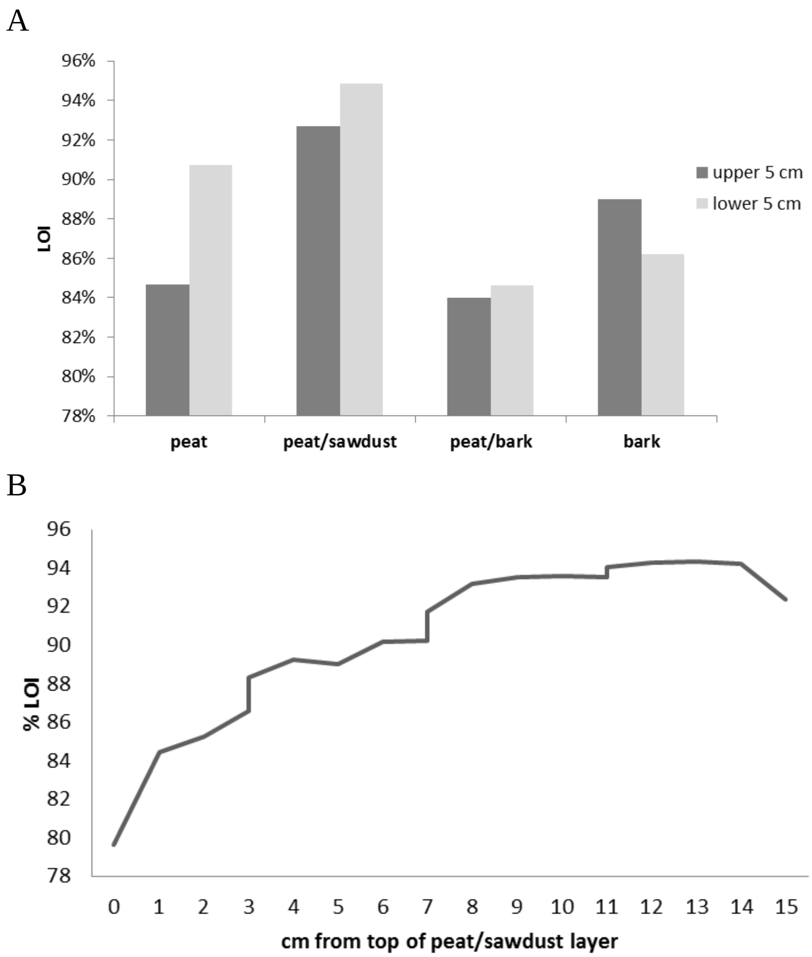


Figure 15*: A- Loss of ignition (LOI) in the top and bottom layer of the different filter media loaded with SBR mixture; B- LOI with depth of the 15 cm of peat/sawdust filter column loaded with settled blackwater.*

Clogging issue on organic filters

In the experiment using SBR mixture the filter columns started to clog (visual ponding of water on the filter surface) after 200 flushes for peat (8 days), 250 flushes for peat/sawdust and peat/bark and 400 flushes for bark. According to literature, SBR sludge has a lower fraction of small particles (>10 µm) and also a tendency to form flocks (Govoreanu et al., 2003). Larger flocks and particles will to a lesser degree penetrate into the media. In the experiment using settled BW all filter columns started to clog after 65 flushes (3 days). One reason for the lower hydraulic performance of peat/sawdust which used settled BW may be different retention mechanisms. Particles in settled BW, however, seem to penetrate into the media especially in the upper 5 cm (Figure 15A) and thereby clog the pores of the media. For the columns loaded with settled BW, hydraulic filter regeneration was obtained by mixing the uppermost 5 cm of filter media after clogging had started to occur. Despite the regeneration clogging occurred again after 15-20 loadings so that the filters needed to be regenerated on a daily basis. However, no statistically significant effect ($p=0.989$) on the filter performance could be determined comparing TSS reduction rates before and after filter regeneration. These findings indicate that mixing might prolong the organic filters life, without having a significant impact on the TSS filter performance, as application for filtering of concentrated wastewater effluents. However, more research is needed to develop an appropriate mechanical unit to automate this process. It may even be possible to automate both regeneration of clogging and renewal of filter media.

Clogging in a vertical flow filter such as experimental columns is a complex interaction between particle accumulation and biofilm development. Those processes seem to be strongly dependent on the content of organic matter and particle size distribution of the influent (Zhao, 2009). At the start of the experiment the biofilm needs to be established. This takes 2-3 days according to experience from biofilm reactors (Henze, et al. 2008). After media regeneration (stirring of the top layer) the biomass is dispersed, but ready to grow without a lag phase of 2-3 days resulting in a more rapid clogging development. Although secondary sludge contains active biomass, biofilm development may take more time than in the BW loaded columns due to lack of substrate for biofilm growth.

Regardless the discrepancies between SBR mixture and settled BW, the relatively short time of operation before clogging is a critical issue. The hydraulic loading rate of this experiment was 710 mm/day. This is extremely high compared to 76 mm/day applied by Kõiv et al. (2009) and 100 mm/day applied by Lens et al. (1994). According to Jenssen and Siegrist (1991), clogging increases exponentially with the reduction of the loading rate. Thus, larger filter areas could obtain substantially longer filter operation before maintenance is needed as well as mixing, scraping or changing of filter media. Application of compost worms in the organic filter media could be an option to improve filter operation as it has been reported by

Taylor et al. (2004) that worms are an effective measure to counteract clogging and to obtain higher loading rates and longer maintenance intervals.

Based on market availability and laboratory results, peat which had a removal efficiency of 75% and a preliminary hydraulic resistance of 8 days, was the organic filter material chosen to serve as solid separation unit (PF) for BW prior to downstream treatment steps in this sanitary treatment system.

4.1.2 Blackwater liquid fraction treatment in organic filters, biofilter and solar evaporation tank – pilot scale experiment

The system used in this study was an "interrupted" flow system: the BW from vacuum toilets (faeces, urine and toilet paper, 0.8 l per flush) and urine from dry urinals were ducted in a buffer tank; from the buffer tank 37 flushes per day simulating the toilet flushes were released into the PF by electro-valves controlled by the PLC. In the PF the separation of liquid from solid took place. In BF the liquid fraction was circulated for two hours over expanded clay (Figure 4 and 8).

Total suspended solids and particles distribution in organic filters and biofilter

Differences of TSS in BW in low and high input periods for the whole sampling period are presented in Figure 16. Samples were taken once per week for a period of 7 months. The TSS measurements show evident differences between low and high input periods in PF and BF.
In the low input periods in the PF TSS ranged from 431-890 mg/l with an average of 721 mg/l in influent, while in the effluent TSS ranged from 142-900 mg/l with an average of 401 mg/l. In the high input periods, the BW influent concentration in the PF showed TSS loads in range of 1043-3650 mg/l with an average of 1677 mg/l, and in the effluent from the PF a range of 326 to 4500 mg/l with an average of 884 mg/l.

The results for the influent of BW in this system located in a touristic facility where tourists have a short stationary time are not as expected in the range with the TSS results in the influent from the laboratory experiment (chapter 3.1), when the BW was collected from a student dormitory. In the laboratory experiment the input ranges were 403-1590 mg/l, while in the field at pilot scale experiment the TSS ranges were 431-3650 mg/l with an average of 1199 mg/l (Figure 16). These results show that location of the system has a significant impact on the BW TSS load and peaks frequency.

The TSS concentrations in BF outflow showed small differences. In low period it ranged from 240-1600 mg/l and in high season 240-1700 mg/l. In reducing the TSS, the BF's efficiency was with an average of 11% and a maximum of 62% (all samples were taken after the start of 2 hours of recirculation).

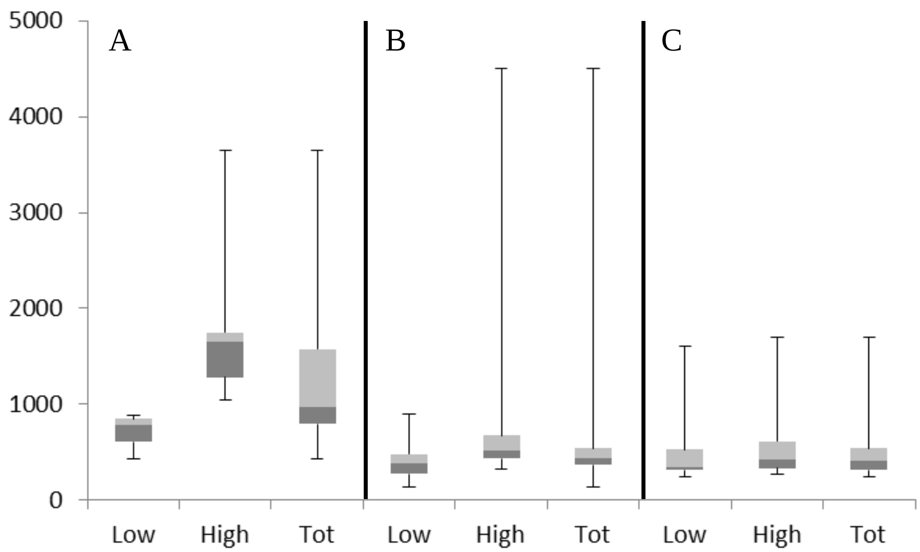


***Figure 16**: Distribution of total suspended solids (TSS) concentrations in different periods at the pilot scale in Salina Nature Park (low, high and the whole period-Tot): A- peat filters inflow; B- peat filters outflow and biofilter inflow; C- biofilter outflow.*

The particle size distribution was measured randomly in BW samples taken at the influent and effluent of the PF and gave valuable information for the particle retaining efficiency. Table 9 shows that larger particles (>1000 and >100 µm) are retained in the PF as expected (96.5% and 88.8%, respectively). The smallest fraction (<10 µm) was not retained in the PF. More than 40% of the influent concentration was washed out from the PF. This can be explained via sedimentation of incoming BW over peat filter especially big particles, interactions of bacteria with peat filter which cleaved big organic particles, small particles which flowed out with the filtrate, and a small fraction which was represented by active motile bacteria which did not interact with peat but were filtered and passed through the outflow.

Table 9*: Blackwater particle distribution in inflow and outflow of peat filters at the pilot scale in Salina Nature Park (average, standard deviation, minimum and maximum, n=8).*

Particle size µm	IN %	OUT %	Reduction efficiency
>1000	33.8±24.0	1.2±1.3	96.5 %
	1.6 66.5	0.1 3.4	
>100	17.8±17.3	2.0±1.5	88.8 %
	2.7 41.8	0.3 5.0	
>10	38.5±23.7	64.3±31.0	-40.2 %
	6.6 76.2	20.7 92.6	

Nutrient reduction in organic filters and biofilter

The pathway of the liquid phase of BW in our study was the waste from toilets, passing the PF where solid particles were deposited and recirculation of the filtrate in BF. The aim was to prepare liquid fraction as pathogen free and with minimum content of nutrients.

During the operation of the system in Salina Nature Park (March-October) and based on physical and chemical measurements conditions were rather stable, except for the expected unstable starting period until the system started to operate at nearly optimum conditions such as adjustment of microbial communities (Figures 18-23).

In the continuation the measured parameters in the liquid fraction of BW are presented with the special emphasis on the nitrogen cycle.

The measured temperature in the BW liquid fraction in PF and BF generally followed the trend of ambient temperature (Figure 17) which is reasonable as no additional insulation was applied in the system.

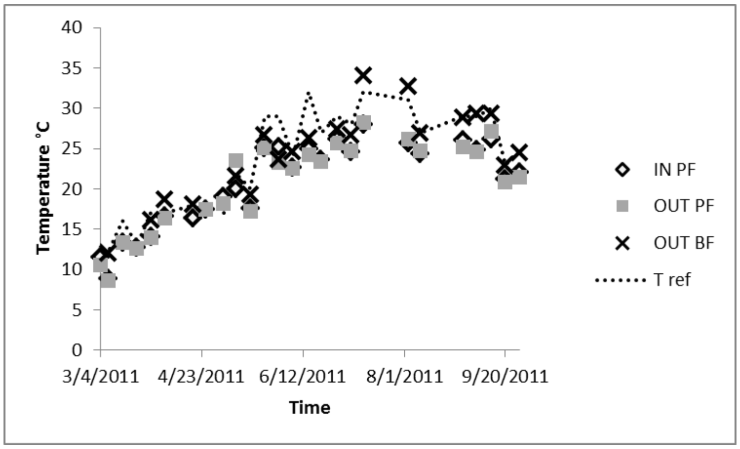
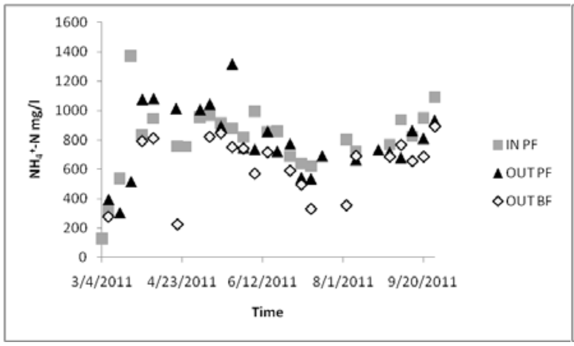


***Figure 17**: Temperature in blackwater liquid fraction over the whole experimental period at pilot scale in Salina Nature Park.*

In laboratory conditions, temperatures above 25°C affect the nitrification process because the ammonium oxidizers can out-compete the nitrite oxidizers, and at temperature >15°C the nitrite oxidizers have a slower growth (Paredes at el., 2007) (Figure 17). In the system where suboptimal conditions prevailed, at temperatures below 25°C (March-May) the NH_4^+-N in influent was on the average of 722 mg/l, in the effluent of PF on the average of 916 mg/l and in BF 628 mg/l. At higher temperatures than 25°C (i.e. 26, 27 and 28°C) starting with May, NH_4^+-N values increased compared with previous period in the buffer tank (average of 830 mg/l), at the effluent of PF (average of 723 mg/l) and BF (average of 637 mg/l) (Table 11). NO_3^--N concentrations at temperatures below 25°C were in the range of 11 and 63 mg/l, but after 100 days of experimental period (already in the period above 25°C) they were in a range of 142 and 510 mg/l. The increase of NO_3^--N after 100 days coincided with the sudden raise of NO_2^--N (1 mg/l NO_2^--N in inflow, 15 mg/l NO_2^--N in effluent from PF). Starting with the next measurement till the end of experimental period the NO_2^--N concentrations (Figure 20B) decreased to the initial basal levels (average of 0.4 mg/l), but NO_3^--N remained higher (in average 9 times higher than in the period before sudden raise) (Figure 18B, 20B). Interestingly, concentrations of NH_4^+-N before this event (sudden increase of NO_2^--N and NO_3^--N concentrations) were higher and then they suddenly dropped. After certain "lag" phase they started slowly to increase (Figure 18A). These results indicate that after "this event", around 100 days of system functioning, the nitrification started to be more effective (higher

NO_3^--N and lower values of NH_4^+-N) which can be attributed to establishment of effective nitrification biofilm.

A B



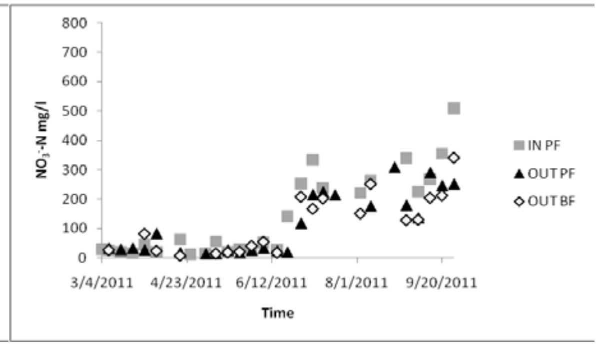


***Figure 18**: Changes of physical and chemical parameters over the whole treatment period in peat filters and biofilter in inflow and outflow at pilot scale in Salina Nature Park: A- NH_4^+-N mg/l, B- NO_3^--N mg/l.*

In the BW inside of the buffer tank the pH was in a range of 7.3-9.4 with an average of 8.6 (Figure 19A). For first 67 days pH was alkaline (9.1-9.4) then pH started to drop until it reached the neutral pH (min 7.3) in the middle of the testing period. From day 67 until end of September (end of experiment) pH again increased and reached pH 8.2. In the effluent of PF the pH was in a range of 7.7-9.2, in alkaline range for first 61 days - less time than in BW inflow. In the BF effluent pH fluctuation was less. The pH was in a range of 8.2-9.2 with an average of 8.5, indicating that alkaline range lasted first 41 days. EC in the PF influent (for 11 days in a range of 2.1-5.0 mS/cm) and effluent (for 11 days in a range of 4.7-5.3 mS/cm) was lower at the beginning of system operation. Similar trend was found in the BF. For the rest of experimental period, EC had higher values, a maximum of 9.7 mS/cm in the PF influent, 10.5 mS/cm in the PF effluent and 8.7 mS/cm in the BF. The fluctuations of EC in the BW are possibly due to incoming flushes composition (urine and faeces ratio) and load variations (Figure 19B, Table 11).

As Figure 1 shows, an optimum nitrification take place when pH was between 7.0 and 8.0 because pH between 7.9 and 8.2 is an optimum range for ammonia oxidizers and between 7.2 and 7.6 is an optimum range for nitrite oxidizers. The ranges of pH from Figure 19 proved to have three effects on the nitrification process: activation-deactivation of nitrifying bacteria, nutritional effects and inhibition through NH_3 and NO_2^-. Nutritional effects are associated with mineral carbon availability which is a carbon source for the nitrifying bacteria. In the system, many times high values of NH_4^+-N were detected particularly in the buffer tank and to a lesser extent also in other modules (e.g. peak of 1320 mg/l in the buffer tank and in BF 1600 mg/l). High values of NH_4^+-N can be explained as result of pronounced ammonification process. In the conditions of higher pH (e.g. in the buffer tank 9.1-9.4 and BF 9.1-9.2), equilibrium of NH_3/NH_4^+ is in favour of NH_3 in water solution (Camargo and Alonso, 2006). High values of NH_3 inhibit nitritation in the downstream process (Figure 1). The high pH of BW from the buffer tank (inflow into the PF) was lowered in the PF due to acid nature of peat (pH= 4-5). The system itself allows gradual decrease of pH towards the values optimal for nitrification (pH= 7-8) in BF.

A B

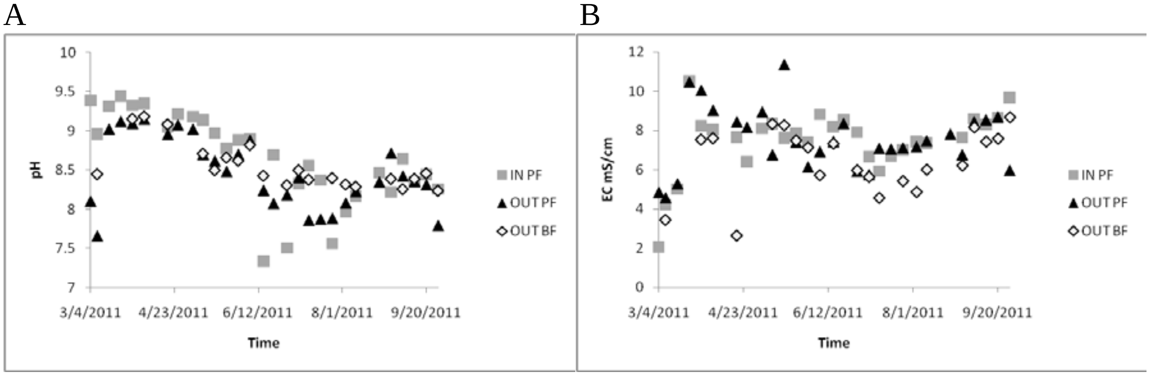


***Figure 19**: Changes of physical and chemical parameters over the whole treatment period in peat filters and biofilter in inflow and outflow at pilot scale in Salina Nature Park: A- pH, B- EC mS/cm.*

The oxic conditions are characterized by presence of oxygen while the sub-oxic regions are present between oxic and anoxic conditions, where nitrate is serving as the major terminal electron acceptor in the oxidation of organic matter (denitrification). In these sub-oxic regions, DO is very low (< 0.32 mg/l) (Murray at al., 1995), also characterized by an evidence of nitrate reduction or denitrification, or by a negative nitrate anomaly or by the presence of appreciable concentrations of nitrite (Rue et al., 1997).

DO in the buffer tank (inflow) was highest in the first week (3.4 mg/l). In weeks which followed until the end of the experiment, the measured DO was much lower ranging from 0.07

to 0.4 mg/l (Figure 20A), so the conditions were nearly anaerobic. The tank received BW every day, what means that each flush introduced certain amount of DO. The introduced DO amount was probably low because of the small incoming flush with BW from vacuum toilets which contained approx. 60% of faeces and urine of the whole flush amount (0.8 l of water per flush and approx. 0.5 l urine and faeces). In the outflow of PF the DO had similar oxygenic conditions (0.06-0.9 mg/l). In the effluent from the BF the oxygen was high till mid period of the experiment when it ranged from (3.7-8.3 mg/l) and low in the last two months of the experiment ranging from 0.05 to 1.6 mg/l (Figure 20A). Despite water recirculation, the results showed that DO became limited to the end of the experiment. This can be explained through the fact that in the bottom of the BF microbial biofilm and organic residues were formed/accumulated which represented an obstacle (clogging) for the oxygen diffusion (Figure 20A). Another explanation is the growth of microbial mass in time which requires higher oxygen consumption.

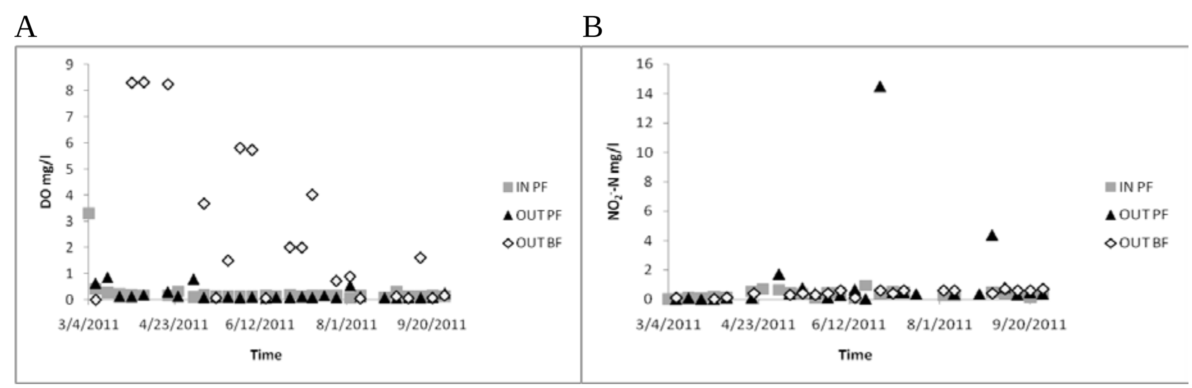


Figure 20*: Changes of physical and chemical parameters over the whole treatment period in peat filters and biofilter in inflow and outflow at pilot scale in Salina Nature Park: A- DO mg/l, B- NO_2^--N mg/l.*

The switch from the oxic to sub-oxic or nearly anaerobic conditions (<0.32 mg/l DO) coincided with rise of NH_4^+-N (peak of 1320 mg/l) in the collecting tank (Figure 20A, 22A) after one week of experiment. After 100 days of experiment the NH_4^+-N values coincided with a rise of NO_3^--N values in buffer tank (inflow) and outflow from PF in nearly anaerobic conditions. Similar trend was observed also in BF, and expected notable increase of nitrification was not observed although with the liquid recirculation additionally oxygen was introduced in BF.

The key to successful operation of a BF is uniform distribution of the BW liquid over the filter media and intermittent dosing (Heistad et al., 2001). In the BF, due to recirculation, nitrification was expected in a higher extent. The DO ranged from 0.05 to 8.3 mg/l (Figure 20A). A maximum reduction of NH_4^+-N in this module was from 758 mg/l NH_4^+-N to 225 mg/l NH_4^+-N. There were few occasions in the first period of experiment when NO_3^--N was accumulated (day 25 - 82.2%, day 32 - 20%, day 88 - 46.4% and day 95 - 5.3%) coincided with DO at maximum values - 8.3 mg/l for day 25 and 32, 5.8 mg/l at day 88 and 5.7 mg/l at day 95 (Figure 18B, 20A).

Researches on the nitrification through DO were carried out in different type of systems such as suspended biomass and biofilm systems (Paredes et al., 2007). In the case of systems with suspended growth biomass a limited oxygen supply enable a conversion of ammonium into NO_2^-, while under no oxygen limitation sludge age was the limiting factor for nitrification. For biofilm systems, as in the case of suspended growth systems, low DO led to NO_2^- accumulation, and even more, DO limited/generated by the diffusion resistance within the biofilm limits the nitrite oxidizers. At low DO concentrations, nitrite oxidizers are out-competed. Based on FISH (Fluorescence *In Situ* Hybridization) analysis it was observed that ammonium oxidizers are densely present outside the biofilm, and in deeper layers of the biofilm nitrite oxidizers are prevailing. It was concluded that nitrite oxidizers, due to their spatial distribution, are more exposed to oxygen limitations than ammonia oxidisers (Paredes et al., 2007). In the BF from this system (Figure 20A) which can be considered as a biofilm system, DO was high for 5 months when ranged from (3.7-8.3 mg/l) and low in the last two months of the experiment ranging from 0.05 to 1.6 mg/l. Although small particles and organic matter accumulated in the bottom and low values of oxygen in the last experimental period occurred, formation of NO_2^- wasn't completely inhibited (Figure 20B). This means that oxygen was a limiting factor for the microbial communities, oxidation continued until the final oxidation product which is NO_3^-, but not to the expected levels as NH_4^+ remained on average above 500 mg/l during the whole experimental period.

Orthophosphate represented the biggest fraction of TP in the inflow from the collecting tank, on average 86.6%, in the effluent from PF 87.0% and 77.2% in the effluent from BF (Figure 21A). These results show that BW can be considered as a rich source of phosphate, partly used for microbial metabolism, although a huge fraction still remains available in the solution. From the total concentration of TP in the influent on average 21.2% was retained in the PF (Figure 21B) indicating that almost half of the P was available to be recycled through composting process in this system. Compounds associated with microbial biofilm on the expanded clay in BF can represent an important source of P saved from human excrements and applied as fertilizers in agriculture.

A B

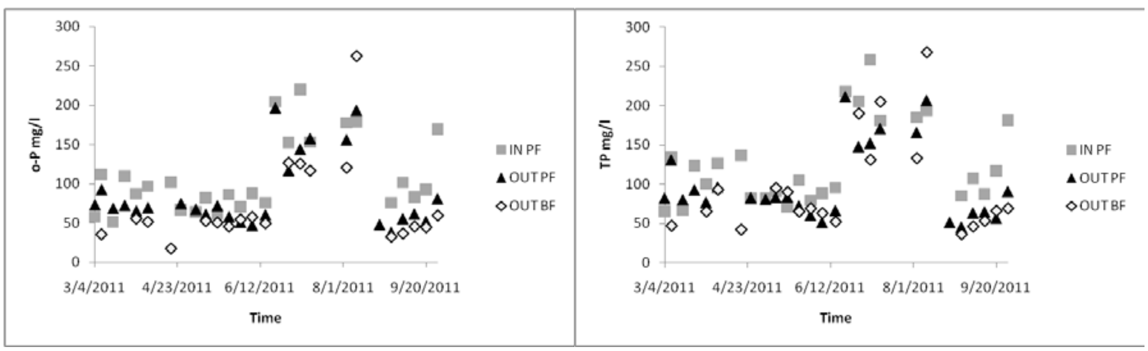


***Figure 21**: Changes of physical and chemical parameters over the whole treatment period in peat filters and biofilter in inflow and outflow at pilot scale in Salina Nature Park: A- o-P mg/l, B- TP mg/l.*

The average ratio BOD_5/COD of 0.66 indicated a high biodegradability and therefore a feasible biological treatment of BW (van Voorthuizen et al., 2008). Over the whole period BOD_5/COD ratio in PF was 0.67 and in BF 0.58 (after 2 hours of recirculation) what can be considered a satisfactory result regarding the biological treatment of BW. In the inflow, the COD values were in range of 739-5267.5 mg/l (Figure 22A). In the effluent of PF, COD was on average 1800 mg/l. High values of COD in BW can be caused by low amount of water per flush (1 litre) (Kujawa-Roeleveld et al., 2005). The overall removal efficiency of COD in the PF was 18.1% and in the BF 38.2%. In low TSS load period COD in effluent was on average in PF 1437.5 mg/l and in high TSS load period 2191.8 mg/l. The BF showed differences of COD values in effluent in different TSS load periods, 932.8 mg/l in low and 1259.5 mg/l in high periods (Table 12).

A B

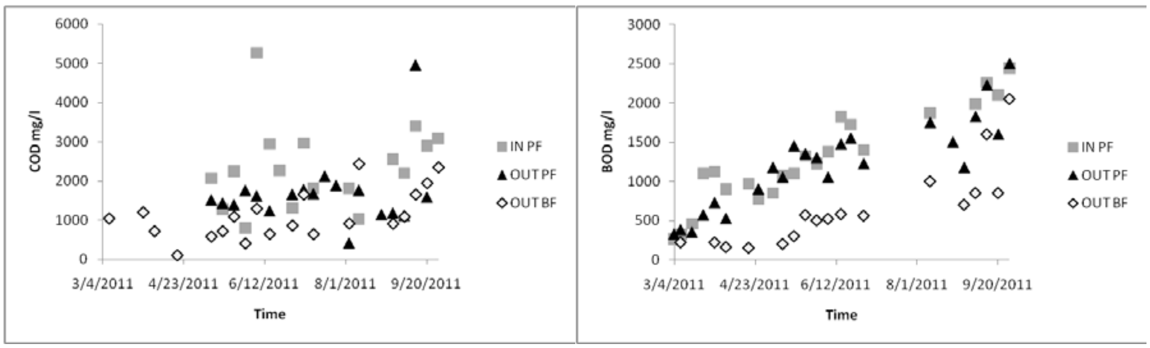
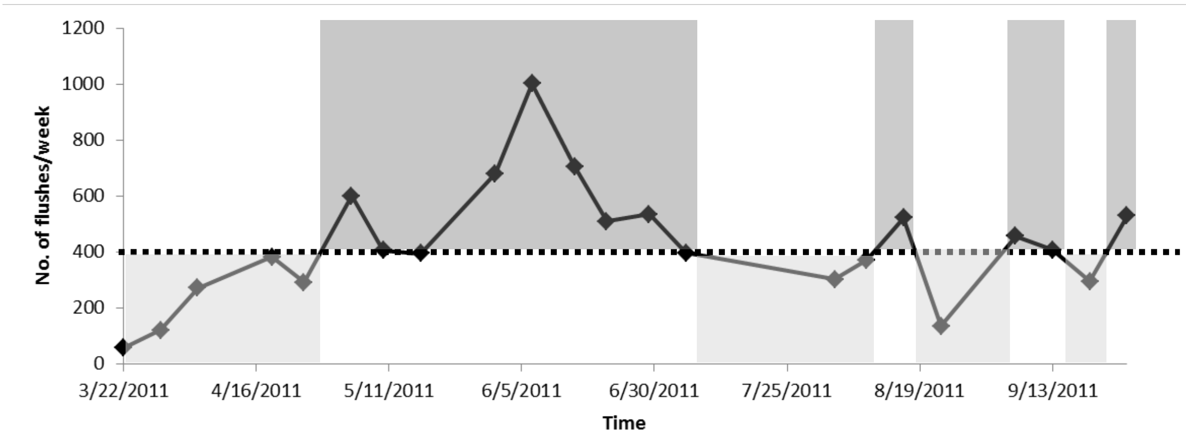


Figure 22*: Changes of physical and chemical parameters over the whole treatment period in peat filters and biofilter in inflow and outflow at pilot scale in Salina Nature Park: A- COD mg/l, B- BOD$_5$ mg/l.*

System in low and high total suspended solids input periods

The composition of BW varied according to tourist visits. The system efficiency was evaluated during the period of high BW input compared to low period (occasional visits of the toilets). Two approaches were used: the first approach was based on number of flushes in the whole study period. The 400 number of flushes in the toilets per week was selected as a threshold to define low and high load of BW in the system (Figure 21A). The second approach was based on the TSS load for the same period. The threshold of 1000 mg/l TSS (1000≈970 mg/l TSS, 970 mg/l TSS represented median value for the TSS load during the whole experimental period) was selected to define the low and high input of BW into the system (Figure 23B). Figure 23A an shows that high number of flushes (Figure 23B) do not corespond to high TSS input of BW into the system. For further analyses we used the threshold of 1000 mg/l TSS and above defining "high load period".

A



B

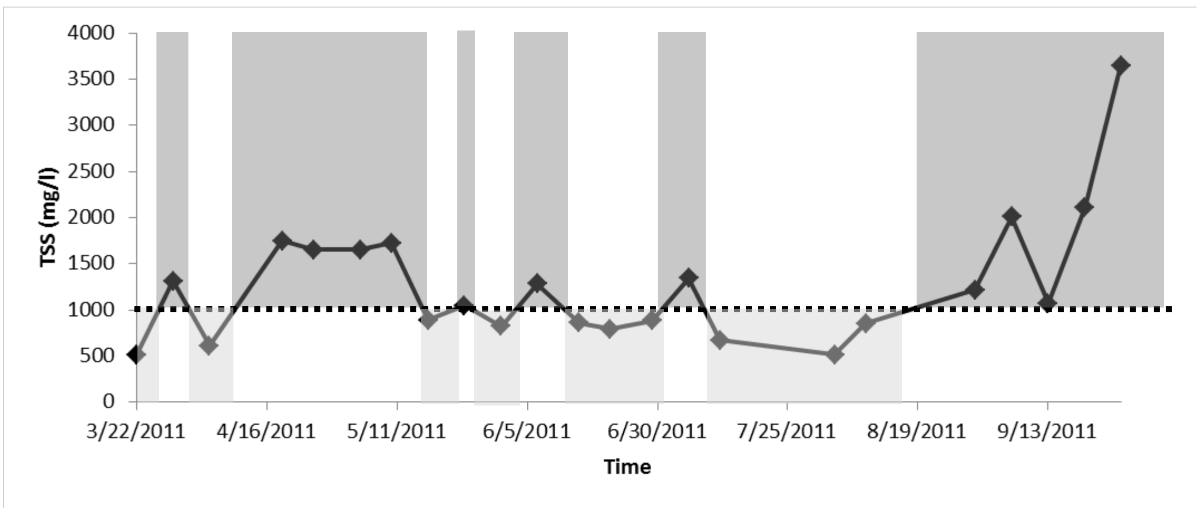


Figure 23*: Various periods of BW input into the system in Salina Nature Park; low input period (light grey) and high input period (dark grey); low and high input periods based on number of flushes (A); low and high input periods based on TSS load (B); threshold is set for 400 flushes (A) and 1000 mg/l (B).*

Physical and chemical parameters in low and high input periods in organic filters and biofilter

Based on TSS changes low and high input periods was defined (Figure 23). Here are shown the relations between individual measurements of TSS and physicochemical parameters at the influent and effluent of PF and BF (Figures 24-28). Measured parameters showed differences in high and low TSS load periods that indicated different biological processes in different modules such as ammonification, nitrification and P solubilisation (Figures 26-27).

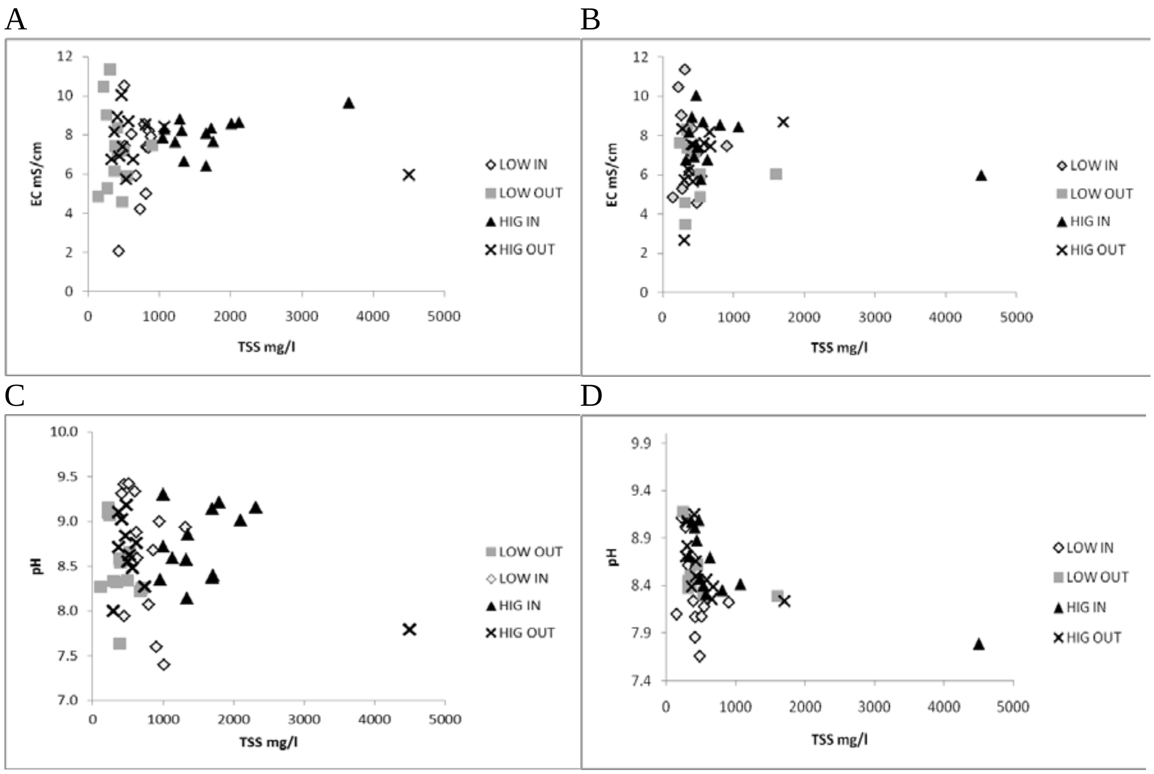


Figure 24*: Distribution of physical and chemical parameters in relation with TSS loads in low and high season at pilot scale in Salina Nature Park: A- EC mS/cm, C- pH in PF; B- EC mS/cm, D- pH in BF.*

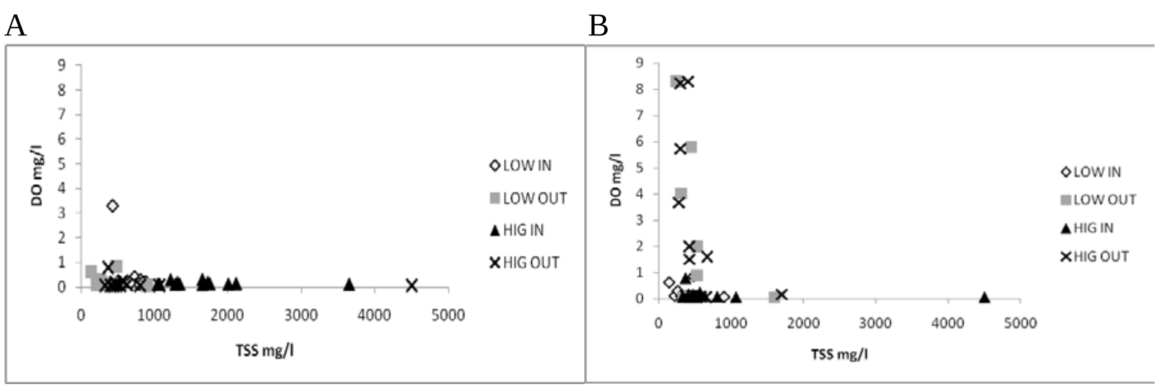


Figure 25*: Distribution of physical and chemical parameters in relation with TSS loads in low and high season at pilot scale in Salina Nature Park: A- DO mg/l in PF; B- DO mg/l in BF.*

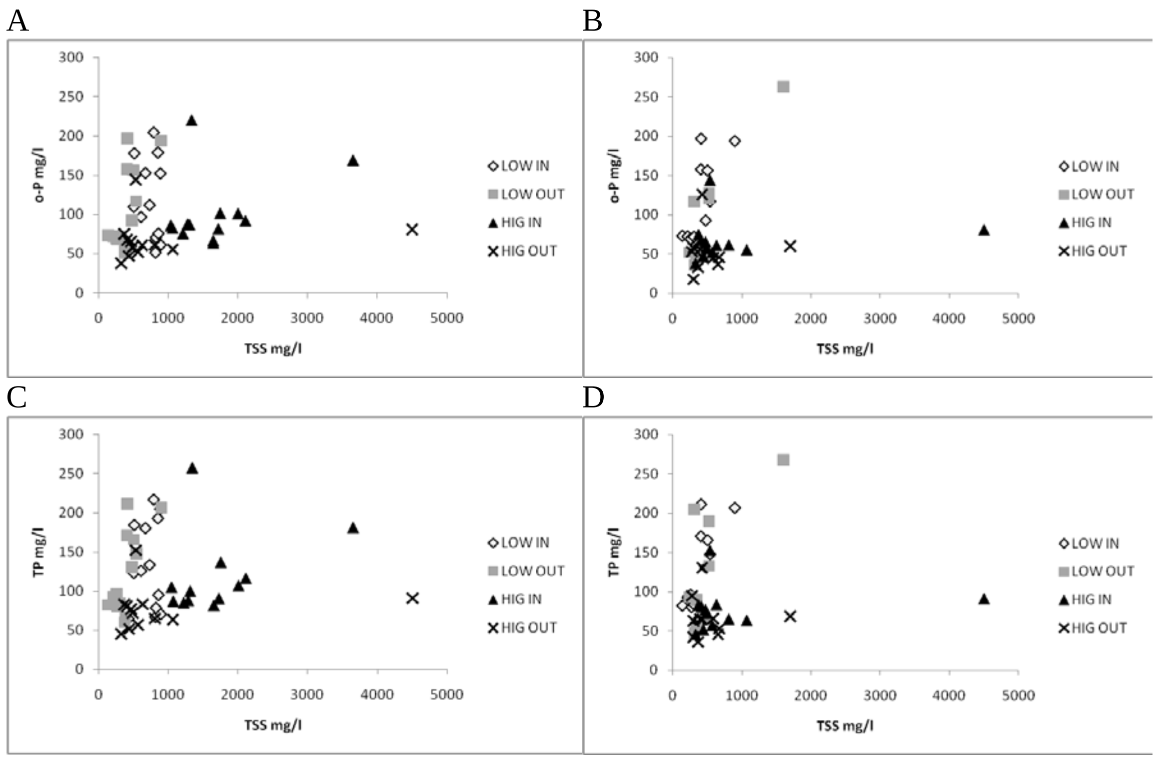


Figure 26*: Distribution of physical and chemical parameters in relation with TSS loads in low and high season at pilot scale in Salina Nature Park: A- o-P mg/l, C- TP mg/l in PF; B- o-P mg/l, D- TP mg/l in BF.*

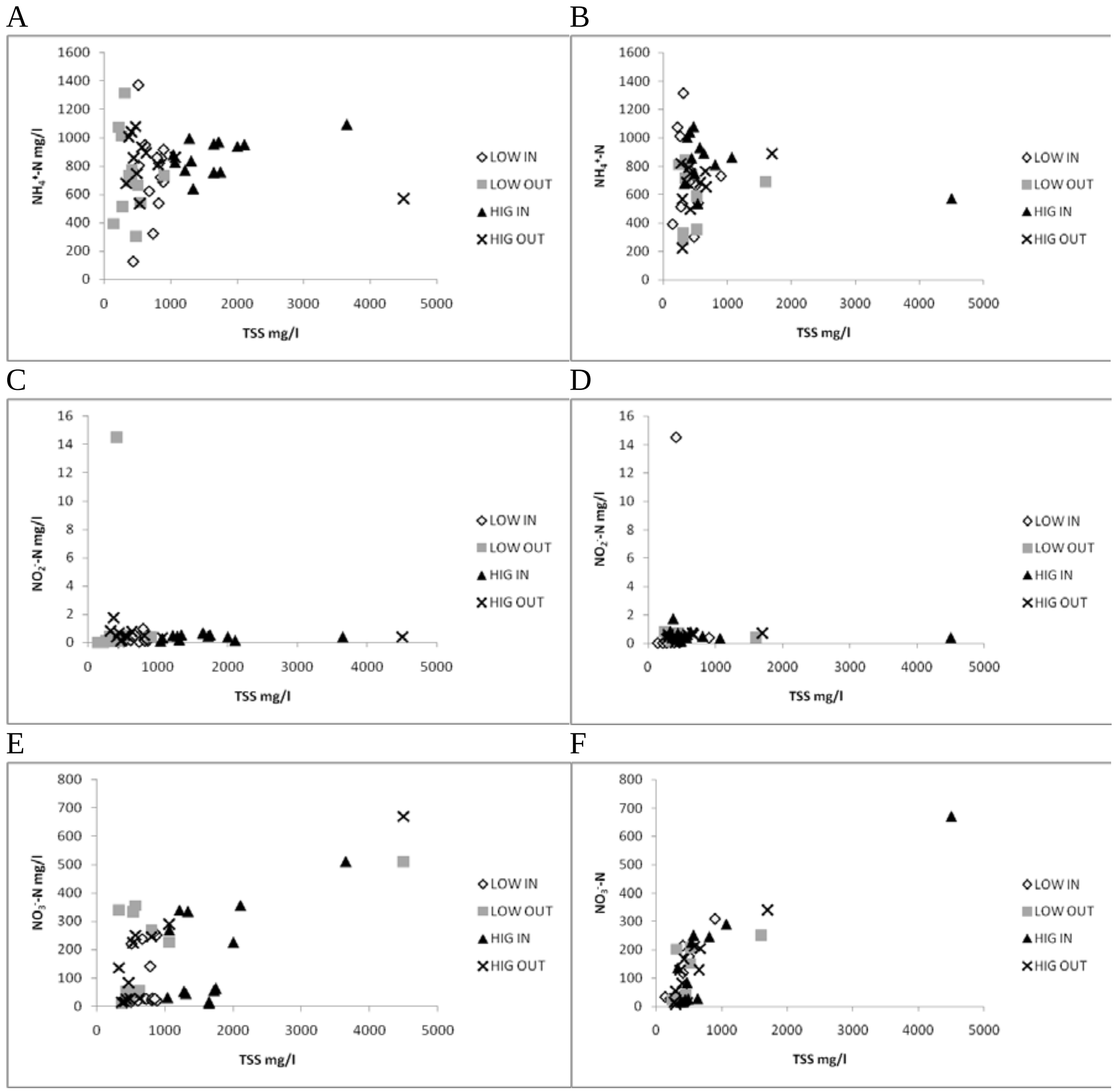


Figure 27*: Distribution of physical and chemical parameters in relation with TSS loads in low and high season at pilot scale in Salina Nature Park: A- NH_4^+-N mg/l, C- NO_2^--N mg/l, E- NO_3^--N mg/l in PF; B- NH_4^+-N mg/l, D- NO_2^--N mg/l, F- NO_3^--N mg/l in BF.*

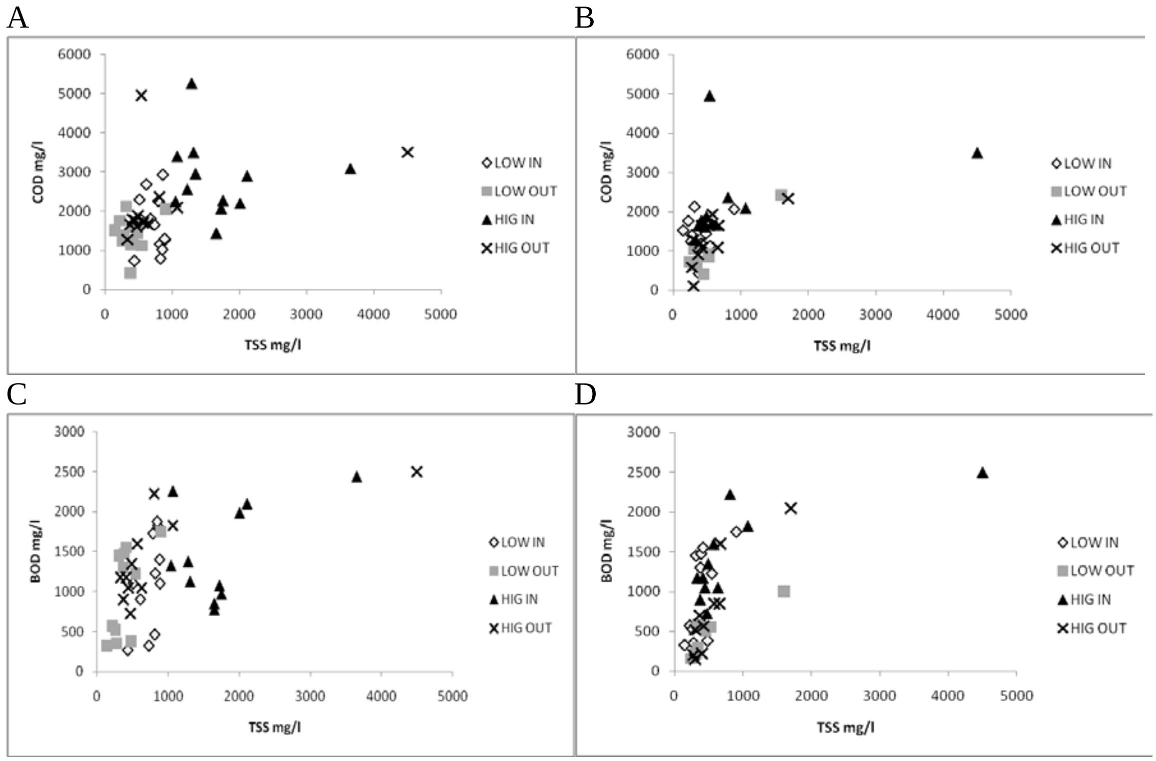


Figure 28*: Distribution of physical and chemical parameters in relation with TSS loads in low and high season at pilot scale in Salina Nature Park: A- COD mg/l, C- BOD mg/l in PF; B- COD mg/l, D- BOD mg/l in BF.*

The correlations of TSS in different periods (low period TSS<1000 mg/l, high period TSS>1001 mg/l) with different parameters were analysed to observe its effects on characteristics in individual modules (Table 10).

In the buffer tank (inflow) TSS showed no correlations with measured parameters. The low TSS input period is connected with low faeces input. In this period was more abundant load of only urine and toilet paper.

In the high TSS input period (TSS>1000 mg/l) an increase in TSS reflected the increase of NH_4^+-N in the buffer tank. High loads of organic matter (>1000 mg/l) in the system was indicated by strong positive correlations between TSS and N compounds (NO_3^--N, BOD_5, COD), especially with NO_3^--N, and a moderate positive correlation with NH_4^+-N in the inflow of PF. High TSS load resulted also in decreasing trend of pH indicated by a strong negative correlation in effluent from PF/inflow of BF. As in low TSS input period, BOD_5 and COD showed strong positive correlations in the effluent from BF. In both TSS load periods, the most numerous correlations were in the BF where components of liquid fraction were much more mineralized and “diluted” compared with PF (Table 10).

Table 10*: Summary of statistical significant Pearson’s correlation (bold, $p<0.05$) between total suspended solids in low (light grey) and high (dark grey) input periods (TSS low in-inflow; TSS low out-outflow; TSS high in-inflow; TSS high out-outflow) and physicochemical characteristics in peat filters (PF) and biofilter (BF) at pilot scale in Salina Nature Park.*

		PF				BF			
TSS low in < 1000 mg/l						NO_3^--N	o-P	TP	BOD_5
	r					0.816	0.681	0.693	0.622
	p					**0.001**	**0.010**	**0.009**	**0.041**
TSS low out < 1000 mg/l		NO_3^--N	o-P	TP	BOD_5	COD	o-P	TP	BOD_5
	r	0.816	0.681	0.693	0.622	0.931	0.915	0.719	0.885
	p	**0.001**	**0.010**	**0.009**	**0.041**	**0.001**	**0.001**	**0.029**	**0.008**
TSS high in > 1000 mg/l		NH_4^+-N				NO_3^--N		pH	BOD_5
	r	0.591				0.886		-0.734	0.729
	p	**0.033**				**0.001**		**0.007**	**0.011**
TSS high out > 1000 mg/l		NO_3^--N		pH	BOD_5	NO_3^--N		COD	BOD_5
	r	0.886		-0.734	0.729	0.845		0.714	0.878
	p	**0.001**		**0.007**	**0.011**	**0.001**		**0.014**	**0.001**

Table 11*: Physical and chemical parameters (average, standard deviation, minimum and maximum) in the influent (inf) and effluent (eff) of peat filters (PF) and biofilter (BF) at pilot scale in Salina Nature Park.*

	Low season				High season				Low season		High season	
	PF inf		PF eff		PF inf		PF eff		BF eff		BF eff	
TSS mg/l	721.5±158.5		400.9±189.8		1676.7±682.3		884.4±1157		516.4±418.3		554.1±404.6	
	431.0	890.0	141.5	900.0	1042.5	3650	327.5	4500	240.0	1600.0	275.0	1700.0
pH	8.7±0.7		8.4±0.5		8.8±0.4		8.6±0.4		8.5±0.3		8.6±0.3	
	7.3	9.4	7.7	9.2	8.2	9.3	7.8	9.1	8.3	9.2	8.2	9.2
EC mS/cm	6.9±2.2		7.3±2.1		8.1±0.4		7.7±1.3		6.1±1.6		6.9±1.7	
	2.07	10.52	4.6	11.4	6.4	9.7	5.8	10.1	3.5	8.3	2.7	8.7
DO mg/l	0.4±0.9		0.2±0.2		0.2±0.1		0.2±0.2		2.7±3.1		2.9±3.2	
	0.07	3.31	0.07	0.9	0.1	0.3	0.1	0.8	0.1	8.3	0.1	8.3
ORP	-70.6±8.0		-75.6±5.9		-91.1±9.8		-87.4±17.5		-85.7±2.5		-90.1±5.8	
	-76.3	-65.0	-79.8	-71.5	-104.6	-79.6	-107.9	-59.4	-87.5	-83.9	-98.9	-83.6
o-P mg/l	115.7±52.3		106.4±52.2		101.5±44.2		67.2±27		96.9±71.8		52.5±27.3	
	51.9	204.5	51.0	197.0	64.0	220.5	45.5	152.5	35.9	263.0	18.0	126.0
TP mg/l	134.2±56.5		122.9±52.8		116.8±50.8		76.9±27.6		127.4±77.4		66.5±26.7	
	65.2	217.5	60.5	211.5	81.5	258	45.5	152.5	46.9	268.0	36.0	131.0
NH_4^+-N mg/l	737.3±304.8		728.8±277.7		874.0±124.0		834.4±175.6		594.7±218.6		666.4±184.4	
	127.0	1370.0	303.0	1315.0	640.0	1092.5	535.0	1080.0	277.0	845.0	225.0	890.0
NO_2^--N mg/l	0.3±0.3		1.3±4.0		0.4±0.2		0.6±0.4		0.6±0.4		0.5±0.2	
	0.03	1.0	0.03	14.5	0.1	0.7	0.1	1.8	0.1	1.8	0.02	0.7
NO_3^--N mg/l	100.2±105.1		95.9±99.3		177.3±168.3		167.3±190.4		104.6±96.9		123.8±102.6	
	15.0	262.5	18.8	308.0	10.9	510	14.5	670	18.0	251.0	8.0	340.0
COD mg/l	1675.9±709.1		1437.5±445.2		2718.8±1009.3		2191.0±1034.7		932.8±588.3		1259.5±624.2	
	739.0	2940.0	421.5	2125	1436.0	5267.5	1282.5	4955	412.0	2425.9	110.0	2340.0
BOD_5 mg/l	1108.6±581.1		990.9±557.3		1480.2±604.4		1415.9±562.4		474.3±285.6		771.0±620	
	265.0	1875	325	1750	775.0	2442.0	725.0	2500.0	160.0	1000.9	150.0	2050.0

Microbial analyses in organic filters, biofilter and solar evaporation tank

The microbial analyses in the liquid fraction in the buffer tank and PF were based on RIDA®COUNT plates. For detailed results see chapter 3.4.

After two hours of daily recirculation in the BF, the liquid fraction was ducted into the solar evaporation tank to be sanitized at >60°C and released in the environment with a minimum impact, by evaporation. To calculate the microbial removal efficiency of the liquid fraction from the solar evaporation tank, the liquid fraction coming from the BF represented the inflow. The microbial parameters identified in the liquid fraction in the solar evaporation tank are shown in Table 12. Removal efficiencies were calculated based on differences between bacterial concentrations in BF and solar evaporation tank. Sampling in BF was performed at same time as in the evaporation tank. Less than 10 total coliforms were determined in solar evaporation tank while removal of total coliforms was 99.9%. Evaporation tank was highly efficient in reducing total bacterial count, removal efficiency of faecal enteroccoci and staphylococci was above 92.2%, while *E. coli* was not found.

***Table 12**: Bacteriological parameters in evaporation tank at pilot scale in Salina Nature Park.*

Parameter	Biofilter (CFU/ml)	Evaporation tank (CFU/ml)	Removal (%)
E. coli	ND	0	
Faecal enteroccoci	3.0×10^{5}	1.1×10^{3}	99.6
Staphylococci	ND	1	92.2
Total bacterial count	3.0×10^{5}	6.0×10^{3}	98.0
Total coliforms	3.0×10^{4}	<10	99.9

ND-not done

The concentrations of monitored compounds (TSS, N and P compounds) at the exit point from the biofilter were above the permitted values to discharge the liquid in environment (Ur.l. RS 64/2012) however the system was created to close the loop, to recycle and preserve nutrients from liquid and solid fraction (reason for high values of nutrients at exit points), and to evaporate water phase.

4.2 Blackwater solid fraction treatment

4.2.1 Composting of blackwater solids efficiency with selected bulk materials – a laboratory scale experiment

This laboratory experiment comprised by a bench scale experiment and tested the composting of BW solids with a combination of selected bulk materials as follows: (1) cereal bran, oat husks, bark; (2) cereal bran, oat husks; (3) cereal bran, oat husks, peat; (4) cereal bran, oat husks, sawdust; (5) peat, shrimp waste; (6) BW solids as control. The objective of this experiment was to compare effectiveness of different mixtures of bulk materials with BW in a self-heating mode and no stirring with a small quantity of composting material (maximum 1.125 kg of compost per composting reactor). The composting process was observed by performing physicochemical and microbial analyses into a special designed set-up.

The compost in bench scale reactors did not reach the thermophilic phase (>45°C). The maximum registered temperature was 41.5°C in mixture OCS (Figure 29). The temperature was in the mesophilic range, in mixture OCS between days 7 and 30, in OCB between days 7 and 31, in OCP between days 7 and 35, in OCE between days 12 and 24, and in PSW between days 12 and 17. In the CON (control) maximum temperature of 33.5°C was registered. At day 29 the reactors were opened to add water because of repeated low value of CO_2 (<0.5%), high values of O_2 (>17%) and no condensate on the exhaust tubes (see drop of temperature, Figure 29).

The temperature development during composting is influenced by the size of a reactor and consequently by the amount of composting material (Holtze and Backlund, 2003). Thermophilic conditions are unlikely to occur in ventilated composting systems with small masses of composting material (Del Porto and Steinfeld, 2000), which is the case in the laboratory experiment presented here.

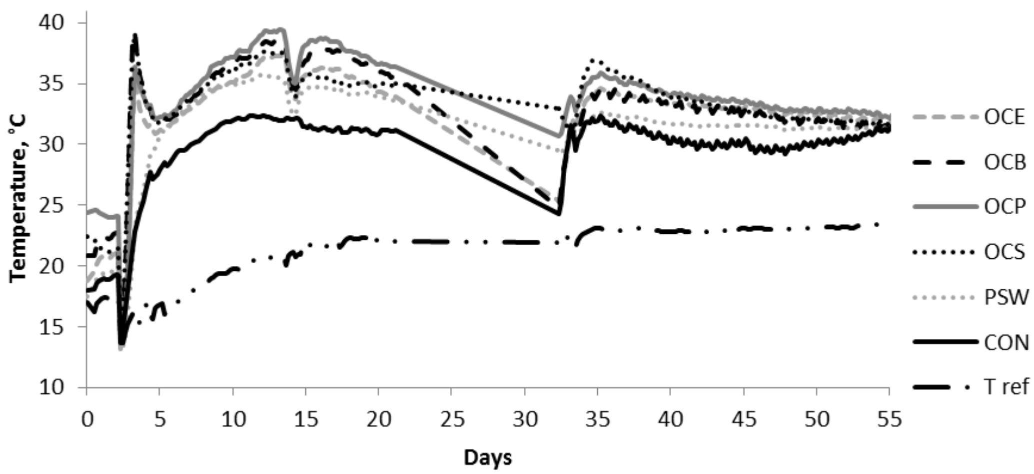


***Figure 29**: Temperature profiles for the different composting mixtures in the bench scale experiment. The figure shows the average values within the duplicate/triplicate of each mixture. OCE- oat husks, bran, blackwater; OCB- oat husks, bran, bark, blackwater; OCP- oat husks, bran, peat, blackwater; PSW- peat, shrimp waste, blackwater; CON- blackwater, T ref-ambient temperature.*

Table 13 shows results from physical and microbial analyses, conducted before and after composting, of the five different substrate mixtures and the reference mixture.

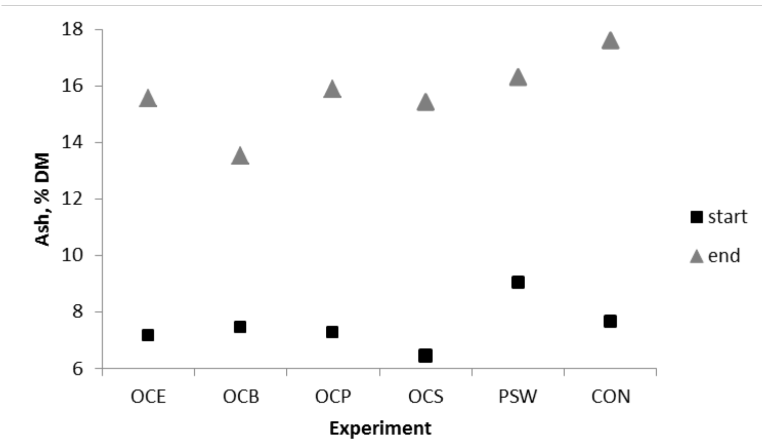
During composting considerable mass reduction was achieved, the highest in PSW (88%), followed by OCS (60%), OCP (56%), OCB (49%) and CON which exhibited only 41%. The VS content was still high in all mixtures at the end of the experiment, at the beginning was in the range of 91-94% and at the end around 84% for all mixtures (Table 13).

The compost experiment was aimed to reach a TS content of approximately 20% in the mixtures with the calculated ratio of BW solids and bulking materials. TS content was similar in mixtures OCS, OCB, OCP and OCE (approximately 22-24%), but was considerably lower in mixture PSW (approximately 15%) and CON 14% (Table 13).

The ash content during the composting process is increasing due to the mineralization of organic matter (Rekha et al., 2005).The ash increase over the experimental period (Figure 30A) ranged from 13.5% in OCB, a change compared to the beginning of experiment of

81.4%, to 17.6 in CON -a change of 129.5% compared to the beginning of experiment as well. At the end of the composting experiment, in OCS the ash increase was 15.4%- the highest change of 139.0% compared with the beginning of experiment; OCP-15.9%- change of 118.7%; OCE-15.5%- a change of 117.0; PSW- 16.3% -the lowest change of 80.3%. As well, at the end of the composting experiment which lasted 62 days, the mineralization rate was ranging in all mixtures from 44.5% (PSW) to 58.2% (OCS) (Figure 30B).

A



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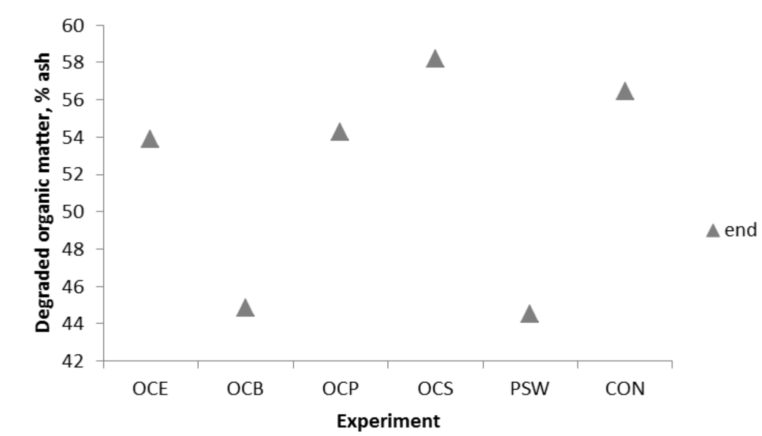


***Figure 30**: Ash content at start and the end of bench scale composting experiment (A) and mineralisation at end of composting (B) in six mixtures: OCE- oat husks, bran, blackwater; OCB- oat husks, bran, bark, blackwater; OCP- oat husks, bran, peat, blackwater; PSW- peat, shrimp waste, blackwater; CON- blackwater.*

At the beginning and at the end of the experiment, pH did not differ notably and ranged from 6.8-7.2 (Table 13) which is favourable for the substrate degrading microorganisms, but not for pathogen reduction (Holtze and Backlund, 2003).

Table 13*: Characteristics of the compost mixtures before (Start) and after (End) bench scale composting experiment (OCB- oat husks, bran, bark, blackwater; OCE- oat husks, bran, blackwater; OCP- oat husks, bran, peat, blackwater; OCS- oat husks, bran, sawdust, blackwater; PSW- peat, shrimp waste, blackwater; CON- blackwater).*

Mixture	OCB		OCE		OCP		OCS		PSW		CON	
Parameter	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
TS (%)	24.2	32.9	22.3	23.4	22.5	19.7	24.3	22.3	14.7	17.9	14.3	19.6
ASH (%)	7.4	13.5	7.2	15.5	7.3	15.9	6.5	15.4	9.0	16.3	7.7	17.6
VS (% of dry weight)	92.6	86.5	92.8	84.5	92.7	84.1	93.5	84.6	91.0	83.7	92.3	83.4
pH	6.8	6.9	6.8	6.8	7.1	6.9	6.8	7.2	7.1	6.7	ND	6.8
TC (%)	45.6	43.7	44.0	41.1	44.7	42.3	45.3	42.3	45.7	41.5	ND	39.2
TN (%)	1.6	2.7	2.1	3.1	1.8	3.0	1.7	2.7	2.6	2.7	1.5	3.0
C/N ratio	27.2	15.5	28.0	16.0	24.5	13.9	20.8	13.4	17.6	15.5	ND	13.1
NH_4^+-N (mg/kg)	2736	2497	3001	1979	3454	3588	2791	2448	4799	1235	1156	745
NO_3^--N (mg/kg)	<5.0	0.0	<5.0	0.0	<5.0	112.0	<5.0	42.0	<5.0	596.0	5.0	323.5
KTOT (g/kg)	7.1	9.2	8.0	12.1	8.5	10.7	6.4	10.5	5.2	5.0	ND	6.2
TP (g/kg)	13.1	22.9	16.5	29.1	17.6	25.3	16.3	25.8	20.0	26.9	ND	35.8
Mass (kg)	1.06	0.54	1.00	0.53	1.06	0.47	1.06	0.43	0.69	0.08	1.00	0.59
Mass reduction (%)		49.1		47.0		55.7		59.4		88.0		40.6
OM (% ash)		44.9		53.9		54.3		58.2		44.5		56.4
Faecal streptococci (10^6 CFU/g)	54.8	0.9	64.2	0.04	65.0	0.06	14.3	0.19	17.3	0.25	2.1	7.6
E. coli (10^3 MPN/g)	160	0.2	54.0	0.8	160	0.3	14.3	35.0	28.0	5.40	18.0	2.40

DM- dry matter, VS- Volatile solids, TC- Total carbon, TN- Total nitrogen, KTOT- total potassium, TP- total phosphorus, OM- organic matter degradation, ND- Not done.

C/N ratio at the beginning of composting ranged from 21-28:1 in the mixtures OCS, OCB, OCP and OCE. In PSW mixture was lower, 18. During composting C/N-ratio decreased in all mixtures, at the end of the process was between 13 and 16. C/N-ratio changes were in OCB- 43.0%, OCE- 42.9%, OCP- 43.3%, OCS- 35.6% and PSW- 11.9% (Table 13). A C/N ratio below 15 indicates stable compost recommended for direct agricultural use (Hait and Tare, 2011; Stratton and Rechcigl, 1998).

Total phosphorus (TP) increased during the experimental period in all mixtures. Concentration of TP in faeces have been reported to vary from 13.4 to 17.0 g/kg TS (Del Porto and Steinfeld, 2000; Vinnerås, 2001; Wrisberg et al., 2001). Before the experiment start the concentration of TP in the composting mixtures were within or close to this range.

Similarly to TP, KTOT in all mixture increased except in PSW. At the beginning of composting, concentrations of KTOT were 5.2-8.5 mg/kg. This was lower than reported for KTOT concentrations in faecal matter which range from 13.4-37.0 g/kg (Del Porto and Steinfeld, 2000; Vinnerås, 2001; Wrisberg et al., 2001) what can be attributed to added bulk material (bark, cereal bran, oat husks, peat, sawdust, shrimp waste) in the mixtures.

The nutrient and dry matter (DM) losses were estimated based on ash content calculations (Luebbe et al., 2011) and are listed in Table 14. The lowest nutrient and DM losses were estimated in OCB mixture, with a small notable N loss of 7.5%. The highest N and KTOT loss was found in PSW, while the highest TC and DM loss was noticed in OCS. TP was lost in the bigger percentage in OCP (34.2%). As no free leachate was observed, N was probably lost through volatilization of ammonia (measurements not done in this research). The N losses were in the range with those found by Himanen et al. (2011) in bio-waste compost (5%) and anaerobic sludge compost (around 40%).

***Table 14**: Effect of composting on dry matter (DM), total carbon (TC), nitrogen (N), total phosphorus (TP), and total potassium (KTOT) in all mixtures of the bench scale composting experiment. The table shows the average measured values within the duplicate/triplicate of each mixture.*

	Loss, % ash				
Experiment	DM	TC	N	TP	KTOT
OCE	51.8	57.0	33.4	18.5	29.9
OCB	24.9	47.2	7.5	3.8	28.0
OCP	59.8	56.7	23.9	34.2	42.5
OCS	61.5	61.0	31.7	34.0	31.5
PSW	32.5	49.6	42.7	25.6	46.4
CON	46.5	ND	14.4	14.4	ND

ND-not done

After 55 days of composting NH_4^+-N concentration decreased from 4799.0 mg/kg DM to 1235.5 mg/kg DM (Figure 31A). The increase of NH_4^+-N (ammonification) was noticed in OCP (from initial level of 3454.0 mg/kg DM to 3588.7 mg/kg DM to the end of experiment). In OCE concentration of soluble NH_4^+-N was 3001.0 mg/kg DM and decreased to 1979.5 mg/kg DM after 55 days of composting. OCS and OCB had a similar NH_4^+-N values and low reduction, from 2791.0 to 2448.0 mg/kg DM and respectively 2736.0 to 2497.3 mg/kg DM.

Nitrification was generally weak in all the BW compost mixtures (Figure 31B). The highest level of NO_3^-N was detected on average at the end of the compost experiment (day 62) in PSW- 596.0 mg/kg DM. In CON NO_3^--N was 323.5 mg/kg DM, in OCP- 112.0 mg/kg DM and in OCS 42.0 mg/kg DM. In OCE, OCB NO_3^-N was under the detection limit. The weak nitrification in this case was possibly connected with high moisture and less available oxygen and in consequence a lower microbial activity.

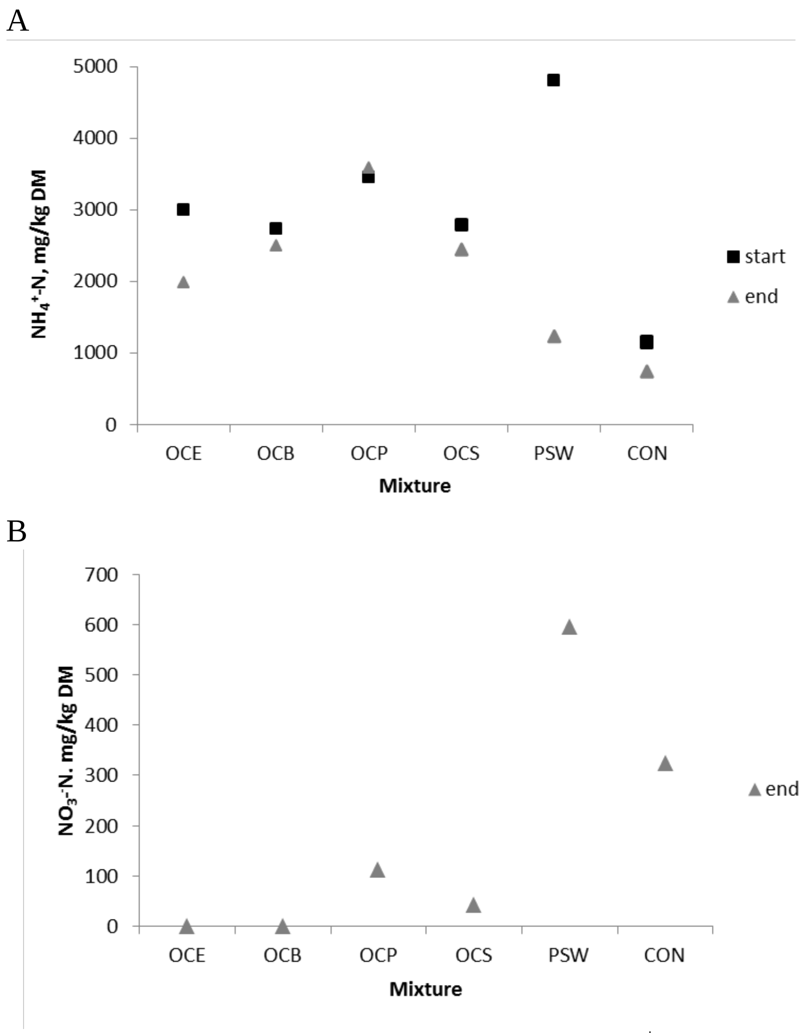


***Figure 31**: Concentrations of water soluble NH_4^+-N at the beginning and end of bench scale composting experiment (A) and NO_3^-N at the end (B). The figure shows the average measured values within the duplicate/triplicate of each mixture. OCE- oat husks, bran, blackwater; OCB-oat husks, bran, bark, blackwater; OCP- oat husks, bran, peat, blackwater; PSW- peat, shrimp waste, blackwater; CON- blackwater.*

The highest detected concentrations of CO_2 were noticed, with small differences, in OCE- 17.4 vol% with a peak in one of the triplicates of 19.9 vol% in first day, OCP- 17.1 vol%-peak of 18.7 vol% in the second day. The lowest emissions were in OCS- 13.8 vol% and CON- 14.7 vol%. Their peaks in one of the triplicates were reached in first day-14.8 vol% and respectively 14.1 vol% in the fifth day. In all the mixtures the highest CO_2 emissions were noticed in first 7 days of the experiment (CO_2 concentrations over 9 vol%), followed by a gradual decrease till day 27. During these 7 days, PSW showed a slower decrease in CO_2

concentrations compared with other mixtures but a faster decrease till the end of experiment (Figure 32). In the day 27 in all compost reactors an increase CO_2 concentration was detected at values over 4 vol%, with a peak of 10.8 vol% in one of OCS triplicates, lasting till day 33 for OCS, OCP, OCB, but lower for OCE and CON- 3.5 vol% and PSW- 2.0 vol%. In days 28 and 29 a fast drop in the CO_2 concentration was registered due to the drying of material and because of adding of water in individual compost reactors of all mixtures with the exception of OCP (Figure 32). The differences of CO_2 emissions between mixtures can be explained by the different degree of organic matter degradation in feedstocks. Methane was detected in all experiments up to 1.1 % and a value of 2.6 in CON. Methane in small percentage is formed possibly as result of aerobic oxidation reactions.

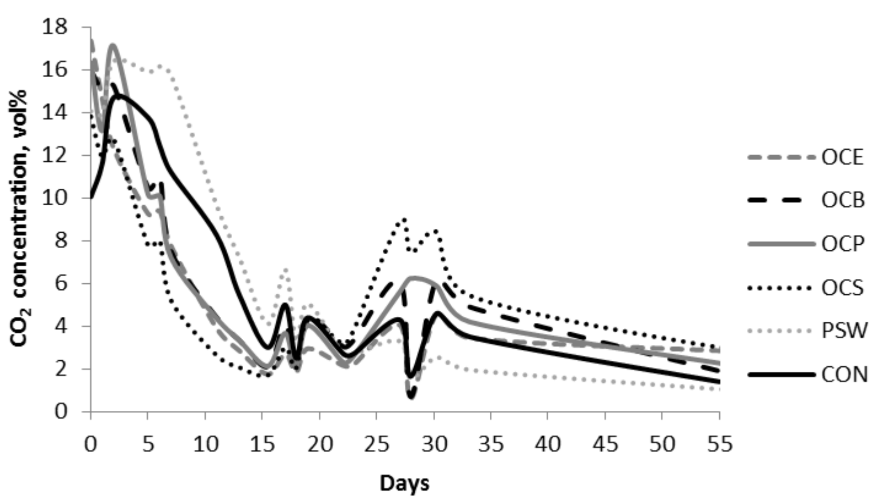


Figure 32*: Dynamics of carbon dioxide concentration measured in the mixtures in bench scale composting experiment (OCE- oat husks, bran, blackwater; OCB- oat husks, bran, bark, blackwater; OCP- oat husks, bran, peat, blackwater; PSW- peat, shrimp waste, blackwater; CON- blackwater).*

The total production of CO_2 ml/kg was calculated. The highest production of CO_2 was in PSW- 0.75 ml/kg, and the lowest in OCE- 0.36 ml/kg. In the CON CO_2 production was 0.45 ml/kg followed by OCB and OCP- 0.40 ml/kg and OCS- 0.39 ml/kg (Figure 33).

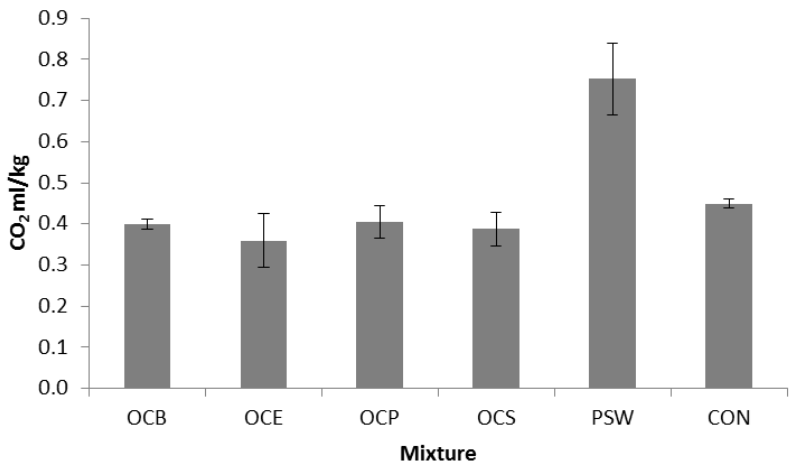
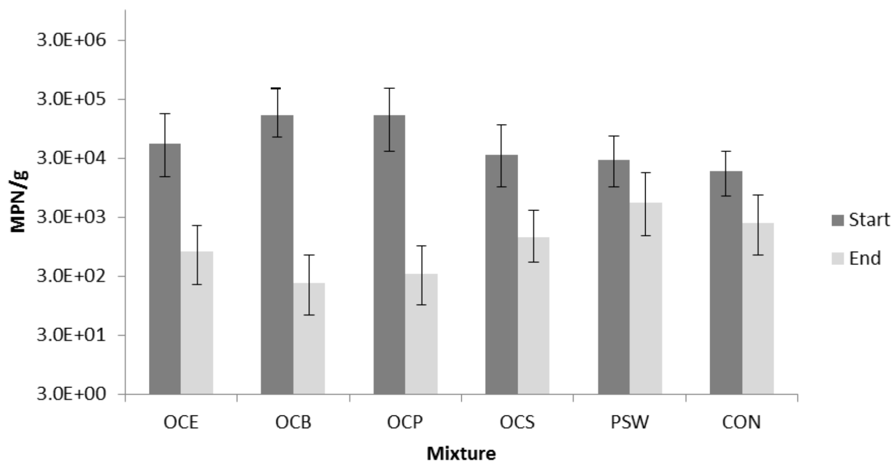


***Figure 33**: Total CO_2 production in blackwater compost mixtures in bench scale experiment. Error bars are standard deviation of triplicate determination.*

Even the composting process did not reach thermophilic temperatures in any of the mixtures the results showed that composting had a considerable reducing effect on *E. coli* at the end of experiment, as follows: OCB 99.9%, OCP 99.8%, OCE 98.5%, OCS 96.0%, CON 86.7% and PSW 80.7% (Figure 34A). Reduction of *E. coli* would be probably even higher if the moisture content had been lower towards the end of experiment, and the developed temperature reached thermophilic conditions. The moisture content in all the mixtures was in the range of 76-86%, which is higher than the reported optimum moisture conditions. Del Porto and Steinfeld (2000) found optimum moisture content for the composting process at 45-70%, (Boisen, 1995; Kalkoffen et al., 1995). High humidity may have caused limited oxygen access, resulting in reduced microbial activity in the composting mixtures (Holtze and Backlund, 2003).

A



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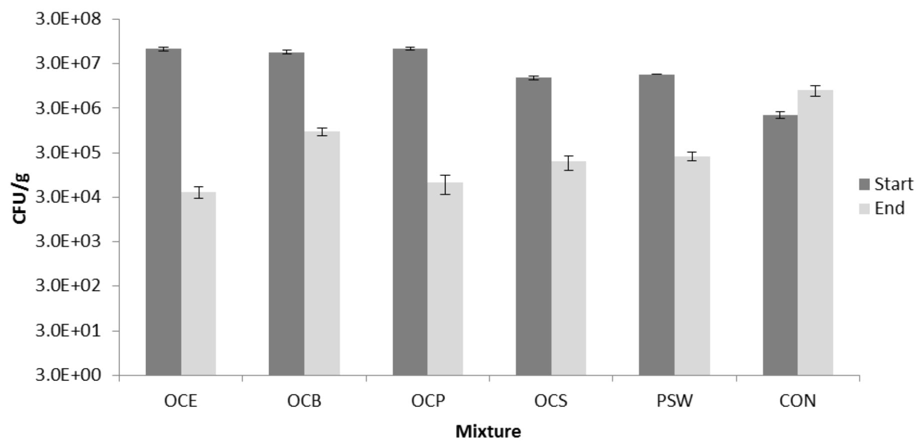


Figure 34: *Microbial analyses in bench scale composting laboratory experiment: A- E. coli; B- faecal streptococci.*

Composting had a substantial effect on the reduction of faecal streptococci (Figure 34B). The biggest reduction occurred in OCE and OCP (99.9%), followed by OCS 98.7%, PSW 98.5%, and OCB 98.4%. No reduction of this microbial indicator group was detected in CON. Even in composting toilet systems without development of high temperatures, the reductions of pathogen and compost volume occur, but slower (Holtze and Backlund, 2003). The volume reduction in such systems is related to activity of slower growing microorganisms while reduction of pathogens can occur due to competition and antagonism (Holtze and Backlund, 2003). In a case of composting of larger volume during secondary composting, higher temperatures may develop up to 61-72°C in 3-11 days in frosty weather, although secondary composting is generally characterized by lower biological activity and lower pile temperatures (Holtze and Backlund, 2003).

4.2.2 Composting of blackwater solids efficiency – a pilot scale experiment

Physical and chemical analyses

In this chapter are discussed the effects of this system set up and of a combination of bulk materials on BW composting process, differences of nutrient quantity and quality in the top, middle and bottom layers of the BW compost, phytotoxicity disappearance and eradication/inactivation of pathogens and parasites. This experiment lasted 19 weeks and two independent compost reactors were used. One way ANOVA showed the same trend of spatial and temporal distribution of measured parameters in both reactors.

The BW compost reached the thermophilic phase (>45°C) in the middle layer within 3 days and lasted until week 3 with a maximum temperature of 58°C. In the first 3 days and in the weeks 4, 5 and 6 respectively, the middle layer was in the mesophilic phase (35-45°C). The top layer had only mesophilic conditions which was reached on the third day and lasted until week 7 (max. 43.5°C). Top and middle layers reached the ambient temperature in week 7 and 6, respectively (Figure 35). Further, the temperature had a long-lasting gradually decrease until the end of the experiment (week 19). In both layers a similar trend was observed, but with longer a lag phase and after 6 weeks a higher mesophilic temperature in the top layer in comparison with the middle layer. Higher temperature in the middle layer can be explained by outer layers of a compost pile functioning as insulation for the inner part. Similar trends of temperatures were described in other compost studies with small differences in the duration of the thermophilic phase and the maximum temperature reached (Himanen et al., 2011). Niwagaba et al. (2009a, b) found out that in a compost mixture of 216 l (faeces/ash, 75 mm insulation) the thermophilic phase (≥50°C) was maintained for 4-12 days and in a mixture of 78 l (25 mm insulation) for a period of up to 3 days only. In our case the thermophilic phase lasted for 3 weeks without interruption with a volume of 30 l (6 cm insulation) starting material. Insulation played a crucial role to satisfy temperature requirements for sanitation and organic material degradation, which is necessary for small quantities even in tropical areas (Karlsson and Larsson, 2000).

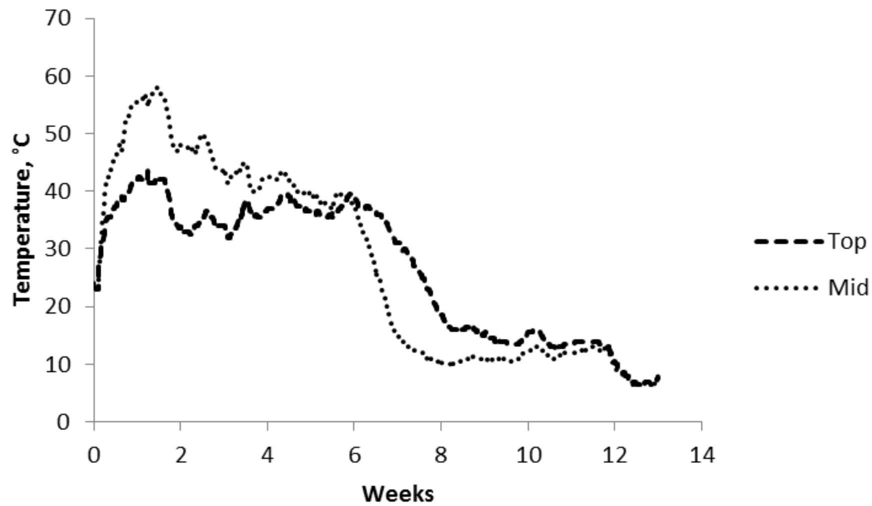


Figure 35*: Temperature measured during composting process at pilot scale in Salina Nature Park (Top- top layer, Mid- middle layer).*

During the composting pH varied in the different layers, but by the end of the experiment, all three layers reached a similar pH: top-7.3, middle-7.2 and bottom-7.3 (Figure 36A). In the top layer pH varied between 7.3 and 7.8 with two measurements of 8.4 and 8.7 in weeks 2 and 3, respectively. The middle layer showed other dynamics than the top, with a minimum value of 4.9 in first week and maximum values of 8.2 and 8.3 in the week 3 and 5. The bottom layer had a slower rise in week 1 and 2 (4.9 and 5.7) to a maximum of 8.4 in week 5.

According to findings of Niwagaba et al. (2009 b) in compost of faeces/ashes materials with a pH of ≤6 cannot attain thermophilic temperatures. This, however, was not confirmed by this study in which in the middle layer a pH lower than 6 was measured during the thermophilic period of the composting process. Two of the reasons for a low pH, particularly in the initial phase of the composting, are the production of organic acids as a result of the biodegradation process (Beck-Friis et al., 2003) and the presence of acidic peat as bulk material.

Electrical conductivity (EC) in the top layer was slightly lower (range 0.4-0.8 mS/cm) than in the middle and bottom layers (range 0.6-0.8 mS/cm) (Table 15). The trend of increased EC towards the bottom is caused by a possible leaching within the material. As ASCP guidelines (2001) demand that the EC of end product compost should be lower than 2.5 mS/cm, the studied layers were within acceptable levels in terms of safe application in agriculture.

***Table 15**: Analyses of compost at pilot scale in Salina Nature Park (DM-dry matter; ASH-ash; EC- electrical conductivity; >2 mg/l- detection limit for NO_3^-).*

Week	DM (%)			ASH (%)			EC (mS/cm)			NO_3^- (mg/l)		
	Top	Mid	Bot	Top	Mid	Bot	Top	Mid	Bot	Top	Mid	Bot
1	44.1	34.7	35.0	13.3	10.0	8.0	0.6	0.8	0.7	>2.0	5.0	>2.0
2	48.2	38.3	38.0	13.0	11.0	ND	0.4	0.8	0.8	>2.0	>2.0	>2.0
3	60.7	37.1	36.2	15.0	11.0	9.0	0.7	0.8	0.8	>2.0	>2.0	>2.0
5	41.57	41.4	57.0	ND	13.0	19.0	0.8	0.7	0.7	70.0	21.0	43.0
19	24.74	23.6	18.4	19.9	21.0	17.82	0.7	0.6	0.6	54.0	65.0	41.0

ND-not done

The C/N ratio is an indicator of organic matter mineralization and of the stabilization rate during composting. A C/N ratio of or below 15 indicates a stable compost recommended for direct agricultural use (Hait and Tare, 2011; Stratton and Rechcigl, 1998). In this study, the C/N ratio in BW compost had a decreasing trend during the composting process: in the top layer at the end of experiment was 11.2 (a decrease of 29.1%), in the middle 8.9 (a change of 48.7%) and bottom layer showed 9.9 (the highest decrease of 60.3%).

Values of TN and carbon over the 19 weeks of composting experiment had different trends. TN changed compared with the starting value by an increase of 0.96% in the top layer (2.6% in DM in week 19), 48.6% in the middle layer (3.7% in DM) and with 52.5% in the bottom layer (2.8% in DM) (Table 16). The increasing of nitrogen (N) and other nutrients was expected in lower layers, because the measurements showed that CO_2 was produced during organic matter degradation and emitted of the system faster than other partly volatile elements. N accumulation can be influenced here by several factors such as the mineralization rate, the initial amount of N present (average of 2.31% in all three layers), the pH variations (range 5.32-8.81), and leaching. A decrease in pH values can influence the N accumulation, while an inverse reaction of a high pH (>8) produces the loss of N as volatile ammonia (Brock et al., 1994). In this study, the increase of TN corresponded to a decrease of pH more visibly in the bottom and the middle layers, than in the top layer. TC decreased for 28.4% in the top (29.7% at the end of experiment or 19 weeks of composting), 23.7% in the middle (32.5% at the end of experiment) and for 39.5% in the bottom (27.6% at the end of experiment) layer.

For sulphur it was registered a decrease in the top layer of 4.5% (0.4% at the end of experiment), and an increase in the middle layer of 70.1% (0.5%) and in the bottom layer of 88.8% (0.4%) indicating that available sulphur concentrates in lower compost strata (Table 16). TP and KTOT in BW compost had an increasing trend in all three layers after 19 weeks of composting, with a maximum in the middle: TP dynamics showed a 12.14% increase (33.1 g/kg DM towards the end of the experiment) and KTOT of 9.1% (15.8 g/kg DM). The increasing concentration of P is caused by the mineralization of organic matter during the composting process.

***Table 16**: Quantity of nutrients at the end of compost experiment (g/kg DM) and relative change of individual nutrient compound during the process of composting (%) in top (Top), middle (Mid) and bottom (Bot) layers: total carbon (TC), total nitrogen (TN), total phosphorus (TP), sulphur (S) and total potassium (KTOT).*

Layer [position]	TC [g/kg DM]/Δ%	TN [g/kg DM]/Δ%	TP [g/kg DM]/Δ%	S [g/kg DM]/Δ%	KTOT [g/kg DM]/Δ%
Top [1/10]	296.7/-28.44	26.4/0.96	321.5/4.53	3.7/-4.45	153.1/8.67
Mid [4/10]	324.9/-23.70	36.7/48.62	331/12.14	5.1/70.13	158.4/9.08
Bot [9/10]	275.8/-39.45	28.0/52.45	291.5/11.51	3.9/88.78	156.5/8.63

The composting process can also be disadvantageous due to the loss of nutrients such as N (Eghball et al., 1997). In the study, the concentration of some important nutrients was increased in all three layers (TN, TP, KTOT). Nutrient losses were estimated using ash as an internal marker (Larney and Buckley, 2007, Luebbe et al., 2011). The results are summarized in Table 17.

At the end of the experiment, a loss of N of 29.2% was estimated in the middle layer, followed by 31.6% in the top, and 45.2% in the bottom. As some free leachate was observed, part of N was probably lost in liquid form. An accumulation of TP was observed in the middle (5.8%) and of KTOT in the top layer (1.2%). The smallest estimated losses were registered for KTOT. As the required long term average of nutrients for maintenance of soil fertility is in the level N: 170 kg/ha, P: 26 kg/ha, K: 133 kg/ha, Ca: 177 kg ha, Mg: 18 kg/ha, S: 20 kg/ha (Hammer and Clemens, 2007) application rate of compost from blackwater, which contains 32 kg/t P, is thus on average limited to around 800 kg/ha DM.

Table 17*: Effect of composting on dry matter (DM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), sulphur (S) and total potassium (TK) in Top (top), Cor (core), Bot (bottom) layers at pilot scale in Salina Nature Park.*

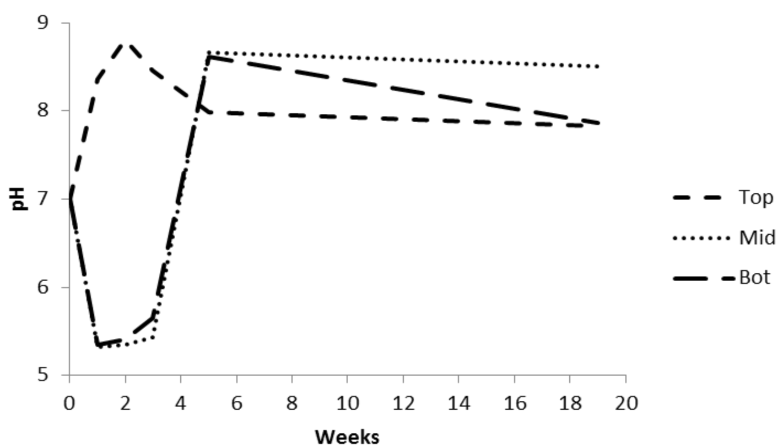
Parameter	Loss, % ash					
	DM	TC	N	TP	S	KTOT
Top	69.6	61.2	45.2	22.2	48.2	-1.2
Mid	67.6	63.7	29.2	-5.8	19.0	9.4
Bot	76.4	72.8	31.6	3.2	15.3	16.6

During composting the top layer had the highest values of DM ranging from 24.7-60.7% (Table 15). The middle layer was less fluctuating with a range of 23.6-41.4% and the bottom layer with 18.4-57.0%. The dry matter content varied until week 5, afterwards it decreased gradually. The middle layer was the closest to the preferred content of dry matter in compost, which should be between 30 and 50% (McFarland, 2000). Too high moisture content could fill pores and cause oxygen supply insufficiency (Marcinkowski, 2010). Probably due to bulk material combination (bark, peat and bran) and its hydraulic or moisture capacity, the leachate quantity could be limited to approximately one litre.

Rekha and his co-authors (2005) reported that the ash content increased from 49.4% to 61.9% due to the mineralization of organic matter. In this study, the ash content also increased at the end of the experiment by 84% (range 10.3%-19%) compared to the start of the experiment in the top layer, by 110% (range 10%-21%) in the middle and by 122% (range 8%-17.8%) in the bottom layer (Table 14). The organic matter mineralization at the end of the composting experiment was 45.7% in the top layer, 52.4% in the middle and 55.0% in the bottom layer.

The NH_4^+ levels were highest in top and bottom layers and started to decrease after the fifth week (Figure 36B). The peaks of NH_4^+ were reached at different time points for the different layers: in the top, the peak was reached in the third week (6560 mg/kg DM), in the middle in the fifth week (5330 mg/kg DM) and in the bottom in the second week, with 6150 mg/kg DM. At the end of the compost experiment NH_4^+ decreased to 3690 mg/kg DM in the top, to 3280 mg/kg DM in the middle, and to 4920 mg/kg in the bottom layer. In an experiment of Himanen et al. (2011) when sludge from municipal wastewater treatment plants was aerobically composted, a comparable concentration of NH_4^+ was reached after 20 weeks of composting (>2000 mg/kg).

A



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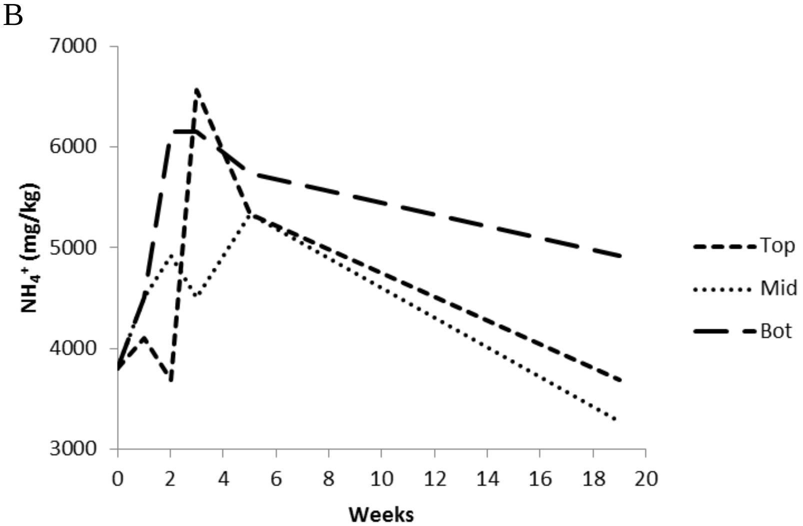

Figure 36*: pH (A) and ammonium concentrations (B) measured in top (Top), middle (Mid) and bottom (Bot) layers in compost at pilot scale in Salina Nature Park.*

In all three layers detectable levels of NO_3^- were observed after week 5. At the end of 19 weeks of composting NO_3^- was 2214 mg/kg DM in the top layer, 2665 mg/kg DM in the middle and 1681 mg/kg in the bottom layer (Table 15). At the end of the experiment the molar ratio NH_4^+/NO_3^- was the lowest in the middle layer, with 4.2 (top 5.7 and bottom 10.1) indicating that nitrification was highest in the middle layer. The NH_4^+/NO_3^- ratio provides a useful parameter to assess the degree of maturity (Compost maturity index, 2001). By the end of the experiment NH_4^+/NO_3^- was between 0.5-3 which according to the Compost maturity index (2001) is classified as mature compost.

Microbial and parasitological screening

One important requirement for applying compost in the field is the absence of pathogens. Human faecal material is the most important source of infection and contaminated water and/or food can cause serious epidemics (WHO, 2006). In comparison, contaminated (often cross-contaminated) urine only plays a minor role as a source of infections agents (Höglund et al., 1998).

Compost was screened for the presence of *E. coli*, overall coliforms and Enterobacteriaceae. It was shown, that 40 days (5.7 weeks) of composting is sufficient for complete removal of *E. coli* and for a significant reduction of coliforms and enterobacteria, i.e. to <0.03% (see chapter 3.4 and Oarga et al., 2012). Thus, from the sanitation point of view the produced compost was acceptable for agricultural use.

Compost was also screened for parasites. Although the prevalence of enteric parasites has become very low in the Central European population within the past 50 years, it was important to include parasites in the analyses, as protozoan cysts and helminth eggs are particularly resistant against temperature and desiccation and can thus remain viable in the environment for a very long time. Moreover, in this study set-up the faecal material derived from a touristic site, so that infection rates with parasites in the toilet users might have been very different to the average Central European population.

Nevertheless, all BW compost samples investigated were negative for human parasites (protozoa and helminths) by microscopic examination and all samples were also negative for *Entamoeba histolytica* and for *E. dispar* by realtime PCR. Other authors have found viable ova of *Ascaris lumbricoides* in BW compost produced from composting toilets, showing that their inactivation is not always successful. Yet, McKinley et al. (2012) report a successful inactivation of *Ascaris* ova within 2 weeks via high levels of ammonia in BW compost mixed with stored urine and ash. Jensen (2009) showed that the amount of ammonia (urine) in faeces is the most critical parameter for the reliable inactivation of helminth eggs, indicating that longer storage periods are needed if urine is separated from the excreta. In this study BW was collected and stored for a period of 4 months with high ammonium at the inflow, moreover BW compost had high values of ammonium from the very beginning indicating that inactivation of helminth eggs occurred during collecting and composting. These findings underline that this system was efficient in inactivating helminth eggs that is why the proper set up is crucial for sanitizing the compost.

Phytotoxicity test

The Index of Germination (GI) revealed toxicity of immature BWS compost and differences in the duration of the phytotoxic period. The measured parameters (germination and roots length) were affected in a different way in compost of different incubation times and in different layers (Figure 37).

The germination rate in the control was in average 96% and the average root length was 10.40±3.75 mm. In the top layer the GI showed a minimum of 64% in the first week of composting and a maximum of 89% in the last week. In the middle layer the minimum was 17.7% in first week and the maximum 111.4% after five weeks. In the bottom layer the GI showed a minimum of 18.5% in the second week and a maximum of 86.9% in the last week.

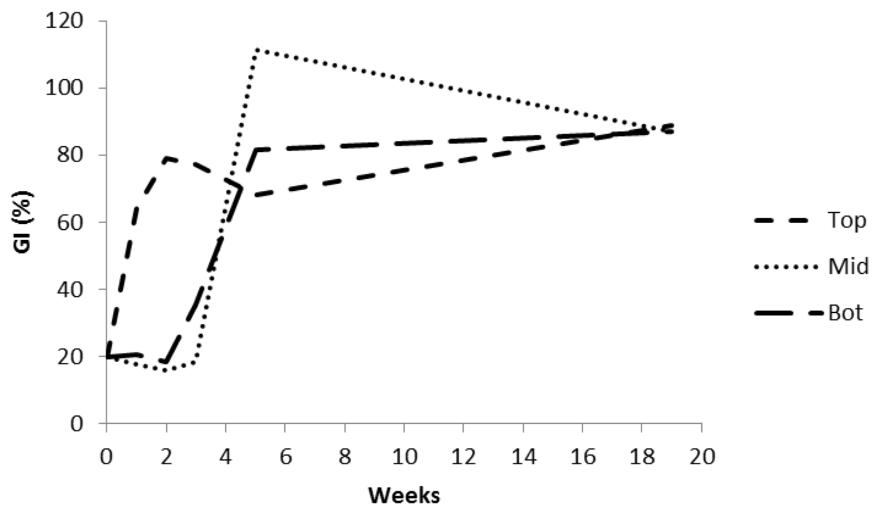

Figure 37*: Dynamics of phytotoxicity during composting in three layers (Top-top, Mid-middle, Bot-bottom) with species Lepidium sativum. Results of germination assay are expressed as ratio from root length and seed germination in blackwater compost extract and control (de-ionized water); mean of three replications. IG>80% is considered as a non-toxic compost.*

It has been suggested (Zucconi, 1981) that a germination index >0.5 indicates from the phytotoxicity point of view that the compost is usable as an organic fertilizer in agriculture. A GI ≥0.8 is considered non-toxic when coming into direct contact with roots of growing plants (Baca et al., 1990; Zucconi et al., 1981). The GI shows that in all three studied layers the phytotoxicity disappeared after five weeks of composting (≥0.8) and in the top layer the level was above 0.5 already from the first week.

Among the many reasons for the toxicity of immature compost are high values of ammonia, oxygen depletion, heavy metals and high concentrations of volatile organic acids and salts (Britto and Kronzucker, 2002; Adriano et al, 2006). The application of immature compost on the field would suppress seed germination and affect the root and plant growth (Gao et al., 2010). Pearson's correlation analysis was used to correlate the data on GI with physical and chemical parameters. Analyses of GI as a measure of phytotoxicity and physical and chemical parameters in the top layer showed no correlation, but in the middle layer it was shown that KTOT, TC and pH have an important impact ($p<0.05$). In the bottom layer important parameters influencing the germination were ASH, EC, TP and KTOT (Table 18). Similar patterns (pH and KTOT) were observed for middle and for bottom layers. Within the range of pH measured, a close to neutral pH was most supportive to seed germination and root growth of tested plants. A pH neutral to alkaline reduces or eliminates the toxicity and supports the plants growth (Figures 36A, 37; Biocycle, 1994). More available KTOT diminished the phytotoxicity. The negative correlation of TC in the middle layer can be attributed to the highest TC of all layers measured (Table 16), which can be used in microbial metabolism (respiration and fermentation) that resulted in lower pH and higher phytotoxicity. In the bottom layer compared with other layers, the conditions are very likely different due to the effect of sedimentation, formation of leachate and altered oxygen availability what can explain the statistically significant correlations between ASH, EC and TP and phytotoxicity.

Table 18*: Pearson's correlation coefficients (r/****p<0.05****) of Index of Germination (GI) to physical and chemical parameters (ASH- ash; EC- electrical conductivity; TC- total carbon; KTOT- total potassium; TP- total phosphorus) through time. Tested species- Lepidium sativum. Studied layers were Top (top), Mid (middle), Bot (bottom).*

Layer			GI		
Cor	pH	TK	TC		
	0.987/**0.002**	0.883/**0.047**	-0.895/**0.040**		
Bot	pH	TK	ASH	EC	TP
	0.966/**0.008**	0.958/**0.001**	0.982/**0.018**	-0.894/**0.041**	0.992/**0.001**

4.3 Bacteriological monitoring

The results showed a notable reduction of bacteria and fungi after the treatment process in the tested systems (Table 19). As RIDA®COUNT E. coli/coliforms kit test covers different subgroups of bacteria compared to RIDA®COUNT Enterobacteriaceae kit test, the numbers of coliforms and enterobacteria in some cases differed, for example in samples BW2, BW4, GW1. This is one of the parameters indicating changes in the abundance of a certain bacterial group. A reduction of bacteria was also observed in the GW module, although *E. coli* was present in the last treatment wetland at the outlet. This unexpected result can be attributed to the high summer temperature of stagnant water after accidental spilling from the BW module. In the samples from municipality wastewater treatment plant 91% of total bacteria, 91% of

Enterobacteriaceae, and 71% of *E. coli* were reduced at the outflow from the system. 40 days of composting was successful in complete removal of *E. coli* and reduction of coliforms and enterobacteria, i.e. <0.03%.

Table 19*: Ranges of counts of cultivable microorganisms expressed as CFU/ml in liquid and as CFU/g in solid wastes; blackwater treatment at pilot scale, BW1-BW4; greywater in hybrid constructed wetland at pilot scale, GW1-GW3; municipality wastewater treatment plant, MW1-MW2; compost from blackwater at pilot scale, SW1-SW2. Percentages of indicator group E. coli, total coliforms and Enterobacteriaceae compared to total bacteria are presented in brackets and referred to all samplings.*

Sample	Bacteria CFU/ml	*E. coli* CFU/ml (% of total)	Coliforms CFU/ml (% of total)	Enterobacteriaceae CFU/ml (% of total)	Yeast and moulds CFU/ml
BW1	4.5×10^{6}-1.9×10^{7}	1.3×10^{5}-1.9×10^{5} (1.02-3.43)	3.6×10^{5}-1.5×10^{6} (4.60-13.00)	2.8×10^{5}-8.3×10^{5} (3.51-13.47)	1.7×10^{3}-2.5×10^{4}
BW2	4.9×10^{6}-1.5×10^{7}	1.2×10^{4}-9.3×10^{4} (0.24-0.97)	6.8×10^{4}-9.7×10^{6} (0.94-10.14)	3.8×10^{4}-3.2×10^{5} (0.78-3.30)	1.9×10^{3}-1.4×10^{5}
BW3	1.6×10^{7}-1.9×10^{7}	2.4×10^{3}-4.3×10^{4} (0.01-0.26)	4.2×10^{4}-1.7×10^{5} (0.21-1.00)	8.8×10^{4}-2.4×10^{5} (0.52-1.26)	8.8×10^{2}-6.8×10^{3}
BW4	3.5×10^{3}-2.3×10^{6}	1.1×10^{1}-9.4×10^{2} (0.00-0.32)	1.1×10^{1}-2.0×10^{3} (0.01-0.32)	0-1.6×10^{3} (0.00-0.07)	2.0-4.8×10^{2}
GW1	2.2×10^{4}-1.8×10^{8}	0-5.6×10^{3} (0.00-0.00)	1.3×10^{4}-2.0×10^{5} (0.11-57.17)	5.6×10^{3}-2.4×10^{5} (0.13-25.00)	1.7×10^{2}-3.1×10^{2}
GW2	1.00×10^{3}-5.6×10^{4}	0-5.1×10^{1} (0.00-0.09)	1.2×10^{1}-4.5×10^{2} (0.39-6.84)	1.1×10^{1}-3.1×10^{2} (0.55-3.83)	1.0-1.6×10^{2}
GW3	3.3×10^{2}-7.9×10^{4}	0-4.9×10^{2} (0.00-0.62)	7.8-1.0×10^{3} (1.00-2.33)	7.8-7.1×10^{2} (0.90-2.33)	5.6-6.3×10^{1}
MW1	6.06×10^{5}	6.83×10^{4} (11.28)	2.02×10^{5} (33.30)	2.55×10^{5} (42.11)	2.67×10^{3}
MW2	6.53×10^{4}	2.33×10^{3} (3.57)	2.98×10^{4} (45.58)	2.99×10^{4} (45.83)	5.67×10^{2}
SW1*	1.62×10^{8}	2.81×10^{6} (1.74)	$2.85\text{x}10^{6}$ (1.76)	2.84×10^{6} (1.75)	7.88×10^{3}
SW2*	2.33×10^{7}-5.57×10^{8}	0	2.22×10^{1} -7.11×10^{3} (<0.01)	0-7.43×10^{3} (<0.03)	0

* microbial counts expressed as CFU/g

To get a better insight into changes in BW communities, the following indicators were used: ratio of total coliform bacteria vs. total bacteria, ratio of Enterobacteriaceae vs. total bacteria, and ratio of *E. coli* vs. total bacteria. During the treatment, reduction of *E. coli* in the system is clearly evident (Figure 38). Different sets of RIDA®COUNT plates showed significant changes in the bacterial community, for example in the samples BW2 and BW3 when percentage of bacterial subgroups of coliforms and enterobacteria notably differed (Figure 38).

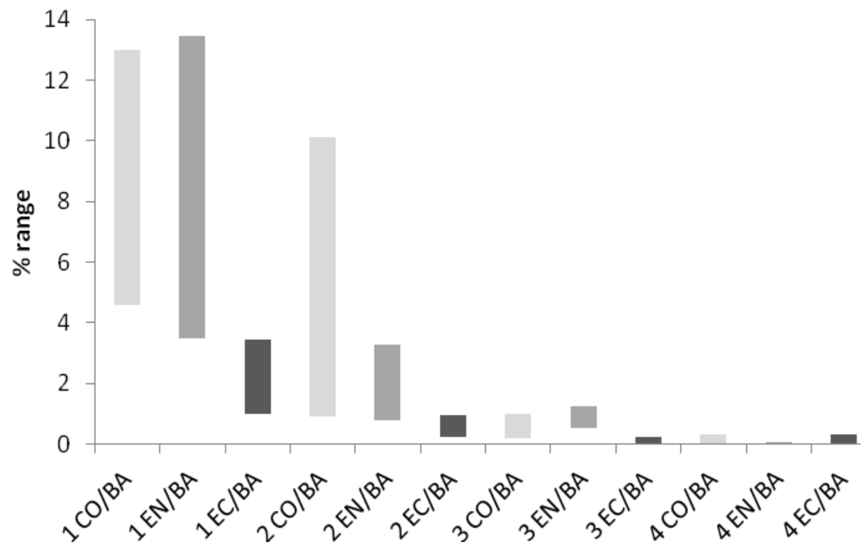

***Figure 38**: Ranges of microbial indicators during blackwater treatment at input (1-BW1), separation from solid phase (2-BW2), treatment of liquid (3-BW3), and ultimate phase of liquid treatment (4-BW4) at pilot scale in Salina Nature Park; CO/BA- ratio of coliform bacteria vs. total bacteria, EN/BA- ratio of Enterobacteriaceae vs. total bacteria, EC/BA- ratio of E. coli vs. total bacteria*

The only cells that are able to form colonies on RIDA®COUNT plates are those that can grow under the conditions of the test (incubation media, temperature, time and oxygen conditions). In bacteriology, nutrient agar is frequently used for nutrient non-demanding species to grow (Tanner, 2007), which is why was compared the retrieval of cultivable bacteria on RIDA®COUNT medium sheets and on nutrient agar plates from BW liquid (BW1) and compost at the beginning of the process (SW1). The average values of bacterial counts on RIDA®COUNT test plates after plating ten sheets with the compost indexed were 100.0 (RSD 17.2%); the streak plating on nutrient agar for compost was 146.0 (RSD 29.4%) and for pour plating on agar plates it was 122.9 (RSD 33.3%). This comparative agar method retrieved 22.9

to 46.0% more bacterial colonies than RIDA®COUNT plates. RIDA®COUNT plate counts were in the range of 7.27×10^7 and 1.11×10^8 CFU/g, streak plating 8.51×10^7 and 3.93×10^8 CFU/g, and pour plating in the range of 6.22×10^7 and 2.29×10^8 CFU/g. When BW liquid was analysed, streak plating on agar plates retrieved 61.7% more bacterial isolates (RSD 16.4%) but pour plating with nutrient agar gave 59.0% (RSD 22.2%) less bacterial colonies (Table 20). RSD for RIDA®COUNT plates was 27.9%. RIDA®COUNT plate counts were in the range of 1.08×10^7 and 1.43×10^8 CFU/ml, streak plating between 1.64×10^7 and 2.51×10^7 CFU/ml and pour plating in the range of 3.20×10^6 and 6.80×10^6 CFU/ml.

Table 20*: Relations between CFU counts on RIDA®COUNT Total and NA indexed on counts on RIDA®COUNT Total.*

Average counts of 10 replicates	RIDA®COUNT Total index	NA pour plating	NA streak plating
BW	100.0	41.0	161.7
SW	100.0	122.9	146.0

BW-blackwater; SW-compost

To demonstrate the eventual use of RIDA®COUNT plates in microbial ecology research in complex systems, the results of physical and chemical analyses in the BW module (BW1, BW2, BW3) were correlated with microbial groups. Physical and chemical parameters were fluctuating between different sampling campaigns. Temperature ranged from 20.9 to 28.8°C, pH from 8.00 to 8.71°C, DO from 0.05 to 6.8 mg/l, EC from 2.3 to 8.1 mS/cm, ORP from -107.7 to -71.4 mV, TSS from 295 to 2,625 mg/l, NH_4^+-N from 170 to 755 mg/l, NO_2^--N 0.1 to 0.8 mg/l, NO_3^--N from 47 to 282 mg/l, o-P from 24 to 263 mg/l, TP from 30 to 268 mg/l, COD mg/l from 910 to 2885 mg/l and BOD_5 from 600 to 2400 mg/l. Variations in the environmental parameters were high, probably due to occasional extremely high loads in the system. Pearson's correlation analysis was used to correlate the data on abundance of microbial groups, *E. coli* (EC), non-*E. coli* enterobacteria (NECEN, calculated as number of *E. coli* colonies subtracted from enterobacterial counts), non-*E. coli* non enteric bacteria (NECNENBA, defined as bacterial group which does not include EC and NECEN), and yeasts and moulds (Y&M) to environmental variables, and microbial subgroups. This analysis showed that there were no statistically significant correlations between microbial groups and the following measured environmental parameters, BOD_5, DO, NO_3^--N, o-P and TP. Analyses of microbial groups detected by RIDA®COUNT and environmental parameters in BW1 characterized by fresh BW inflow, showed that COD, ORP, pH and TSS had an important impact ($p<0.05$). In BW2 (output from the PF) important parameters which influenced the communities were: NH_4^+-N, NO_2^--N, ORP, pH and SEC. Temperature, pH and SEC had significant impact on some microbial groups in BW3 (liquid fraction after recirculation in BF). Correlations between abundances of different microbial groups were also observed in modules; furthermore, in all three studied BW modules, positive correlations were observed between total bacteria and non-*E. coli* non enteric bacteria, and coliforms and non-*E. coli* coliforms (Table 21).

Table 21: Summary of statistical significant Pearson's correlation (bold, p<0.05) between microbial subgroups (BA, EC, CO, EN, NECCO, NECEN, NECNENBA, Y&M) and physicochemical characteristics and microbial parameters in three different blackwater treatment modules (BW1, BW2, BW3).

Group	BW1		BW2				BW3		
BA	pH 0.998 **0.037**	NECNENBA 1.000 **0.016**	NH_4^+-N 1.000 **0.019**	NECNENBA 1.000 **0.013**			T -0.999 **0.025**	NECNENBA 0.999 **0.026**	
EC	ORP -0.999 **0.033**	COD 0.998 **0.04**							
CO	NECCO 1.000 **0.008**		NO_2^--N 0.998 **0.045**		NECCO 0.998 **0.038**	Y&M 1.000 **0.009**	SEC -1.000 **0.009**	NECCO 0.998 **0.038**	Y&M 1.000 **0.01**
EN	NECEN 1.000 **0.019**		pH 0.997 **0.05**	SEC -0.998 **0.031**					
NECCO	CO 1.000 **0.008**		NO_2^--N 1.000 **0.007**	CO 0.998 **0.038**	Y&M 0.997 **0.047**		SEC 0.997 **0.048**	CO 0.998 **0.038**	Y&M 0.999 **0.029**
NECEN	EN 1.000 **0.019**		pH 0.997 **0.046**	ORP -1.000 **0.008**			pH 0.999 **0.032**		
NECNENBA	BA 1.000 **0.016**		NH_4^+-N **0.999** **0.032**	**BA** 1.000 **0.013**			T -1.000 **0.002**	BA 0.999 **0.026**	
Y&M	TSS -0.998 **0.039**		NECCO 0.997 **0.047**	**CO** 1.000 **0.009**			NECCO 0.999 **0.029**	CO 1.000 **0.01**	SEC -1000 0.019

BA- total bacteria, EC- *E. coli*, CO- coliforms, EN- enterobacteria, NECCO- non-*E. coli* coliforms, NECEN-non- *E. coli* enterobacteria, NECNENBA- non-*E. coli* non enteric bacteria, Y&M- yeast and moulds, shaded relations between microbial groups.

For this study wastewater treatment systems with high (BW module) and moderate high organic input (municipal wastewater) were selected. We demonstrated the use of overlapping RIDA®COUNT culture media in organically enriched wastewater treatment systems. The plates used in the study revealed only a portion of the heterotrophic microorganisms. Use of cultivable enrichment techniques has a high value where selective media are required to demonstrate the presence and abundance of particular organisms in the system, for example *E. coli*, *Salmonella* spp., coliforms, enterobacteria. *E. coli* is the most common representative of the coliform group and is an indicator for the presence of other pathogenic genera (Tallon et al., 2005). Total cultivable microbial counts decreased during the natural treatment process but, more specifically, the indicator group for enterobacteria (*E. coli* and total enterobacteria counts) demonstrated the efficient removal of pathogens of enteric origin. In the treatment system for municipal wastewaters, the reduction of *E. coli* in the treatment process was 71% (BOD_5 in the input 330 and output 13 mg/l), in the case of BW treatment the removal rate was 99.4%, and in the compost it reached 100%. A similar trend was observed when Enterobacteriaceae was used as an indicator group. Yeasts and moulds were present in all samples with the highest counts of 1.4×10^5 CFU/ml, except in the compost sample SW2 after 40 days of composting. The absence of fungal colonies in SW2 sample on RIDA®COUNT Yeast&Mold Rapid plates is not necessarily linked to the absence of this microbial group - more probably, slow growing fungi do not successfully thrive on this medium even after prolonged cultivation of 72 hours.

The retrieval of cultivable bacteria on RIDA®COUNT Total Aerobic Count medium sheets compared to nutrient agar plates from BW liquid can be considered satisfactory: the RIDA®COUNT test plates can substitute agar cultivation for organically rich solid and liquid wastes for monitoring and quick screening of the samples (Table 20).

Although commercially available kits, such as RIDA®COUNT do not cover a wide range of different bacterial groups, simultaneous use of different selective plates, in combination with total bacterial counts for the same sample, give comprehensive information on microbial status. Data on abundance of an individual microbial group obtained by cultivation can be correlated with environmental parameters. The results of correlation analyses of the BW modules in the Salina Nature Park indicated very complex conditions in the source separation treatment system. The bacteria of non-enteric origin frequently correlated with physical and chemical parameters in the BW modules, but bacteria of enteric origin to a lesser extent (Table 21). This can be attributed to the fact that conditions in the system are not favourable for enteric indicators which are practically eliminated downstream in the system - for example, *E. coli* becomes less relevant in the process, but it is still a good indicator for the treatment efficiency (Figure 38). It is worth stressing that in the mineralization processes, other bacteria in the community which have not been retrieved on the RIDA®COUNT test plates, have important roles in energy and matter fluxes. Human waste contains on average $10^{11.66\pm0.23}$ by direct microscopic count (wet weight) per stool sample (Gosslingand and Slack, 1974). In general, a large proportion, (93%) of the microscopic clump counts from human stool can be cultivated in anaerobic conditions (Moore and Holdeman, 1974). In BW liquid on RIDA®COUNT plates, bacterial counts were up to 1.9×10^7 CFU/ml. In the treatment systems conditions are different - for example, conditions in compost samples resemble more closely the soil environment, where only 1 to 10% of microorganisms can be cultivated (van Elsas et al., 2006).

RIDA®COUNT test kits were not originally designed to be used for wastewaters, but these results showed that these plates can indeed be an effective substitute for routine use with such complex environmental samples. Cultivation of bacteria on RIDA®COUNT test plates average the conditions of streaked plates when microorganisms have normal available concentrations of oxygen, and poured plates when exchange of oxygen between atmosphere and the media is slowed-down microaerophillic conditions. This study shows that RIDA®COUNT plates can be used successfully as an alternative method to agar counting, and can be used as a research tool. With only basic training in microbiology, this method can also be used for robust routine monitoring at municipal wastewater treatment plants, before treated water is released into the environment. There are other more sensitive methods available to identify and quantify microorganisms from the environment, such as PCR and its derivatives (Bartlett and Stirling, 2003).

5. CONCLUSIONS

Prior to treatment in downstream modules the use of organic filter media (peat, bark, peat/sawdust, peat/bark) as pre-treatment through filtration and total suspended solids (TSS) retention capacity, was assessed on a laboratory scale.

- Organic filter media are suitable for filtering blackwater (BW) prior to further treatment and peat/sawdust and peat/bark as well as plain peat showed better filtering properties than bark only.
- The reduction of TSS achieved by the organic filters was: peat/sawdust 81%, peat/bark 76%, peat 74% and bark 74%.
- The columns loaded with SBR mixture started to clog after 200 flushes for peat (8 days), 250 flushes for peat/sawdust and peat/bark and 400 flushes for bark. Regeneration (mixing) of filters could prolong the filters life, without having a significant impact on the TSS filter performance.
- These results showed that filter increasing thickness of organic filters, from 15 to 30 cm did not give significantly better performance in terms of particle (TSS) removal.

On a field scale, peat filter had an effective impact on BW treatment in retaining bigger particles (retaining efficiency of particles >1000 µm was of 96.5% and particles >100 µm 88.8%), and nutrients (e.g. 21.2% of total phosphorus from which 98.1% orthophosphate retained in peat filters). During functioning of the PF no clogging appeared.

- The peat filters (PF) and biofilter (BF) needed a period of approximately 100 days to achieve efficient nitrification.
- Nearly anaerobic conditions prevailed in the buffer tank and peat filters (PF). Nitrification in the biofilter (BF) was achieved as expected. To optimize nitrification conditions, input of oxygen in the system should be increased in the buffer tank (inflow). The retention and recirculation time in the BF should be prolonged to a minimum of 4 hours. Further research is needed to address this question.

- The system self-regulated high pH in the BW from the inflow (pH>9) in the PF that is acidic by nature (pH=5). As a consequence, the BW in BF reached pH close to the nitrification optimum (pH=7-8)
- High temperatures in Mediterranean areas can have an inhibiting effect on nitrification process at temperatures above 25°C. In the system at a maximum temperature of 28°C of the BW NO_3^--N reached maximum values. Oxygen concentration dropped in all treatment modules.
- The system effectively treated the BW in low and even at high TSS load periods before the remained water was released in environment by evaporation.

By composting, the nutrients and solids from BW accumulated in the PF were successfully treated and recycled.

- The experimental results proved the system set-up as suitable for BW composting.
- Low flush toilets, collection and storing of faeces and urine for six months and filtering over a peat filter-acted as a pre-treatment and resulted in potential parasites inactivation, and reduction of bacterial indicators of pathogenicity (*Escherichia coli* and enterobacteria).
- The compost was after five weeks of composting process odourless, free of phytotoxines, and rich in nutrients, especially phosphorus; (%) N: P: K = 3.0: 3.2: 1.6.
- The first five weeks were the most crucial in obtaining good quality of compost. To the end of 19 weeks of composting a maximum loss of nitrogen (45.2%) was estimated for the top layer (1/10 depth: 0-5 cm) of the compost and a minimum for the middle layer- 4/10 depth: 20-30 cm (29.2%).
- An initially inhomogeneous mixture because of the nature of the material is not the main parameter that influences composting, but also leaching, aeration rate and pre-treatment conditions play significant roles.
- Further improvements in composting should aim at better retention of N and sulphur.
- The study showed that the composted solid fraction of the toilet waste can serve as useful end-product and additional source of P for agriculture.

In the study a monitoring procedure using ready to use RIDA®COUNT test plates was tested on the field where minimum laboratory equipment is available. Total counts of heterotrophic aerobic bacteria, *E. coli*, total coliforms, Enterobacteriaceae, and counts of cultivable yeasts and moulds in BW, greywater (hand washing water), compost derivated from BW solids and additional municipal wastewater, were studied and compared.

- Simultaneous use of different RIDA®COUNT test plates in wastewater research and monitoring provided reliable results to observe: reduction of bacterial pathogens, and fluctuation of cultivable microorganisms. RIDA®COUNT plates can be used for routine monitoring in wastewater treatment plants, as well as for checking efficiency of sequential treatment modules for segregated effluents in terms of reduction of biological indicator parameters.
- The test kits also have a potential for preliminary analysis but not enough to complete microbiological studies in environmental research.

The source separation system which was located at the Adriatic Sea in Salina National Park, Slovenia, had been tested for performance on BW treatment for the first time. System by itself is a passive one and during the operation does not require a lot of maintenance and energy consumption. The prototype successfully passed the first trial by adopting different approaches to observe treatment efficiency, nutrient cycles and pathogens removal. The design of the system still offers a frame to improve the treatment process by simple (automatic/mechanical) adjustments. It should be emphasized that this system is particularly suitable for low- to medium concentrated BW at the source which in particular represents tourist destinations where tourists stay for a short period (less than three hours tours).

6. SUMMARY

Toilet waste or blackwater (BW) which is released untreated in environment cause not only severe pollution and represent biohazard, but also mean significant loss of nutrients and energy. The separate treatment of BW fractions (liquid and solid) is an efficient method for nutrient recycling with minimal risk of contamination. In the study components and processes of a sanitary treatment system situated in a tourist facility in a sensitive protected area (coastal Mediterranean area in Salina Nature Park, Slovenia) were tested. The tested system is based on separate treatment of black- and greywater and aim at closing the material flows by end products suitable for use in agriculture.

The BW treatment module consisted of the following components: buffer tank (inflow), peat filters (PF) and biofilter (BF). In this study the particulate matter retention in PF, removal of phosphorus and nitrogen from BW liquid fraction and composting of BW solids were studied.

Research questions regarding treatment of solid and liquid BW fractions were addressed on a laboratory level prior to conducting full scale experiments. The main focus of the research was the fate of BW solids in the novel system tested. Only satisfactory solutions from the laboratory scale were adopted at the full scale in Salina Nature Park.

One of the biggest challenges of source separating sanitary systems is treatment of concentrated BW with a high concentration of total suspended solids (TSS). Traditional mechanical treatment methods such as settling tanks are not sufficient to reduce TSS to the level that is appropriate for further treatment. On the laboratory scale, TSS retention capacity of different organic filter media such as peat, bark, peat/bark and peat/sawdust, as a potential additional filtration step for BW prior further downstream treatment was assessed. The experiments used mixture of raw sewage and secondary sludge (SBR mixture), for simulating potential storm loads, and settled BW filtered through 30 cm thick organic materials. For this purpose TSS was measured on daily base, a randomly selected number of samples have been analysed for particle size distribution and the accumulation of mineral material was assessed with the loss of ignition (LOI). The highest TSS reduction (81%) was reached with a

peat/sawdust mixture, followed by pure peat and peat/bark mixture (74% and 76%, respectively), while the TSS retention capacity of pure bark was only 58%. Filters performance depended on the inlet of TSS concentrations. The results showed that most of TSS accumulated in the upper layers of the filters, and the thickness of the filter material over 15 cm had no significant effect on the treatment performance. Organic filters have a key role as a BW pre-treatment media for removal of TSS.

Based on the laboratory results and market availability possibilities, peat was applied as organic filter for BW treatment in the system that was located in Salina Nature Park. BW from low flush vacuum toilets was filtrated over peat filters (PF). The PF had an effective impact on BW treatment in retaining big particles and nutrients, and during the testing period of 7 months no clogging appeared. Buffer tank and mainly urine content in BW were key factors for long period (7 months) of PF functioning without clogging.

General characteristics of liquid fraction of BW were studied. The BW was collected in a buffer tank from low flush vacuum toilets, filtered over PF, recirculated in a biofilter (BF) for two hours and evaporated using an evaporation module. The goal was to retain nutrients (N and P) in PF and BF, to reduce ammonia in PF and BF, to enhance nitrification in the BF, in order to evaporate the remaining liquid of BW into environment with the smallest possible impact. In the BW liquid fraction, the following parameters were measured; TSS, temperature, pH, electrical conductivity, dissolved oxygen, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, biological oxygen demand, chemical oxygen demand, total phosphorus, orthophosphate. The processes in the BF were oxygen limited. Oxygen input in the buffer tank (inflow) and in BF could probably improve the treatment efficiency in the BF. Microbial analyses showed that removal of total heterotrophic bacteria, faecal enterococci and staphylococci was above 98% while *Escherichia coli* was not detected.

The further step in this study was treatment of BW solids collected in the PF by composting. Composting of toilet waste is a suitable way to sanitize the material, to recycle nutrients, to eliminate odors and to reduce disposal areas. The goal of this experiment was to assess the efficiency for collection and treatment of BW. The field scale experiment was based on set of laboratory experiments. In the laboratory mixtures of BW and various combinations of bulk materials such as bark, peat, cereal bran, oat, sawdust, biological waste and shrimp waste were tested to observe the composting process. Analyses of physicochemical parameters were carried out (total solids, volatile solids, ashes, temperature, pH, ammonia, nitrite, nitrate, total phosphorus, total potassium). Daily CO_2 emissions using gas chromatography were measured as an indicator of organic matter degradation. During composting microbial indicators (faecal streptococci and *E. coli*) were monitored.

In the full scale experiment the BW solids collected in the PF were mixed with wheat bran and pine bark, and were composted. The composting process was monitored in terms of temperature development, reduction of pathogenic microbes, nutrient composition, phytotoxicity, and inactivation of parasites. The highest microbial activity was detected in the middle of the compost where three weeks of thermophilic conditions occurred, along with high levels of ammonia production. Detectable levels of NO_3^- were observed after week 5, when also the phytotoxicity disappeared (index of germination ≥0.8). By the end of the experiment (19 weeks) NH_4^+/NO_3^- was between 0.5-3 one criteria that defines mature compost. Total loss of dry matter was 71.2 %, 35.3 % of N, 27.5 % of S, 6.5 % of P, and 8.3% of K. The concentration of nutrients, especially P and K increased during the composting process. The experimental results proved the system set-up as suitable for toilet waste composting; the end-product was sanitized as indicated by parasites inactivation and killing of enterobacterial pathogens and a final phytotoxines-free condition. The compost was rich in nutrients, especially P. N: P: K ratio was 3.0: 3.2: 1.6. Such product is suited as an additional source of P for agriculture.

In this study total counts of heterotrophic aerobic bacteria, *E. coli*, total coliforms, Enterobacteriaceae, and counts of cultivable yeasts and moulds were studied using RIDA®COUNT test plates. Test plates were tested on samples from (treatment system in Salina Nature Park), greywater and compost derived from BW solids, and additionally from municipal wastewater from Postojna Treatment Plant. Microbial indicator groups based on RIDA®COUNT plates were studied to observe microbial fluxes in relation to environmental parameters, and to observe their reduction during the treatment process (e.g. *E. coli* and

Enterobacteriaceae) before treated wastes are release in the environment. Using ready-to-use RIDA®COUNT test plates was successful on the field when minimum laboratory equipment is available. The bacteria of non-enteric origin in the BW treatment system frequently correlated with physical and chemical parameters ($p<0.05$), but bacteria of enteric origin to a lesser extent. The RIDA®COUNT test plates can be used as a substitute for agar plating, to a certain level, for microbial investigation of organically rich solid and liquid wastes. Relative standard deviations for total bacterial counts on RIDA®COUNT were for compost 17.2% and for BW 27.9%, respectively.

All the approaches used for this research (i.e. filtration through peat filters, composting, recirculation of the liquid fraction and evaporation) proved that this source separation system was efficient for BW treatment: the nutrient cycle was closed by composting, liquid fraction was evaporated without negative impact into environment, and represented no biohazard.

Keywords: *sustainable sanitation, wastewater separation, blackwater, recycling, nutrients, closed loop*

POVZETEK

Odplake iz stranišč ali črna voda (ČV) ki jih neočiščeno spuščamo v okolje, ne povzročajo le resnega onesnaženja in biološkega tveganja, temveč pomenijo tudi pomembno izgubo hranil in energije. Ločeno čiščenje frakcije ČV (tekoče in trdne) je učinkovita metoda za kroženje hranil z minimalnim tveganjem onesnaženja. V raziskavi smo testirali komponente in procese sanitarnega čistilnega sistema, ki je bil postavljen na turistični lokaciji v občutljivem zaščitenem območju (obala Mediterana v Krajinskem parku Soline, Slovenija) za zapiranje snovnih tokov s pomočjo ločevanja vode na izvoru. Testirani sistem je bil zasnovan kot ločevalni sistem za črno in sivo vodo z namenom zaključevanja snovnih tokov in pridobivanja za kmetijstvo uporabnih končnih proizvodov. Enota za čiščenje ČV je bila sestavljena iz naslednjih komponent; usedalnik (dotok), šotni filtri (ŠF) in biofilter (BF). Omenjena raziskava je bila namenjena proučevanju zadrževanja delcev v ŠF, zmanjševanju fosforja in dušika v tekoči frakciji ČV in kompostiranju trdne frakcije ČV.

Raziskovalna vprašanja, ki se nanašajo na čiščenje trdnih in tekočih frakcij ČV, smo pred začetkom pilotnih poskusov najprej obravnavali na laboratorijski ravni. Raziskava je bila osredotočena na usodo trdnih frakcij ČV. Zadovoljive rešitve iz laboratorija smo prenesli na pilotni objekt v Krajinskem parku Soline.

Eden največjih izzivov pri ločevanju snovi v sanitarnih sistemih je čiščenje koncentrirane ČV, z visoko koncentracijo celokupne suspendirane snovi (TSS). Običajne mehanične metode čiščenja, kot je usedalnik, niso dovolj učinkovite pri zmanjšanju TSS na raven, ki je primerna za nadaljnje čiščenje. Z laboratorijskimi poskusi smo ocenili sposobnost zadrževanja TSS v različnih organskih filtrih, kot so šota, lubje, šota/lubje in šota/žaganje kot potencialni dodatni korak filtracije ČV pred nadaljnjo obdelavo v sistemu. Pri poskusih smo uporabili mešanico surovih komunalnih odplak in sekundarnega blata (SBR mešanica), pri čemer smo simulirali možne obremenitve ob nevihtah. V dveh neodvisnih poskusih smo sekundarno blato (SBR mešanica) in ČV iz usedalnika filtrirali skozi 15 cm in 30 cm debel sloj organskega materiala. V ta namen smo dnevno spremljali količino TSS; v naključno izbranem številu vzorcev smo analizirali porazdelitev velikosti delcev in ocenili akumulacijo mineralnih snovi z izgubo ob

žarenju (LOI). Največje zmanjšanje TSS (81%) smo dosegli z mešanico šota/žaganje, sledila sta čista šota in mešanica šota/lubje (74% oziroma 76%), medtem ko je bila sposobnost zadrževanja TSS pri čistem lubju le 58%. Rezultati so pokazali, da se je večina TSS akumulirala v zgornjih plasteh filtrirnega materiala in da debelina filtrirnega materiala večja od 15 cm ni imela učinka na učinkovitost čiščenja. Rezultati so tudi pokazali, da imajo lahko organski filtri ključno za predhodno stopnjo čiščenja ČV glede zmanjševanja TSS.

Na podlagi ugotovitev v laboratoriju in glede na razpoložljive možnosti na trgu, smo uporabili šoto kot organski filter za čiščenje ČV v postavljenem sistemu v Krajinskem parku Soline. ČV iz vakuumskih stranišč z nizko porabo vode smo filtrirali skozi šotne filtre (ŠF). ŠF so učinkovito vplivali na čiščenje ČV z zadrževanjem večjih delcev in hranil, zato med 7 mesečnim delovanjem sistema ni prihajalo do mašenja filtrov. Usedalnik in pretežna vsebnost urina v ČV sta bila ključna dejavnika za dolgo (7 mesecev) obdobje delovanja ŠF brez mašenja.

V raziskavi so bile proučevane splošne značilnosti tekoče frakcije BW. Iz vakuumskih stranišč se je BW sprva akumulirala v usedalniku, nato je sledila njena filtracija preko PF, dveurna recirkulacija v biofiltru (BF) in končno izhlapevanje v evaporacijskem modulu. Delovanje sistema je bilo naravnano na zadrževanje hranil (N in P) v ŠF in BF, zmanjšanje amonija v ŠF in BF, pospeševanje nitrifikacije v BF, in reduciranje mikroorganizmov tako, da je izhlapevanje preostale tekočine predstavljalo najmanjši možni učinek na okolje. Tekom poskusnega obratovanja sistema so bili v ČV izmerjeni različni fizikalno-kemijski parametri (TSS, temperatura, pH, električna prevodnost, raztopljeni kisik, amonijski dušik, nitritni dušik, nitratni dušik, BPK, KPK, celokupni fosfor, ortofosfati). Procesi v BF so bili omejeni s koncentracijo raztopljenega kisika. Povečan vnos raztopljenega kiska v usedalniku (dotok) in BF bi lahko verjetno povečal učinkovitost delovanja BF. Mikrobiološke analize so pokazale da je prišlo do več kot 98% zmanjšanja koncentracije celokupnih heterotrofnih bakterij, fekalnih enterokokov in stafilokokov, medtem ko *Escherichia coli* ni bila zaznana.

Nadaljnji korak v raziskavi je bil čiščenje trdne frakcije ČV, ki se je zbrala v ŠF, s kompostiranjem. Kompostiranje straniščnih odpadkov je primeren način za sanitacijo materiala, odstranitev neprijetnih vonjav in zmanjšanje območja za odlaganja odpadkov. Cilj poskusa je bil oceniti učinkovitost pričujočega sistema za zbiranje in čiščenje Poskus na terenu je temeljil na setu predhodnih laboratorijskih poskusov. V posebej načrtovani eksperimentalni postavitvi v laboratoriju smo testirali mešanice ČV in različnih kombinacij razsutih materialov, kot so lubje, šota, žitni otrobi, oves, žaganje, biološki odpadki in odpadki kozic, z namenom raziskovanja procesa kompostiranja. Opravili smo različne analize fizikalno-kemijskih parametrov (skupne trdne snovi, hlapne trdne snovi, pepel, temperatura, pH, amonij, nitrit, nitrat, celokupni fosfor, celokupni kalij). Dnevno smo merili emisije CO_2 z uporabo plinske kromatografije kot indikatorja za razgradnjo organskih snovi. Dodatno smo med kompostiranjem spremljali tudi mikrobiološke parametre (skupno število aerobnih bakterij, fekalni streptokoki in *E. coli).*

V okviru poskusa na terenu smo trdno frakcijo ČV, zbrano v ŠF, zmešali s pšeničnimi otrobi in borovim lubjem ter jo kompostirali. Med procesom kompostiranja smo spremljali rast temperature, zmanjšanje števila patogenih mikrobov, sestavo hranil, fitotoksičnost in inaktivacijo parazitov. Najvišjo mikrobno aktivnost smo glede na dvig temperature zaznali v srednjem sloju, kjer so bili tri tedne termofilni pogoji in visoke vrednosti amoniaka. Vrednosti NO_3^- smo lahko zaznali po 5 tednih, ko je izginila tudi fitotoksičnost (indeks kaljivosti ≥ 0.8). Do konca poskusa (19 tednov) je bilo razmerje NH_4^+/NO_3^- med 0.5-3, kar definira zrel kompost. Skupna izguba suhe snovi je bila 71.2 %, 35.3 % dušika, 27.5 % žvepla, 6.5 % fosforja in 8.3 % kalija. Koncentracije hranil, še posebej fosforja in kalija, so bile med kompostiranjem opazno povečane. Rezultati poskusov so dokazali, da je postavljen sistem primeren za kompostiranje straniščnih odpadkov; končni proizvod je bil sanitiran glede na inaktivacijo parazitov, odstranitev enterobakterijskih patogenov in odsotnost fitotoksinov. Kompost je bil bogat s hranili, še posebej s fosforjem; razmerje N:P:K je bilo 3.0: 3.2: 1.6. Takšen proizvod lahko služi kot dodatni vir fosforja za uporabo v kmetijstvu.

V raziskavi smo opravili štetje heterotrofnih aerobnih bakterij, *E. coli*, skupnih koliformnih bakterij in Enterobacteriaceae ter kvasovk in plesni z uporabo RIDA®COUNT testnih plošč. Testirali smo vzorce s čistilne naprave z decentraliziranim ločevanjem snovi, in sicer ČV, sivo vodo in kompost iz trdne frakcije ČV, ter dodatno vzorce komunalne odpadne vode s komunalne čistilne naprave Postojna. Proučili smo skupine mikrobioloških indikatorjev na podlagi RIDA®COUNT za ugotavljanje povezave med mikrobiološkimi tokovi in okoljskimi parametri ter njihovo zmanjšanje med procesom čiščenja (npr. *E. coli* in Enterobacteriaceae), preden so očiščeni odpadki odloženi v okolje. Uporaba RIDA®COUNT testnih plošč je bila primerna za terenske analize, kjer je na voljo le minimalna laboratorijska oprema. Bakterije ne-enteričnega izvora v čistilnem sistemu za ČV so bile pogosto korelirane s fizikalnimi in kemijskimi parametri ($p<0.05$), bakterije enteričnega izvora pa le v manjšem obsegu. RIDA®COUNT testne plošče lahko do določene mere nadomestijo agar plošče za mikrobiološke analize trdnih in tekočih odpadkov, ki so bogati z organskimi snovmi. Relativna standardna odklona za skupno število mikroorganizmov na RIDA®COUNT ploščah sta bila 17.2% za kompost in 27.9% za ČV.

Vsi pristopi, uporabljeni v tej raziskavi (npr. filtracija skozi šotni filter, kompostiranje, kroženje tekoče frakcije in evaporacija), so pokazali, da je bil sistem za ločevanje snovi učinkovit pri čiščenju ČV: kroženje hranil smo zaključili s kompostiranjem, tekoča frakcija pa je evaporirala brez negativnega vpliva na okolje in ni predstavljala biološkega tveganja.

Ključne besede: *trajnostna sanitacija, ločevanje odpadnih voda, črna voda, reciklaža, hranila, zaokrožena zanka*

7. REFERENCES

Adriano D.C., Chang A.C., Pratt P.F., Sharpless R. 1973. Effect of soil application of dairy manure on germination and emergence of some selected crops. Journal of Environmental Quality, 2: 396-399.

Ahn H.Y. 2006. Sustainable nitrogen elimination biotechnologies: a review. Process Biochemistry, 41: 1709-1721.

Anastasi E.M., Matthews B., Gundogdu A., Vollmerhausen T.L., Ramos N.L., Stratton H., Ahmed W., Katouli M. 2010. Prevalence and Persistence of *Escherichia coli* Strains with Uropathogenic Virulence Characteristics in Sewage Treatment Plants. Applied Environmental Microbiology, 76, 17: 5882-5886.

Anastasi E.M., Matthews B., Stratton H.M., Katouli M. 2012. Pathogenic *Escherichia coli* Found in Sewage Treatment Plants and Environmental Waters. Applied Environmental Microbiology, 78, 16: 5536-5541.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater; 2005. Washington DC.

Ariasd C.A., Heistadc A., Jonssone L., Hellströme D., Brix H., Markku Yli-Halla, Vrålec L., Valveg M. 2010. Filter bed systems treating domestic wastewater in the Nordic countries - Performance and reuse of filter media. Ecological Engineering, 36: 1651-1659.

ASCP Guidelines, 2001. Quality Criteria for Composts and Digestates from Biodegradable Waste Management. Association of Swiss Compost Plants (ASCP) in Collaboration with Swiss Biogas Forum. http://www.vks-asic.ch (10 Feb. 2012).

ASTM D7348 - 08e1, 2008. Standard Test Methods for Loss on Ignition (LOI) of Solid Combustion Residues, ASTM International, West Conshohocken, PA. DOI: 10.1520/D7348-08E01.

Baca M.T., Delgado I.C., Sanchez-Raya A.J., Gallardo-Lara F. 1990. Comparative use of cress seed germination and physiological parameters of *Heliantus annuus L*. to asses compost maturation. Biological Wastes, 33, 4: 251-262.

Ban Z., Dave G. 2004. Laboratory studies on recovery of N and P from human urine through struvite crystallisation and zeolite adsorption. Environmental Technology, 25, 1: 111-121.

Bartlett J. M.S., Stirling D. 2003. A short history of the polymerase chain reaction. PCR Protocols, 226, 3-6.

Baumayer A. 2004. New Toilets for Indian Slums. Nutrients Mass Balance of a Co-Composting Plant in Bangalore, India. BSc thesis, Hochschule Wädenswil, Switzerland.

Baur R., Prasad R., Britton A. 2009. Struvite harvesting to reduce ammonia and phosphorus recycle, International Conference on Nutrient Recovery from Wastewater Streams, 351-360.

Beck-Friis B., Smårs S., Jönsson H., Eklind Y., Kirchmann H. 2003. Composting of source separated household organics at different oxygen levels: gaining an understanding of the emission dynamics. Compost Science and Utilization, 11, 41-50.

Blessmann J., Buss H., Nu PA, Dinh B.T., Ngo Q.T., Van A.L., Alla M.D., Jackson T.F., Ravdin J.I., Tannich E. 2002. Real-time PCR for detection and differentiation of Entamoeba histolytica and Entamoeba dispar in fecal samples. Journal of Clinical Microbiology, 40, 12: 4413-7.

Biocycle, 1994. Composting Source Separated Organics, Journal of composting and recycling, The JG Press, Emmaus.

Blodgett R. 2010. Bacteriological Analytical Manual Appendix 2 Most Probable Number from Serial Dilutions. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm109656.htm (20 Sep. 2010).

Boisen T. 1995. Alternativ håndtering af spildevand og humant affald. Ph.d. projekt. Energigruppen. Fysisk Institut. Technical University of Denmark.

Britto D.T., Kronzucker H.J. 2002. NH_4^+ toxicity in higher plants: a critical review. Journal of Plant Physiology, 159, 567-584.

Brix H. 1997. Do macrophytes play a role in constructed treatment wetlands? Water Science and Technology, 35, 5: 11-17.

Brock D.T., Madigan M.T., Martinko J.M., Parker J. 1994. Biology of Microorganisms, 7th ed., Prentice-Hall International, New Jersey.

Bulc G.T. 2006. Long term performance of a constructed wetland for landfill leachate treatment. Ecological Engineering, 26, 365-374.

Bulc G.T., Klemenčič K.A., Oarga A., Uršič M., Kompare B., Todt D., Jenssen P. 2010. Treatment of sanitary wastewater with constructed wetland for remote located tourist facilities at sensitive coastal area. In Proceedings of 12th International Conference on Wetland Systems for Water Pollution Control, International Water Association, Venice. Italy, 4-9 October 2010.

Bulc G.T., Klemenčič K. A., Kompare B., Oarga A., Babič R., Uršič M., Smolar-Žvanut N., Jensen P., Todt D., Schumacher G., Marées H., Oberdieck A. 2011. Prototype of SANBOX Mediterranean modification with technical descriptions and user manual - final report: project SANBOX – Development of an innovative sanitation and wasterwater treatment system for remote located tourist facilities: seventh framework programme, capacities, research for the benefit of SMEs. Ljubljana: Faculty of civil and geodetic engineering: Institute of sanitary engineering: Faculty of health sciences, Department of sanitary engineering, 96 p.

California Compost Quality Council, 2001. Compost maturity index. http://www.ccqc.org/ (14 March 2012).

Camargo J.A., Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment International, 32: 831-849.

Corcoran E., Nellemann C., Baker E., Bos R., Osborn D., Savelli H. 2010. Sick water? the central role of wastewater management in sustainable development. A rapid response assessment. The Hague, UN-Habitat/UNEP/GRID-Arendal.www.grida.no (3 June 2012).

Corley M., Rodgers M., Mulqueen J., Clifford E. 2006. The performance of fibrous peat biofilters in treating domestic strength wastewater. Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering, 41, 5:811-824.

Cordell D., Dranger J.O., White S. 2009. The story of phosphorus: global food security and food thought. Global Environmental Change, 19: 292-305.

Couillard D. 1994. The use of peat in wastewater treatment. Water Research, 28, 6: 1261-1274.

Decree of emission of substances in discharge of waste water from small communal treatment plants ("Uredba o emisiji snovi pri odvajanju odpadne vode iz malih komunalnih čistilnih naprav" OG RS 98/2007).

Decree on the treatment of biodegradable waste (Uredba o obdelavi biološko razgradljivih odpadkov). Uradni list RS, 62/2008.

Decree on the emission of substances and heat in the discharge of wastewater into waters and public sewage system (Uredba o emisiji snovi in toplote pri odvajanju odpadnih vod v vode in javno kanalizacijo). Uradni list RS, 64/2012.

Del Porto D., Steinfeld C. 2000. What About Graywater, The Composting Toilet System Book. The Center for Ecological Pollution Prevention (CEPP), Concord, Massachusetts, 167-193.

DIN 11540, 2005. Peats and peat products for horticulture and landscape gardening. Test methods, properties, specifications. Deutsches Institut für Normung e.V., Beuth Verlag GmbH, Berlin.

Eaton A.D., Clesceri L.S., Rice E.W., Greensberg A.E., Franson M.A.H. 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington.

Ecosanres, 2005. Fact sheet No 4: closing the loop on phosphorus. Ecological Sanitation Research/SIDA, Sewden.
http://www.ecosanres.org/PDF%20files/Fact sheets/ESR4lowres.pdf (9.11.2012).

Eghball B., Power J.F., Gilley J.E., Doran J.W. 1997. Nutrient, carbon, and mass loss of beef cattle feedlot manure during composting. Journal of Environmental Quality, 26: 189-193.

Engel A., Kunz A., Blanke M. 2001. Effects of compost and wood chippings on soil nutrients and on vegetative and reproductive growth and fruit quality of apple to overcome the replant problem. Erwerbsobstbau, 43, 6: 153-160.

Directive 91/271/EEC of the Council Directive of 21 May 1991 concerning urban waste water treatment. Official Journal of the European Communities. L 135/40-52.

European Committee for Standardization 1999a. Soil Improvers and Growing Media – Determination of pH. Standard CEN EN 13037:1999 E. European Committee for Standardization, Brussels.

Gajurel D.R., Benn O., Li Z., Behrendt J., Otterpohl R. 2003. Pre-treatment of domestic wastewater with pre-composting tanks: evaluation of existing systems. Water Science and Technology, 48, 11-12: 133-138.

Gao M., Liang F., Yu A., Li B., Yang L. 2010: Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. Chemosphere, 78: 614-619.

Gariglio N.F., Buyatti M.A., Pilatti R.A., Gonzalez Russia D.E., Acosta M.R. 2002. Use of a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. New Zealand Journal of Crop and Horticultural Science, 30, 2: 135-139.

Gerba C.P., Smith J. E Jr. 2005: Sources of Pathogenic Microorganisms and Their Fate during Land Application of Wastes. Journal of Environmental Quality, 34:42-48.

Gossling J., Slack J.M. 1974. Predominant gram-positive bacteria in human feces: numbers, variety and persistence. Infeciton and Immunity, 9, 4: 719-729.

Govoreanu R., Seghers D., Nopens I., De Clercq B., Saveyn H., Capalozza C., Van der Meeren P., Verstraete W., Top E., Vanrolleghem P.A. 2003. Linking floc structure and settling properties to activated sludge population dynamics in an SBR. Water Science and Technology, 47, 12: 9-18.

Grove S.F., Lee A., Lewis T., Stewart C.M., Chen H., Hoover D.G. 2006. Inactivation of Foodborne Viruses of Significance by High Pressure and Other Processes. Journal of Food Protection®, 69, 4: 957-968.

Guterstam B. 1991. Ecological engineering for wastewater treatment: theoretical foundations and practical realities. In: Etnier C., Guterstam B. (Eds.), Ecological engineering for wastewater treatment; proceedings of International Conference Stensund. Bokskogen, Sweden, 38-54.

Hait S., Tare V. 2011. Optimizing vermistabilization of waste activated sludge using vermicompost as bulking material. Waste Management, 31, 3: 502-511.

Heeb J. 2007. Ecosan concept – closing the loop.

http://www2.gtz.de/Dokumente/oe44/ecosan/cb/en-indian-training-closing-the-loop-concept-2007.pdf (5 Nov. 2011).

Heinonen-Tanski H., van Wijk-Sijbesma C. 2004. Human excreta for plant production. Bioresource Technology, 96, 4: 403-411.

Heistad A., Jenssen P.D., Frydenlund A.S. 2001. A new combined distribution and pretreatment unit for wastewater soil infiltration systems. In: Mancl K. (Ed.), Onsite wastewater treatment; proceedings of the 9th International Conference on Individual and Small Community Sewage Systems. ASAE, Fort Worth, Texas, 2001, 200-206.

Henze M., Comeau Y. 2008. Wastewater Characterization. In: Henze M., van Loosdrecht M.C.M., Ekama G.A., Brdjanovic D. (Eds), Biological Wastewater Treatment. IWA Publishing, London, pp. 33-52.

Himanen M., Hänninen K. 2011: Composting of bio-waste, aerobic and anaerobic sludges - Effect of feedstock on the process and quality of compost. Bioresource Technology, 102, 2842-2852.

Höglund C., Stenström T.A., Jönsson H., Sundin A. 1998. Evaluation of faecal contamination and microbial die-off in urine separating sewage systems. Water Science and Technology, 38, 6: 17-25.

Holtze A., Backlund, A. 2003. Økologisk byfornyelse og spildevandsrensning, nr. 39- Kompostering og efterkompostering af humarne restprodukter indeholdt i afvandet "sort" spildevand. Storstrøms Amt, Miljøstyrelsen 2003.

Hotta S., Terazawa M., Funamizu N. 2004. Ammonia volatilization from sawdust matrix in composting toilet. Environmental Engineering Research, 41: 79-86.

Jensen P.K., Phuc P.D., Konradsen F., Klank L.T., Dalsgaard A. 2009. Survival of Ascaris eggs and hygienic quality of human excreta in Vietnamese composting latrines. Environmental Health, 8:57.

Jenssen P.D., Siegrist R.L. 1991. Integrated Loading Determination for Wastewater Infiltration System Sizing. On-site waste water treatment. Proceedings of 6th National Symposium on Individual and Small Community Sewage Systems, Chicago, Illinois, USA, 1991, 16-17 December 2001.

Jenssen P.D., Skjelhaugen O.J. 1994. Local ecological solutions for wastewater and organic waste treatment - a total concept for optimum reclamation and recycling. Proceedings of

7th International Symposium on Individual and Small Community Sewage Systems, Atlanta, USA, 11-13 December 1994.

Jenssen P. D., Bergstrøm C., Vatn A. 2009. From crap to food. The New Economy, Sommer 2009.

Jenssen P.D., Heyerdahl P.H.,Warner W.S., Greatorex J. 2003. Local recycling of wastewater and organic waste - a step towards the zero emission. Community. In Lekkas T.D. (ed.), proceedings of 8th International Conference Environmental Science and Technology, Lemnos Island, Greece, 8-10, September 2003.

Jenssen P.D., Greatorex G.M., Warner W.S 2004. Sustainable wastewater management in urban areas. Weiterbildendes Studium Wasser und Umwelt, Universität Hannover, pp.78.

Jenssen P.D., Krogstad T., Paruch A.M., Mæhlum T., Adam K., Arias C.A., Heistad A., Jonsson L., Hellström D., Brix H., Yli-Halla M., Vråle L., Valve M. 2010. Filter bed systems treating domestic wastewater in the Nordic countries – Performance and reuse of filter media. Ecological Engineering, 36, 1651-1659.

Johansson M., Jönsson H., Gruvberger C., Dalemo M., Sonesson U. 2001. Urine separation-closing the nutrient cycle. Stockholm Water Company. http://stockholmvatten.se/pdf_arkiv/enghlish/Urinsep_eng.pdf (15 Feb.2011).

Johannesen E., Ovell L., Eikum A.S. 2007. Optimal organisering og funksjonskontroll av renseanlegg i spredt bebyggelsen.

Johannessen E. 2012. Optimizing phosphorus removal in onsite wastewater treatment facilities. Doctoral thesis. Norwegian University of Life Sciences, Ås.

Jönsson H., Vinnerås B., Hoeglund C., Stenstroem T.A. 1999. Source separation of urine. Wasser Boden, 51, 11:21-5.

Kadlec R.H., Knight R.L. 1996. Treatment Wetlands, Lewis Publishers, Boca Raton, La Florida.

Kadlec R.H, Wallace S. 2009. Treatment Wetlands, 2nd ed., CRC Press, Taylor and Francis Group, New York.

Kalkoffen J., Fiedler D., Kludt R. 1995. Komposttoiletten–verschiedene Bauarten–Analyse und Bewertungen der Kompostqualität. Fachgebiet Siedlungswasserwirtschaft. Technische Universität Berlin.

Karlsson, J., Larsson, M. 2000. Composting Latrine Products in Addis Ababa, Ethiopia. Minor Field Studies 32, Luleå University of Technology, Luleå.

Khopkar S.M. 2004. Environmental Pollution Monitoring and Control, New Age International, New Delhi.

Kim J., Song I., Jong J., Lee S., Kim P., Jeong E., Park J., Choung Y. 2010. Influence of aeration rate on nitrogen during blackwater composting. Desalination, 251, 298-307.

Kõiv M., Vohla C., Mõtlep R., Liira M., Kirsimäe K., Mander Ü. 2009. The performance of peat-filled subsurface flow filters treating landfill leachate and municipal wastewater. Ecological Engineering, 35, 2: 204-212.

Kovod, 2012. Čistilne naprave. http://www.kovodpostojna.si (20 Aug. 2012).

Krogstad T., Sogn T.A., Asdal Å., Sæbø A. 2005. Influence of chemically and biologically stabilized sewage sludge on plant-available phosphorus in soil. Ecological Engineering, 25: 51-60.

Kujawa-Roeleveld K., Zeeman G. 2006. Anaerobic treatment in decentralised and source-separation-based sanitation concepts. Reviews in Environmental Science and Biotechnology, 5, 1: 115-139.

Kujawa-Roeleveld K., Fernandes T., Wirayawan Y., Tawfik A., Visser M., Zeeman G. 2005. Performance of UASB septic tank for treatment of concentrated black water within DESAR concept. Water Science Technology, 52, 1-2: 307-313.

Larney F.J., Buckley K.E. 2007. Dry matter mass balance estimates for composted feedlot manure. Compost Science and Utilization, 15: 222.

Lens P.N., Vochten P.M., Speleers L., Verstraete W.H. 1994. Direct treatment of domestic wastewater by percolation over peat, bark and woodchips. Water Research, 28, 1: 17-26.

Lopez Zavala M.A., Funamizu N., Takakuwa T. 2005. Biological activity in the composting reactor of the bio-toilet system. Bioresource Technology, 96, 7: 805-812.

Luebbe M.K., Erickson G.E., Klopfenstein T.J., Greenquist M.A., Benton J.R. 2011. Composting or stockpiling of feedlot manure in Nebraska: nutrient concentration and mass balance. Faculty Papers and Publications in Animal Science, Paper 768.

Marcinkowski T.A. 2010. Composting of Waste from Poultry Breeding – Biological Analysis. Chemical and Biochemical Engineering Quaterly, 24, 3: 377-382.

Masi F., El Hamouri B., Shafi H. A., Baban A., Ghrabi A., Regelsberger M. 2010. Treatment of segregated black/grey domestic wastewater using constructed wetlands in the Mediterranean basin: the zer0-m experience. Water Science and Technology, 61, 1: 97-105.

McFarland M.J. 2000. Biosolids Engineering. McGraw-Hill, New York.

McKinley J.W., Parzen R.E., Guzmán Á.M. 2012. Ammonia inactivation of Ascaris ova in ecological compost using urine and ash. Applied Environmental Microbiology, 78, 15: 5133-5137.

Meinzinger F., Oldenburg M. 2009. Characteristics of source-separated household wastewater flows: a statistical assessment. Water Science and Technology, 59, 9: 1785-1791.

Mitsch W.J., Jørgensen S.E. 1989. Ecological Engineering: an Introduction to Ecotechnology. John Wiley & Sons, New York.

Moore W.E.C., Holdeman V.L. 1974. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Applied Microbiology, 27, 5: 961-979.

Morgan P. 2007. Toilets That Make Compost: Low-Cost, Sanitary Toilets That Produce Valuable Compost for Crops in an African Context. Stockholm: Stockholm Environment Institute. Link: http://www.ecosanres.org/pdf_files/ToiletsThatMakeCompost_lowres_greyscale.pdf (31 Nov. 2012).

Murray J.W., Codispoti L.A., Friederich G.E. 1995. Oxidation-reduction environments: the suboxic zone in the Black Sea. In: Aquatic Chemistry: Interfacial and Interspecies ProcesseS. Huang C.P., O'Melia C.R., Morgan J.J. (Eds.). ACS Advances in Chemistry Series, 224: 157-176.

Niwagaba C., Nalubega M., Vinnerås B., Sundberg C., Jönsson H. 2009a. Bench-scale composting of source-separated human faeces for sanitation. Waste Management, 29, 2: 585-589.

Niwagaba C., Nalubega M., Vinnerås B., Sundberg C., Jönsson H. 2009b. Substrate composition and moisture in composting source separated human faeces and food waste. Environmental Technology, 30, 5: 487-497.

Novoselova L.Y., Sirotkina E.E. 2008. Peat-based sorbents for the purification of contaminated environments: a review. Solid Fuel Chemistry, 42, 4: 251-262.

Oarga A., Bulc G.T., Jenssen D.P., Mulec J. 2012: Monitoring of microbial indicator groups in organically heavily loaded wastewater treatment systems by using RIDA®COUNT kits. Fresenius Environmental Bulletin, 21, 12a: 3886-3893.

Paredes D., Kuschk P., Mbwette T.S.A., Stange F., Müller R.A., Köser H. 2007. New aspects of microbial nitrogen transformations in the context of wastewater treatment - a review. Enggineering for Life Sciences, 7, 1: 13-25.

Peter-Fröhlich A., Bonhomme A., Oldenburg M., Gnirss R., Lesjean B. 2007. Sanitation Concepts for Separate Treatment of Urine, Faeces and Greywater (SCST) - Results. Berlin, Kompetentzzentrum Wasser Berlin gGmbH.

R-Biopharm, 2012. Microbiology/hygiene. http://www.rbiopharm.com/product_site.php?product_range=Food%20and%20Feed%20Analysis&&product_class_one=TWljcm9iaW9sb2d5IC8gSHlnaWVuZQ== (10 Aug. 2012).

Rekha P., Suman Raj D.S., Aparna C., Hima Bindu V., Anjaneyulu Y. 2005. Bioremediation of contaminated lake sediments and evaluation of maturity indicies as indicators of compost stability. Internal Journal of Environmental Research and Public Health, 2, 2: 251-262.

Rezanezhad F., Quinton W.L., Price J.S., Elliot T.R., Elrick D., Shook K.R. 2010. Influence of pore size and geometry on peat unsaturated hydraulic conductivity computed from 3D computed tomography image analysis. Hydrological Processes, 24, 21: 2983-2994.

Rosemarin A. 2004. The precarious geopolitics of phosphorous. Down to Earth, 30: 27-34.

Rue E. L., Smith G.J., Cutter G. A., Bruland K.W. 1997. The response of trace element redox couples to suboxic conditions in the water column. Deep-Sea Research I, 44, 1: 113-434.

Ruiz G., Jeison D., Rubilar O., Ciudad G., Chamy R. 2006. Nitrification-denitrification via nitrite accumulation for nitrogen removal from wastewaters. Bioresources Technology, 97, 2: 330-335.

Rules on the treatment of biodegradable waste for compost (Pravilnik o predelavi biološko razgradljivih odpadkov v compost). Uradni list RS, 42/2004.

Safapour N., Metcalf R. H. 1999. Enhancement of solar water pasteurization with reflectors. Applied and Environmental Microbiology, 65, 2: 859-861.

Skjelhaugen O. J. 1999. Thermophilic aerobic reactor for processing organic liquid wastes Water Resource, 33: 1593-1602.

Smith D.C., Beharee V., Hughes J.C. 2001. The effects of composts produced by a simple composting procedure on the yields of Swiss chard (*Beta vulgaris L*. var. *flavescens*) and common bean (*Phaseolis vulgaris L.* var *nanus*). Scientia Horticulture, 91: 394-406.

Smith J.E. Jr., Perdek J.M. 2003. Assessment and management of watershed microbial contaminants. Critical Reviews in Environmental Science and Technology, 33:1-27.

Stenstrøm T.A. 2002. Reduction efficiency of index pathogens in dry sanitation compared with traditional and alternative wastewater treatment systems. In Proceedings of 1st International Conference on Ecological Sanitation, EcoSanRes, Nanning, China. http://www.ecosanres.org/Nanning_Conf_Proceedings.html (5 March 2012).

Stratton M.L., Rechcigl J.E. 1998. Organic Mulches, Wood Products, and Composts as Soil Amendments and Conditioners. In: Handbook of Soil Conditioners: Substances that Enhance the Physical Properties of Soil. Wallace A., Terry R.E. (eds.), New York, Springer: 43-96.

Tallon P., Magajna B., Lofranco C., Kam Tin Leung 2005. Microbial indicators of faecal contamination in water: a current perspective. Water, Air, and Soil Pollution, 166: 139-166.

Tanner R.S. 2007. Cultivation of Bacteria and Fungi. In Manual of Environmental Microbiology. Hurst C.J., Crawford R.L., Garland J.L., Lipson D.A., Mills A.L., Stetzenbach L.D. (eds.), Washington DC, ASM Press: 69-78.

Taylor M., Clarke W.P., Greenfield P.F., Swain G.J. 2004. Characterizing the physical and chemical properties of a vermicompost filter bed. Compost Science and Utilization, 12 4: 383-391.

Tchobanoglous G., Burton F.L., Stensel D.H. 2003. Wastewater Engineering: Treatment Disposal and Reuse, 4th ed., McGraw-Hill, New York.

Todt D., Jenssen P.D., Bulc T.G. 2010. Greenhouse wetland technology for blackwater treatment in high alpine locations. In Proceedings of 12th International Conference on Wetland Systems for Water Pollution Control, International Water Association, Venice. Italy, 4-9 October 2010.

Todt D., Jenssen P. D., Oarga A., Bulc T. G. submitted. Improvement options for organic filters applied as post polishing for package treatment plants and filtration of blackwater. Wastewater Science and Technology.

Triastuti J., Sintawardani N., Irie M. 2009. Characteristics of composted bio-toilet residue and its potential use as a soil conditioner. Indonesian Journal of Agricultural Science, 10, 2: 73-79.

UN WCED 1997. Brundtland Report. New York: United Nations. World Commission on Environment and Development (UN WEDC). http://www.sswm.info/library/5299 (12.06.2012).

van Elsas J.D., Torsvik,V., Hartmann,A., Øvreås,L., Jansson J.K. 2006. The Bacteria and Archaea in Soil. In: Modern Soil Microbiology. van J.D. Elsas, J.K. Jansson, J.T. Trevors (eds.). Boca Raton, CRC Press: 83-105.

van Voorthuizen E., Zwijnenburg A., van der Meer W., Temmink H. 2008. Biological black water treatment combined with membrane separation. Water Research, 42, 16: 4334-4340.

Vinnerås B. 2001. Faecal separation and urine diverting for nutrient management of household biogradable waste and wastewater. Licentiate Thesis. Swedish University of Agricultural Sciences Department of Agricultural Engineering, Uppsala, Report 244.

Vinnerås B. 2002. Possibilities for Sustainable Nutrient Recycling by Faecal Separation Combined with Urine Diversion. Doctoral Thesis. Swedish University of agricultural Sciences, Uppsala.

Vinnerås B., Palmquist H., Balmér P., Weglin J., Jensen A., Andersson Å., Jönsson H. 2006. The characteristics of household wastewater and biodegradable waste - a proposal for new Swedish norms. Urban Water, 3: 3-11.

Voet D., Voet J.G. 1995. Biochemistry, 2nd ed., John Wiley & Sons, Inc., New York.

Water quality, 1998. Determination of suspended solids by filtration through glass-fibre filters, ISO 11923 (E).

Wendland C. 2008. Anaerobic Digestion of Blackwater and Kitchen Refuse. Doctoral Thesis. Institut fuer Abwasserwirtschaft und Gewaesserschutz (AWW), Technische Universitaet Hamburg-Hamburg (TUHH), Hamburg.

Werner Ch., Panesar A., Bracken P., Huba-Mang E., Gerold E., Demsat S., Eicher I. 2004. An ecosan source book for the preparation and implementation of ecological sanitation projects. 3rd draft. GTZ.

WHO 2006. Guidelines for the Safe use of Wastewater, Excreta and Greywater. Volume 4. Excreta and Greywater Use in Agriculture. ISBN 92 **4** 154685 9.

Wilcoxon F. 1945. Individual Comparisons by Ranking Methods. Biometrics Bulletin, 1: 80-83.

Winker M., Vinnerås B., Muskolus A., Arnold U., Clemens J. 2009. Fertiliser products from new sanitation systems: their potential values and risks. Bioresource Technology, 100: 4090-4096.

Wolgast M. 1993. Recycling System. Brochure produced by WM-Ekologen, AB, Stockholm, Sweden.

Wrisberg S., Eilersen A.M., Nielsen S.B., Clemensen K., Henze M., Magid J. 2001. Vurdering af muligheder og begrænsninger for recirkulering af næringsstoffer fra husholdninger fra by til land. Miljøprojekt/Aktionsplanen for økologisk omstilling og spildevandsrensning. Miljøstyrelsen.

Yri A., Hensel G.R., Aasen R., Mæhlum T. 2007. Undersøkelse av mindre avløpsanlegg i normal drift. Bioforsk Rapport, 2,146.

Zar J. H. 1984. Biostatistical Analysis, 2nd ed., Prentice-Hall, Englewood Cliffs, New Jersey.

Zhao L., Zhu W., Tong W. 2009. Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. Journal of Environmental Sciences, 21, 6: 750-757.

Zucconi F., Forte M., Monaco A., De Bertoldi M. 1981. Biological evaluation of compost maturity. BioCycle, 22: 27-29.

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