Optimization of Nitrogen Removal in Various Vertical Flow Constructed Wetland Designs and Application of Treated Wastewater for Reuse in Irrigation in Jordan

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By

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Optimierung der Stickstoffentfernung in verschiedenen vertikalen Strömungspflanzenkläranlage Designs und Anwendung von gereinigtem Abwasser zur Wiederverwendung in Bewässerung in Jordanien

Von der Fakultät für Umweltwissenschaften und Verfahrenstechnik der Brandenburgischen Technischen Universität Cottbus zur Erlangung des Akademischen Grades eines Doktors der Philosophie (Ph.D.) in Umweltwissenschaften genehmigte Dissertation

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Summary

In arid countries, reclaimed water in irrigation is a widespread practice. Therefore, robust treatment designs are prerequisite to obtain effluent quality that conforms to the legal requirements and guidelines for reuse and health standards. Vertical flow constructed wetlands (VFCWs) are attractive decentralized treatment plants in many countries and communities. VFCWs are capable of providing adequate treatment for organic and solids removal, even though there are limitations on nutrient and pathogen removal. Within the context of the SMART project, various VFCW systems were investigated, in Germany and Jordan, to optimize nitrogen removal using sustainable and low cost options to guarantee the safe reuse and conform to the reuse standards in Jordan.

In Germany at Langenreichenbach research facility, two-stage VFCWs planted (*Phragmites australis*) and unplanted were evaluated and modified to compare the role of plants over two years. Generally, there was no significant role of plants on the treatment performance. Both systems showed high removal efficiency for TOC, BOD_5, and TSS over the study period. On the other hand, during the first year of the study, effluent TN concentrations ranged from 60 – 61 mg/L in both systems as a result of high effluent NO_3^--N concentrations (50 - 52 mg/L). In the second year, the systems were modified by adopting a saturated layer in the 1st stage to enhance denitrification. Average effluent TN concentrations were reduced to 45 mg /L in both systems. In addition, the operational modifications optimized the *E. coli* removal such that both systems achieved 4 log concentration reduction instead of 2 log concentration reduction during the first year of the study.

In Jordan at the Fuhais research facility, two VFCW systems were investigated considering category-A (TN: 45 mg/L and NO_3-N: 30 mg/L) in the Jordanian Standards (JS) for reuse in irrigation (JS 893/2006). Recirculating (ECO-1) and Multi-stage (ECO-2) VFCW designs have shown high removal efficiency of COD, TSS, and BOD_5 over three years of monitoring. ECO-1 is a modified VFCW system, combing simultaneous nitrification and denitrification by recycling portion of nitrified effluent (circulation ratio 3:1) into the recirculation tank. However, effluent TN and NO_3^--N concentrations were 55 and 44 mg/L, respectively, that the system conformed to the JS category-B (TN: 70 mg/L and NO_3-N: 45 mg/L) during monitoring phase. Therefore, ECO-1 was modified by installing plastic media in the recirculation tank that attached growth increases the abundance and activity of microorganisms. TN concentration was reduced effectively of 40 mg/L, conforming to the JS category-A, whereas, NO_3^--N concentration was reduced to 37 mg/L, conforming to the JS category-B. However, over the study period, *E. coli* concentrations were not compatible with the JS (category-A: 100 MPN/100 mL and category-B: 1000 MPN/100 mL), but it was conformed to the JS category-C (more than 1000 MPN/100 mL).

ECO-2 consists of two unsaturated VFCWs in series; single-pass unplanted filter followed by planted filter (*Phragmites australis)*. *E. coli* removal was relatively high before operational modification that the effluent conformed to the JS category-B, achieving 4.4 log concentration reductions. The effluent TN and NO_3^--N concentrations did not conform to the JS of 77 and 76 mg/L, respectively, due to insufficiency of carbon source to promote denitrification (high BOD_5 removal in VFCW) during monitoring phase of the study. Thus, ECO-2 was modified adopting

raw wastewater step-feeding strategy that a specific volume of raw wastewater was mixed with 1st stage effluent in the mixing tank. TN and NO_3^--N concentrations were reduced to 52 and 50 mg/L, respectively; conforming to the JS category-B. Whereas, *E. coli* removal was influenced by *E. coli* ingress from raw step-feeding, achieving 3.5 log concentration reductions, conforming to the JS category-C.

The short-term impact of irrigation with different water quality and quantity was also investigated at the Fuhais research site. Soil physical, chemical, and biological properties in three parallel experimental reuse plots at Fuhais site were investigated. The plots were cultivated with lemon trees. The irrigation water was supplied via a subsurface irrigation system. Each plot received water from a different source (tap water, ECO-1 and ECO-2 effluent). Moreover, each plot was divided into two sub-parts (A and B) whereby one plot received 11 mm/day of irrigation water and the other subplot received 6 mm/day. In the end of the experiment, using treated effluent and tap water showed the same trend of increased soli salinity (ECs). Significant difference in ECs, SAR, Mg^{+2}, Ca^{+2}, and Na^+ were observed at 0 - 20 cm as a result of high evaporation and capillary rise that increased salts accumulation in the topsoil. However, using more water in subsurface irrigation system reduced the salts accumulation in sub soil layers due to continuous leaching. On the other hand, results showed no significant variation in soil physical properties (texture, structure, moisture, and infiltration rate) among reuse plots and subparts. In addition, results revealed an absence of total coliform, fecal coliform, and *E. coli* in the irrigated soils, indicating the effectiveness of using subsurface irrigation as a disinfection step for reuse.

Keywords: Constructed wetlands; Nitrogen; Nitrification; Denitrification.

Zusammenfassung

In ariden Ländern ist die Verwendung von wiederaufbereitetem Wasser zur Bewässerung eine weit verbreitete Praxis. Daher sind widerstandsfähige Wasseraufbereitungsanlagen Voraussetzung für das Erreichen einer Wasserqualität, die den gesetzlichen Anforderungen und Richtlinien für die Wiederverwendung und Gesundheitsnormen entspricht.

Vertikal durchströmte Pflanzenkläranlagen (VFCW) stellen für viele Länder und Gemeinden attraktive dezentrale Abwasserreinigungstechnologien dar. VFCWs sind in der Lage, eine ausreichende Entfernung organischer Stoffe und Feststoffe zu gewährleisten, obgleich Einschränkungen bei der Entfernung von Nährstoffen und Krankheitserregern bestehen.

Im Rahmen des SMART-Projekts wurden in Deutschland und Jordanien verschiedene VFCW-Systeme untersucht und hinsichtlich ihres Leistungsvermögens zur Stickstoffentfernung optimiert, um mit nachhaltigen und kostengünstigen Anlagen eine sichere Wiederverwendung des gereinigten Abwassers unter Einhaltung der jordanischen Standards (JS) gewährleisten zu können.

In der Forschungseinrichtung des Helmholtz-Zentrums für Umweltforschung Leipzig in Langenreichenbach (Deutschland) wurden zwei zweistufige VFCWs (einmal mit *Phragmites australis* bepflanzt und einmal unbepflanzt) beprobt und modifiziert, um auch die Rolle der Pflanzen in einem Zeitraum von zwei Jahren zu vergleichen. Dabei wurde festgestellt, dass in den untersuchten Systemen die Bepflanzung keine signifikante Rolle bei der Reinigungsleistung spielt.

Beide Systeme zeigten im Verlauf der Studie eine hohe Reinigungsleistung für TOC, BSB_5 und TSS. Während des ersten Untersuchungsjahres lag die Konzentration an Gesamtstickstoff (TN) im Ablauf der VFCW´s im Bereich von 60 mg/L und 61 mg/L, da die NO_3^--N-Konzentrationen (50 mg/L und 52 mg/L) relativ hoch waren. Zur Verbesserung der Denitrifikation wurden die Systeme im zweiten Untersuchungsjahr modifiziert, indem die erste Stufe eingestaut betrieben wurde. Die durchschnittliche Konzentration an Gesamtstickstoff im Ablauf konnte so in beiden Systemen auf 45 mg/L reduziert werden. Darüber hinaus konnte durch diese Betriebsänderung die Reduktion der *E. coli* so erhöht werden, dass in beiden Systemen im zweiten Jahr der Studie 4 log Stufen, statt der 2 log Stufen im ersten Jahr entfernt wurden.

In der Forschungseinrichtung der Universität AlBalqa in Fuhais (Jordanien) wurden zwei VFCW-Systeme untersucht und mit dem Ziel, Kategorie A (TN: 45 mg/L und NO_3-N: 30 mg/L) des jordanischen Standards zur Wiederverwendung von gereinigtem Abwasser zur Bewässerung (JS 893/2006) zu erreichen, modifiziert. Das einstufige VFCW mit Rezirkulation (ECO-1) und das mehrstufige VFCW (ECO-2) zeigten in einem Untersuchungszeitraum von drei Jahren hohe Reinigungsleistungen in Bezug auf COD, TSS und BSB_5.

ECO-1 ist ein modifiziertes VFCW-System, welches eine gleichzeitige Nitrifikation und Denitrifikation durch ein anteiliges Rückführen des nitrifizierten Ablaufs (Rücklaufverhältnis 3:1) in den Rezirkulationstank ermöglicht. Während der Beobachtungsphase betrugen die TN und

NO_{3}-N -Konzentrationen im Ablauf des Systems 55 mg/L und 44 mg/L, dh. diese entsprachen der Kategorie B (TN: 70 mg/L und NO_3-N: 45 mg/L) des jordanischen Standards. Daher wurden mit dem Ziel, die Menge und Aktivität der Mikroorganismen zu erhöhen und so das Wachstum zu steigern, Plastikteile in den Rezirkulationstank von ECO-1 eingebaut. Auf diese Weise wurde die TN-Konzentration effektiv auf 40 mg/L reduziert (entspricht Kategorie-A des JS), während die NO3--N -Konzentration nur auf 37 mg/L (entspricht Kategorie B der JS) reduziert werden konnte. Im Verlauf der Studie waren die *E. coli*-Konzentrationen nicht mit dem JS (Kategorie A: 100 MPN/100 mL bzw. Kategorie B: 1000 MPN/100 mL) konform, sondern entsprachen der JS-Kategorie C (mehr als 1000 MPN/100 mL).

ECO-2 besteht aus zwei nacheinander geschalteten ungesättigten VFCWs. Die erste Stufe des Systems besteht aus einem unbepflanzten Bodenfilter, die nachfolgende zweite Stufe besteht aus einem mit *Phragmites* australis bepflanzten Bodenfilter. Die Reinigungsleistung bezüglich E. coli war auch ohne Betriebsänderung relativ hoch. Der Ablauf der Anlage erreichte eine Reduktion der E.coli um 4,4 log Stufen, was der Kategorie B des jordanischen Standards entsprach.

Während des Untersuchungszeitraumes entsprachen die TN und NO3--N -Konzentrationen des Ablaufs des Anlage nicht dem JS von 77 mg/L und 76 mg/L, da nur unzureichend Kohlenstoffquellen zur Förderung der Denitrifikation in der zweiten Stufe (generell hohe BSB5 Entfernung in VFCW) zur Verfügung standen. Daher wurde ECO-2 so modifiziert, dass dem Ablauf der 1. Stufe in einem Mischbehälter eine bestimmte Menge an Rohabwasser (Kohlenstoffquelle) zu gemischt wurde. So konnte im Ablauf der 2. Stufe eine Reduzierung der TN und NO_3^--N -Konzentrationen auf 52 mg/L und 50 mg/L entsprechend Kategorie B des jordanischen Standards erreicht werden. Allerdings wurde durch die Zufuhr von frischen E. coli aus dem Rohabwasser in den Mischbehälter, die Entfernung von *E. coli* aus der Gesamtanlage negativ beeinflusst und erreichte nur noch 3,5 log Stufen, was der Kategorie C des jordanischen Standards entspricht.

Die kurzfristigen Auswirkungen der Bewässerung von Böden mit unterschiedlichen Wasserqualitäten und -mengen wurden auf dem Forschungsgelände in Fuhais untersucht.

Physikalische, chemische und biologische Bodeneigenschaften wurden in drei parallelen Versuchsparzellen untersucht. Die Parzellen wurden mit Zitronenbäumen bepflanzt. Das Bewässerungswasser wurde über ein unterirdisches Bewässerungssystem verteilt. Jede Parzelle erhielt Wasser aus einer anderen Quelle (Leitungswasser, Ablauf ECO-1, Ablauf ECO-2). Darüber hinaus wurde jede Parzelle in zwei Unterparzellen (A und B) aufgeteilt, wobei eine Unterparzelle 11 mm/Tag Bewässerungswasser und die andere Unterparzelle 6 mm/Tag erhielt. Am Ende des Experiments mit behandeltem Abwasser und Leitungswasser zeigte sich die gleiche Tendenz einer erhöhten Bodenversalzung (ECs, SAR). Ein signifikanter Unterschied von ECs, SAR, Mg^{+2}, Ca^{+2}, und Na^{+} wurde bei 0-20 cm auf Grund hoher Verdunstung und hohen Kapillarkräften beobachtet, welche die Salzanreicherung im Oberboden erhöhten. Allerdings konnte die Verwendung von mehr Wasser bei der unterirdischen Bewässerung die Salzanreicherung in den unteren Bodenschichten durch kontinuierliche Auslaugung reduzieren.

Auf der anderen Seite zeigten die Ergebnisse keine signifikanten Unterschiede der physikalischen Bodeneigenschaften (Textur, Struktur, Feuchtigkeit und Infiltrationsrate) innerhalb der Parzellen und ihrer Unterparzellen. Darüber hinaus konnte festgestellt werden, dass keine Gesamtcoliformen, Fäkalcoliformen und *E. coli* in den bewässerten Böden vorhanden waren, was auf die Effektivität der unterirdischen Bewässerung als Desinfektionsschritt bei der Wiederverwendung von gereinigtem Abwasser hinweist.

Schlagwörter: Pflanzenkläranlagen, Stickstoff, Nitrifikation, Denitrifikation.

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ANOVA	**An**alysis **O**f **Va**riance
BOD_5	**B**iochemical **O**xygen **D**emand over **five** days
C°	Degrees Celsius
Ca^{2+}	Calcium
CFU	**C**olony **F**orming **u**nit
Cl^-	Chloride
cm	centimeter
CW	**C**onstructed **W**etland
COD	**C**hemical **O**xygen **D**emand
DO	**D**issolved **O**xygen
dS/m	**d**eci**s**iemens per **m**eter
DWWT	**D**ecentralized **W**astewater **T**reatment
E	**E**vaporation
EC	**E**lectrical **C**onductivity of water
ECs	**E**lectrical **C**onductivity of **s**oil
E. coli	***E**scherichia **coli***
ECO-1	Recirculating vertical flow constructed wetland design
ECO-1M	Modified recirculating vertical flow constructed wetland design
ECO-2	Two-stage vertical flow constructed wetland design
ECO-2M	Modified two-stage vertical flow constructed wetland design
ET	**E**vapo**t**ranspiration
ESP	**E**xchangeable **S**odium **P**ercentage in soil
FAO	**F**ood and **A**griculture **O**rganization
g	**g**ram
HFCW	**H**orizontal **F**low **C**onstructed **W**etland
HLR	**H**ydraulic **L**oading **R**ate
HCO_3^-	Bicarbonate
ICARDA	**I**nternational **C**entre for **A**griculture **R**esearch in **D**ry **A**reas
JS	**J**ordanian **S**tandards for Reuse in Irrigation
K^+	Potassium
LRB	**L**angen**r**eichen**b**ach research site
m	**m**eter
MCM	**M**illion **C**ubic **M**eters
meq/L	**m**illi**eq**uivalents per **L**iter
Mg^{2+}	Magnesium
mg/L	**M**illigram Per **L**iter
µS/cm	**m**icro **S**iemens per **cm**
MPN	**M**ost **P**robable **N**umber
MWI	Jordanian **M**inistry of **W**ater and **I**rrigation

Na^+	Sodium
NH_4^+-N	Ammonium-nitrogen
NO_3^--N	Nitrate-nitrogen
NO_2^--N	Nitrite nitrogen
OM	**O**rganic **M**atter
P1	Control reuse plot
P2	ECO-2 effluent reuse plot
P3	ECO-1 effluent reuse plot
pH	Hydrogen Ion Activity
PO_4^{3-}	phosphate
SAR	**S**odium **A**dsorption **R**atio
SD	**S**tandard **D**eviation
SI	**S**aturation **I**ndex
SMART	**S**ustainable **M**anagement of **A**vailable Water **R**esources with innovative **T**echnologies
SSF	**S**ub**s**urface **F**low
SSVF	**S**ub**s**urface **V**ertical **F**low
SO_4^{2-}	Sulfate
SOM	**S**oil **O**rganic **M**atter
TN	**T**otal **N**itrogen
TP	Phosphorous
TOC	**T**otal **O**rganic **C**arbon
TSS	**T**otal **S**uspended **S**olids
UNEP	**U**nited **N**ations **E**nvironment **P**rogram
USEPA	**U**nited State **E**nvironmental **P**rotection **A**gency
VFCW	**V**ertical **F**low **C**onstructed **W**etland
WHO	**W**orld **H**ealth **O**rganization

1. Introduction

In arid and semi-arid regions, increasing demand for water in irrigation and domestic usage has put the water sector under notable pressure. Therefore, treated wastewater can and should be considered as an alternative water resource in agricultural irrigation to secure food production. However, the main concern with the reuse of treated wastewater is its composition (chemicals, nutrients, and pathogens) which can cause health and environmental hazards. In particular, discharging inadequately treated wastewater releases nutrients and waterborne pathogenic microorganisms into soils or receiving water bodies, which can cause environmental problems such as eutrophication, groundwater deterioration, transmission of disease, as well as contamination of soils and plants.

To mitigate these risks, wastewater should undergo a proper treatment before reuse application (WHO, 2006). Thus, international standards have been issued to regulate different reuse options. Decentralized wastewater treatment (DWWT) technologies can help protect water resources by providing an appropriate treatment for reuse on a local scale (Tchobanoglous *et al.*, 2004, Brown *et al.*, 2010)

DWWT is implemented to treat and dispose wastewater from rural areas and small settlements instead of constructing or upgrading centralized wastewater treatment. Constructed wetlands (CWs) are appropriate for DWWT in unsewered villages and settlements due to their technical simplicity, high treatment efficiency, cost effectiveness, and successful application in developed countries.

For decades, CWs have been utilized in developed countries as primary and secondary treatment systems (Wallace & Knight, 2006, Brix *et al.*, 2007). However, implementing treatment wetlands in arid regions can be challenging when reuse is a main goal.

Within the framework of the **SMART** research project (**S**ustainable **M**anagement of **A**vailable Water **R**esources with Innovative **T**echnologies), the research in this study focused on various vertical flow constructed wetlands (VFCW) designed for decentralized wastewater treatment. VFCW systems were investigated to optimize nitrogen removal using sustainable and low cost options to guarantee the safe reuse and conform to Jordanian national standards for reuse of treated wastewater. This study consists of three parts. The first part in Germany, two identical pilot-scale VFCWs (planted and unplanted) were implemented and modified to compare the role of plants. The second part in Jordan, two pilot-scale VFCWs were implemented and modified to produce effluents according to the Jordanian Standards (JS 893/2006) for reuse in irrigation. The third part in Jordan, reuse of treated wastewater was investigated using the effluents from the two pilot-scale VFCWs. Although reuse has a long-term impact; but the effects of using different water quantity and quality were evaluated over three years (short-term) of watering citrus trees (lemon trees) in the irrigation field.

Over all, the study investigated the constructed wetlands treatment efficiencies under different conditions such as climatic conditions, wastewater qualities, vegetation, reuse standards, and effective path for treatment optimization.

1.1 Research Questions and Aims

The main research questions of this work are:

- **Can the various VFCW systems remove nitrogen effectively under different conditions (wastewater quality and climatic conditions)?**
- **Do the effluents of VFCWs meet the Jordanian standards for reuse in irrigation?**
- **How can the systems be optimized for TN removal?**
- **What are the impacts of using treated wastewater via subsurface irrigation system with different irrigation regimes in Jordan?**

To answer these questions, two research phases have been realized. The first phase was to assess the ability of different VFCW designs to produce effluents that comply with the Jordanian standards for reuse of treated wastewater. The second phase was designed to optimize the treatment performance. In this regard, the study also aims at assessing the treatment efficiency with respect to wetland vegetation (*Phragmites australis*) by comparing planted and unplanted VFCW systems in Germany.

A third aim was to evaluate the effects of utilizing different water quality and quantity on soil and plants by reusing of treated wastewater via subsurface irrigation system in Jordan.

1.2 Research Background

In the Middle East, the critical water situation and water conflict pools the Arab World. This situation strongly connected to population growth, agriculture, development, changes in environment and climate (Oron *et al.*, 2008). Treated wastewater has been considered as a valuable water resource rich with natural fertilizers for irrigation (Metcalf & Eddy, 2003b, Guest *et al.*, 2009, IWA, 2011).

In most developing countries, major cities are served by centralized sanitation systems (Mara, 2013). Implementing centralized systems may be less suitable for places such as rural areas with low population density (UNEP/GPA, 2000, Bakir, 2001). The majority of low-income dwellings discharge their wastewater without treatment into the environment, damaging their nature and available resource.

DWWT is a promising strategy which reduces the mass of pollutants discharged to the local environment and local water cycle (Gikas & Tchobanoglous, 2009). Wastewater is collected, treated and disposed or reused at or near the source (Tchobanoglous *et al.*, 1998). As suitable decentralized treatment designs, constructed wetlands can be employed to provide adequate treatment for organic and solids removal, even when there are limitations on nutrients and pathogen removal in some designs (UNEP/GPA, 2000, Friedler, 2001). These systems are fast growing and there existence are more effective for reuse instead of conventional centralized plants (Bakir, 2001).

Jordan, as an arid country, has limited water resources and has unpredictable rainfall in winter season which ranges from around 660 mm in the northwest to less than 130 mm in the east (Mohsen, 2007). The kingdom's potable water availability is only 145 m^3/capita/year, which is below "water poverty line" of 1000 m^3/capita/year (MWI, 2009).

The government in Jordan recognizes the value of treated water. Jordan´s water strategy adopted by the Jordanian Council of Ministers stating: "Wastewater shall not be managed as waste; it shall be collected and treated to standards that allow its use in unrestricted agriculture and other non-domestic purposes, including groundwater recharge." With this change in the strategies, where reuse application is included in the design of a wastewater facility, it must be ensured that effluent quality complies within the legal requirements and guidelines for reuse and health standards. Thus, this strategy is being pursued through developing proper treatment systems, increasing water recycling, and improved irrigation techniques in order to reduce water losses.

1.3 Guidelines and Standards for Reuse

International guidelines, standards, and policies have been issued to control wastewater utilizations. The major objective of these guidelines is to reduce health and environmental risks that associated with wastewater reuse. Some of the more well-known guidelines are:

- The World Health Organization (WHO, 2006, 1989): "Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture". The latter guidelines in 2006 consider the treatment process, irrigation system, and type of crops.
- The United State Environmental Protection Agency (USEPA) (USEPA, 2004) "Guidelines for Water Reuse".
- The Food and Agriculture Organization (FAO) (Ayers & Westcot, 1985, Pescod, 1992), "Wastewater Treatment and Use in Agriculture". These standards determine the degree of suitability of a given effluent of irrigation.

Strategies and Guidelines for Reclaimed Water in Jordan

The Jordanian Ministry of Water and Irrigation (MWI) has formulated standards and guidelines for water reuse to maximize the amount of wastewater in irrigation to reach 232 MCM by 2020, especially in the Jordan Valley (Wardam, 2004). Many Mediterranean countries have formulated their standards based on the WHO guidelines (WHO, 1989) or the USEPA guidelines (USEPA, 2004). However, these guidelines have been adjusted in many arid countries (Choukr-Allah, 2010), adding a guideline for irrigation methods to their national guidelines for reuse in irrigation (Blumenthal & Peasey, 2002).

For decades, Jordan has issued and applied its old national standards and guidelines (JS 893/1995) for different reuse applications for treated wastewater (irrigation, artificial groundwater recharge, and discharging to wadis or streams). In 2002, the national standards were updated (JS 893/2002), prohibiting the use of treated wastewater for irrigation of vegetables eaten raw or recharging aquifers for potable use. In 2006, further revisions took place providing less restriction for BOD, COD, and *E. coli* than what was stated in the previous guidelines (JS 893/2006) (JISM, 2006).

The current JS 893/2006 guidelines for reuse of treated wastewater were written for centralized wastewater treatment plants. Nevertheless, decentralized wastewater treatment plants have no explicit standards and are thus assumed be held to the regulations for centralized wastewater treatment plants. **Table 1-1** shows the current standards for reuse in irrigation consisting of four categories (A, B, C, and D). Each category shows water quality for different irrigated crops, however, they do not address the level of treatment (primary, secondary, tertiary or advanced).

Reclaimed water contains nutrient that can be utilized in agricultural irrigation. In the JS 893/2006, total nitrogen is limited to 45 mg/L in class A, and 70 mg/L in B, C and D classes, depending on the sensitivity of the plants in each group.

The standards are also based on removal of pathogens such as *E. coli,* Intestinal nematode eggs and Helminth eggs. The concentration of *E. coli* is widely used as indicator of pathogenic microorganisms, which is a member of the faecal coliform group of bacteria. The *E. coli* concentrations in untreated municipal wastewater ranges from 10^5 to 10^8 MPN/100 mL. Concentrations of 10^6 to 10^{10} MPN/100 mL have been shown to cause disease in humans (Asano *et al.*, 2007). In the JS 893/2006 guidelines for reuse of treated wastewater standards, *E. coli* numbers should be less than 10^2 MPN/100 mL for crops in class A, 10^3 MPN/100 mL for crops in class B and 1.1 MPN/100 mL for cut flowers (class D). In addition, the JS 893/2006 recommends that the concentration of intestinal nematodes should be less or equal to 1 egg/L for reuse in irrigation as recommended in the WHO 1989 and 2004 guidelines.

The strict *E. coli* standards in the JS 893/2006 guidelines require very high levels of treatment in order to ensure the public and environmental health. The standards force designers and operators of wastewater treatment plants to improve existing treatment facilities, but the stringent *E. coli* standards, for example, can be achieved cost-effectively for centralized wastewater treatment plants, but are not economically feasible for small and decentralized wastewater treatment plants.

Table 1-1: The current Jordanian standards for treated wastewater reuse in irrigation (JS 893/2006) (After JISM, 2006).

Parameter	**Cooked Vegetables, Parks, Playgrounds and sides of Roads within city limits**	**Fruit Trees, sides of roads outside city limits and landscape**	**Field crops Industrial Crops and Forest Trees**	**Cut Flowers**
	A	**B**	**C**	**D**
BOD_5 [mg/L]	30	200	300	15
COD [mg/L]	100	500	500	50
DO [mg/L]	> 2	-	-	> 2
TSS [mg/L]	50	150	150	15
pH	6 - 9	6 - 9	6 - 9	6 - 9
Turbidity [NTU]	10	-	-	5
NO_3-N [mg/L]	30	45	45	45
TN [mg/L]	45	70	70	70
E. coli [MPN/100 mL]	100	1000	-	< 1.1
Intestinal Helminth eggs [egg/L]	< or = 1	< or = 1	< or = 1	< 1
Grease, oils and fats [mg/L]	8	8	8	8

E. coli: *Escherishia Coli.*

2. Literature Review

2.1 Wastewater Composition and Treatment

The composition of wastewater varies widely and depends on the socioeconomic level of the communities and density of industrial and commercial activity. **Table 2-1** shows the classification of domestic raw wastewater based on its composition as reported by Pescod (1992). Wastewater contains solid and soluble organic matter, nutrient, inorganic matter or dissolved minerals, gases, toxins, pathogens, non-pathogenic bacteria, and pharmaceutical drugs (Metcalf & Eddy, 1991). In Jordan, water consumption is low in comparison with north American and European countries (90 L/day per person in Jordan compared to 150 L/day in Germany). As a result of low water consumption in Jordan, the wastewater tends to be strong (Pescod, 1992).

Table 2-1: Type of domestic raw wastewater based on main constituents concentrations (Pescod, 1992).

Parameters	Strong	Medium	Weak
BOD_5 [mg/L]	300	200	100
Total suspended solids (TSS) [mg/L]	1200	700	350
Total dissolved solids (TDS)* [mg/L]	850	500	250
Suspended solids (TSS) [mg/L]	350	200	100
Chloride (Cl) [mg/L]	100	50	30
Alkalinity (as $CaCO_3$) [mg/L]	200	100	50
Nitrogen (as N) [mg/L]	85	40	20
Phosphorus (as P) [mg/L]	20	10	6
Grease [mg/L]	150	200	100

* TDS is a measure of all substances contained in a liquid (molecular, ionized, or solid particles).

Conventional wastewater treatment technologies are widely used in many countries in the world (EPA, 2004). Wastewater treatment combines physical, chemical, and biological processes to improve the wastewater quality. Pettygrove and Asano (1984) described different levels of wastewater treatment, which are preliminary, primary, secondary, and tertiary or advanced wastewater treatment.

- **Preliminary treatment** is the first step in the treatment, screening and/or grinding coarse solids and other large materials from raw sewage.
- **Primary (mechanical) treatment** removes the suspended and floating solids from wastewater by several mechanical processes (sedimentation, skimming, and flocculation). It can reduce the biochemical oxygen demand (BOD_5) of the wastewater by 20 - 30 % and the TSS by 50 - 60 %. This level of treatment is considered the minimum level required for reuse to irrigate orchards and crops that are not consumed by humans (Pettygrove & Asano, 1984).
- **Secondary (biological) treatment** removes the residual dissolved organic and suspended matter degradation by microbes. About 85 % of TSS and BOD_5 can be removed by this step of treatment. CWs are designed to achieve this level of treatment. Biological

treatment is the required level of treatment for safe reuse when moderate risk is expected from reuse (Pettygrove & Asano, 1984).

- **Tertiary (advanced) treatment** removes nutrients (nitrogen and phosphorus) and pathogens from wastewater, producing high quality effluents. In this step of treatment, treated wastewater can be disinfected by many paths such as adding chlorine or using ultraviolet light.

2.2 Constructed Wetland (CW)

CWs are engineered wastewater treatment systems that are based natural functions of vegetation, substrate, and microorganisms for water quality improvement (Hammer, 1989). The emergence of constructed wetland research started from Germany in the early 1950s. This ecological design has been implemented and developed for treating various sources of water pollution (Seidel, 1955), and has been earning more interest as an efficient technology for secondary treatment (Luederitz *et al.*, 2001, Stefanakis & Tsihrintzis, 2009).

For the purpose of water quality improvement, CWs (also referred to as treatment wetlands) can be implemented in a variety of designs and hydrologic modes (Kadlec & Wallace, 2008). CWs have been designed to mimic many of the processes that occur in natural wetlands. However, the technology is more controlled than natural wetlands, specifically due to the well-defined composition of substrate, vegetation and water regime (Brix, 1993). CW designs are flexible in size, and range from small treatment plants (which serve small settlements) to municipal facilities that serve entire communities. This ecotechnology is a preferred option for decentralized treatment due to low operations and maintenance requirements and fact that constructed wetlands do not require technological components such as chemical feeds (Crites & Tchobanoglous, 1998).

Different substrates (clay, silt, sand, and gravel) have been applied as a filter matrix for CWs. The soil material strongly influences the hydraulic conductivity through the wetlands. Generally, the use of fine gravel or coarse sand increases the permeability of filter and minimizes the risk of clogging (Brix & Arias, 2005b). The filter medium acts as both a fixed surface for attached biofilm growth and a rooting base for plants.

Treatment wetlands can be planted with emergent wetland plants such as *Phragmites australis* (common reed), *or Typha spp.* (cattail). In fact, the second common name for subsurface flow constructed wetlands in Europe is “Reed-bed” treatment systems, which is because *Phragmites australis* is the most commonly used plant in treatment wetlands in Europe . Plants can stimulate the microbial activity by releasing oxygen, expanding the surface area for microbial attachment, and in some cases salts uptake via roots (Brix, 1994a).

There are several different types of treatment wetlands based on groups presented in **Table 2-2**. The various treatment wetland designs can be subdivided based on two main physical attributes in the proposed hierarchical classification system (Fonder & Headley, 2010):

1. Hydrology and
2. Vegetation characteristics

Table 2-2: The different classes of treatment wetland within the classification hierarchy (after Fonder and Headley, 2010).

Physical Attribute	Specific Trait	Description	Defined Classes for each Trait	Sub-Class
Hydrology	a. Water position	Position of water surface relative to soil or substrate	Surface flow [a]	-
			Subsurface [b]	-
	b. Flow direction	Predominant direction of flow through system	Horizontal	
			Vertical [c]	Down
				Up
				Mixed
	c. Saturation of media [c]	Degree of saturation in media-based systems	Free-draining	-
			Intermittent	-
			constant	-
	d. Surface flooding [c]	Type of surface flooding in media-based systems	None	-
			Ephemeral	-
			Permanent	-
Vegetation	a. Sessility [d]	Location of the roots: Attached in the benthic sediments or floating	Sessile (benthic bound)	-
			Floating	-
	b. Growth form	Dominant growth form of the vegetation in relation to the water	Emergent	Herbaceous
				woody
			Submerged [d]	-
			Floating leaved [d]	-
			Free-floating [d]	-

[a] majority of flow through a column of water overlying a benthic substrate.
[b] majority of flow through a porous media.
[c] only relevant to Subsurface Flow systems (by virtue of design, all surface flow wetlands have horizontal flow, are constantly saturated and with a permanently inundated substrate).
[d] only relevant to surface flow systems (subsurface flow excludes submerged or floating plants).

Constructed wetlands are categorized into two main groups according to water position in treatment wetlands, as shown in **Figure 2-1**:

- **Free water surface wetlands (FWS)**: the water is flowing over substrate or organic soils, and the vegetation can be emergent, submerged, or floating depending on the treatment application (Fonder & Headley, 2010). These sorts of systems are similar to natural wetlands in appearance, and they are utilized for advanced treatment (Kadlec & Wallace, 2009).

- **Subsurface wetlands or “Reed-bed” treatment systems (SSF)**: the water is passing through a porous medium in the filter, the top layer remains dry (Fonder & Headley, 2010). A wide range of purification processes are performed by attached microbes.

FWS wetlands are not used as much as the SSF wetlands solely in the old designs in Europe (Vymazal, 2001, Brix, 1993). Furthermore, SSF wetlands are more suitable for decentralized wastewater treatment plants in rural areas than FWS wetlands.

Reed-bed treatment systems are sub-classified based on water flow direction into two groups: horizontal flow constructed wetlands (HFCWs) and vertical flow constructed wetlands (VFCWs).

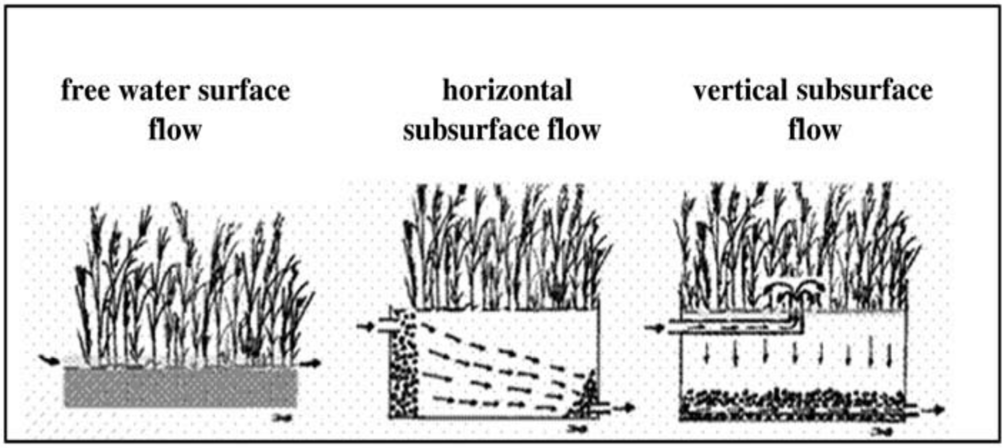


Figure 2-1: Types of treatment wetland free water surface and subsurface CWs according to water flow (Ghermandi *et al.*, 2007).

HFCWs have limited ammonium nitrogen removal due to their predominantly anaerobic subsurface environmental conditions (Vymazal, 2005a). HFCWs also require relatively large land area, whereas VFCW requires less land (Brix & Arias, 2005b, Nivala *et al.*, 2013) and are capable of consistent nitrification. Thus, VFCWs are increasingly implemented as a decentralized wastewater treatment due to their high oxygen transfer capacity, high organic matter removal, and high nitrification rate.

Another special group of amended CWs is intensified or hybrid wetlands, which include a mix of HFCW and VFCW unit designs, in order to achieve higher treatment efficiency (Johansen & Brix, 1996).

2.2.1 Vertical Flow Constructed Wetlands (VFCWs)

VFCWs have been used for wastewater treatment in Europe and the United States (Kadlec & Wallace, 2009) as a single-pass bed that receives intermittent loading (ÖNORM B 2505., 1997). This system is suitable for situations in which the risks of human contact with the wastewater should be minimized. The treatment efficiency of VFCW depends on design criteria and operating parameters that manipulate the elimination processes such as wastewater quality, hydraulic loading rate, intervals between loadings, hydraulic retention time within the system, physical properties of the filter material, thickness of the filter, vegetation, climate, and other factors.

After wide usage of single-stage VFCWs, many investigations have been carried out in order to develop VFCWs in different countries (Cooper, 1999, Platzer, 2000, Luederitz *et al.*, 2001, Prochaska & Zouboulis, 2009, Laber *et al.*, 1997, Brix & Arias, 2005b). Many studies have reported on nitrogen removal efficiency and various design modifications (Rogers *et al.*, 1991, Morris & Herbert, 1997, Lee *et al.*, 2009, Fuchs & Vincent, 2010, Zhang *et al.*, 2005, Laber *et al.*, 1997, Langergraber, 2007, Brix, 1994a).

The VFCW system is designed to receive a primary treated effluent, thus, septic tank is included in the system to separate solid materials, faeces, and grease or oil from the bulk liquid. Generally, the septic tank removes about 70 % of the TSS and 65 % of the BOD_5, which minimizes clogging problems and prolongs the filter life. Brix and Arias (2005b) recommend pre-treating the raw wastewater in a two or three chamber sedimentation tank (septic tank) prior to loading on the CW in order to prolong the filter life.

In VFCWs, primary treated wastewater is distributed over the whole filter surface via perforated inlet distribution pipes (**Figure 2-2**). Researchers have shown that the vertical hydraulic regime is a determining parameter for removing ammonium from wastewater in laboratory and field scales (Breen, 1990, Farahbakhshazad & Morrison, 1997, Farahbakhshazad *et al.*, 2000). In contrast, little research has been conducted on upflow wetlands, which may have the advantage of saturated, anaerobic conditions beneficial for denitrification (Langergraber, 2008). The wastewater is usually applied in small pulses (intermittent loading), and the water percolates downward through the filter, allowing the pores to fill up with air between the loadings (Brix, 1994b). In most VFCWs, the water is completely drained before the beginning of the following pulse (Schwager & Boller, 1997). Therefore, aerobic and anaerobic processes occur in this design.

The dosing regime (frequency and volume) is an important operational parameter for VFCWs (Headley *et al.*, 2004, Molle *et al.*, 2006, Torrens *et al.*, 2009a, Olsson, 2011). Headley *et al.* (2004) observed that NH_4^+ removal in pilot-scale VFCWs was higher when wastewater applied in 12 doses/day instead of 48 doses/day. Thus, a larger dose volume can lead to poorer pollutant removal because a larger amount of water will have a shorter contact time with the biomass (Molle *et al.*, 2006). Despite that, Olsson (2011) reported that there was no difference by applying different dosing regimes (4 mm hourly and 8 mm bi-hourly) in six VFCWs (planted and unplanted), however the systems were still in start-up when the study was conducted. However, the internal samples showed that smaller and many frequent doses provided better pollutant removal in the upper part of the filters.

The filter substrate is composed of gravel, sand, or both. The VFCWs require more concern in the construction regarding media selection compared to other CW designs (Brix, 1994b). Sand, as a fine medium, is usually preferred in VFCWs where pollutant removal rates are higher than in coarser medium (Brix & Arias, 2005b). In addition, the sand grains should be relatively well-graded and free of clay and silt to maximize the hydraulic loading rate and lower the risk of clogging (Brix & Arias, 2005b, Nivala *et al.*, 2013).

In a coarser (gravel) filter, water percolates through the filter faster, which generally results in lower treatment performance (Brix & Arias, 2005b). However, gravel has been shown high removal rate as filter substrate in trickling filters for removal of organic matter, suspended solids and NH_4^+ (Sasse, 1998, Newton & Wilson, 2008, Tekerlekopoulou *et al.*, 2010). That is mainly related to the depth of filter that increases the treatment performance. The average depth of VF designs is in the range of 0.6 - 1.0 m (Crites & Tchobanoglous, 1998). It has been debated whether deeper or shallower filter beds give a better treatment performance (Torrens *et al.*, 2009a, Brix & Arias, 2005b). Torrens *et al.* (2009a) they found that the overall treatment performance was significantly better in deep (0.65 m) than in shallow sand filters (0.25 m).

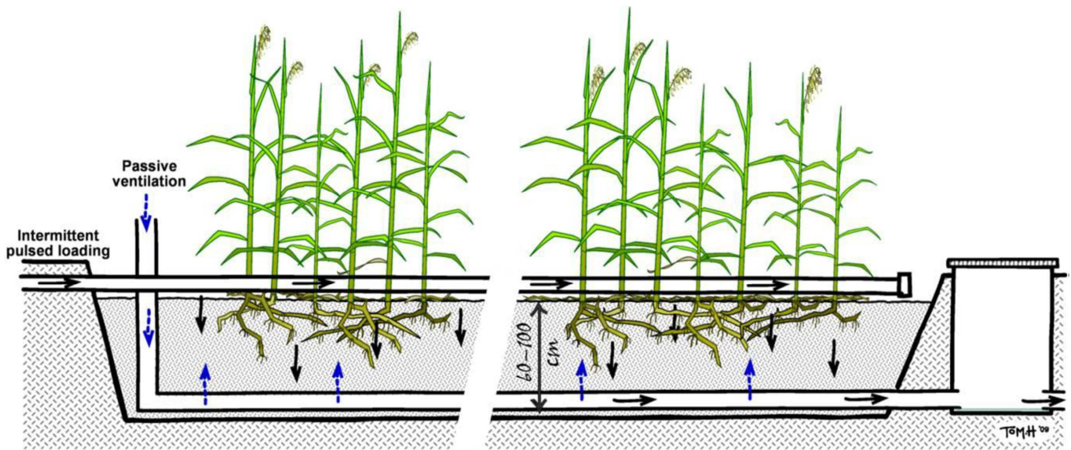


Figure 2-2: Schematic diagram of a VFCW design, showing inlet distribution pipes in the top layer of the filter, and drainage pipes at the bottom connected to vertical pipes for passive ventilation (Headley & Tanner, 2012).

The presence of plants has been shown to influence the treatment performance in HFCWs (Stottmeister *et al.*, 2003), however, the role of plants is not as clear in VFCWs. Many studies have shown that plants improve nitrogen removal (ammonia, nitrate, and nitrite) in VFCWs (Keffala & Ghrabi, 2005, Stefanakis & Tsihrintzis, 2009). Keffala and Ghrabi (2005) reported higher nitrogen removal in planted wetlands (19 % for planted and 6 % for unplanted).

2.2.2 Treatment Processes in VFCWs

The water in VFCW systems is treated by a combination of physical treatment processes (sedimentation and filtration), chemical processes (ion exchange, adsorption and chemical oxidation processes), and biological processes (nitrification, denitrification, microbial degradation, predation, natural die-off, and plant uptake) (Brix, 1993, Vymazal, 2007, Kadlec & Wallace, 2009).

The majority of studies on VFCWs have been carried out with a "black box" approach (Von Felde & Kunst, 1997, Prochaska & Zouboulis, 2009) simply comparing the influent and effluent water quality. Therefore, little is known about the actual treatment processes and how they are connected to the design (depth) and operation process in the filter.

Removal of settleable and suspended solids are primarily happening by filtration and settling within the top layers of wetland, whereas soluble organics are degraded aerobically or anaerobically by microbes (Vymazal, 1999). Brix and Arias (2005b) reported that several studies have shown that most of removal processes take place in the upper few centimeters of the filter. Further studies have shown that 80 - 95 % of the microbial biomass and activity can be found in the upper 10 cm of VFCWs (Tietz *et al.*, 2007).

Organic matter is reduced biologically within the wetland system. Microorganisms are responsible for biological degradation of pollutants. Bacteria in wastewater attach to the soil media through percolation forming a biofilm (Gray, 1989). The biofilm is responsible for organic matter removal (Kadlec & Wallace, 2009). However, biofilms may affect the hydraulic conditions in filters by forming filamentous colonies or aggregates (Knowles *et al.*, 2011). The biofilm is most abundant near the inlet of the wetland because it has the highest concentration load of organics (Brix & Arias, 2005b). However, accumulation of organic matter and suspended solids may cause clogging, especially in the upper part of the bed where a large part of these substances may be trapped (Knowles *et al.*, 2011).

Some studies also have reported lower treatment performance for organic matter at lower temperatures (Stefanakis & Tsihrintzis, 2009), while others have found no significant effect of temperature on organic matter removal.

The most common tests used to measure the organic content in water are the BOD_5, chemical oxygen demand (COD), and total organic carbon (TOC). The high treatment performance in BOD_5, COD, and TOC in the VFCWs is connected with high aerobic degradation levels (Brix & Arias, 2005b). Carbon compounds in aerobic conditions are oxidized via respiration by microorganisms. Whereas, in anoxic (low dissolved oxygen) or anaerobic (no dissolved oxygen) conditions carbon compounds are degraded via fermentation, denitrification, and iron or sulfate reductions (Mitsch & Gosselink, 1993, Kadlec & Wallace, 2009). In addition, plants can improve the COD and BOD removal by providing oxygen through the root zone (Tanner, 2001).

Phosphorus is mainly conserved within the system. Phosphorus detention mechanisms are physical, chemical (precipitation, settling, filtration, and sorption), with minor biological process (plant and bacterial uptake) (Kadlec & Wallace, 2009). Orthophosphates (PO_4^{-3}-P) are the dominant form with some polyphosphates and organic phosphate. Most studies on phosphorus cycle in wetlands have shown that removal of phosphorus is limited unless media with high sorption capacity are used (Vymazal, 2011). Plant uptake can also result in phosphorus retention, but it is not significant as physical processes. A study conducted by Tanner *et al.* (1999), compared phosphorus uptake over two years with planted and unplanted constructed wetlands. Average TP accumulations in planted wetlands ranged from 52 - 100 g/m^2, while it was ranged from 40 to 51 g/m^2 in unplanted wetlands. Harvesting plants from constructed wetlands would be an ineffective method of phosphorus removal.

In VFCW designs, the removal of settleable and suspended solids and organic matter removal is relatively high but total nitrogen removal is somewhat limited. Many researchers have reported the negative effects of excessive nitrogen concentrations on receiving waters (Galloway *et al.*, 2003). Optimizing nitrogen removal is thus an important objective, because nitrogen compounds are responsible for negative phenomena such as eutrophication, algal blooms, groundwater contamination, and depletion of dissolved oxygen levels in receiving water bodies.

Nitrogen compounds are gaining more concern in wastewater that high ammonium concentrations in water have been reported to cause depletion in dissolved oxygen in receiving waters. Unionized ammonium is toxic to aquatic organisms. Furthermore, high nitrate concentrations in drinking water can cause methemoglobinemia in infants. Thus, many studies have been optimized the nitrogen removal process in CWs (Arias *et al.*, 2005, Brix *et al.*, 2003, Stefanakis *et al.*, 2011, Li *et al.*, 2014). The nitrogen removal mechanisms in the VFCWs include ammonification, nitrification, denitrification, plant uptake, and physicochemical routes such as sedimentation, ammonia volatilization, and ion exchange (Kadlec, 1999a).

Nitrogen compounds exist in wastewater in form of ammonia and organic nitrogen with minor to no nitrate and/or nitrite. Ammonia has the highest concentration of the nitrogen forms in raw wastewater. Organic nitrogen can be converted into ammonia-nitrogen via ammonification. Ammonification can occur under aerobic or anaerobic conditions, and it is relatively fast in aerobic zones (Kadlec & Wallace, 2009). The rate of aerobic ammonification doubles when temperatures increase of 10°C (Reddy *et al.*, 1989). The optimum pH range for this process is 6.5 - 8.5 (Vymazal, 2007). In addition, filter media texture and structure can also affect ammonification rates (Reddy *et al.*, 1984).

Volatilization is a significant path for nitrogen removal in constructed wetlands required open water surface that algal assemblages can raise up the pH values during the day through their photosynthetic activity (Brix, 1990). When ammonia (NH_3) is unionized, it will be volatized to the atmosphere, if the pH values exceed 8.0 (Reddy *et al.*, 1984). Vymazal (1995) reported that the ammonia volatilization rate is dependent on the NH_4 concentration in water, temperature, wind velocity, solar radiation, aquatic plants (type and density) in the system, and the pH values. Reddy and Patrick (1984) documented that loss of ammonia via volatilization from flooded soils and sediments is significant if pH exceeds 7.5. It was reported that 9.3 is the optimal pH for volatilization (Vymazal, 1998). **Table 2-3** shows the biogeochemical transformation of nitrogen in wetlands (Vymazal, 2007).

Nitrification, the conversion of NH_4^+ into nitrate (NO_3^-), is autotrophic. Nitrification is executed by autotrophic bacteria (nitrifiers) in two steps; first the oxidation of NH_4^+ to nitrite (NO_2^-), and second the oxidation of NO_2^- to NO_3^- by facultative aerobic bacteria (Nitrobacter).

Table 2-3: Biogeochemical transformation of nitrogen in wetlands (after Vymazal, 2007).

Process	Transformation
Volatilization	$NH_{3\ (aq)} \longrightarrow NH_{3\ (g)}$
Ammonification	$\text{Organic-N}_{(aq)} \longrightarrow NH_{3\ (aq)}$
Nitrification	$2NH_4^+{}_{(aq)} + 3O_2 \longrightarrow 2NO_2^-{}_{(aq)} + 2H_2O_{(aq)} + 4H^+{}_{(aq)}$
Nitrification	$2NO_2^-{}_{(aq)} + O_2 \longrightarrow 2NO_3^-{}_{(aq)}$
Denitrification	$2NO_3^-{}_{(aq)} \longrightarrow 2NO_2^-{}_{(aq)} \longrightarrow 2NO_{(g)} \longrightarrow N_2O_{(g)} \longrightarrow N_{2\ (g)}$
Biological Assimilation	$NH_{3\ (aq)}$, $NO_2^-{}_{(aq)}$, $NO_{3\ (aq)} \longrightarrow \text{Organic-N}_{(aq)}$
Ammonia Adsorption	$NH_{3\ (aq)} \longrightarrow NH_3\ (s)$
ANAMMOX	$NH_{3\ (aq)} + NO_2^- \longrightarrow N_{2\ (g)}$

Nitrification is an aerobic process, which requires 1.14 g O_2/g NH_3^+-N (Kadlec & Wallace, 2009). Based on the nitrification equation, 4.3 mg/L of O_2 is required to oxidize 1 mg/L of NH_3^+-N to NO_3^--N and 8.64 mg/L of bicarbonate is utilized. That illustrates the drop in pH values during nitrification. The sufficient amount of oxygen in the filter medium provides microorganisms with the required oxygen for the biological activities. Cooper (2005) reported that oxygen concentration in air is about 250 mg/L at 20°C, oxygen transfer rate in VFCWs is at least 28 g/m².day. However, diffusion of oxygen into VFCWs is fast between dosing events and it is passively aerated (by ventilation pipes) through a drainage perforated pipe system at the bottom of the bed. The actual amount of oxygen requirement for total oxidation of ammonia after considering the ammonia used for cell synthesis is 4.25 g (Metcalf & Eddy, 2003a). Wezernak and Gannon (1967) found that the actual total oxygen consumption was 4.33 g O_2/ g N.

The rate of nitrification is also influenced by temperature, pH, alkalinity, inorganic carbon source, moisture, microbial population, and concentrations of NH_4^+-N (Vymazal, 2007). The optimum temperature for nitrification ranges from 25 to 40 °C in water and soils. VFCWs are very suitable for nitrification, by converting up to 99.9 % of the NH_4^+ from the influent to nitrate (Langergraber *et al.*, 2007).

Denitrification is the subsequent reduction of NO_3 to nitric oxide (NO), nitrous oxide (N_2O) and nitrogen gas (N_2) (Hauck, 1984, Jetten *et al.*, 1997). Denitrification is accomplished by heterotrophic denitrifying bacteria under anoxic conditions with sufficient organic carbon (Gable & Fox, 2003). Nitrogen oxides (ionic and gaseous) serve as terminal electron acceptor in place of oxygen for electrons that are originated from organic matter, reduced sulfur compounds, or hydrogen source (Vymazal, Brix, *et al.*, 1998a). Denitrification is illustrated by **Equation 2-1**:

$$6(CH_2O) + 4NO_3^- \rightarrow 6CO_2 + 2N_2 + 6H_2O \qquad \text{Equation 2-1}$$

However, denitrification is influenced by many factors, including NO_3^- concentration, density of denitrifying bacteria, type, and quality of organic carbon source, retention time, anoxic conditions, pH value, redox potential, temperature, plants, soil type, and water level (Vymazal, 1996, Bastviken *et al.*, 2005, Sirivedhin & Gray, 2006). The optimum pH for denitrification is in the range of 7 - 8. During the process of denitrification, 1 mole of alkalinity is produced per mole of nitrate reduced to gaseous nitrogen that can results in a slight rise in pH. Denitrification is strongly temperature dependent, if the temperature falls below 5°C it precedes at very slow rates (Vymazal *et al*., 1998). Lack of carbon in wastewater can limit the denitrification so the optimum carbon requirement is 3.02 g BOD/ NO_3-N (Kadlec & Wallace, 2009). In CWs, added carbon source can be a methanol, glucose and other organic matter, which is illustrated by **Equation 2-2**:

$$84.8NO_3^- + (CH_2O)_{106}(NH_3)_{16}(H_3PO_4) \rightarrow 42.4N_2 + 106CO_2 + 16H_2O + H_3PO_4 \qquad \text{Equation 2-2}$$

In some cases, for the denitrification process to proceed, the use of an external organic carbon source such as plant detritus, step feeding of raw or primary-treated wastewater, or chemicals (e.g. methanol) is necessary. Many studies on CWs have considered the usage of plant biomass as additional carbon source (Gersberg *et al.*, 1983, Hume *et al.*, 2002). This practice is unsustainable due to the associated extra costs and need of specific dosing equipment, which is, in case of decentralized treatment plants, practically and economically infeasible. A number of researchers have studied denitrification systems, by adding granular activated carbon to packed beds (Lee *et al.*, 2009). On the other hand, a step-feeding approach can effectively provide a carbon source to promote denitrification (Stefanakis *et al.*, 2011), especially after nitrification is achieved. However, few research studies have focused on this method in VFCWs. Burgoon

(2001) provided carbon by feed-forward of un-nitrified influent to wetlands receiving nitrified waters. This operation has been also proposed by other researchers (US EPA, 1988, Dialynas *et al.*, 2002).

Many studies evaluated the effects of recirculation of nitrified effluent back to sedimentation tank or a septic tank in order to enhance denitrification (Brix & Arias, 2005b, Brix *et al.*, 2002). Arias *et al.* (2005) found in their experiment that TN removal optimized to 52 %, 66 %, and 68 % at 100, 200, and 300 % recycling rate, respectively. However, high recirculation rates increased the hydraulic and negatively affected the nitrification performance.

Numerous studies have shown that nitrogen removal is improved in VF designs when plants are present (Keffala & Ghrabi, 2005, Stefanakis & Tsihrintzis, 2009, Cui *et al.*, 2010, Chen *et al.*, 2011). Cui *et al.* (2010) reported that additional 33 % TN was removed in VF planted beds (*Canna indica*) than in unplanted beds. In addition, Stefanakis and Tsihrintzis (2009) found the presence of *Phragmites australis* and *Typha* improved the removal of both TN and organic matter. De Feo (2007) reported that the contribution of plants in nitrogen removal with the highest loads was greater than filtration processes. In contrast, other studies have reported minor or no effect of plants on the removal of nitrogen (Tietz *et al.*, 2007), organic matter (Tietz *et al.*, 2008, Zhao *et al.*, 2010) and bacteria (Vacca *et al.*, 2005, Keffala & Ghrabi, 2005, Torrens *et al.*, 2009a).

The uptake of ammonia and nitrate by macrophytes converts inorganic nitrogen forms into organic compounds, as basic units for cells and tissues (Vymazal, 1995). The uptake and assimilation rate of nutrients by plants depend on the nutrient concentration of their tissues. On the contrary, during autumn and winter, plants may release much of their accumulated nitrogen back into the water during the winter season (Vymazal, 2007).

The ANAMMOX process (ANaerobic AMMonium OXidation) provides a possible alternate pathway for improving total nitrogen removal. The oxygen requirement for ANAMMOX to occur is 1.94 g O_2/g NH_3-N (Kadlec & Wallace, 2009). In particular, the Anammox process has been reported to produce higher removal efficiency of TN (Dong & Sun, 2007, Sun & Austin, 2007, Saeed & Sun, 2012).

2.2.3 Pathogen Removal in VFCWs

Pathogenic organisms (e.g. bacteria, viruses, fungi, protozoa, and helminthes) are common components of domestic wastewater. In developing countries, pathogen reduction is strongly required for safe reuse. Pathogen removal can be accomplished by adding disinfection steps in conventional wastewater treatment plants (Metcalf & Eddy, 1991, Crites & Tchobanoglous, 1998). The most common disinfection processes are chlorination, ozonation, and ultraviolet irradiation. However, some wetland treatment technologies have the ability to reduce pathogens, depending on design and operation specifications.

Coliform has been used as an indicator for the fecal contamination in water (Gerba, 2000). In particular, *E. coli* (which is a subgroup of fecal coliforms) has become the preferred indicator. This is also demonstrated in the WHO (1989, 2004) guidelines for unrestricted irrigation using treated wastewater.

Many researchers have studied the removal of pathogens and *E. coli* in VFCWs, and have found that removal is very high in a well-designed, operated, and maintained system. In VFCWs, bacteria and viruses are removed via physical, chemical, and biological processes. The main physical processes include filtration through filter matrix, aggregation, and sedimentation, whereas chemical processes include oxidation and adsorption to organic matter. Among the biological removal mechanisms are predation by protozoa, nematodes and rotifers, attack by viruses and natural die-off due to starvation (Gersberg *et al.*, 1989, Decamp & Warren, 2000a, Wand *et al.*, 2007).

The removal of pathogens and *E. coli* in VFCWs depends on the water retention time in the filter (Brissaud *et al.*, 1999), which depends on filter depth and the dose volume. Tawfik *et al.* (2004) observed the removal rate of *E. coli* under aerobic conditions (DO of 3.3 - 8.7 mg/L) was significantly higher than anaerobic conditions. Similar results were also reported by Headley *et al.* (2013) who observed that the highest *E. coli* removal was achieved in aerated HFCWs and a reciprocating design. In addition, they reported that plants did not affect the removal of bacteria. Therefore, the aerobic condition in system can play an important role for pathogen inactivation, beside adsorption to biofilms, sedimentation, and other die-off-processes. Wand *et al.* (2007) deduced that predation is the dominant mechanism of bacterial removal in laboratory-scale planted and unplanted sand filters vertical flow constructed wetlands.

There are many studies showing the efficiency of pathogens treatment processes (Stevik *et al.*, 2004, Vymazal, 2005b, Wand *et al.*, 2007). Asano *et al.* (2007) reported that the *E. coli* concentrations in effluent wastewater range from 10^5 to 10^8 MPN/100 mL, where it is 10^6 to 10^{10} MPN/100 mL as concentration causes disease in man. Cooper P *et al.* (1996) showed the concentrations of *coliform* bacteria in wastewater have fluctuations rate in different CWs. Stevik *et al.* (2004) pointed out that the two mechanisms straining (the physical blocking of movement of bacteria) and adsorptions are responsible for immobilization of pathogens in wastewater. Removal of Faecal coliform depends on hydraulic retention time and matrix grain size (García *et al.*, 2003). Headley *et al.* (2013) found that poorest reduction observed in the gravel VF with *E. coli* concentrations (geometric mean effluent concentration of 6.4 - 8.9 × 10^5 MPN/100 mL). Tanner *et al.* (1998) also reported that the removal of pathogens increased with increased hydraulic residence time.

Overall, CW designs show various treatment performance in different countries. There are some experiences in arid countries such as Egypt, Morocco, Tunisia, and South Africa (Vymazal & Kröpfelová, 2008).

2.3 Reuse of Wastewater in Agriculture

Treated wastewater is used worldwide for agricultural irrigation directly and indirectly (Carr *et al.*, 2011, Westcot, 1997). In Jordan, treated wastewater is reused indirectly; treated water is discharged into surface water (wadis, dams, rivers, and aquifers) to be mixed with freshwater and used by farmers. Abu-Madi *et al.* (2002) showed that 87 % of farmers in Jordan use treated water in irrigation and religion is not seen as a factor to prohibit water reuse. Many researchers also found that farmers willingly use reclaimed water and consider its economic benefits (Kilelu, 2004, Keraita *et al.*, 2010, Ouedraogo, 2002). Generally, the use of treated wastewater in irrigation can reduce the total expenses associated with treatment process and protect the environment (Eriksson *et al.*, 2002).

Many studies have reported the advantages and disadvantages of utilizing treated wastewater in irrigation for different crops (Reboll *et al.*, 2000). Papadopoulos (1988) reported increased yield production in Cyprus, which was a result of the high nutrient level such as nitrogen and phosphorus in the treated wastewater (Pescod, 1992).

Several reports have documented and investigated the impacts of reuse on soil physiochemical properties, using various irrigation methods such as flood irrigation (Bowman & Rice, 1986, Jaynes *et al.*, 1988, Westcott & Vines, 1986), furrow irrigation (Hornbuckle *et al.*, 1999, Kang *et al.*, 2000, Walker & Humpherys, 1983, Sojka & Lentz, 1997) and sprinkler irrigation (Sammis, 1980, Merriam & Keller, 1978, Aase *et al.*, 1998, Pair, 1970).

In Jordan, drip irrigation is used as preferable irrigation method in the Jordan Valley (Molle *et al.*, 2008). Many researchers found that salts accumulated on the soil surface and along the soil profile when drip irrigation is applied (Yaron *et al.*, 1972, Al-Nakshabandi *et al.*, 1997, Shatanawi, 1987). A study by Rusan *et al.* (2008) investigated the effects of reuse in irrigation using treated water in cut flowers (ornamental) plants. The plants showed higher flower yields and better flower quality within few days, and that was correlated to higher macro and micro nutrients concentrations in leaves of rose plants (Rusan *et al.*, 2008). Abedi-Koupai *et al.* (2006) they reported the impacts on soil chemical and physical properties in an arid region. They noticed that irrigation system had potential effects on infiltration rate, bulk density, and porosity of soil.

Subsurface irrigation is a relatively new method in Jordan, where little information is available on the impact of irrigation water on chemical and physical soil characteristics and plants. On the other hand, subsurface irrigation system is a suitable method for irrigation using treated wastewater, because it minimizes the risk of human exposure to pathogenic organisms. Oron *et al.* (1999) reported that pear yield was higher with fresh water using subsurface irrigation system at 30 cm. The subsurface irrigation is in some way more effective because nutrients and water are applied directly to the root zone, where sprinkler irrigation may pose a risk of human exposure to pathogenic organisms and/or cause leaf burn on sensitive crops. Thus, subsurface irrigation is not only beneficial to securing human health but plant health as well. Bohrer (2000) studied the performance of six subsurface drip irrigation systems with an adjustment during winter months. The results showed that the nitrogen levels in the shallow soil samples were similar to the nitrogen levels in the original soil samples. In contrast, different irrigation methods showed increased nutrient concentrations (nitrogen and phosphorus) and heavy metals in the soil over time.

2.3.1 Soil Salinity

In most of the Mediterranean countries, irrigated land affected by soil salinity and it was estimated in Jordan about 16 % of the irrigated area (Hamdy & Lacirignola, 1999). Furthermore, treated wastewater in arid countries is typically slightly saline (Feign *et al.*, 1991).

Heidarpour *et al.* (2007b) showed the effects of treated wastewater reuse on soil chemical properties by using different irrigation methods. The most important concern was the increase of salinity in the top soil layer with subsurface irrigation. Saline water exacerbates the salinity problem within the soil matrix, especially when drip irrigation is applied. The salt content increases on the soil surface within the root zone layer, and influences water availability, fertility of the soil and crop yields (NCARTT, 2003).

Many researchers have studied the impacts of irrigation water on the soil chemical and physical properties; including soil salinity problems (Aiello *et al.*, 2007, Rusan *et al.*, 2007, Kiziloglu *et al.*, 2008, Travis *et al.*, 2010, Pereira *et al.*, 2011, Lado *et al.*, 2012, Lado & Ben-Hur, 2009, Tzanakakis *et al.*, 2011, Al-Shdiefat *et al.*, 2009). Salinity effects depend on many factors such as climate conditions, soil characteristics, agricultural practices, and water quality and quantity (Katerji *et al.*, 2003). Increased salinity, as indicated by Electrical Conductivity (EC) measurements, increases the water stress on the plant through its effect on the osmotic potential of the soil water (Ayers & Westcot, 1985). With increasing salinity, the osmotic potential decreases, as does the water available for the plant, resulting in increased water stress, which negatively affects leaf growth and photosynthesis. To neutralize the salinity concentration frequent leaching with good water quality is necessary.

According to the (Ayers & Westcot, 1985), negative effects of salinity were not anticipated with water EC less than 0.7 dS/m. Although, high EC (0.7 - 3 dS/m) showed a slight to moderate salinity issue. Water EC higher than 3 dS/m showed harmful impacts on soil and crops (Pettygrove & Asano, 1984).

Salinity effects on soil pH and EC have been observed in graywater reuse, where changes in pH ranged from 6.9 to 7.9 and in the EC from 126.2 µS/cm to 306.3 µS/cm (Pinto *et al.*, 2010). The reuse of wastewater with a pH < 8 increases soil alkalinity (pH), which reduces the availability of useful cations, anions, and micronutrients for plants (Christova-Boal *et al.*, 1996).

2.3.2 Soil Sodicity

In general, increased soil salinity and sodium accumulation in soil are expected as reuse issues after long-term irrigation with treated wastewater. Salts can affect the soil structure and reduce the hydraulic conductivity and the infiltration rate.

Sodium adsorption ratio (SAR) is the ratio of sodium concentration to calcium and magnesium concentrations in meq/L. High SAR can damage the soil structure, reduce soil permeability, and reduce the crop yields due to toxic and osmotic effects (Bouwer & Chaney, 1974, Quirk, 1994, Oster, 1994, Oster & Shainberg, 2001). Patterson (1994) showed loss of soil permeability begun when SAR reached a value of three. The maximum recommended level of SAR is 6, which is capable to keep the soil permeability and structural stability (Patterson, 1994). According to ANZECC (1992), SAR = 8 was suggested as the higher limit for irrigation.

Lado and Ben-Hur (2009) found that increasing of soil sodicity depends on soil and irrigation water quality. They observed that a sandy soil with high sodicity, under precipitation conditions, showed a reduction in infiltration rate because of salt accumulation (soil clogging). While, under same conditions, a calcareous soil did not show runoff or soil loss, because of a release of Ca in

$CaCO_3$ dissolution so Ca replaced the Na and neutralized the soil sodicity (Lado & Ben-Hur, 2009).

2.3.3 Microbial Contamination

Many studies showed that soil acts as a filter and purification system for various pathogens in wastewater (Oron *et al.*, 2001, Idelovitch & Michail, 1984, Bouwer, 1991, Bales *et al.*, 1991, Bitton & Harvey, 1992).

Soil provides additional treatment that pathogenic microorganisms in reclaimed water can be removed by several physical and chemical processes (Oron *et al.*, 2001). Bacteria are eliminated by sedimentation, adsorption, straining and natural die-off during water movement through soil matrix (Oron *et al.*, 2001). Many researchers have found that factors such as temperature, soil moisture, soil texture, soil pH, organic matter content, and soil salinity affect pathogen transport in soils (Bales *et al.*, 1991, Bitton & Harvey, 1992, Gannon *et al.*, 1991).

A study by Lance and Gerba (1984) reported a reduction in concentration of microorganisms when the water percolated through the soil. Keswick and Gerba (1980) observed that the transport of coliforms in course soils (sand and gravel) is higher than fine sand. On the other hand, Zhang *et al.* (2009) indicated that pathogenic bacteria transmission to plants by its root is quite limited. Subsurface drip irrigation can also contribute to pathogen removal. A study by Oron *et al.* (2001) in Israel, showed that using subsurface drip irrigation gave higher reduction of microorganisms in comparison with using surface irrigation.

2.3.4 Plant Toxicity

Reuse of treated wastewater in irrigation has been studied in many crops such as alfalfa, wheat, and corn (Campbell *et al.*, 1983, Feizi, 2001, Al-Jaloud *et al.*, 1995, Hussain & Al-Jaloud, 1995), cotton (Oron & DeMalach, 1987, Zwart & Bastiaanssen, 2004), forages (Mohammad & Ayadi, 2004, Mohammad & Mazahreh, 2003, Mohammad Rusan *et al.*, 2007), and other vegetables (Al-Nakshabandi & Khalil, 1983, Al-Nakshabandi *et al.*, 1997, Hanson *et al.*, 2006). Many studies have investigated reuse on citrus trees (Lapeña *et al.*, 1995, Reboll *et al.*, 2000, Pedrero & Alarcón, 2009), but there is a little information about irrigation of citrus trees with treated wastewater.

Each crop has a threshold for increases in soil salinity and sodicity. Plant toxicity occurs when the concentrations of different parameter exceed these thresholds. Ayers and Westcot (1985) reported that sodium, chloride, and boron are the common toxic ions in treated water. Chloride and sodium increase from domestic usage (Pettygrove & Asano, 1984). The sensitivity to irrigation with treated wastewater differs from crop to crop (Ayers & Westcot, 1985). Many researchers have determined crop reaction to salinity by measuring crop yields. Katerji *et al.* (2003) studied the effects of salinity on different crops and reported that salinity affected yield, evapotranspiration rate, and leaf size). Salinity caused a yield reduction by affecting the number and weight of grains, tubers, and fruit. For citrus, the acceptable EC levels in the irrigation water should be less than 1.7 dS/cm (GTZ, 2006).

In the United States, specifically in Florida, reuse of treated water in irrigation is widely implemented for citrus plants (Morgan *et al.*, 2008). Studies documented higher concentrations of Mg and B in citrus after reuse of treated water in irrigation (Morgan *et al.*, 2008, Pedrero & Alarcón, 2009). However, because chloride is not adsorbed by soils, its effects depend on the plant sensitivity. In citrus trees, high chlorides concentration can cause a reduction in vegetative growth (Walker *et al.*, 1982). For citrus, the acceptable levels of chloride in the irrigation water is from 6.7 meq/L (237 mg/L) to 16.6 meq/L (588 mg/L) for sensitive crops and less sensitive crops having a threshold as high as 27 meq/L (956 mg/L) (Ayers & Westcot, 1985).

Researchers have claimed that reuse of treated wastewater is an important source of nitrogen for citrus trees (Zekri & Koo, 1994, Legaz *et al.*, 1995). However, long-term use of treated water can cause salts and metals accumulation in the soil and plants (Madyiwa *et al.*, 2004, Wiel-Shafran *et al.*, 2006).

The previous studies have been applied with variable conditions for instance the period of irrigation (long-term or short-term), irrigation water quality and quantity, and sorts of crops. However, the outcomes of these previous experimental research studies express the increasing of soil salinity as a major effect of reclaimed water in comparison with the control plot.

3. Langenreichenbach Research Facility

3.1 Site Description

The Langenreichenbach (LRB) treatment wetland research and demonstration site is located in Langenreichenbach, Germany, approximately 50 km northeast of Leipzig (**Figure 3-1**). The experimental site was rebuilt in 2009 - 2010, to include different kinds of subsurface CW designs (horizontal, vertical and intensified). The facility consists of 15 pilot-scale treatment systems, which were constructed in planted and unplanted pairs in order to investigate the role of plants in treatment performance.

The CW systems at LRB treat domestic wastewater. The research facility receives raw wastewater from the wastewater treatment plant for the neighboring villages. The raw wastewater received primary treatment via a septic tank with a residence time of approximately two days. A specific volume of primary-treated wastewater was dosed to each system every 30 - 60 minutes by submersible pumps. The inflow for each system was measured using an electromagnetic flowmeter and recorded by a central control computer in the main control building. The effluent from each filter returned by gravity to the main control building where it was measured by a calibrated 6 L tipping bucket before discharge to the main wastewater treatment plant. An automatic weather station recorded air temperature, rainfall, evaporation, and humidity on a daily basis.

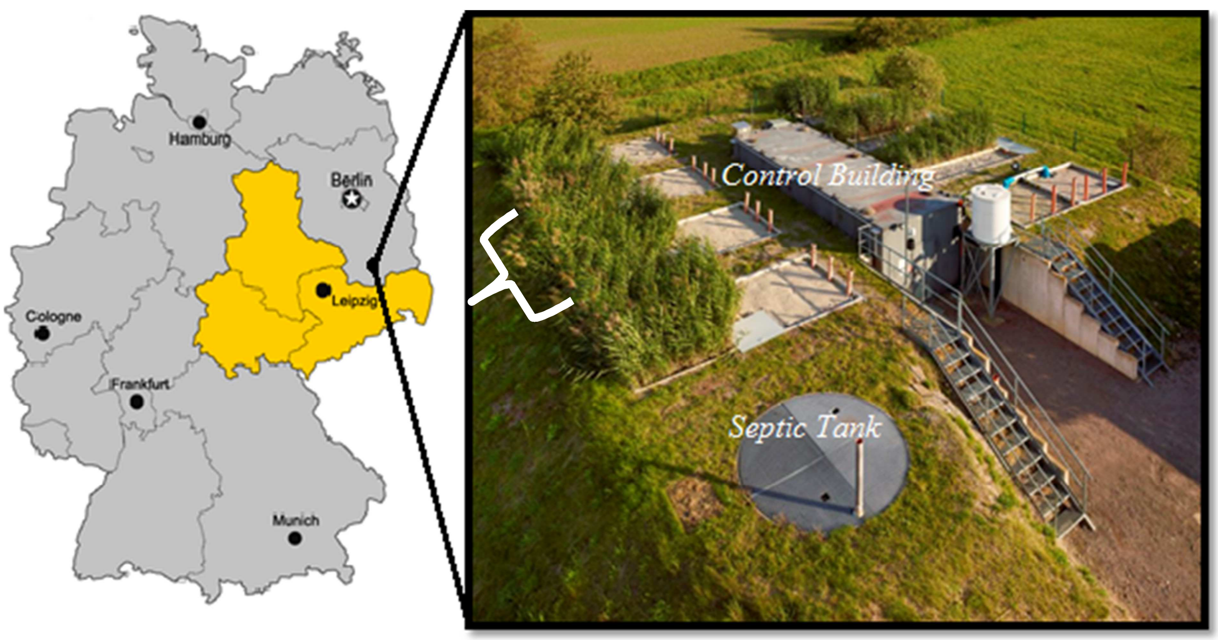


Figure 3-1: The Langenreichenbach wetlands research facility in Germany, showing two investigated VFCW systems.
Map source: http://photos.state.gov/libraries/leipzig/475/public/germany-map.gif
(Areal Photo by Andr´e Künzelmann - UFZ)

3.2 Research Designs and Methodology

3.2.1 Experimental Setup

A pair of two-stage VFCW was investigated in the research facility at LRB. Each system consisted of two unsaturated beds. In the first VFCW design, a planted gravel bed (VGp) was followed by a planted sand bed (VSp). The second system had the same configuration without plants (VG and VS), **Figure 3-2**. The second-stage beds were equipped with sampling pans at 10, 20, and 40 cm depths.

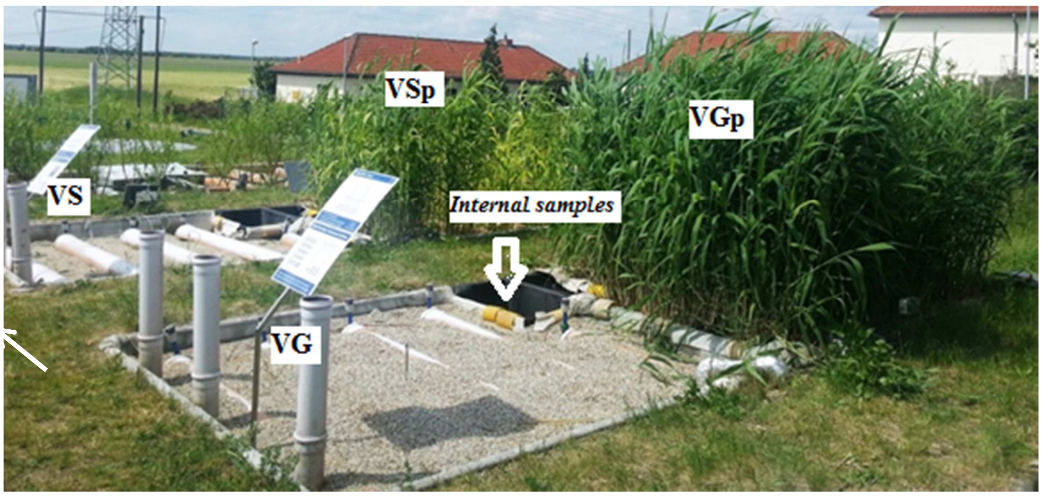


Figure 3-2: The two-stage VFCW systems (VGp - VSp) and (VG - VS) at the experimental site.

Each bed had a surface area of 6.2 m^2 (2.4 m width, 2.75 m length). The details of each VF bed are provided in **Table 3-1**. The 1st stage (VGp and VG) and 2nd stage (VSp and VS) were dosed once every hour in sequence. Overall, both systems received the same average hydraulic loading rate of 95 L/m^2.d.

Figure 3-3 shows the layout of VF beds. The wastewater was dosed to the top of the filter via inlet distribution pipelines through 4.0 mm diameter holes spaced approximately every 0.5 m. The distribution pipelines were covered by half-pipe shield tunnel, ensuring an equal distribution of water, preventing water freezing on the surface in the winter. The distribution pipelines were designed with clean-outs, which can be used to flush any accumulated solids from the pipelines. As depicted by **Figure 3-2**, there are lateral vertical pipes, which connected with drainage system in the bottom of VF bed, promoting a passive aeration. The bottom of each VF bed was covered with geotextile fabric (Liner: PVC soft, 1.0 mm black, and geotextile: 300g/m^2), from KAT GmbH Kunststoff-Abdichtungs-Systeme company, to ensure the basins were water tight.

For vertical internal profile sampling, interception pan lysimeters (length 0.5 m length, 0.12 m width, 0.06 m depth) were installed at 10, 20, and 40 cm depths, **Figure 3-4**. The interception pans were filled with coarse gravel in order to avoid clogging in the pan, **Figure 3-5**.

Table 3-1: The vertical flow constructed wetlands details and setup at LRB.

VFCWs	Depth (m)	Saturation status	Media size (mm)	Hydraulic loading rate ($L/m^2.d$)	Surface area (m^2)	Dosing interval
VGp, VG	0.85	unsaturated	fine gravel (4 - 8)	95	6.2	hourly
VSp, VS	0.85	unsaturated	coarse sand (1 - 3)	95	6.2	hourly

VGp and VSp: planted system with *Phragmites australis*.

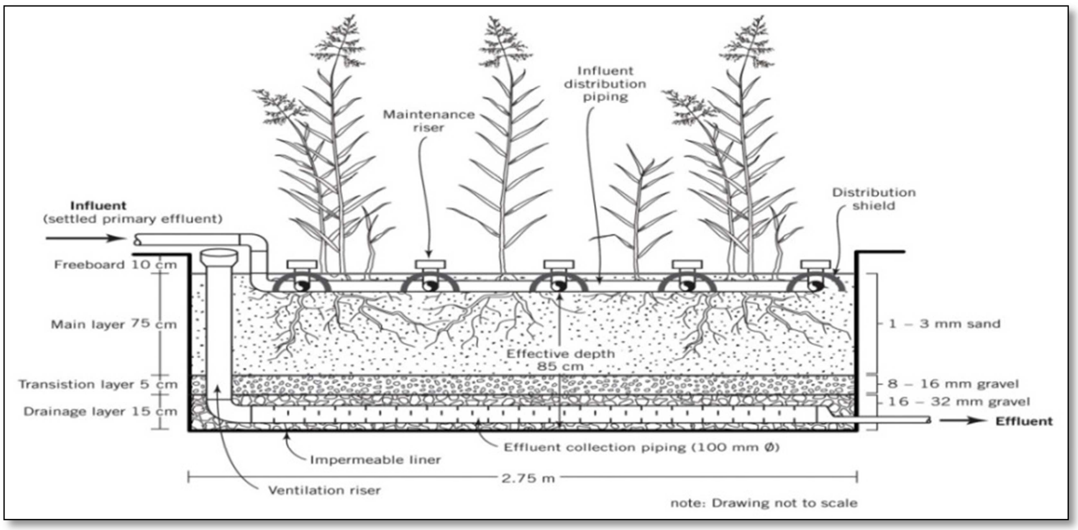


Figure 3-3: The layout of the VF bed cross section (Nivala *et al.*, 2013).

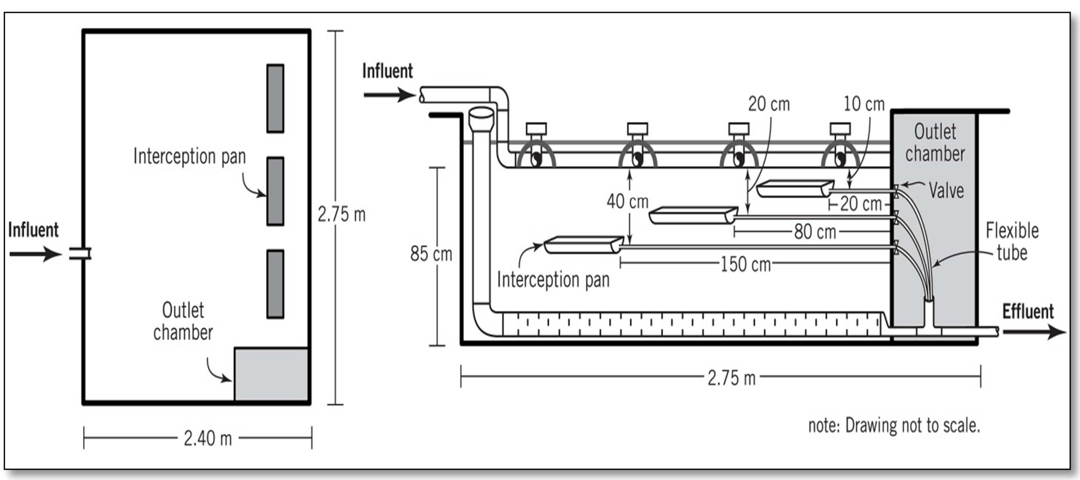


Figure 3-4: layout of the vertical profile at 10, 20 and 40 cm depths in each bed (Nivala *et al.*, 2013).

Figure 3-5 : The interception pans for internal sampling in the VFCWs during construction.
(Photo by Nivala)

3.2.2 Operational Modifications

The two two-stage wetland systems were monitored weekly over the course of one year (steady-state operation). The systems were modified in July 2013. The modification was conducted in order to enhance the nitrogen removal based on the prior results of monitoring under steady state operation. Modification was implemented in the 1st stage of each system (VGp and VG), by saturating the 30 cm at the bottom of the bed. The saturated zone was thought to increase anoxic conditions, thus boosting denitrifying bacteria growth and activity. The technical work of the modification was carried out by adding an elbow connected with a pipe (30 cm height) to keep the water level within 30 cm in the beds, as depicted in **Figure 3-6**. The total saturation volume in both VGp and VG was calculated to be 81.28 L, according to Brassington (1998) in **Equation 3-1**.

$$Substrate\ Saturation = Volume\ of\ substrate \times Porosity = W_A \times S_D \times P \qquad \text{Equation 3-1}$$

Where:
W_A = wetland area = 6.2 m^2
S_D = saturated depth (gravel = 0.3 m)
P = porosity (gravel = 0.437 as measured in the lab)

Figure 3-6: The modified VGP and VG beds with the internal sampling hoses.

3.2.3 Water Sampling Scheme

The systems were monitored from June 2012 until July 2013. Water samples were collected from the inlet and outlet of each bed on a weekly basis. Internal samples were collected fortnightly during the research period. Sampling was canceled in the case of site maintenance or more than 10 mm rain within the 48 hours prior to sampling. Sampling at the research site was not conducted during extreme rain events due to excessive rainwater infiltration into the sewer system connected to the community wastewater treatment plant of Langenreichenbach and surrounding villages. Small and decentralized wastewater treatment systems, which are constructed on site or near to the wastewater source are not subject to excessive rainwater infiltration from the sewer network, thus, for these extreme rain events sampling was not conducted.

The inlet and outlet samples were collected in the control building, after two minutes of dosing to flush the pipes. The inlet sample for the VGp and VG was the effluent from the septic tank, followed by the VGp and VG outlet samples the inlet water for the VSp and VS. The outlet samples of VSp and VS beds were considered as final effluent of the two-stage systems. The field temperatures, for all samples, were measured directly after sampling with a portable thermometer.

The internal samples were taken only from the 2nd stage beds (VSp and VS), from hoses which were connected to valves at the outlet shaft in each bed. The sampling bottles were linked to hoses for various depths (10, 20, and 40 cm). Fresh samples were collected during a dosing pulse of the VSp and VS beds. Weekly, before internal sampling, the hoses were cleaned using a pipe-brush in order to remove any biomass growth in the tubes. The valves were opened all the time and connected to the outlet pipe in order to ensure self-flushing.

3.2.4 Analytical Methods

Field measurements were carried out directly in the on-site laboratory. Subsequently, the samples were transported in iceboxes to the Helmholtz Environmental Research Centre (UFZ) laboratory in Leipzig. TOC, TN, TSS, BOD_5, turbidity, and *E.coli* were analyzed in the laboratory of Environmental and Biotechnology department (UBZ) at the UFZ. Other analyses for NH_4^+-N, NO_2^--N, and NO_3^--N were conducted in the department of Analytical Chemistry at the UFZ in Leipzig. **Table 3-2** shows the conducted analyses for water samples during the study period.

Table 3-2: The scheme of water samples with their measuring parameters.

Parameters	Water samples
Field measurements	VGp, VG, VSp, VS and six internal samples (10, 20 and 40 cm)
TOC	VGp, VG, VSp, VS and six internal samples (10, 20 and 40 cm)
BOD_5	VGp, VG, VSp, and VS
TSS	VGp, VG, VSp, and VS
Turbidity	VGp, VG, VSp, VS and six internal samples (10, 20 and 40 cm)
E.coli	VGp, VG, VSp, VS and six internal samples (10, 20 and 40 cm)
TN, NH_4^+-N,NO_2^--N, and NO_3^--N	VGp, VG, VSp, VS and six internal samples (10, 20 and 40 cm)

Field Measurements

100 mL of each sample were taken to measure lab water temperature, pH, electrical conductivity (EC), redox potential, and dissolved oxygen (DO). All of these parameters were measured using the multi-meter WTW (Multi 350i) and the WTW model pH-96 for pH. 10 mL of the each sample were filtered (using a 0.45 µm filter with syringe) for NH_4^+-N, NO_2^--N, and NO_3^--N analyses.

Physical and Chemical Analyses

The carbonaceous Biological Oxygen Demand over 5 days (BOD_5) was measured using OxiTop® manometric OC100 according to the German standard DIN 38 409 H52. A specific volume from each sample was incubated at 20°C for 5 days. Drops of nitrification inhibitor (N-allylthiourea, 5g/L C4H8N2S) was added to the samples in order to prevent nitrification. The test reflects a pressure measurement caused by oxygen consumed by microorganisms, which produced CO_2 absorbed by sodium hydroxide (NaOH) pellets. The measured pressure is recorded in the OxiTop heads as a BOD value.

Total Organic Carbon (TOC) was analyzed referring to the German Standard DIN EN 1484, using the Total Organic Carbon Analyzer TOC-VCSN from Shimadzu. The test represents the organic carbon in the water samples that it is oxidized to CO_2, the amounts of CO_2 are recorded by the machine as a TOC result. While, inorganic carbon (IC) is acidified and purged to be removed from sample.

Total Suspended Solids (TSS) was analyzed using the mass balance method according to the Standard Methods for Examination of Water and Wastewater (APHA, 1995). The samples were filtered via a filter paper in GF/C glass fiber filter 934 - AH™. The filters were dried at 103°C. The difference between the dry filter weight with and without trapped solids represents the TSS value.

Turbidity results were determined using the Hach 2100AN Turbidimeter in NTU unit, according to the German standard DIN ISO EN 27027. The light of wavelength 455 nm from a tungsten-filament lamp is passed through water sample in a cylindrical glass cell. The intensity of the scattered light indicates the turbidity value.

Nitrogen Forms (TN,* NH_4^+-N, NO_2^--N, and NO_3^--N*)

Total Nitrogen (TN) was measured according to the German standard DIN EN 12660, using the Total Nitrogen Measuring TNM-1 unit from Shimadzu. The nitrogen is transformed into nitrogen monoxide (NO) by combustion at 700°C. The NO reacted with ozone to give nitrogen dioxide (NO_2), which its emissions in the form of visible light easy to be recorded and detected by a chemiluminescence detector in this unit.

Ammonium (NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) were analyzed, using a spectrophotometer (EPOS Analyzer 5060) from Eppendorf, referring to the German standards DIN 38 406 E5, DIN 38 405 D9 and DIN 38 405 D10, respectively. NH_4^+-N was analyzed at 690 nm. The ammonia is extracted from alkaline sample. The NH_3 reacted with a chlorinating agent forming monochloramine, which is reacted with thymol to an indophenol derivate to produce a blue indophenol. This blue substance is determined by absorption spectroscopy as NH_4^+-N.

NO_2^--N was analyzed photometrically at 546 nm. The samples were acidified and reacted with sulfanilamide agent to diazotozate and Naphtyl-ethylendiamindihydrochloride (NED) to form an azo couple. This produces a red dye determining the NO_2^--N concentration.

NO_3^--N was analyzed photometrically at 546 nm. The water sample is acidified and analyzed using to the same approach of NO_2^--N.

Microbial Analysis
E. coli numeration was measured using the IDEXX™ Colilert-18 / Quanti-tray2000 method. The *E.coli* water samples were collected in sterilized and autoclaved 100 mL glass bottles. The samples were diluted according to the analysis range of the method and the dilution was subsequently mixed with the IDEXX nutrient powder (100 µg Colilert-18). The solution was poured in an IDEXX envelope (49 large and 48 small wells) then sealed using a Quanti-Tray® Sealer model 2X. After incubation in 37°C for 18 hours, the number of fluorescent wells is reported under UV light that used in the most probable number (MPN) table to find out the MPN of *E. coli* in a 100 mL solution. The procedure included making triplicate samples from the VSp bed that showed a geometric mean of $1.6*10^3 \pm 2.3*10^2$ MPN/100 mL in order to check accuracy of conducting one sample from each sampling point per sampling event.

3.2.5 Calculation

This section shows all equations that were used to calculate evaporation (E), evapotranspiration (ET), flow rate, hydraulic rate, mass load, removal rate, and removal efficiency of various VFCW systems.

Water Budget
Water budget integrates all the inflows and outflows influencing the hydrology of wetlands design. Wastewater inflows and rainfall are considered as CW water input. On the other hand, there are negative outflows sources such as E, ET and groundwater recharge-discharge (Kadlec, 1983). Some studies measured the ET values of *P. australis* that vary from 0.2 to 57 mm/day, depending on vegetation growth and climatic conditions (Fermor *et al.*, 2001, El Hamouri *et al.*, 2007). E and ET values were calculated per unit of area in each VF bed from the monthly average rainfall, inflow and outflow, using **Equation 3-2** (Armstrong, 1978):

$$Q_i = Q_o + A((ET\ or\ E) - P) \qquad \text{Equation 3-2}$$

Where:
A = total surface area of the wetland (m^2)
E= evaporation rate (mm/day)
ET = evapotranspiration rate (mm/day)
P = daily precipitation rate (mm/day)
Q_i = daily inflow to the wetland (L/day)
$Q_{o\,=}$ daily outflow from system (L/day)

ET is the integrated loss of water from E and plant transpiration. This water loss minimizes the total water volume in the CWs, hereby, increasing the concentration of pollutants in water

(Wallace & Knight, 2006). While, rainfall increases the water volume in the CWs, diluting the concentration of pollutants (Wallace & Knight, 2006).

The Hydraulic Loading Rate (HLR)

HLR is an important variable in designing and assessing the treatment efficiency of treatment wetland (Hammer & Kadlec, 1983). It presents the water volume (inflow) applied per unit area of treatment wetland and per unit of time.

$$HLR = q = {}^{Q}/_{A} \qquad \text{Equation 3-3}$$

Where:
q = Hydraulic Loading Rate (m/day)
Q = inlet flow rate (m^3/day)
A = wetland area (m^2)

The Inlet Mass load (M_i)

M_i presents the chemical loading rate in inflow per unit of area in g/m^2.day (Kadlec & Wallace, 2009). It can be calculated using **Equation 3-4**:

$$M_i = {}^{(Q_i \times C_i)}/_{A} \qquad \text{Equation 3-4}$$

Where:
M_i = inlet mass loading per unit of area (g/m^2.day)

Mass Removal Rates (R_{mass})

R_{mass} represents the average amount of pollutant that removed per unit of area in the CW. It can be calculated in g/m^2.day using **Equation 3-5**.

$$R_{mass} = (Q_i \times C_i - Q_o \times C_o)/A \qquad \text{Equation 3-5}$$

Mass Removal Efficiency (% R_{mass})

It connects the chemical losses and gains to water losses and gains in percentage using **Equation 3-6**.

$$\%R_{mass} = \frac{(Q_i \times C_i - Q_o \times C_o)}{(Q_i \times C_i)} \times 100\% \qquad \text{Equation 3-6}$$

3.2.6 Statistical Methods

Statistical analyses were performed using SigmaPlot software, version 12.0. Results of planted and unplanted VF beds were compared (one-way ANOVA) to assess the role of plants in the treatment performance. Results of the 1st and 2nd phases were statistically compared using Paired *t*-test to examine the impact of partly saturated layers in the 1st stage on pH, EC, DO, turbidity, TSS, TN, NH_4^+-N, NO_3^--N, TOC, BOD_5, and calculated geometric mean for *E. coli.* Monthly pollutants removal mass rate was calculated and compared using one-way ANOVA (statistical significance, $p > 0.05$) for TN, NH_4^+-N, TSS, BOD_5, and calculated geometric mean for *E. coli* for the VGp-VSp and VG-VS during 1st and 2nd year.

3.3 Results and Discussion

3.3.1 Weather Description and VFCWs Water Balance

Inflow, outflow, rainfall, air temperature, E, and ET data are addressed in monthly means in this section. Monthly means are presented from May 2012 to May 2013 (1st phase, steady-state operation) and presented from July 2013 to July 2014 (2nd phase, post-modification operation). Non-representative data were removed from calculation (e.g., operation and maintenance days or days on which the rainfall exceeded 10 mm/day over 48 hours).

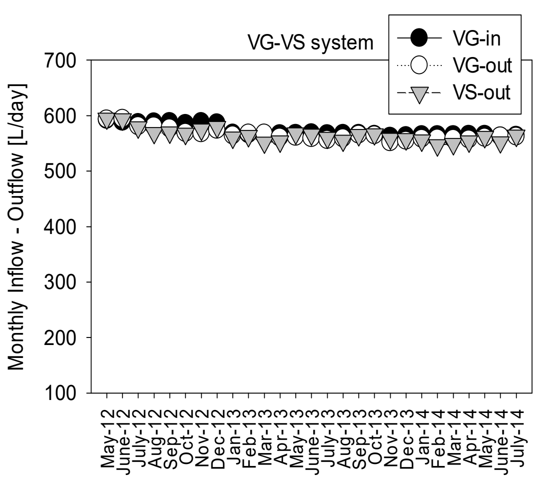
3.3.1.1 Air Temperature and Rainfall

The maximum mean daily air temperature was recorded in July 2014 of 21.1°C, whereas the lowest mean air temperature was approximately -1°C in March 2013.

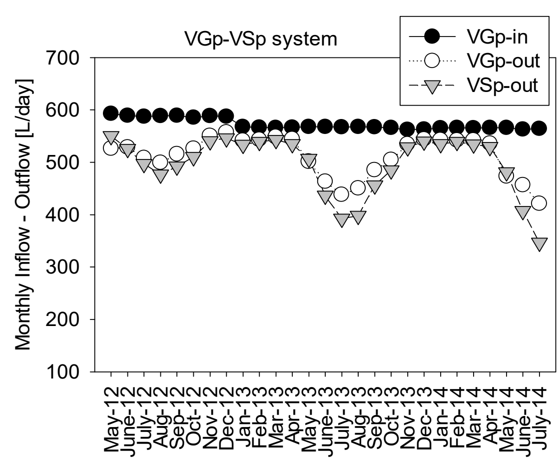
Germany has a relatively high rainfall frequency and magnitude over the year. During the 1st phase, the maximum monthly rainfall observed was 90 mm in June 2012 from a total annual rainfall of 487.5 mm. Around 80 % of the annual rainfall was recorded in May - December 2012, while the remaining 20 % recorded in January - May 2013. During the 2nd phase, higher total annual rainfall was recorded of 528.3 mm. The maximum average rainfall was reported of 95 mm in May 2014. The distribution of average rainfall was 50 % of the annual rainfall recorded in July -December 2013, while the remaining 50 % recorded in January - July 2014.

3.3.1.2 Inflow and Outflow

Monthly average inflow and outflow rates are depicted in **Figure 3-7**. The average inflow in both systems was ranged from 565 - 579 L/day. The outflow values fluctuated between 499 - 557 L/day in VGp and ranged from 492 - 543 L/day in VSp. Water loss was observed in VGp-VSp during the summer and spring due to evapotranspiration. The monthly average inflow and outflow rates for the unplanted system (VG-VS) system showed little to no water loss through evaporation.



a)

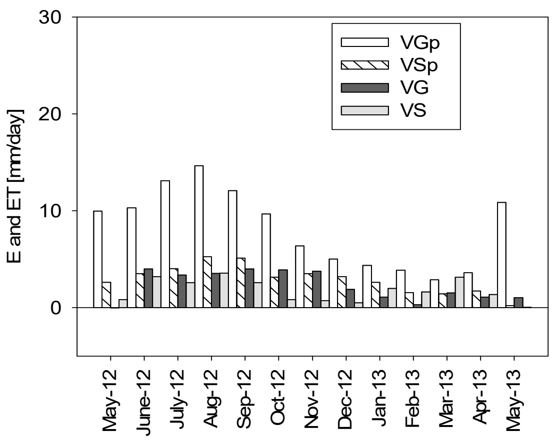


b)

Figure 3-7: Monthly average inflow and outflow data of VGp-VSp and VG-VS over the study period, a) VG-VS, b) VGp-VSp.

3.3.1.3 Evaporation (E) and Evapotranspiration (ET)

Figure 3-8 shows the E and ET rates of VGp-VSp and VG-VS treatment wetlands during the study period. In phase 1, the maximum ET rate was calculated to be 14.7 mm/day from VGp bed in August. High ET rate in the summer was compatible with highest plants growth rate and foliage surface area. However, minus ET rate was resulted of rain ingress that gave higher Q_o than Q_i. Similar results were observed by Schütte and Fehr (1992) that the water loss via ET in CWs in Europe was ranged from 5 to 15 mm/day in the summer. Moro *et al.* (2004) reported that ET of *Phragmites australis* was highest in summer (June), in a wetland in Natural Park in Spain, while it was decreased in winter. In the VG-VS system, the maximum E rate was equated of 3.9 mm/day from VG bed. During the 2nd phase, higher ET rates were recorded due to different weather conditions such as wind speed, rainfall, solar radiation, and humidity.



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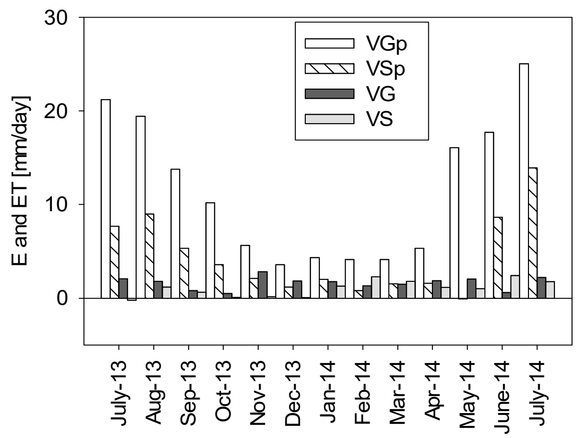


b)

Figure 3-8: Monthly E and ET for VGp-VSp and VG-VS wetlands over the study period, a) 1st phase, b) 2nd phase.

3.3.2 Two-stage VFCWs Treatment Performance

pH, EC, DO, turbidity, TSS, TN, NH_4^+-N, NO_2^--N, NO_3^--N, TOC, BOD_5 and *E. coli* results are presented in this section. Means and standard deviation (SD) of the 1st phase results were calculated from May 2012 - May 2013. Means and SD of the 2nd phase results were calculated from July 2013 to July 2014.

3.3.2.1 Physico-chemical Parameters

Table 3-3 shows the pH, EC, DO, redox potential, TSS and turbidity results. In phase 1, both systems produced slightly acidic effluents, on average pH of 6.6, which was compatible with high nitrification rate in the VF beds (Bitton, 1994, Kadlec *et al.*, 2000). During the 2nd phase, pH values were influenced by the saturated layer in the VGp and VG, and were higher (7.2 and 7.3 in the VSp and VS effluents, respectively).The longer retention time due to the partial saturation of the first stage prolongs the water-substrate interaction, thus, substrate would provide carbonate to water and buffer the pH, which is in accordance with Kadlec and Wallace (2009). Effluent pH values of planted and unplanted VFCWs were statistically similar ($p < 0.05$). While, effluent pH values in phase 1 and 2 were statistically different ($p > 0.05$).

In phase 1, the EC values were reduced gradually in the 1st and 2nd stage effluents in both systems compared with influent EC. The EC reduction can be explained by settlement of suspended particles and elements (Bitton, 1994, Kadlec *et al.*, 2000). Additionally, the removal of macronutrients (NO_3^--N and PO_4^{3-}) from water mitigates the EC value. The VSp and VS effluents had a mean EC of 1214 µS/cm and 1156 µS/cm, respectively. Higher EC value was observed in the planted system due to water loss via ET that increased the salts concentration in water (Morari & Giardini, 2009). During the 2nd phase, effluent EC values were slightly increased due to higher influent EC. The mean EC concentrations for VSp and VS were 1361 and 1239 µS/cm, respectively. In comparison, effluent EC results showed that planted and unplanted VFCWs were statistically similar ($p < 0.05$). While, effluents EC results in phase 1 and 2 were statistically different ($p > 0.001$).

The DO concentration was sharply increased in the 1st and 2nd stage effluents in both systems during phase 1. Effluents DO levels were ranged from 6.4 to 9.7 mg/L in both systems. Highly oxygenated effluent in the VF beds was promoted by gas diffusion from the atmosphere between intermittent hydraulic loads as documented by many authors (Brix & Arias, 2005b, Brix & Schierup, 1990, Saeed & Sun, 2012, Laber *et al.*, 1997). In addition, plants roots could release oxygen within the root zone, increasing the DO levels. However, Effluent DO levels in the planted and unplanted system in this study were statistically similar ($p < 0.05$). In phase 2, depletion of DO content in the VGp and VG effluents was observed because of partial saturated zone. However, effluents DO content in phase 1 and 2 were statistically similar ($p > 0.05$).

The evolution of redox potential over the course of the study is shown in **Figure 3-9**. Redox measurements were also coordinated with DO results. In phase 1, redox values ranged of 236.1 - 251 mV in the effluents, indicating dominant aerobic conditions in the VF beds. In the 2nd phase, the redox decreased progressively to 47.5 mV in VGp and 13.0 mV in the VG bed, revealing anoxic conditions. Therefore, anoxic zones in VFCWs prohibit complete nitrification and improve the TN removal by proceeding denitrification, as suggested by Headley *et al.* (2005). The redox measurements showed that planted and unplanted VFCWs were statistically difference ($p > 0.001$). While, effluents redox values in phase 1 and 2 were statistically different ($p > 0.001$).

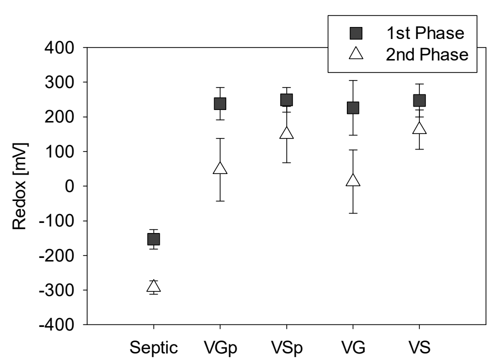


Figure 3-9: Redox mean values with SD (error bars) of each component in the systems over the course of the study.

3.3.2.2 Total Suspended Solid (TSS) and Turbidity

In phase 1, the influent TSS concentration was on average 150 mg/L, which was higher than the suggested influent for CWs from septic tanks of 44 - 54 mg/L (USEPA, 2000). The mean TSS concentrations were reduced to 12.5, 1.4, 16.4, and 1.6 mg/L in the VGp, VSp, VG, and VS, respectively. TSS and turbidity are removed by physical processes such as sedimentation and filtration (Metcalf & Eddy, 1991, Kadlec & Wallace, 2009). The VGp bed showed higher TSS removal compared to the VG, which may be a result of by increased filtration by plant roots (Petticrew & Kalff, 1992). TSS and turbidity performance for planted and unplanted VF beds were statistically similar ($p < 0.05$). During the 2nd phase, the TSS was filtered out and settled effectively in the VGp and VG beds due to higher retention capacity. The mean TSS concentrations in the effluents were 4.4, 1.4, 7.9, and 1.4 mg/L in VGp, VSp, VG, and VS, respectively. The VFCWs are effective in eliminating TSS, similar results were documented by Brix *et al.* (2002). In addition, the 1st and 2nd phase TSS results were statistically similar ($p < 0.05$).

Figure 3-10 shows the turbidity of the VF systems over the study period. In phase 1, the mean turbidity of influent samples was 98.2 NTU. The mean turbidity concentrations were reduced to 6.3, 1.7, 8.6, and 2.1 NTU in VGp, VSp, VG, and VS, respectively. The turbidity removal was higher in VGp, which is in agreement with the findings of Brix (1994a) in which plants roots reduce the water velocity, resulting in better filtering capacity. However, planted and unplanted VFCWs were statistically similar ($p < 0.05$). During the 2nd phase, the mean influent turbidity increased to 223.4 NTU. Saturated layers prolonged the contact time between water and substrate, hereby, decreasing the turbidity in the effluent. The mean turbidity concentrations were 4.7, 0.8, 6.7, and 0.8 NTU in VGp, VSp, VG, and VS, respectively. The 1st and 2nd phase turbidity results were statistically similar ($p < 0.05$).

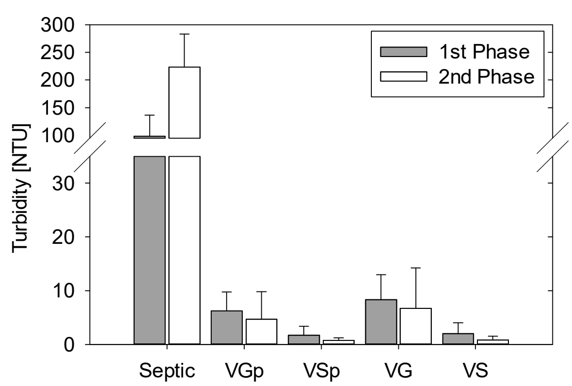


Figure 3-10: Turbidity mean values with SD (error bars) of each component in the systems over the study period.

Table 3-3: Influent and effluent water quality parameters (mean ± SD) and number of samples (N) for the VGp - VSp and VG - VS systems during the 1st and 2nd phase of monitoring.

			Septic		**VGp**		**VSp**		**VG**		**VS**
	Parameter	N		N		N		N		N	
1st Phase	pH	41	7.3 ± 0.2	41	6.9 ± 0.4	41	6.6 ± 0.6	41	7.0 ± 0.3	38	6.6 ± 0.7
	EC [µS/cm]	41	1514.5 ± 298	41	1271.2 ± 135	41	1214.3 ± 144	41	1253.6 ± 154	38	1156.4 ± 171.5
	DO [mg/L]	41	0.6 ± 0.4	41	6.4 ± 2	41	9.7 ± 2.5	41	6.7 ± 0.4	38	9.2 ± 2.1
	Redox potential [mV]	39	-153.3 ± 28	39	238.1 ± 46.7	37	249.4 ± 36	39	236.1 ± 67	35	250.9 ± 46
	TSS [mg/L]	38	150.1 ± 107	40	12.5 ± 11	39	1.4 ± 1.3	40	16.4 ± 17	34	1.6 ± 1.8
	Turbidity [NTU]	37	98.2 ± 38	37	6.3 ± 3.5	37	1.7 ± 1.7	37	8.6 ± 4.6	34	2.1 ± 2.0
2nd Phase	pH	43	7.2 ± 0.2	43	6.7 ± 0.2	43	7.2 ± 0.4	43	7.0 ± 0.2	43	7.3 ± 0.5
	EC [µS/cm]	43	1678.2 ± 186	43	1391.1 ± 170	42	1361.3 ± 185	43	1378.6 ± 134	43	1239.0 ± 113
	DO [mg/L]	39	1.1 ± 0.8	38	5.3 ± 1.0	39	10.1 ± 2.6	38	5.4 ± 1.1	39	9.9 ± 1.7
	Redox potential [mV]	42	-292.2 ± 19.5	42	47.5 ± 90.7	42	148.9 ± 81.2	42	13.0 ± 91.5	40	163.3 ± 56.6
	TSS [mg/L]	43	164.8 ± 69.3	43	4.4 ± 2.3	43	1.4 ± 0.8	42	7.9 ± 10.3	43	1.4 ± 1.0
	Turbidity [NTU]	41	223.4 ± 59.5	40	4.7 ± 5.1	41	0.8 ± 0.5	41	6.7 ± 7.5	41	0.8 ± 0.7

3.3.2.3 Organic Matter

TOC and BOD_5 were measured to evaluate the organic removal in the VFCWs and to determine its influence on the nitrification-denitrification process. The TOC and BOD_5 measurements are summarized in **Table 3-4**.

TOC

Figure 3-11.a shows the TOC values from each component in the systems over the study period. In phase 1, the mean influent TOC concentration was 160 mg/L. Mean effluent TOC concentrations were 23.3, 12.7, 25.2, and 14.6 mg/L in VGp, VSp, VG, and VS, respectively. The treatment performance of planted and unplanted VFCWs were statistically similar ($p < 0.05$), indicating that aerobic degradation was responsible for TOC removal. TOC removal was not greatly influenced by plant uptake, which is in accordance with other studies (Vymazal, 2002, Tietz *et al.*, 2008). During the 2nd phase, the TOC concentrations in VGp and VG were lower. Effluent TOC concentrations were 16.3, 11.7, 18.9, and 11.1 mg/L in the VGp, VSp, VG, and VS, respectively. The removal of TOC improved due to longer residence time in the 1st stage in each system, which is in agreement with Mashauri *et al.* (2000). However, effluent TOC concentrations during the 1st and 2nd phase were statistically similar ($p < 0.05$).

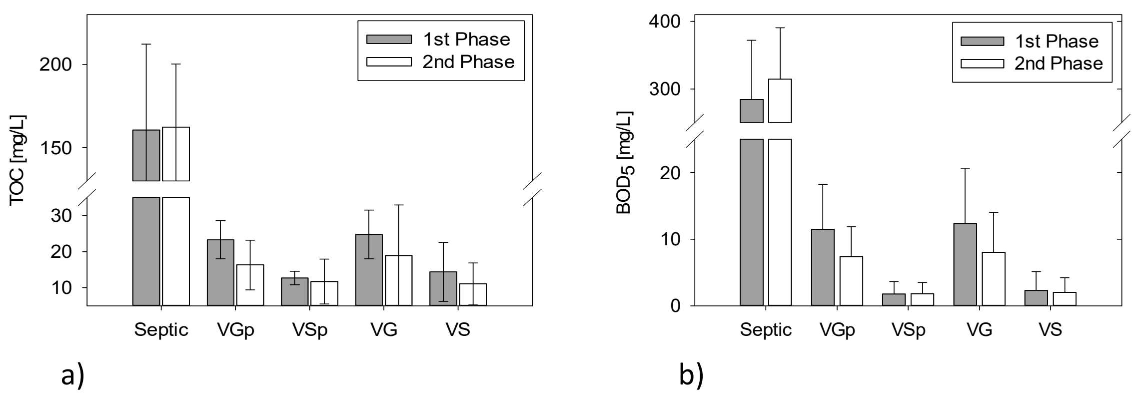


Figure 3-11: Influent and effluent TOC and BOD_5 mean concentrations and SD (error bars) of each component in the systems, a) TOC concentrations during the study period, b) BOD_5 concentrations during the study period.

BOD_5

Figure 3-11.b presents the influent and effluent BOD_5 concentrations over the course of study. In phase 1, the mean influent BOD_5 was 284.1 mg/L that gave an adequate carbon to perform denitrification process mainly in the VGp and VG beds. The BOD_5 concentrations were reduced to 11.5, 1.8, 13.2, and 2.2 mg/L in VGp, VSp, VG, and VS, respectively. The results indicated fast organic degradation rate in VGp and VG by physical and biological mechanisms, which is in agreement with other VFCW studies (Stefanakis & Tsihrintzis, 2009). Similar results documented by Langergraber *et al.* (2009) showed high organic removal was achieved by two-stage VFCWs in Austria.

In phase 2, the mean influent BOD_5 of samples was 314.7 mg/L, which is higher than septic tank effluent as reported by the US EPA (129 - 147 mg/L) (USEPA, 2000). However, BOD_5 concentrations were reduced to 7.4, 1.8, 8.0, and 2.0 mg/L in VGp, VSp, VG, and VS, respectively. The elimination of BOD_5 increased in the 1st stage in each system likely due to longer residence time. The effluent concentrations of planted and unplanted VFCWs did not show significant difference ($p < 0.05$), hereby, plants have no effects on BOD_5 removal. The BOD_5 concentrations of the VFCWs operated in 1st and 2nd phase were statistically different ($p < 0.01$).

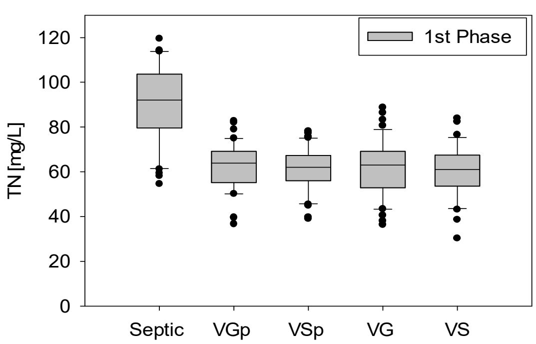
3.3.2.4 Nitrogen Transformations

Nitrogen removal is evaluated by measuring different forms of nitrogen such as TN, NH_4^+-N, NO_2^--N, and NO_3^--N. Means and SD of the investigated nitrogen forms are summarized in **Table 3-4**.

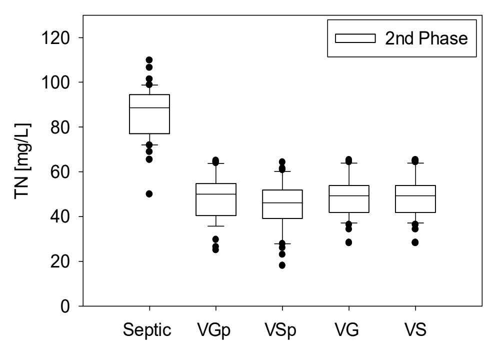
Total Nitrogen (TN)

Figure 3-12.a shows TN mean concentrations in the VFCW filters during the 1st phase. The average TN of influent was 90 mg/L due to high NH_4-N concentration. Effluent TN concentrations were reduced through the VFCW systems to 62.2, 60.9, 62.7, and 61.1 mg/L in VGp, VSp, VG, and VS, respectively. TN concentrations of planted and unplanted effluents did not show significant difference ($p < 0.05$), indicating limited plant role in TN removal, in agreement with Gersberg *et al.* (1983).

Figure 3-12.b shows that TN concentrations decreased in VGp and VG beds under partial saturated zone, indicating that denitrification occurred in these beds. Combing aerobic–anoxic conditions in the VFCWs spur the growth of various microorganisms, hereby, increasing TN removal. In phase 2, the mean influent TN concentration was 86.1 mg/L. The mean TN effluent concentrations were 48.5, 45, 48.7, and 45 mg/L for VGp, VSp, VG, and VS, respectively. The treatment performance of planted and unplanted VFCWs were statistically similar ($p < 0.05$). Plants have no effect on TN removal, while plants affected NO_3^--N concentration based on statistical analysis. NO_3^--N concentration increased in planted system due to oxygenation by roots, which stimulate the growth of nitrifying bacteria (Brix, 1997). TN concentrations of the VFCWs operated in 1st and 2nd phase were statistically different ($p > 0.001$), as shown in **Figure 3-13**.



a)



b)

Figure 3-12: Box-and-whiskers plot of TN concentration of each component in the systems over the study period. Lines in boxes present the means, boundaries of the boxes are the 25th and 75th percentiles, error bars are the maximum and minimum, while the dots represent outliers of the data. a) 1st phase, b) 2nd phase.

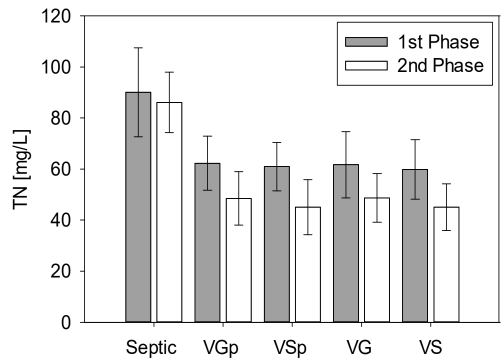


Figure 3-13: TN mean concentrations and SD (error bars) during the study period.

Ammonium Nitrogen (NH_4^+-N)

Figure 3-14 shows the NH_4^+-N influent and effluent concentrations over the course of the study. During the 1st phase, mean NH_4^+-N influent concentration was 72.3 mg/L, which is higher than the reported CW influent from septic tanks (28 - 42 mg/L) (USEPA, 2000). The NH_4^+-N concentrations were reduced to 10.2, 1.1, 15.2, and 2.7 mg/L in VGp, VSp, VG, and VS, respectively. NH_4 is mainly removed under aerobic conditions in the VF beds via nitrification. Moreover, vegetation might have slightly increased nitrification through root oxygenation (Chen *et al.*, 2011). Nevertheless, NH_4^+-N concentrations of planted and unplanted effluents did not show significant difference ($p < 0.05$), hereby, plants have no effects on NH_4^+-N removal.

The NH_4^+-N concentrations increased in VGp and VG effluents in phase 2 of the study due to nitrification reduction under anoxic conditions. In few cases, NH_4^+-N concentrations in VSp and VS effluents were blow detection limit (less than 0.03 – 0.025 mg/L) that illustrates the variation in number of samples. The NH_4^+-N concentrations were reduced to 17.5, 0.8, 29.9, and 1.5 mg/L in VGp, VSp, VG, and VS respectively.

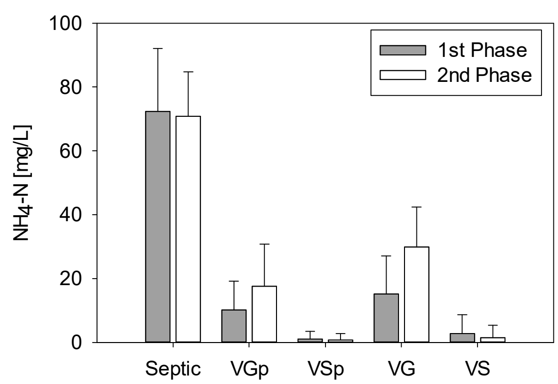


Figure 3-14: Mean NH_4^+-N concentrations and SD (error bars) over the study period.

Table 3-4: Mean and SD of influent and effluent TOC, BOD_5, TN, NH_4^+-N, NO_2^--N, NO_3^--N and *E. coli* with number of samples (N) for the two-stage systems over the study period (1st and 2nd phase).

Time	Parameter	Septic	VGp	VSp	VG	VS
1st Phase	BOD_5 [mg/L]	284.1 ± 87.9	11.5 ± 6.7	1.8 ± 1.8	13.2 ± 8.1	2.2 ± 2.9
	N	40	39	23	37	26
	TOC [mg/L]	160.6 ± 51.6	23.3 ± 5.2	12.7 ± 1.9	25.2 ± 6.6	14.6 ± 8.3
	N	40	40	40	40	36
	TN [mg/L]	90.0 ± 17.4	62.2 ± 10.6	60.9 ± 9.4	62.7 ± 12.4	61.1 ± 10.4
	N	40	40	40	40	36
	NH_4^+-N [mg/L]	72.3 ±19.8	10.2 ± 9.0	1.1 ± 2.4	15.2 ± 11.9	2.7 ± 5.9
	N	40	40	38	41	38
	NO_3^--N [mg/L]	0.2 ± 0.2	44.3 ± 11.3	52.2 ± 10.4	40.1 ± 12.9	52.7 ± 14.8
	N	20	39	40	41	38
	NO_2^--N [mg/L]	0.0 ± 0.0	0.4 ± 0.2	0.1 ± 0.5	0.6 ± 0.3	0.1 ± 0.1
	N	18	39	22	41	21
	E.coli [MPN/100 mL]	$5.5 \times 10^6 \pm 5.1 \times 10^6$	$3.4 \times 10^5 \pm 5.1 \times 10^5$	$1.0 \times 10^4 \pm 8.6 \times 10^4$	$4.5 \times 10^5 \pm 7.5 \times 10^5$	$1.7 \times 10^4 \pm 6.7 \times 10^4$
	N	41	41	41	41	37
2nd Phase	BOD_5 [mg/L]	314.7 ± 75.5	7.4 ± 4.5	1.8 ± 1.7	8.0 ± 6.0	2.0 ± 2.2
	N	43	43	26	41	26
	TOC [mg/L]	162.3 ± 37.9	16.3 ± 6.9	11.7 ± 6.23	18.9 ± 14.2	11.1 ± 5.6
	N	42	42	42	42	42
	TN [mg/L]	86.1 ± 11.8	48.5 ± 10.4	45.0 ± 10.8	48.7 ± 9.5	45.0 ± 9.2
	N	42	42	42	42	42
	NH_4^+-N [mg/L]	70.9 ± 13.8	17.5 ±13.2	0.8 ± 1.9	29.9 ± 12.5	1.5 ± 3.9
	N	43	43	43	43	36
	NO_3^--N [mg/L]	0.1 ± 0.001	28.3± 8.6	41.8 ± 10.2	17.5 ± 11.2	42.2 ± 7.9
	N	3	43	43	41	43
	NO_2^--N [mg/L]	0.0 ± 0.0	0.1 ± 0.03	0.0 ± 0.03	0.1 ± 0.0	0.1 ± 0.1
	N	5	43	21	40	27
	E.coli [MPN/100 mL]	$4.1 \times 10^6 \pm 2.9 \times 10^6$	$1.1 \times 10^5 \pm 2.7 \times 10^5$	$8.1 \times 10^2 \pm 9.0 \times 10^3$	$1.0 \times 10^5 \pm 2.0 \times 10^5$	$7.1 \times 10^2 \pm 4.1 \times 10^3$
	N	43	43	42	43	43

E. coli values are presented in geometric mean.

NH_4^+-N concentrations of the VFCWs operated in 1st and 2nd phase were statistically similar ($p < 0.05$).

In VFCWs, high concentration of oxygen stimulates the nitrification process until NH_4^+-N and NO_2^--N reached low levels. That can be shown by low NO_2^--N mean concentrations in the VFCWs effluent. Some NO_2^--N concentrations in VSp and VS effluents were blow detection limit (less than 0.02 - 0.003 mg/L).

Nitrate Nitrogen (NO_3^--N)

Figure 3-15 shows the NO_3^--N influent and effluent concentrations of the VFCWs operated in 1st and 2nd phase. In phase 1, the mean NO_3^--N influent was 0.2 mg/L. NO_3^--N concentration in some influent samples was below detection limit (0.3 - 0.068 mg/L), indicating that NO_3^--N was produced in the VFCWs via nitrification. **Figure 3-16.a** shows that NO_3^--N concentrations were increased in the VFCW beds to 44.3, 52.2, 40.1, and 50.7 mg/L in VGp, VSp, VG, and VS, respectively. The NO_3^--N concentrations in the 2nd stage of both systems were increased due to continuous nitrification on unsaturated beds. NO_3^--N concentrations of VGp and VG effluents were statistically different ($p > 0.05$). Similar observations reported by Sarafraz *et al.* (2009) and Brix and Schierup (1989) that unplanted gravel bed was effective for NO_3^--N removal than planted gravel bed.

Figure 3-16.b shows that NO_3^--N concentrations were reduced in VGp and VG beds under partial saturated zone, indicating limited nitrification process under anoxic conditions. In phase 2, NO_3^--N concentrations were measured of 28.3, 41.8, 17.5, and 42.2 mg/L in VGp, VSp, VG, and VS, respectively. The treatment performances of planted and unplanted VFCWs were statistically different ($p > 0.05$). Higher NO_3^--N in the VGp would be related to greater surface area provided by plants roots, hereby, higher nitrification process in planted gravel bed. In addition, plants release oxygen via roots and that influenced the biological and redox process (Barko *et al.*, 1991, Sorrell & Boon, 1992).

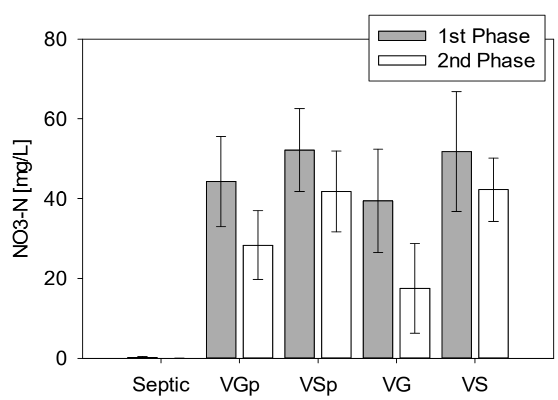
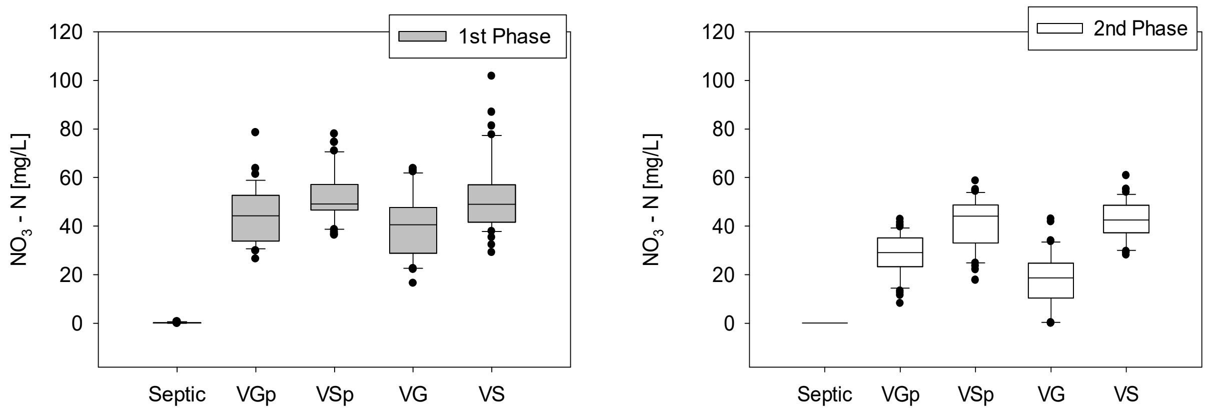


Figure 3-15: NO_3^--N mean concentration with SD (error bars) of influent and effluents during the study period.



a) b)

Figure 3-16: Box-and-whiskers plot of NO_3^--N concentration of each component in the systems over the study period, a) phase 1, b) phase 2.

NO_3^--N concentrations of the VFCWs operated in 1st and 2nd phase were statistically compared. The results from the planted system were significantly different ($p > 0.05$), while, the unplanted system results were statistically different ($p < 0.001$).

3.3.2.5 *E. coli* Reduction

The influent and effluent *E. coli* concentrations over the study period are shown in **Figure 3-17**. In phase 1, the influent *E. coli* geometric mean was 5.5×10^6 MPN/100 mL, which is idealistic for septic tank effluents (Crites & Tchobanoglous, 1998). The *E. coli* concentrations were gradually decreased through filtration in the VFCW beds, achieving around 1 log_{10} reduction through VGp and VG beds of 3.4×10^5 and 4.5×10^5 MPN/100 mL, respectively. An extra 1.2 log_{10} reduction was achieved by VSp and VS beds of 1.0×10^4 and 1.7×10^4 MPN/100 mL, respectively. The treatment performances of planted and unplanted VFCWs were statistically similar ($p < 0.05$). Plants had no effects on *E. coli* reduction, in contrast to other findings that demonstrated higher *E. coli* reduction in planted CWs (Decamp & Warren, 2000b, Merlin *et al.*, 2002, Karathanasis *et al.*, 2003).

E. coli numerations were reduced during partial saturated zone, as results of longer retention time VFCWs (Netter, 1993). In phase 2, septic *E. coli* geometric mean was 4.1×10^6 MPN/100 mL. The *E. coli* concentrations were decreased in VGp and VG beds, achieving around 1 log_{10} reduction, of 1.1×10^5 and 1.0×10^5 MPN/100 mL, respectively. The *E. coli* concentrations were found to be reduced by 3 log_{10} in VSp (effluent concentration of 8.1×10^2 MPN/100 mL) and VS (effluent concentration of 7.1×10^2 MPN/100 mL) beds. Similar results were obtained by Tietz *et al.* (2007) who observed a 3.5 log_{10} reduction in *E. coli* concentration after percolation through a sand-based VF pilot-scale wetland. These findings agree also with Tanner *et al.* (2012) and Headley *et al.* (2013) who observed higher *E. coli* removal efficiency in sand VF compared to gravel VF pilot-scale wetlands. There was no significant effect of plants in the current study ($p < 0.05$). Many studies have reported that plants have no effect on *E. coli* reduction in VFCWs (Tietz *et al.*, 2007, Headley *et al.*, 2013, Torrens *et al.*, 2009b).

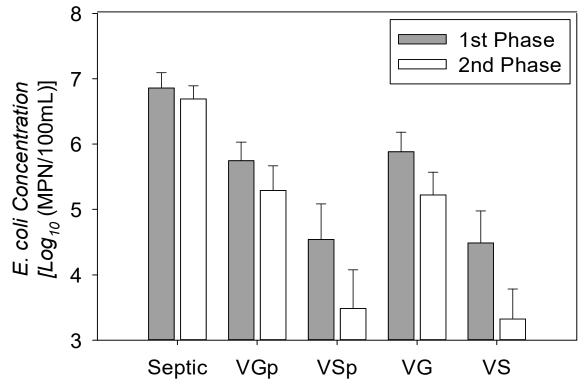


Figure 3-17: Influent and effluents *E. coli* geometric means with SD over the course of the study.

Both systems achieved 4 $\log_{10}$ reduction of *E. coli*, which can be explained that *E. coli* is removed by sedimentation, straining and entrapment in biofilms (Stevik *et al.*, 2004, Kadlec & Knight, 1996), predation by microorganisms such as protozoa (Decamp & Warren, 2000a, Wand *et al.*, 2007), and natural die-off (Wand *et al.*, 2007). *E. coli* concentrations of the VFCWs operated in phase 1 and 2 were statistically different ($p > 0.05$) only in the 2^{nd} stage.

3.3.3 Internal vertical profiles

Results of DO, redox, TN, NO_3^--N and *E. coli* concentrations through vertical flow in VSp and VS beds are presented over the study period in this part.

Figure 3-18 shows the DO and redox potential through the vertical flow in the systems over the study period. In phase 1, the redox values increased in the 1^{st} stage in both systems, then it showed slight changes through vertical flow in the 2^{nd} stage, indicating slow organic matter and nitrogen removal. In phase 2, DO showed a dramatic increase through vertical flow in the 2^{nd} stage. In particular, redox values increase from inlet to outlet due to cumulative degradation of pollutants (Headley *et al.*, 2005).

Both systems showed similar DO profiles over the study period. The DO concentrations increased through vertical profile indicating highly aerobic conditions in the beds.

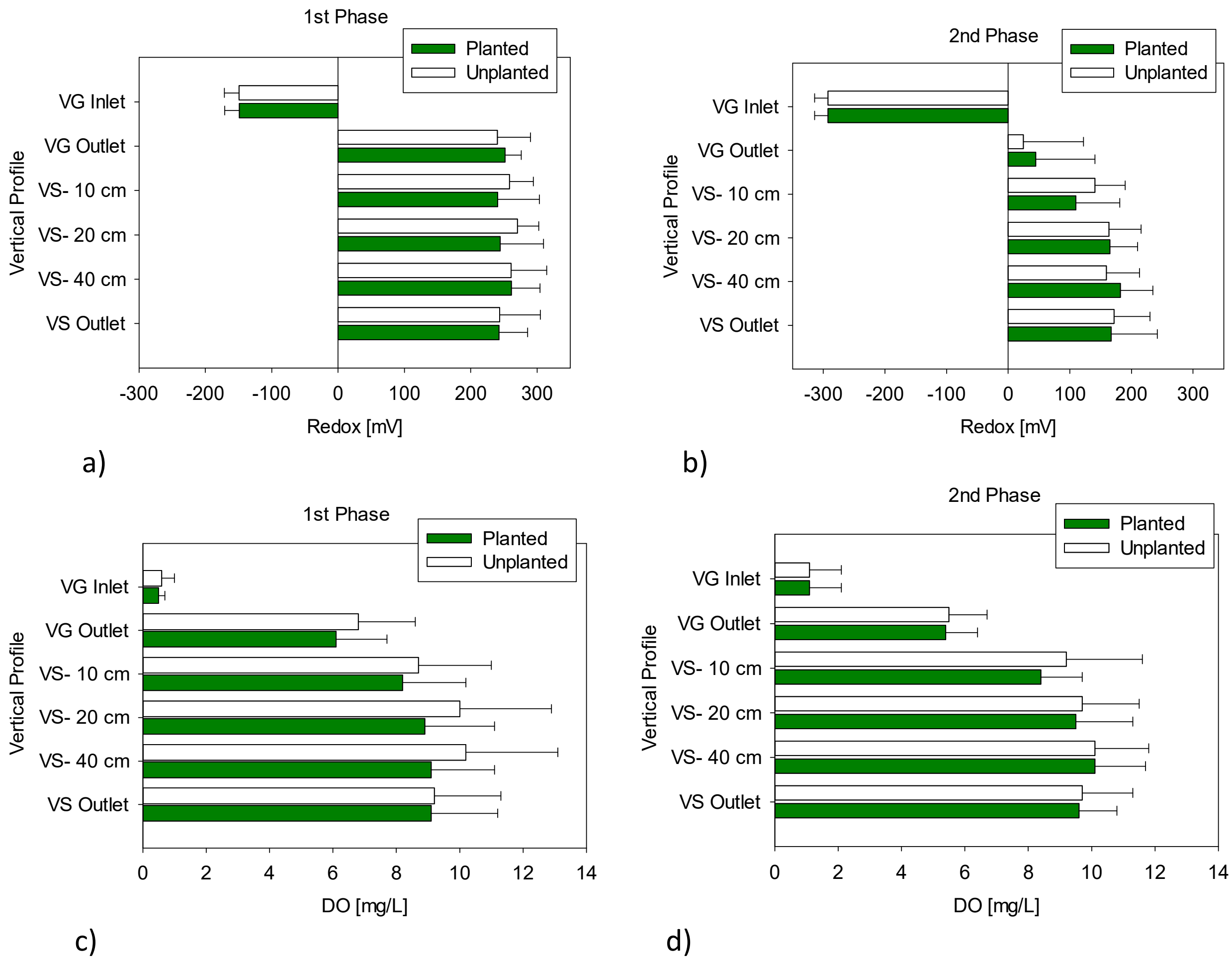


Figure 3-18: Mean DO and redox potential values with SD throughout vertical flow in VGp-VSp and VG-VS systems, a) and b) redox values during phase 1 and 2, c) and d)DO levels during phase 1 and 2.

Nitrogen transformation

Figure 3-19 shows mean TN and NO_3^--N concentrations through vertical flow in the systems. The TN concentrations decreased sharply through VGp and VG beds, indicating fully nitrified effluent. The changes of TN concentrations through VSp and VS beds were constant without variations through vertical flow during phase1. In phase 2, the TN concentrations decreased due to simultaneous nitrification and denitrification in VGp and VG beds. The changes of TN concentrations through VSp and VS beds were low without variations through vertical flow. In general, there was no significant effect of vegetation on TN removal.

On the other hand, the changes of NO_3^--N concentrations observed through vertical flow in the 1st stage and continued throughout infiltration as a result of progressive nitrification in the 2nd stage. In phase 2, NO_3^--N concentrations reduced in VGp and VG due to simultaneous nitrification and denitrification. Significant changes of NO_3^--N concentrations were observed from 10 cm in the 2nd stage, indicating higher nitrification after anoxic conditions. In the planted bed, the NO_3^--N concentration increased sharply from zero to 10 cm that can be explained by higher oxygen content within plants root zone. However, planted and unplanted vertical profile showed similar nitrification rate.

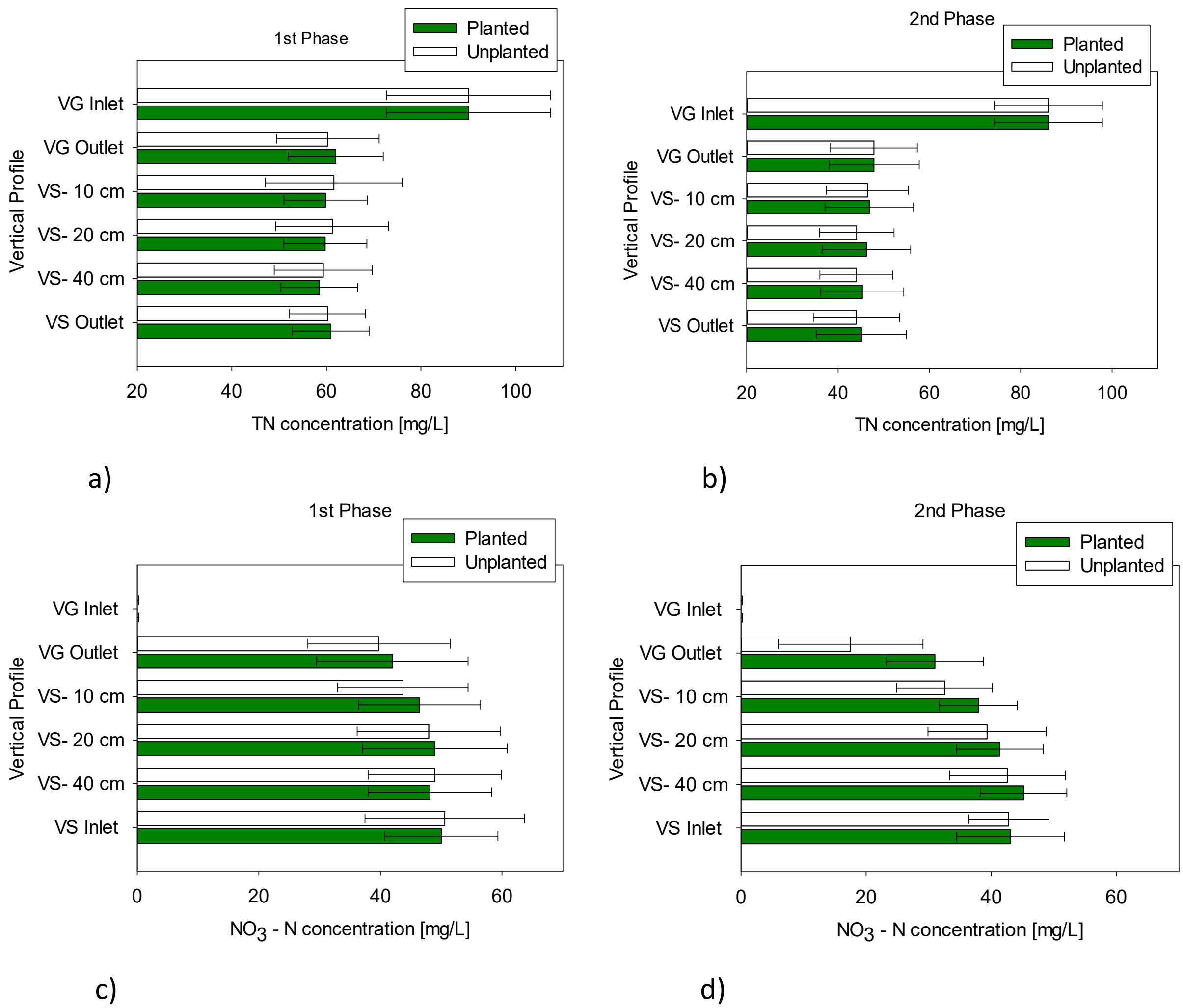


Figure 3-19: Mean TN and NO_3^--N concentrations with SD through vertical profile in the VGp-VSp and VG-VS systems, a) and b) TN concentrations during phase 1 and 2, c) and d) NO_3^--N concentrations during phase 1 and 2.

Figure 3-20 shows *E. coli* geometric means with SD throughout vertical flow in the VGp-VSp and VG-VS systems. The *E. coli* concentrations were gradually decreased through filtration in the VFCW beds, achieving around 1 log_{10} concentration reduction through VGp and VG beds and extra 1.2 log_{10} reduction was achieved by VSp and VS beds. In phase 2, *E. coli* concentrations were decreased throughout VGp and VG beds, achieving 1 log_{10} concentration reduction. Additional 3 log_{10} concentration reductions were accomplished throughout 2nd stage in each system as a result of transferring effluent from anoxic to aerobic beds.

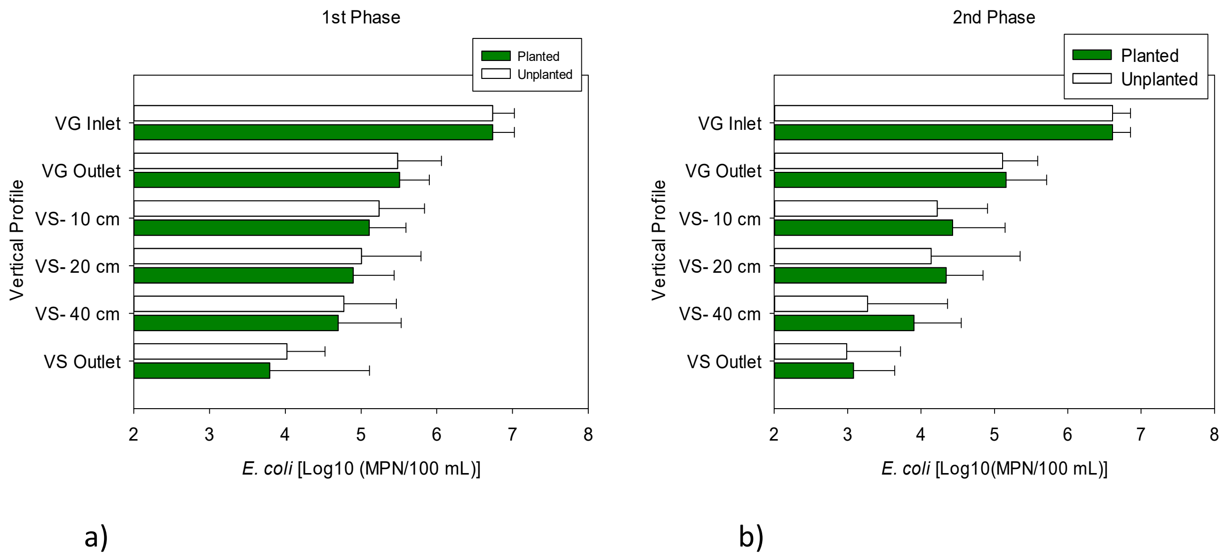


Figure 3-20: *E. coli* geometric means with SD throughout vertical flow in the VGp-VSp and VG-VS systems, a) phase 1 and b) phase 2.

3.3.4 Pollutant Removal Evaluation and Seasonal Variability

TSS, BOD_5, COD, TN, NH_4^+-N, and *E. coli* mass removal rate per unit area of VFCWs during the 1st and 2nd phase are evaluated and compared in this section. In addition, monthly removal mean of the previous parameters is presented over the course of the study to assess the treatment performances under temperature variability.

3.3.4.1 TSS Removal

Figure 3-21 shows high TSS removal rate during the study period. In phase 1, TSS removal was high, on average removal efficiencies of 90 - 99 % and 85 - 99 % in the VGp-VSp and VG- VS, respectively. Nevertheless, higher TSS removal was observed in spring and summer due to higher TSS load during these months. Furthermore, TSS influent concentrations fluctuated due to dilution by rainfall events. Similar findings reported by Kadlec and Wallace (2009). In the planted system, mean TSS mass removal rates were 12.1 and 0.9 g/m^2.day in VGp and VSp, respectively. It was highly compatible with mean mass load of 13.1 and 1.0 g/m^2.day in the 1st and 2nd stage, respectively. In the unplanted VF system, TSS mean removal rates were 13.1 g/m^2.day in VG and 1.4 g/m^2.day in VS, which were highly correlated with mean mass load of 12.9 and 1.5 g/m^2.day in the 1st and 2nd stage, respectively. There was no significant difference observed between planted and unplanted beds with respect to TSS mass removal ($p < 0.05$); illustrating that TSS was removed via higher filtration, regardless of season and/or temperature.

During the 2nd phase, TSS removal efficacy was 97 - 99 % and 95 - 99 % in the VGp-VSp and VG-VS beds, respectively. There was no significant between 1st and 2nd phase results ($p < 0.05$). In planted VF system, TSS mean mass removal rates were 14.7 g/m^2.day in VGp and 0.2 g/m^2.day in VSp, with mean mass load of 15 and 0.4 g/m^2.day in VGp and VSp, respectively. In the unplanted VF system, TSS mean removal rates were 14.7 g/m^2.day in the VG and 0.6 g/m^2.day in the VS, with mean mass load of 15 and 0.7 g/m^2.day in the VG and VS, respectively. In comparison, there was no significant difference on TSS removal ($p < 0.05$) over the study period. The impact of seasonal variations (temperature) was negligible in TSS removal in VFCWs.

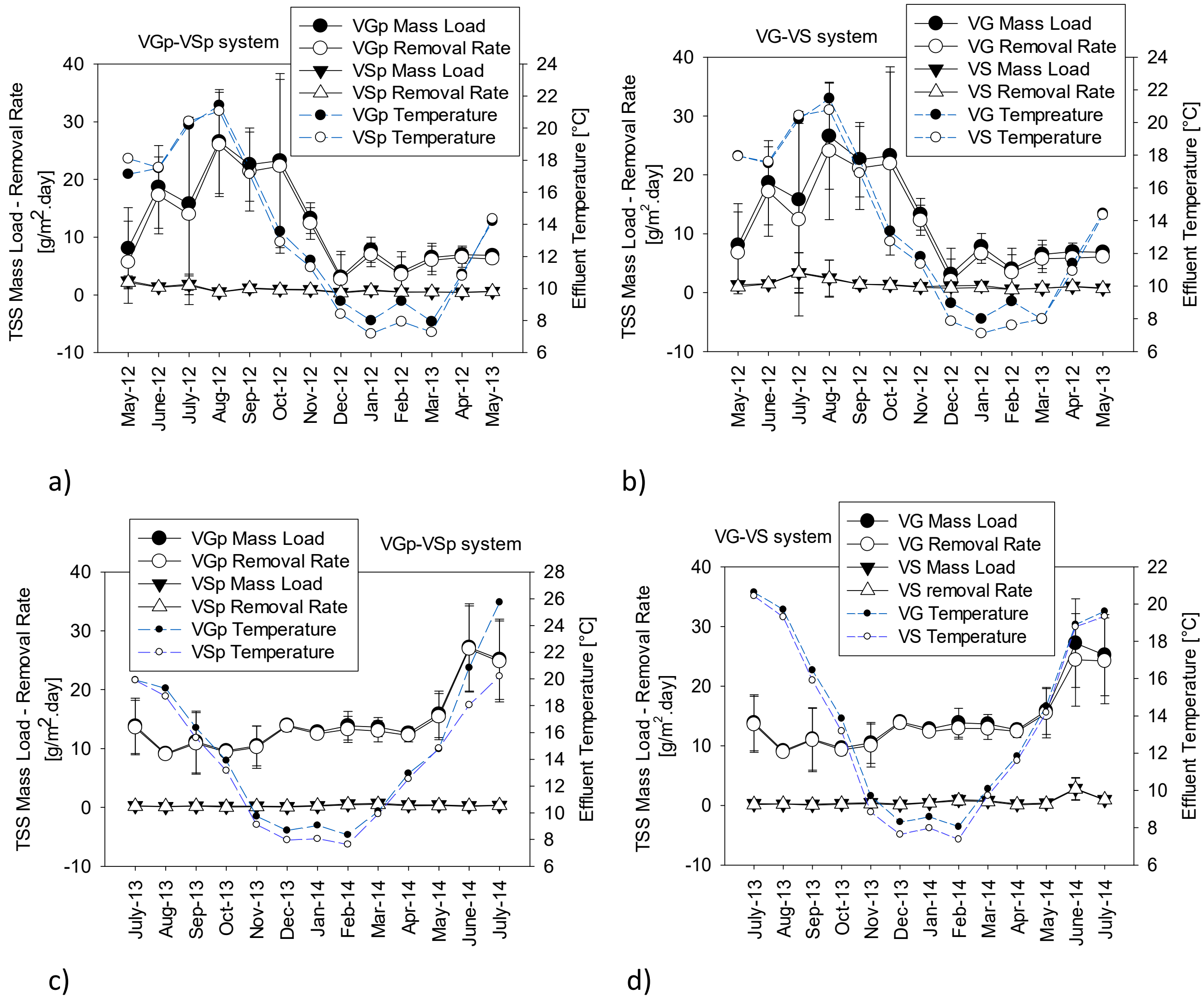


Figure 3-21: Monthly TSS mass load, removal rate, SD (error bars), and effluent temperature. a) and b) phase 1, c) and d) phase 2.

3.3.4.2 Organic Matter (OM) Removal

BOD_5 mass removal was independent on water temperature over the study period, **Figure 3-22**. In phase 1, effective and stable BOD_5 removal rates were observed. Average removal efficiencies of 96 - 99 % and 96 - 99 % were observed for VGp-VSp and VG-VS, respectively. However, VSp and VS showed BOD_5 removal rates similar to TOC removal rates because of many under the detection limit (less than 0.3 mg/L) readings. BOD_5 removal rates did not respond to seasonal changes over the study period. Many other studies have documented negligible temperature influence on organic matter removal in CWs (Vymazal, 1999, Wallace & Knight, 2006). The BOD_5 mean removal rates were 25.1, 0.9, 24.7 and 1.1 g/m^2.day in VGp, VSp, VG, and VS, respectively. Both systems showed high OM removal, whereas, Herouvim *et al.* (2011) reported higher organic matter removal in planted VFCWs due to high oxygen capacity, microbes and bacteria activities within the root zone.

During phase 2, BOD_5 showed a slight unsteady trend lines, on average removal efficiencies of 97.9 - 99.8 % and 97.6 - 99.4 % in VGp-VSp and VG-VS, respectively. The BOD_5 mean removal rates were 28.1, 0.5, 27.8 and 0.8 g/m^2.day in VGp, VSp, VG, and VS, respectively. However, several studies have documented that CWs treatment efficiency is influenced by water temperature (Allen *et al.*, 2002). In addition, Kadlec and Wallace (2009) reported that BOD removal is influenced by seasonal changes (climate, plant biomass cycling and water temperatures) in all wetlands sorts. There was no significant difference on BOD_5 removal rate ($p < 0.05$) over the study period.

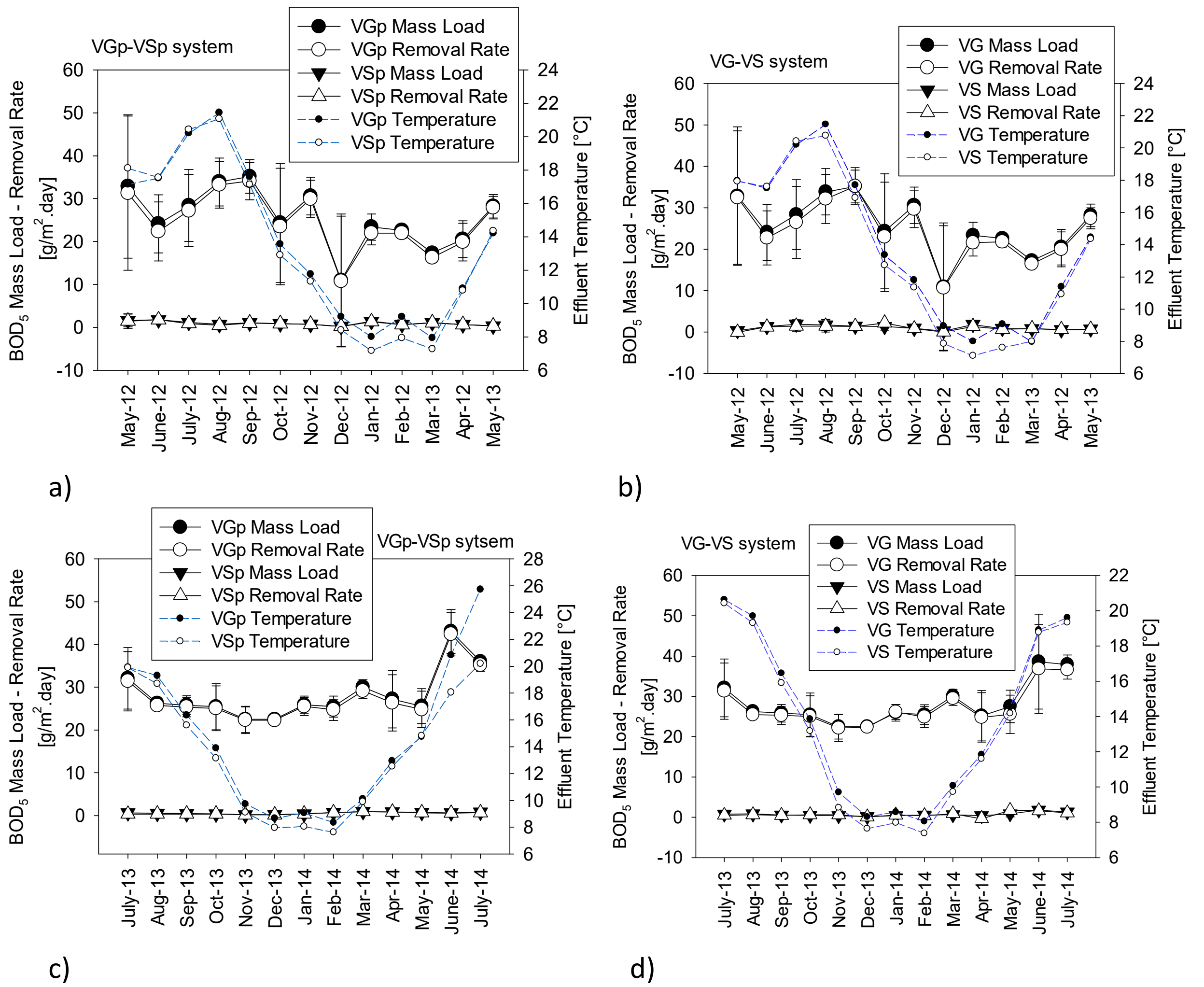


Figure 3-22: Monthly BOD_5 mass load, removal rate, SD (error bars), and effluent temperature. a) and b) phase 1, c) and d) phase 2.

Figure 3-23 shows TOC mass removal over the course of the study period. During phase 1, TOC mean removal efficiencies were ranged of 86 - 92 % and 83 - 91 % in VGp - VSp and VG - VS, respectively. The TOC mean removal rates were 12.7, 0.9, 12.7, 1.1 g/m^2.day in VGp, VSp, VG, and VS, respectively.

During the 2nd phase, the TOC removal efficiency improved in VGp and VG beds, on average removal efficiencies of 90 - 93 % and 88 - 92 % in the VGp-VSp and VG- VS systems, respectively. The TOC mean mass removal rates were 13.2, 0.4, 12.8, and 0.8 g/m^2.day in VGp, VSp, VG, and VS, respectively. Moreover, there was no clear trend that TOC removal is temperature dependent, which is in accordance with Vymazal (2011). These findings are consistent with other studies of CWs for wastewater treatment (Hammer, 1989, Kadlec & Wallace, 2009, Merlin *et al.*, 2002). Planted and unplanted systems were statistically similar ($p < 0.05$). There was no significant difference in TOC removal between 1st and 2nd phase results ($p < 0.05$).

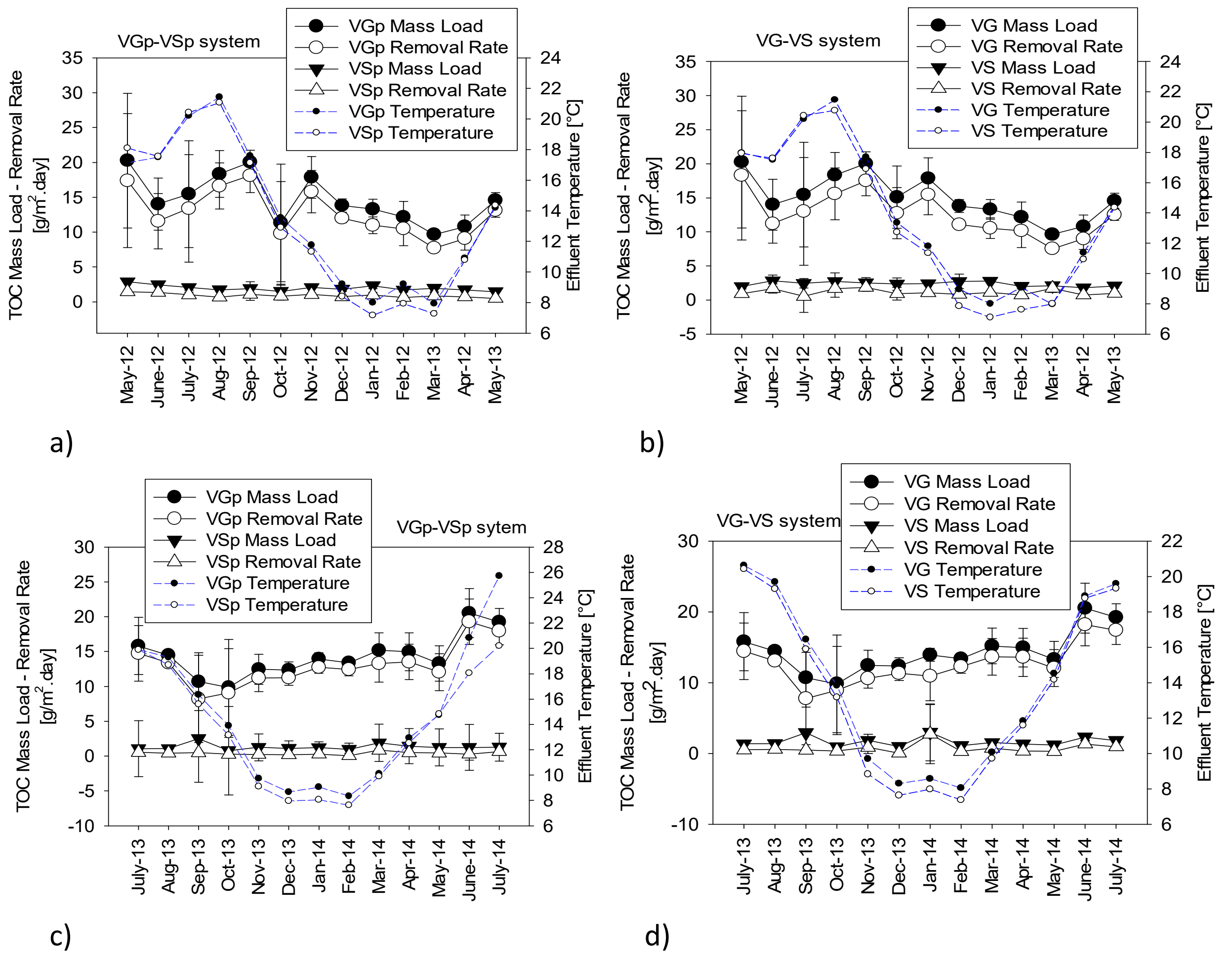


Figure 3-23: Monthly TOC mass load, removal rate, SD (error bars), and effluent temperature, a) and b) phase 1, c and d) phase 2.

3.3.4.3 TN Removal

Figure 3-24 shows TN removal rates in the systems over the study period. In phase 1, TN average removal efficiencies ranged from 36 - 38 % and 30 - 39 % in VGp-VSp and VG- VS, respectively. In the planted VF system, TN mean removal rate was 3.0 g/m^2.day in VGp and 0.2 g/m^2.day in VSp, with mean mass load of 8.2 and 5.2 g/m^2.day in the 1st and 2nd stage, respectively. TN was removed in the 1st stage. NO_3^--N was not removed in the 2nd stage due to the highly aerobic conditions and lack of available carbon source. In the unplanted VF system, TN mean removal rate was 2.6 $g/m^2.day$ in VG and 0.6 $g/m^2.day$ in VS, with mean mass load of 8.4 and 5.8 $g/m^2.day$ in the 1st and 2nd stage, respectively.

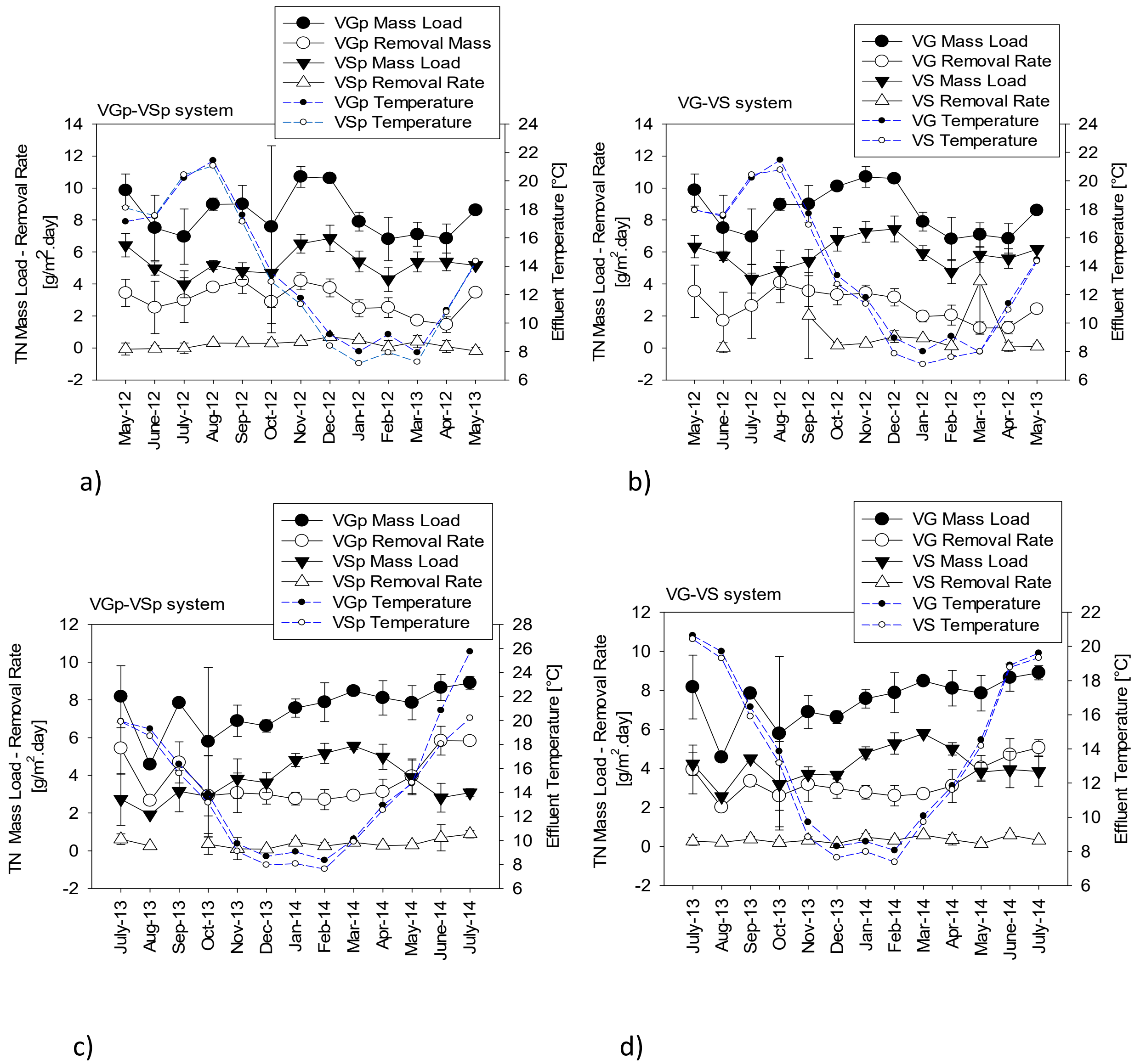


Figure 3-24: Monthly TN mass load removal rate, SD (error bars), and effluent temperature. a) and b) phase 1, c and d) phase 2.

There was no significant difference between planted and unplanted system in TN removal ($p < 0.05$). On the other hand, many studies documented that microbial density and activity are enhanced in plant rhizosphere (Vymazal, 2007, Tanner, 2001). Lin *et al.* (2002) reported that 4 - 11 % of TN removed by plant uptake in a planted wetland. Nevertheless, the results of this study indicate that TN removal by plant uptake is negligible, which is also in accordance with the results of Keffala and Ghrabi (2005).

In phase 2, TN mean removal efficiencies were enhanced to 49 - 54 % and 43 - 48 % in VGp-VSp and VG- VS beds, respectively. TN mass removal rates of VGp and VG beds of 3.8 and 3.4

g/m^2.day, respectively. In comparison, there was no significant difference observed between planted and unplanted VFCWs ($p < 0.05$). There was, however, a statistically significant difference observed between 1st and 2nd phase results for TN mass removal ($p > 0.05$).

NH_4^+-N removal was high throughout the study period, as depicted by **Figure 3-25**. NH_4^+-N average mass removal efficiencies were 85 - 98 % and 77 - 96 % in VGp-VSp and VG- VS beds, respectively. The NH_4^+-N mean mass removal rates were 5.8, 0.8, 5.5 and 1.2 g/m^2.day in VGp, VSp, VG, and VS, respectively, indicating high nitrification capacity. Planted and unplanted systems were statistically similar ($p < 0.05$). There was no significant difference between 1st and 2nd phase results ($p < 0.05$).

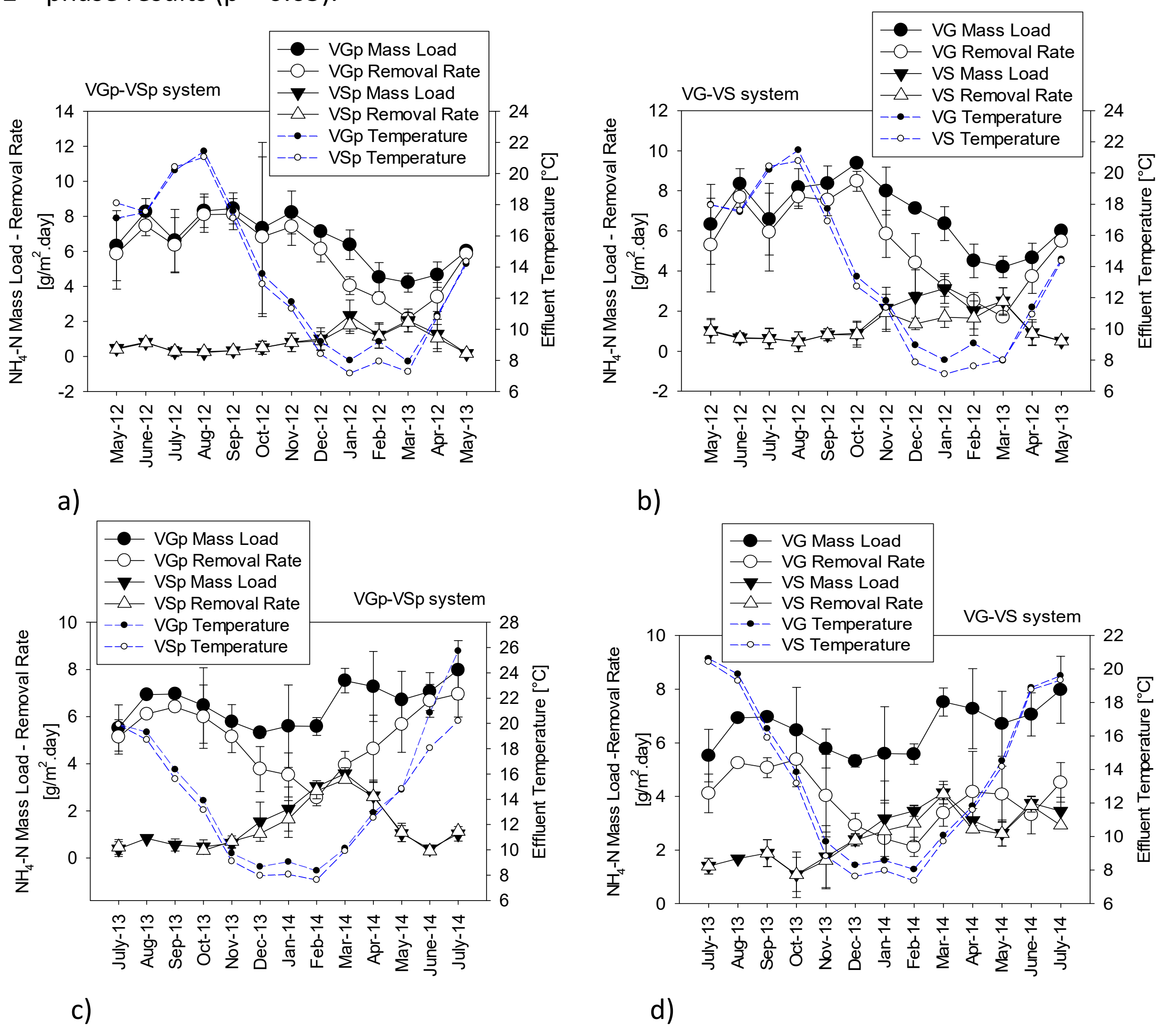


Figure 3-25: Monthly NH_4^+-N mass load removal rate, SD (error bars), and effluent temperature. a) and b) phase 1, c and d) phase 2.

The 30 cm saturated layer in VGp and VG during the second stage of the study decreased NH_4^+-N removal in the 1st stage. The NH_4^+-N mean removal efficiencies were 76 - 99 % and 58 - 98 % in VGp-VSp and VG- VS beds, respectively. The NH_4^+-N mean removal rates were 5.0, 1.5, 3.8 and 2.6 g/m^2.day in VGp, VSp, VG, and VS, respectively. NH_4^+-N mass removal decreased with decreasing temperature. Planted and unplanted systems were statistically similar ($p < 0.05$). There was no significant difference on NH_4^+-N removal between 1st and 2nd phase results ($p < 0.05$).

3.3.4.4 *E. coli* Removal

The *E. coli* areal load removal rates for both planted and unplanted VFCWs are shown in **Figure 3-26**. The *E. coli* removal efficiencies were higher during the 2nd phase in the 2nd stage VSp and VS beds. The *E. coli* removal of 4.4 log units was achieved during phase 2 instead of 2.2 log units during phase 1. Planted and unplanted beds were statistically similar ($p < 0.05$) over the entire course of the study. There was a statistically significant difference between 1st and 2nd phase results ($p > 0.05$).

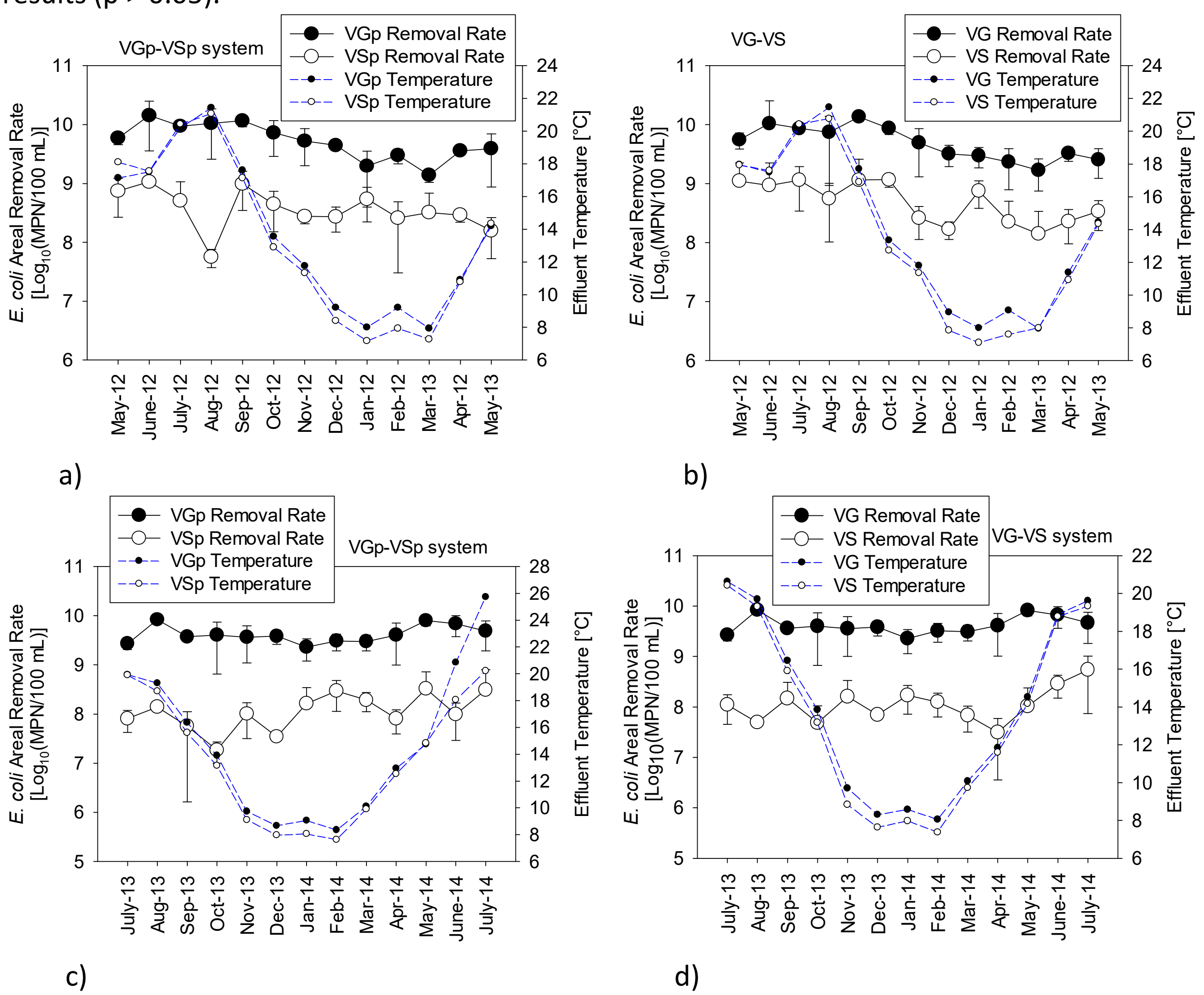


Figure 3-26: Monthly *E. coli* areal load removal rate, SD (error bars), and effluent temperature. a) and b) phase 1, c and d) phase 2.

4. Fuhais Research Facility

4.1 Site Description

The Fuhais research and demonstration site is located at the edge of both Fuhais and Mahis cities, nearby Amman in Jordan, **Figure 4-1**. The site was built in 2009, at the campus of the centralized wastewater treatment plant for Fuhais and Mahes cities, which enables all demonstration technologies to receive municipal wastewater. The demonstration site accommodates various decentralized wastewater treatment technologies (modified septic tanks, sequencing batch reactor, continuous batch reactor, membrane bio-reactor, sludge dewatering reed bed and two different VFCW designs. The site also contains three irrigation fields (plots), as depicted by **Figure 4-2**, where reuse of treated wastewater in irrigation can be studied.

All wastewater plants receive wastewater directly from the Fuhais-Mahes wastewater treatment plant. The raw wastewater is subjected to primary screening (basket screening) at the main plant inlet structure before it is sent to the collection tank at the demonstration site. The raw wastewater is distributed intermittently into each technology by submersible pumps in the collection tank. The inflow and outflow for each technology are measured using an electromagnetic flowmeter and the discharge is controlled using a SIEMENS-SIMATIC S7-200 PLC. The treated effluent from each technology is collected in a separate irrigation tank. For those technologies whose treated effluents are not used in irrigation, effluent is pumped back to the municipal wastewater treatment plant.

Figure 4-1: Fuhais research and demonstration facility for decentralized wastewater treatment technologies and reuse in Jordan.

The research facility is equipped with on-site laboratory where all the water quality analyses are conducted. Meteorological data (air temperature, humidity, wind speed and direction, rainfall and evaporation) were recorded by the nearby Al-Salt Climatic Station.

Figure 4-2: The Fuhais site design scheme shows the two VFCWs, Reuse field and laboratory and control buildings (after Fuhais Poster).

4.2 Research Design and Methodology

4.2.1 Experimental Setup

Two pilot-scale ecotechnologies were designed and constructed at the site in 2009. The first system is a recirculating gravel filter (ECO-1), and the second system is a two-stage vertical flow filter (ECO-2). For ECO-2, the two beds have been connected in series and only the second bed was planted. The design and operational details of the VFCW systems are shown in **Table 4-1**.

Each system has a septic tank, which provides primary treatment to the wastewater before it is dosed to the filter. The inflow and outflow for each system was measured using an electromagnetic flowmeter connected to a PLC system. In addition, effluent from filters was measured by calibrated tipping buckets (40.6 L/tip) mounted at the irrigation tanks. All inflow and outflow readings (from PLC and tipping buckets) were recorded on a daily basis in the morning. This study consisted of two phases; the 1st phase was monitoring the systems in order to identify the suitable options for optimizing the TN removal in these systems; then the 2nd phase was monitoring after implementation of an operational modification.

Table 4-1: Design specifications of the vertical flow constructed wetlands at the site.

VFCWs	**Plants**	**Saturation status**	**Media size (mm)**	**Hydraulic loading rate (L/m^2.d)**	**Surface area (m^2)**
ECO-1	unplanted	unsaturated	Zeotuff gravel (4 - 8)	108	20
ECO-2	1st bed: unplanted 2nd bed: planted (*Phragmites australis*)	unsaturated	Zeotuff gravel (4 - 8)	1st bed : 80 2nd bed: 56	1st bed: 40 2nd bed: 57

4.2.2 Recirculating VFCW System (ECO-1)

The recirculating system consisted of a septic tank, recirculation tank, unsaturated vertical flow filter (VFF) and flow splitting-box, **Figure 4-4**. The system designed to treat 2160 L/day with average hydraulic loading rate of approximately 108 L/m^2.d. The system was dosed intermittently three times every hour (30 L every 20 min).

The raw wastewater received primary treatment in a septic tank (4.6 m^3) with an approximate residence time of two days. The effluent from the septic tank moved passively through a T section to the recirculation tank (4.6 m^3). The water was pumped from the shaft tank to the filter using a submersible pump. The splitting-box placed at the outlet, where the effluent has been passively distributed via a V-notch weir that 25 % of the effluent goes to the irrigation tank and the 75 % flows back to the recirculation tank (3:1 recirculation ratio), **Figure 4-5**.

ECO-1 had a surface area of 20 m^2 (4 m with, 5 m length) and 1 m depth. **Figure 4-6** shows the system layout. The filter was filled with zeotuff gravel (4 - 8 mm) and had a filter depth of 0.8 m (**Figure 4-3**). The zeotuff gravel was chosen because it is locally available in Jordan. The water dosed to the top of the filter via inlet distribution pipelines (perforations every 0.5 m), which was covered by a half-pipe shield tunnel. The distribution pipelines contained risers to enable flushing out of the accumulated solids from the pipelines. Lateral vertical pipes were connected with the drainage system (110 mm diameter) in the bottom of bed, promoting a passive oxygenation. The bottom of each bed was sealed by a geotextile fabric to prohibit any kind of leakage.

Figure 4-3: Zeotuff gravel media in the VFF.

4.2.2.1 Operational Modification (ECO-1M)

This VFCW system was modified in September 2013, in order to enhance nitrogen removal to fulfill the 45 mg TN/L as required by the local JS for irrigation (class A). After a microcosm experiment that was carried out by Al-Zreiqat (2013) at the Fuhais laboratory, modification was implemented in the pilot-scale system. The modification entailed a using of electrical conduit plastic pipes as attached growth media in the recirculation tanks, aiming at promoting growth and retention of fixed biomass. This media has a large surface area and small volume when it is chopped into small pieces, **Figure 4-7**.

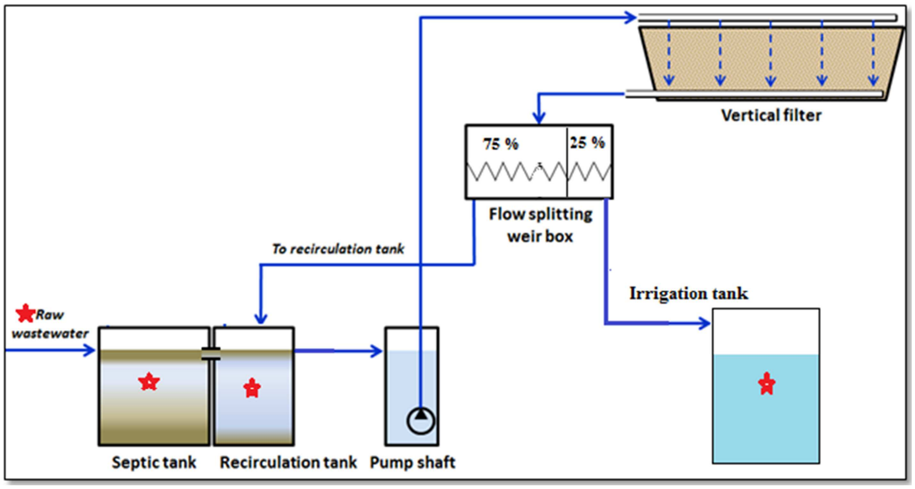


Figure 4-4: The scheme of the recirculating VFCW shows the flow direction and sampling points (stars).

Figure 4-5: The VFF with the flow splitting-box at Fuhais.

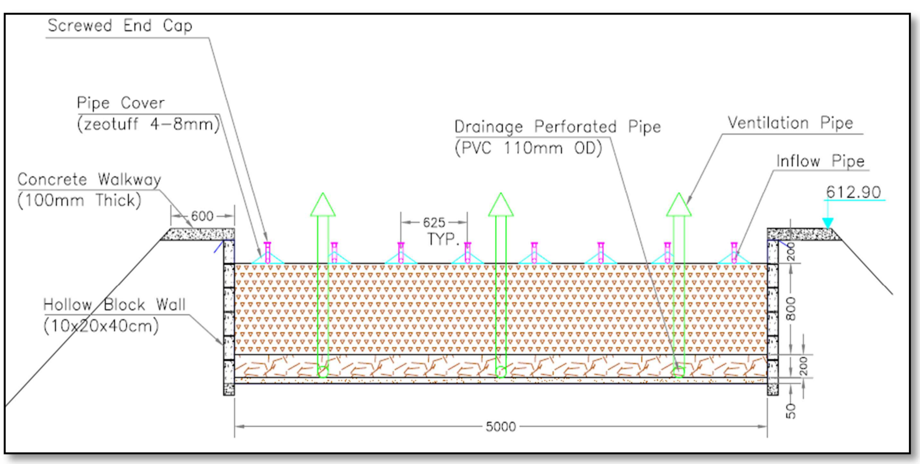


Figure 4-6: The profile view of the unsaturated VFF.

Al-Zreiqat (2013) found that using small pieces of electric conduit as attached growth media enhanced the denitrification process by increasing the density of biofilms. In his experiment, he used 10 L microcosms filled with mixture of raw wastewater and ECO-1 effluent. The mixture ratio was 3:1 as it is in the recirculation ratio of the pilot plant. The microcosms were tested with and without attached growth plastic media. He found out that attached growth media increased the denitrification efficiency up to 99.5 % within 24 hours of contact time.

Figure 4-7: Attached growth plastic media for the ECO-1 modification.

As sustainable, low cost, and available local plastic media, electric conduit pipes (2.3 cm outer diameter, 2.0 cm inner diameter) were used to retain and grow microorganisms in this research work and implemented in as an ECO-1 modification. These conduits were chopped into small pieces (4 cm length each) and confined in plastic textile meshed sacks (120 pieces /sack). Each five sacks were linked together to build up a six series of mobile attached plastic media, as shown in **Figure 4-8**. In total, the surface area of these plastic media was calculated to be 14.796 m^2. The media occupied about 150 L of the recirculation tank volume.

Figure 4-8: One series of the attached plastic media before installation and the installed attached media in the recirculation tank in the right.

Mass of Biofilm Growth

Dry weight of biofilm on the plastic media was measured 10 months after installation, and according to the method described by Bratbak *et al.* (1984), Nouvion *et al.* (1987), and Rittmann *et al.* (1986). Biofilm activity is not prorated to the quantity of fixed biomass, but raises with the density of the biofilm and a specific level of thickness (Kornegay & Andrews, 1968, LaMotta, 1976).

Six samples (random one piece from each series) were collected in plastic bottles (100 mL) and transported to the laboratory at Al-Balqa Applied University. The mass of biofilm was determined by rinsing the fixed biofilm with distilled water using a laboratory water dispenser, **Figure 4-9**. The mixture (biofilm and water from each piece) was transferred to a graduated cylinder. The mixture was blended in order to obtain a homogeneous concentration. The mass was determined as dry weight for each sample and the average was used to obtain the total mass biofilm.

Figure 4-9: The collected attached growth samples.

The total biofilm mass in the plastic media was found to be 3556.8 mg/m^3, and was determined as follows:

$$\text{Total mass} = \text{DW}_{\text{AV}} \times \text{P}_{\text{N}} \times \text{S}_{\text{N}} \times \text{G}_{\text{N}}$$

Where:
DW_{AV} = the average value of dry weights (experimentally it was found to be 988.0 mg/L)
P_N = number of pieces in each sack (120 pieces)
S_N = number of sacks in each set (6 sacks)
GN = number of groups in the recirculation tank (6 group)

4.2.3 Two-Stage VFCW System (ECO-2)

This treatment system consisted of a septic tank and two unsaturated VFCWs in series. The 1^{st} unplanted, single-pass filter was designed in a passive structure so that no pump is needed to proceed with treatment processes, **Figure 4-10**. The 2^{nd} filter was planted with *Phragmites*. The plants were indigenous in the area and obtained from Wadi-Shua´b. **Figure 4-11** shows the ECO-2 diagram. The VFCWs were monitored with and without plants to assess the plant effect on the treatment performance and water balance.

The system was designed to treat 3200 L/day, with an average hydraulic loading rate of approximately 80 and 56 L/m^2.d for the 1^{st} and 2^{nd} filter, respectively. The variation in the hydraulic loading rates related to different surface areas. The 1^{st} bed was designed with a surface area of 40 m^2 (5 m width and 8 m length), whereas, the 2^{nd} bed was designed with a larger surface area of 57 m^2 (6.2 m width and 9.2 m length). **Figure 4-11** and **Figure 4-12** show the layout of the two filters in ECO-2. ECO-2 system was dosed intermittently, three times every hour (47 L every 20 min).

Figure 4-10: The two-stage treatment wetland, 1st stage (single-pass) and 2nd stage (planted bed) at the site.

The main layer of the filters was zeotuff gravel (4 - 8 mm, 0.8 m depth), and the 0.2 m at the bottom of the filter was filled with plastic boxes (0.2 m height) to work as drainage system. The drainage pipes were connected to lateral vertical pipes for passive aeration. The wastewater received primary treatment in a septic tank (6 m^3) with an approximate residence time of two days. The primary treated wastewater dosed to the top of the filter through inlet distribution pipelines (perforations every 0.5 m). As in the previous designs, these distribution pipelines were covered by half-pipe shield tunnel and ended by valve risers. Between loading events, the air fills the space in filters (unsaturated), hereby promotes aerobic treatments (decomposition of organic matter and nitrification).

4.2.3.1 Operational Modification (ECO-2M)

The ECO-2 was modified in September 2013, jointly with the ECO-1 modification (2nd phase). Results from weekly monitoring over 19 months indicated that the unsaturated vertical flow wetland was producing a well-nitrified effluent. However, TN removal was limited due to the low denitrification capacity. Denitrification was hindered due to the limited organic carbon source as a result of efficient carbonaceous removal in the first stage (high reduction in BOD_5 concentration).

Step-feeding of raw wastewater was implemented in the pump shaft tank. Hereby, the 1st stage effluent has been mixed with raw wastewater before being dosed to the 2nd stage, as shown in **Figure 4-13**. The carbon requirement to achieve denitrification is 3.02 g organic matter/g NO_3-N (Kadlec & Wallace, 2009). Two options were considered as step-feeding source points. The carbon could come either from the water in the septic tank effluent, or from the raw wastewater itself. It was calculated that the septic tank step feeding option would require 1900 L/d dosed to the second stage, which was large compared to the design flow of the system. Using raw wastewater, which had a much higher carbon content, would require a volume of 439 L/d. Thus, the raw wastewater step-feeding option was chosen. Details are shown in **Table 4-2**.

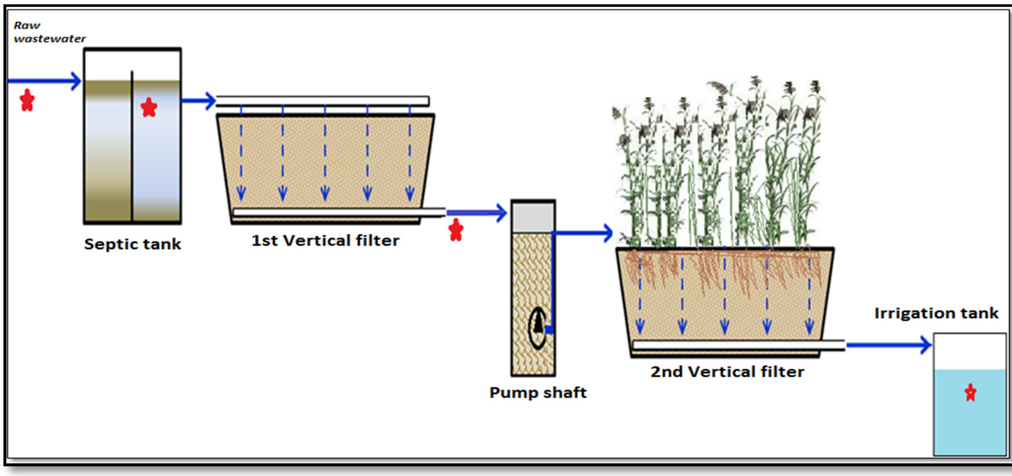


Figure 4-11: ECO-2 system scheme with water sampling points (stars).

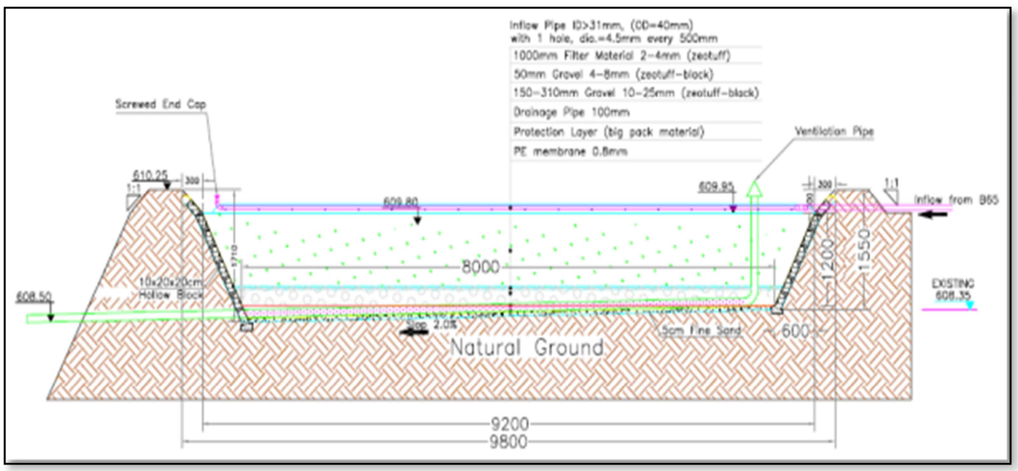


Figure 4-12: Profile view of the 1st stage (single-pass) in the ECO-2 system.

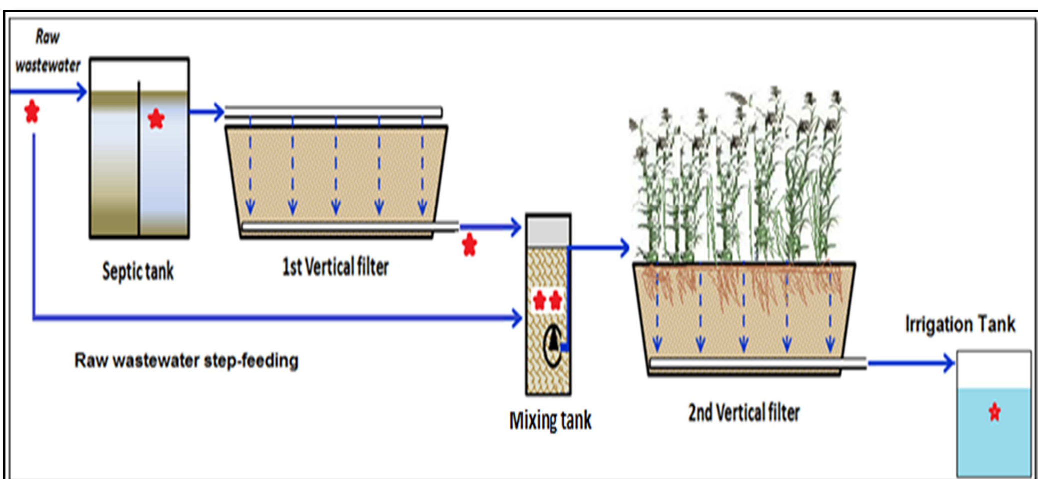


Figure 4-13: Layout of ECO-2 modification with raw step-feeding application.

Table 4-2: The specification of the step-feeding modification in the ECO-2 treatment wetland.

Carbon supplement	**Total inflow (L/d)**	**1st filter effluent (L/d)**	**Step-feeding dose (L/d)**
Raw wastewater	3200	2761	439
Septic tank	3200	1300	1900
Ap. Raw wastewater	3200	2870	330

Ap.: applied raw wastewater step feeing related to pump capacity.

The step-feeding was adjusted based on a used pump capacity that the shaft tank was fed with 330 L raw wastewater/day and 2870 L/day of 1st stage effluent. Raw wastewater step-feeding scheme was intermittent (five times/day) to the mixing tank. The volumetric rate of flow was set to be approximately 66 L/minute every four hours and 45 minutes.

4.2.4 Water Sampling Scheme

From February 2012 to August 2014, water samples were collected weekly from each component of the systems (before and after modification). Additional water samples from ECO-2 mixing tank were collected when it was put in step-feeding operation (during 2nd phase). For this case, the sampling took place at 8:00 AM every time, which was in the middle of the resting time prior to the raw step-feeding dosing. Temperature of all samples was measured directly on-site using a portable thermometer.

The samples were analyzed directly in the laboratory at the demonstration site, including field measurements (pH, EC, turbidity, redox potential and water temperature), as well as COD, BOD_5, TN, NH_4^+-N, NO_2^--N, NO_3^--N, TP, PO_4^{3-}-P, and *E. coli*. Other parameters such as TSS, cations and anions (Na^+, K^+, $Ca^{2+,}$ Mg^{2+}, CL^-, CO_3^{2-} , and HCO_3^-) were measured at the Water Laboratory of Al-Balqa Applied University. The cations, anions, and some heavy elements were measured (bimonthly in 2013 and 2014) solely for the irrigation tanks effluents. Additional water samples were prepared at the demonstration site and sent to the laboratory at the Department of Geology at the University of Jordan to be analyzed for heavy metal content (Fe^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}).

4.2.5 Analytical Methods

Field Measurements

Field measurements were conducted directly after sampling in the on-site laboratory. 500 mL of each sample site was collected to measure water temperature, pH, EC, DO and redox potential. Redox potential and DO were measured using the multi-meter WTW. The WTW ProfiLine-Cond 3110 probe was used to measure the EC, pH and temperature. Subsequently, 10 mL of these samples were filtered (using a 0.45 µm filter with syringe) for the NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P analyses.

Physical and Chemical Analyses

COD, TN, NH_4^+-N, NO_2^--N, NO_3^--N, TP, and PO_4^{3-}-P were measured using *HACH* LANGE test kits referring to the Standards Methods for Examination of Water and Wastewater (APHA, 1995). The kit uses an EPA approved method (Approved General-Purpose Methods, 2012).

COD analysis was measured using different COD kits, LCK 514 (100 - 2000 mg/L), LCK 114 (150 - 1000 mg /L), and LCK 314 (15 - 150 mg/L). A sample of two mL was allowed to react with cuvette solution (sulphuric acid-potassium dichromate) at 148 ^{o}C. After two hours of reaction, the cuvettes were cooled down to room temperature in order to measure the COD concentration using HACH spectrophotometer model 2800.

TN was measured using LATON test kit LCK 338 (20 - 100 mg/L). Samples were digested with Peroxodisulphate in the reaction tube at 100 ^{o}C for 1 hour. The TN concentration was determined using HACH spectrophotometer model 2800.

Phosphorus (TP) was measured using LCK 350 (2 - 20 mg/L) and LCK 348 (0.5 - 5.0 mg/L) test kits. Two mL of water samples were digested in acidic solution with molybdate and antimony ions at 100 ^{o}C over one hour. Phosphormolybdenum blue was measured in the cuvette test using HACH spectrophotometer model 2800 representing TP concentration.

NH_4^+-N was analyzed using LCK 302 (47 - 130 mg/L), LCK 303 (2.0 - 47 mg/L) and LCK 304 (0.015 - 2.0 mg/L) test kits. Ammonium ions from sample react with hypochlorite at pH 12.6, in the presence of sodium nitroprusside as a catalyst in the cuvette. The NH_4^+-N concentration was measured using HACH spectrophotometer model 2800.

NO_3^--N was measured using LCK 340 (5.0 - 35.0 mg/L) and LCK 339 (.023 -13.5 mg/L) Hach kits. Effluent water samples were diluted to be within the LCK 340 range. Nitrate ions from samples react with cuvette solution (dimethylphenol to form 2.6-Dimethylphenol). The NO_3^--N concentration was measured using HACH spectrophotometer model 2800.

NO_2^--N was analyzed using LCK 342 (0.6 – 6.0 mg/L) and LCK 341 (0.015 – 0.6 mg/L) test kits. The water samples were reacted in acidic solution with primary aromatic amines to form diazonium salts. This reaction formed intensive azo dyes. After 10 minutes of reaction, the nitrite concentration was measured using HACH spectrophotometer model 2800.

PO_4^{3-}-P was analyzed using LCK 350 (2 - 20 mg/L) and LCK 348 (0.5 - 5.0 mg/L) test kits. Water sample of two mL is allowed to react in acidic solution with molybdate and antimony ions. An antimonyl phosphomolybdate complex is reduced by ascorbic acid to phosphommolybdenum blue. Phosphormolybdenum blue was measured in the cuvette using HACH spectrophotometer model 2800 representing PO_4^{3-}-P concentration.

BOD_5 was measured using OxiTop® manometric OC 100, following the German standard DIN 38 409 H52.

TSS was analyzed using the gravimetric method according to the Standards Methods for Examination of Water and Wastewater (APHA, 1995). Turbidity was measured in Nephelometric Turbidity Unit (NTU) using turbidity meter, model TU-2016.

On bimonthly basis and during sampling events, cations, anions and trace elements were analyzed for irrigation water tanks according to (APHA, 1995). Sodium (Na^+) and potassium (K^+)

were measured by flame photometry at 589 and 767 nm wavelength, respectively. The Flame Photometer was first calibrated with a set of Na and K standards (5, 10, 20, and 30) and with a blank sample (deionized water). The emission readings of filtered wastewater samples on the Flame Photometer were then used to calculate the Na and K concentrations from the standard calibration curve.

Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) give the total hardness of water. Ca^{2+} was measured by titration with 0.01 N EDTA. A water sample of 10 mL was diluted with deionized water, then 2 - 3 mL of 2N NaOH solution was added to rise up the pH in order to precipitate Mg^{2+}. Ammonium purpuate (Murexide) used as indicator. During titration, the color will be changed from red to lavender, indicating the end point of titration. Ca^{2+} concentration is calculated in meq/L according to the consumed volume of EDTA and EDTA normality. Mg^{2+} was calculated from the difference between total hardness and Ca^{2+}. Total hardness was measured by titration with 0.01 N EDTA. As Ca^{2+} analysis, a water sample of 10 mL was diluted with deionized water, then 3 - 5 mL of buffer solution with a few drops eriochrome black indicator. During titration, the color will be changed from red to blue, indicating the end point titration. The total hardness is calculated using **Equation 4-1**.

$$\mathrm{Ca\ or\ (Ca+Mg)}\left(\frac{\mathrm{meq}}{\mathrm{L}}\right) = (\mathrm{V_1} \times \mathrm{N} \times 1000)/\mathrm{V} \qquad \text{Equation 4-1}$$

Where:
V_1= volume of EDTA titrated for the sample (mL)
N = normality of EDTA solution
V = volume of water sample used for measurement (10 mL)

Carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) were measured by titration with sulfuric acid solution (0.01 N H_2SO_4). One drop of phenolphthalein indicator was added to 10 mL of water sample. If a pink color develops, the water sample should be titrated by H_2SO_4 until the color disappears. The titrant volume will be recorded as (Y) for CO_3^{2-} concentration. For HCO_3^- determination, the titration can be continued using H_2SO_4, after adding 2 drops 0.1 % of methyl orange indicator. The titration stops when the color turns to orange (the end point). The titrant volume will be used to calculate the HCO_3^- concentration. The actual concentrations were calculated using **Equation 4-2** and **4-3**.

$$CO_3\left(\frac{meq}{L}\right) = (2Y \times N \times 1000)/V \qquad \text{Equation 4-2}$$

$$\mathrm{HCO_3}\left(\frac{\mathrm{meq}}{\mathrm{L}}\right) = ((\mathrm{T}-2\mathrm{Y}) \times \mathrm{N} \times 1000)/\mathrm{V} \qquad \text{Equation 4-3}$$

Where:
2 = valance of CO_3
Y = volume of titration for CO_3 (mL)
T = volume of titration for HCO_3 (mL)
V = volume of water sample (10 mL).
N = normality of H_2SO_4 solution.

Chloride (Cl^-) was measured by Mohrs's titration, using 0.01 N Silver Nitrate Solution ($AgNO_3$). 10 mL of water sample were mixed with 4 drops of potassium chromate (K_2CrO_4) solution.

Subsequently, the sample was titrated with $AgNO_3$ solution until a reddish-brown color appeared (the end point). The Cl^- concentration was calculated using **Equation 4-4**.

$$\mathrm{Cl}\left(\frac{\mathrm{meq}}{\mathrm{L}}\right) = (\mathrm{V_1} \times \mathrm{N} \times 1000)/\mathrm{V_{AgNO3}} \qquad \text{Equation 4-4}$$

Where:
V_1 = volume of $AgNO_3$ solution titrated the sample (mL)
N = normality of $AgNO_3$ solution
V = volume of water sample (10 mL)

Sulfate (SO_4^{2-}) was measured by the turbidimetric method. Specific volume from sample was taken into a 250 mL flask. 1 mL 1:1 HCl solution and 2 - 3 drops methyl orange were added to the sample that the color of sample turned into pink. 10 mL of 1 N $BaCl_2.2H_2O$ solution were added in order to precipitate SO_4-S as barium sulfate ($BaSO_4$). The absorption of light by precipitated suspension was measured by spectrophotometer at 420 nm, and SO_4^{2-} concentration is calculated using **Equation 4-5**.

$$\mathrm{SO_4}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = (\mathrm{SO_{4\,(mg)}} \times 1000)/\mathrm{V} \qquad \text{Equation 4-5}$$

Where:
$SO_{4\,(mg)}$ = SO_4 reading by spectrophotometer
V = volume of sample (mL)

Some heavy metals (Fe^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) were measured using Flame Atomic Absorption Spectrometry. The water samples were filtered and preserved in a fridge after adding two mL of diluted HCl (1:10). 20 mL of each sample were taken for analysis. The total concentrations were determined at wavelengths, λ: Fe^{2+} = 372.0 nm; Pb^{2+} = 217.0 nm; Mn^{2+} = 279.5; Zn^{2+} = 213.9 nm and Cu^{2+} = 324.8 nm.

Microbial Analysis
E. coli was measured in MPN/100 L using the IDEXX™ Colilert-18 Quanti-tray method, according to the manufacturer's specifications.

4.2.6 Statistical Methods

Statistical analyses were performed using SigmaPlot software, version 12.0. The analysis of variance, using one way ANOVA, was applied between calculated yearly means of pH, EC, DO, turbidity, TSS, TN, NH_4^+-N, NO_3^--N, TOC, BOD_5 and calculated geometric mean for *E. coli* for the 1st and 2nd phase in order to investigate the effects of modification in each system. Monthly means of TN, NH_4-N, TSS, BOD_5, and monthly geometric mean of *E. coli* mass removal rates were calculated using one way ANOVA (statistical significance, $p > 0.05$) and results from 1st and 2nd year were compared.

4.3 Results and Discussion

4.3.1 Weather Description and VFCWs Water Balance

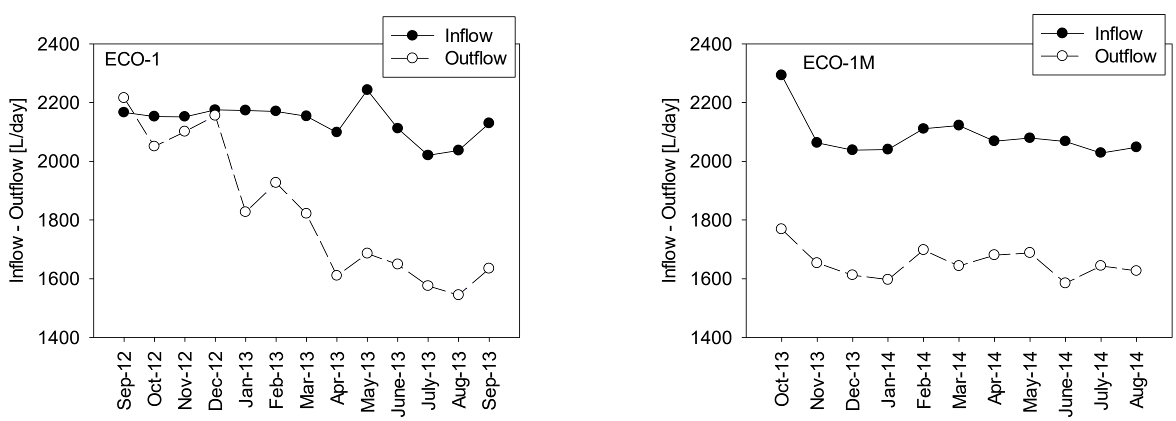
Water balance and seasonal changes of the operated VFCW systems were investigated. Monthly inflow, outflow, rainfall, air temperature, E and ET are presented over the study period in this part. Non-representative data were removed out, therefore, inflow and outflow data were neglected in case of maintenance and operation work or flow meter error. Monthly means are shown from September 2012 to September 2013 (1st phase of monitoring) and means during modification applications (2nd phase) are shown from October 2013 to August 2014.

4.3.1.1 Air Temperature and Rainfall

The climate of the area is semi-arid with a dry, hot summer and winter precipitation with a few degrees during the night. The meteorological data was taken from Al-Salt climatic station as a long-term monthly averages. During the study, the rainy season was from October to May, with an average annual precipitation of 495 mm (Climate-Data.gov). The maximum monthly rainfall mean was 156.7 mm in January, while the minimum mean was 5.8 mm in May. The maximum mean monthly temperature was 29.6 ^{o}C in July and August, while the minimum mean temperature was 4.8 ^{o}C in February.

4.3.1.2 Inflow and Outflow of VFCW systems

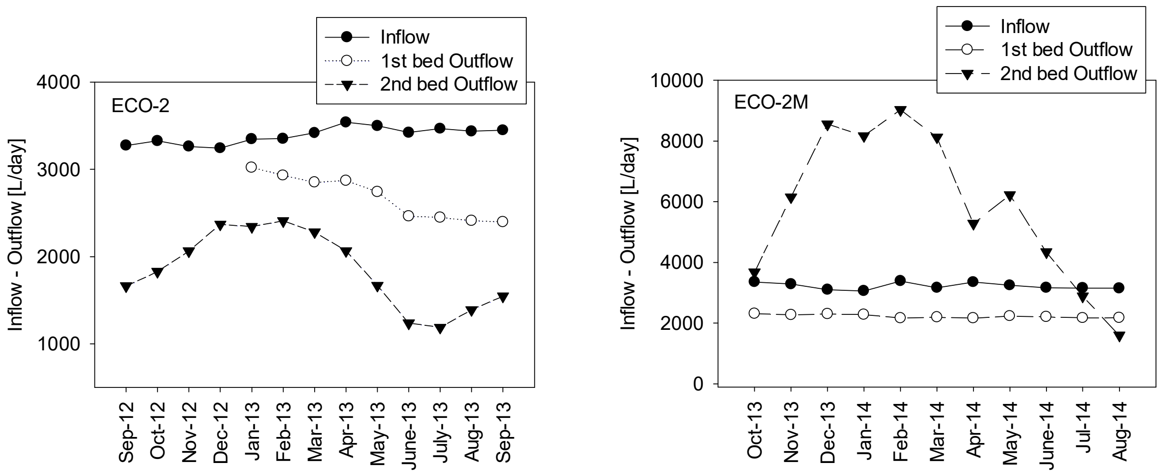
Recirculating system (ECO-1 and ECO-1M) inflow and outflow data over the study period are depicted by **Figure 4-14.a** and **b**. In the 1st phase of operating the system, the average inflow was 2145 L/day and the outflow was 1877 L/day. The outflow values were increased in winter due to rainfall. Stable inflow and outflow values were measured during attached growth application (ECO-1M), on average inflow of 2091 L/day and outflow of 1652 L/day.



a) b)

Figure 4-14: Monthly ECO-1 and ECO-1M inflow and outflow data over the study period; a) phase 1, b) phase 2.

Two-stage system (ECO-2 and ECO-2M) inflow and outflow data during the study period are shown in **Figure 4-15.a** and **b**. In the 1st phase of operation, the average inflow was 3375 L/day and the outflow from 2nd bed was 1900 L/day. The outflow values were increased in winter due to rainfall. During step-feeding application, in theory, the setup of inflow was modified and reduced to be 2870 L/day in order to dose 330 L/day from raw to the mixing tank. In contrast, modification setup was inaccurate (hydraulic overloading); that the average inflow load was recorded of 3229 L/day that ECO-2M was overloaded. The average outflow from 1st bed was 2225 L/day and the outflow was 5891 L/day, which means too much water was dosed to the 2nd stage via step-feeding process.



a) b)

Figure 4-15: Monthly ECO-2 and ECO-2M inflow and outflow values over the study period; a) phase 1, b) phase 2.

4.3.1.3 Evaporation (E) and Transpiration (ET) in VFCWs

In arid and semi-arid countries, saving water from ET is considered a priority when treated effluent is an invaluable resource for reuse. Treated water can be affected negatively by loss of water, which increases the salinity of water (Morari & Giardini, 2009). At Al-Salt climatic station, E is determined from Class A-pan evaporation and ET was measured as a potential evapotranspiration. The maximum E value was recorded in July of 8.75 mm/day, whereas the lowest E value was recorded of in December of 2.2 mm/day, **Figure 4-16**. The maximum potential evapotranspiration as a monthly mean was 5.47 mm/d in August, while the minimum potential evapotranspiration monthly mean was 1.14 mm/d in December.

However, E and ET were calculated per unit of area of the VFCW beds, as depicted in **Figure 4-17** and **Figure 4-18**. During the 1st phase of ECO-1 monitoring, the maximum average E rate was calculated of 28.1 mm/day in May, while, the minimum E rate was equated of 4.4 mm/day in December. High E rate rates in ECO-1 could have been in part attributed to the fact that the splitting box was open to the atmosphere. During the 2nd phase, ECO-1M did not show the same trend that high E rate was almost high over the winter and summer. The E differentiations were probably influenced by other weather conditions such as wind speed, solar radiation, and humidity. That high wind speed and solar radiation with low relative humidity could increase the E rate.

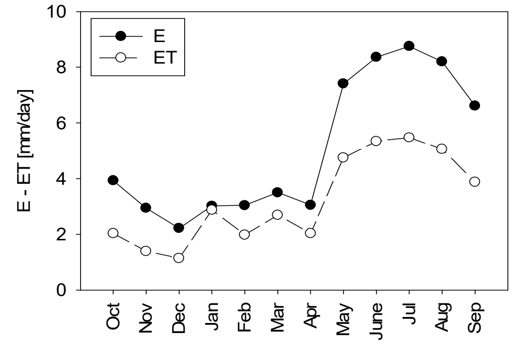
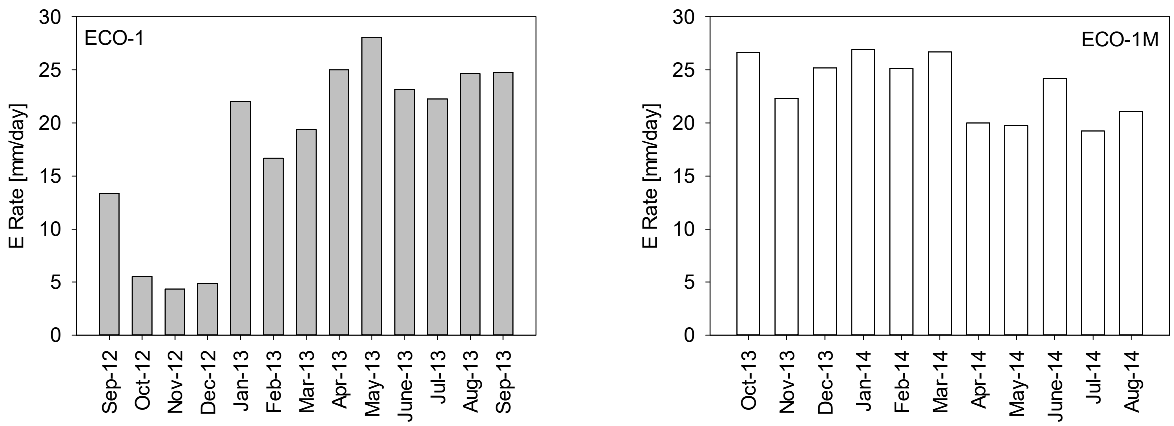


Figure 4-16: Monthly measured A-pan evaporation and potential evapotranspiration from Al-Salt climatic station (1985 – 2012).

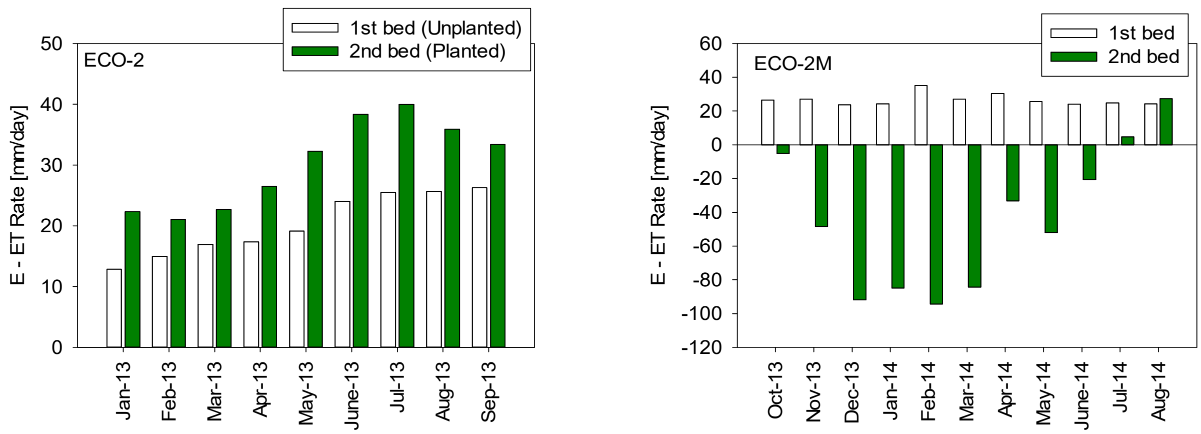


a) b)

Figure 4-17: Calculated E rates for recirculating VFCW system over the study period. a) phase 1, b) phase 2.

During the 1st phase of ECO-2 monitoring, the maximum ET rate was calculated of 40 mm/day for the planted bed (2nd bed) in July, while, the minimum ET rate was equated of 12.9 mm/day in January, **Figure 4-18**. Similar results were observed by Moro *et al.* (2004) that water loss via ET of *Phragmites australis* was highest in summer (June), in a wetland in Natural Park in Spain, due to head plant growth rate and foliage surface area, while, ET rate was decreased in October. During phase 2, inaccurate HLR, consequently, E rate was increased in 1st bed on average water loss of 27.2 mm/day. Low rate of ET was equated of - 94 mm/day, during step-feeding application, as results of hydraulic overloading.

These high ET rates were in agreement with many CW studies planted with *Phragmites* under similar climatic conditions (El Hamouri *et al.*, 2007, Borin *et al.*, 2011, Milani & Toscano, 2013). El Hamouri *et al.* (2007) observed the highest ET rate in HFCW system on average of 57 mm/day in Morocco, while E was 7 mm/day for the unplanted filter. In southern Italy, Milani and Toscano (2013) reported high ET rate in a pilot-scale HFCW system during June - August, on average 32.8 mm/day.



a) b)

Figure 4-18: Calculated E and ET rates for two-stage VFCW system during the experimental study. a) Phase 1, b) Phase 2.

During phase 2, inaccurate HLR, consequently, E rate was increased in the 1st bed on average water loss of 27.2 mm/day. Low rate of ET was equated, during step-feeding application, with rate as low as - 94 mm/day in the 2nd bed as results of hydraulic overloading.

4.3.2 ECO-1 Treatment Performance

The treatment performance of the system was evaluated by the conformity of effluents to the national JS (class A) for reuse in irrigation. The system treatment efficiency is presented in two phases: 1st phase of monitoring system in a steady state (February 2012 to September 2013) and 2nd phase of monitoring of modified system, attached growth biofilm in a recirculation tank, (October 2013 to August 2014). Biofilm development took approximately three weeks. The results of 1st and 2nd phases were statistically compared to examine the influenced parameters with modification adopting.

4.3.2.1 Physico-chemical Parameters

Results of pH, EC, DO, redox potential, turbidity, and TSS were summarized (Mean and SD) in **Table 4-3**. The mean values of the previous parameters were statistically similar ($p < 0.05$) during phase 1 and 2.

The pH values were ranged from 7.1 to 7.6, which conform to the Jordanian Standard (pH values of 6 - 9). There was no significant change in pH values during treatment process compared with raw wastewater pH, indicating a normal biological nitrification in the system.

The effluent EC was reduced gradually during treatment process that can be explained by settlement of suspended particles and elements (Bitton, 1994, Kadlec *et al.*, 2000). The VF effluent had a mean EC of 1546 µS/cm and 1365 µS/cm during the 1st and 2nd phase, respectively. During the 2nd phase, more EC reduction was observed due to higher NO_3^--N removal and it was compatible with influent EC values. The EC results conform to the JS (0.7 - 3000 µS/cm) over the study period.

The effluent DO concentration of ECO-1 was 6.2 mg/L. The raw wastewater had a DO concentration of 0.6 mg/L. The effluent was highly oxygenated and fulfilled the Jordanian Standard (DO greater than 2 mg/L). This result was promoted by gas diffusion from atmosphere between intermittent hydraulic loads as documented by many authors (Brix & Arias, 2005b, Brix & Schierup, 1990, Saeed & Sun, 2012, Laber *et al.*, 1997).

The evolution of redox potential over the study period is shown in **Figure 4-19**. In phase 2, redox measurements were dramatically increase of 54.8 and 143.1 mV in the recirculation tank and VFCW effluent, respectively.

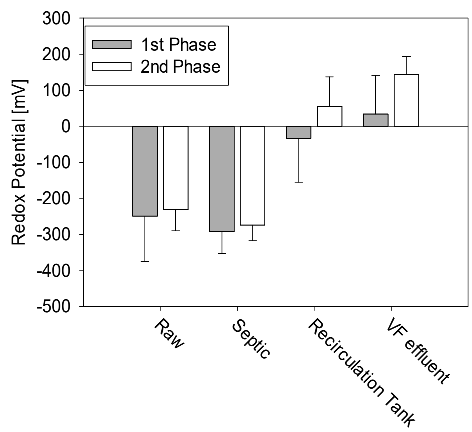


Figure 4-19: Influent and effluent redox mean values with SD (error bars) over the course of the study (phase 1 and 2).

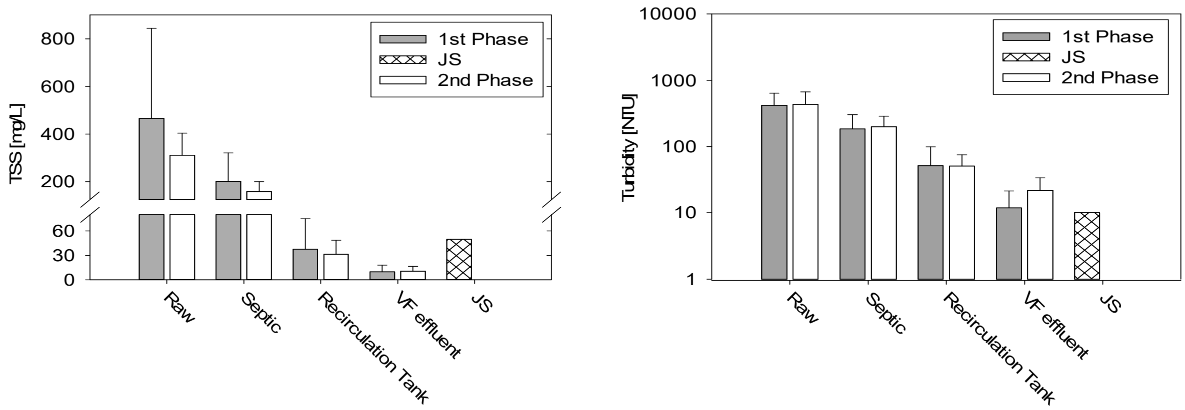
4.3.2.2 Total Suspended Solids (TSS) and Turbidity

TSS and turbidity were typically high in the raw wastewater over the study period. TSS was reduced from 466 to 10 mg/L during phase 1 and was reduced from 311 to 11 mg/L during phase 2. TSS and turbidity are removed by physical processes such as sedimentation and filtration (Kadlec & Knight, 1996, Metcalf & Eddy, 1991), and non-settling solids can be removed by microorganisms (Vymazal, Brix, Cooper, Haberl*, et al.*, 1998) or adsorption (Stowell *et al.*, 1981). Effluent TSS concentrations were compatible with the Jordanian Standards (less than 50 mg/L), **Figure 4-20.a**. Comparing 1st and 2nd phase, effluent TSS concentrations were statically similar ($p < 0.05$).

Turbidity was reduced from 417 to 12 NTU during phase 1. During phase 2, turbidity were reduced from 435 to 22 NTU, hereby, effluent turbidity did not conform to the Jordanian Standards (less than 10 NTU), **Figure 4-20.b**. In the recirculation tank, turbidity values were constant during suspended and attached growth. Therefore, increasing turbidity due to attached growth sloughing is negligible. Turbidity was increased substantially in the VFCW bed in phase 2 as a result of low filtration and extracted suspended particles by bed matrix or low adsorption process over time. Comparing 1st and 2nd phase, effluent turbidity was statically different at $p < 0.05$.

Table 4-3: Influent and effluent physico-chemical parameters (means ± SD) and number of samples (N) for each component in the recirculating VFCW system during the course of study (phase 1 and 2).

		Raw		Septic Tank		Recirculation Tank		VF Effluent	
	Parameter	N		N		N		N	
1st Phase	pH	65	7.4 ± 0.2	65	7.1 ± 0.2	65	7.2 ± 0.2	65	7.3 ± 0.2
	EC [µS/cm]	65	1810.9 ± 220.5	65	1910.7 ± 220.3	65	1641.7 ± 210.9	65	1546.2 ± 245
	DO [mg/L]	65	0.7 ± 0.7	65	0.9 ± 0.6	65	2.0 ± 1.2	65	6.2 ± 1.4
	Redox potential [mV]	65	-250.1 ± 126.1	65	-291.9 ± 61.3	65	-33.7 ± 121.8	65	34.7 ± 108.1
	TSS [mg/L]	62	465.5 ± 379	56	201.2 ± 119.3	55	37.8 ± 36.9	56	10.1 ± 8.1
	Turbidity [NTU]	56	417.1 ± 219.7	56	182.3 ± 119.1	56	52.4 ± 47.1	56	12.0 ± 9.4
2nd Phase	pH	44	7.6 ± 0.3	44	7.2 ± 0.3	44	7.4 ± 0.2	44	7.4 ± 0.2
	EC [µS/cm]	44	1617.7 ± 179.7	44	1741.3 ± 184.8	44	1544.9 ± 493.3	44	1364.9 ± 136.1
	DO [mg/L]	44	0.6 ± 0.5	44	0.9 ± 0.5	44	2.6 ± 0.9	44	6.2 ± 0.9
	Redox potential [mV]	41	-231.6 ± 58.8	41	-274.8 ± 43.7	41	54.8 ± 82.4	41	143.1 ± 50.3
	TSS [mg/L]	41	311.0 ± 92.5	41	157.5 ± 42.1	41	31.3 ± 17.3	41	10.8 ± 5.6
	Turbidity [NTU]	44	434.8 ± 236.9	44	199.9 ± 88.8	44	50.7 ± 23.9	44	21.8 ± 12.5



a) b)

Figure 4-20: Influent and effluent TSS and Turbidity mean concentrations and SD(error bars) of each component in the system with the JS (class A). a) TSS concentrations over the study period, b) Turbidity concentrations over the study period.

4.3.2.3 Organic Matter (OM)

Table 4-4 summarizes the COD and BOD_5 results of each component in the system.

COD

COD concentrations conformed to the JS (A: less than 100 mg/L), **Figure 4-21.b**. COD concentrations were reduced from 1138 to 55 mg/L during phase 1 and were reduced from 777 to 48 mg/L during phase 2. On a few occasions, during fasting (Ramadan) and feast periods, high COD concentrations (1750 - 3224 mg/L) was treated, illustrating a sharp deviation in COD values in raw wastewater. The highest COD removal was observed in the septic tank where the suspended solids are retained with OM, which is agreed with several VFCW studies (Stefanakis & Tsihrintzis, 2009). However, high COD reduction was observed in the filter that OM mostly removed via aerobic degradation in accordance with other studies (Vymazal, 2002, Tietz *et al.*, 2008). Moreover, recirculation of effluent enhances the treatment performance as a result of dilution, which was in agreement with Prost-Boucle and Molle (2012). No significant difference in COD concentrations was observed during 1^{st} and 2^{nd} phase ($p < 0.05$).

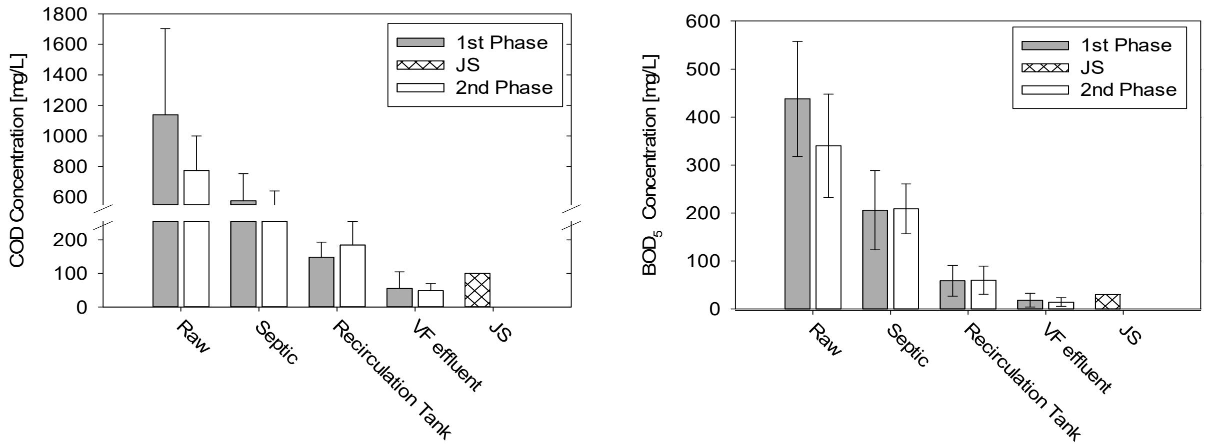
BOD_5

Results of BOD_5 concentrations conform to the JS (A: less than 50 mg/L), **Figure 4-21.b**. BOD_5 concentrations were reduced from 438 to 18 mg/L during the 1^{st} phase and were reduced from 340 to 14 mg/L during the 2^{nd} phase, as a results of aerobic and anaerobic degradation. In addition, OM can be removed via adsorption of solids and dissolved organics in zeotuff due to its large pores, which is agreed by Stefanakis and Tsihrintzis (2012). Similar results were reported by Prost-Boucle and Molle (2012). No significant difference in BOD_5 concentrations was observed during 1^{st} and 2^{nd} phase ($p < 0.05$).

Table 4-4: Recirculating system influent and effluent water quality (means ± SD) and number of samples (N) of each component during the experimental study (1st and 2nd phase).

	Parameter	N	Raw	N	Septic Tank	N	Recirculation Tank	N	VF Effluent
1st Phase	BOD_5 [mg/L]	57	437.6 ± 119.9	59	206 ± 82.8	59	58.8 ± 32.2	59	18.3 ± 14.5
	COD [mg/L]	61	1137.9 ± 564.4	64	575.3 ± 176.3	64	148.6 ± 44.8	64	54.9 ± 50.4
	TN [mg/L]	64	107.3 ± 47.7	64	95.5 ± 36.5	64	54.3 ± 18.5	64	54.9 ± 22.2
	NH_4^+-N [mg/L]	61	60.7 ± 19.5	63	72.2 ± 19.1	63	29.9 ± 14.4	63	2.2 ± 3.9
	NO_3^--N [mg/L]	54	0.5 ± 0.5	63	0.4 ± 0.6	62	9.8 ± 9.6	62	43.9 ± 18.1
	NO_2^--N [mg/L]	65	0.05 ± 0.08	56	0.03 ± 0.01	62	4.3 ± 5.2	62	0.9 ± 1.0
	E. coli [MPN/100 mL]	47	$8.5 \times 10^6 \pm 7.2 \times 10^6$	49	$3.1 \times 10^6 \pm 3.2 \times 10^6$	48	$5.4 \times 10^5 \pm 1.4 \times 10^6$	49	$6.4 \times 10^4 \pm 2.6 \times 10^5$
2nd Phase	$CBOD_5$ [mg/L]	43	340.1 ± 107.5	43	208.4 ± 51.9	43	60.3 ± 29.3	43	14.3± 9.2
	COD [mg/L]	43	777.4 ± 225.8	42	511.7 ± 127.6	42	184.5 ± 69.7	42	48.4 ± 20.9
	TN [mg/L]	44	84.3 ± 26.8	43	89.6 ± 19.9	44	43.6 ± 10.2	44	40.3 ± 8.9
	NH_4^+-N [mg/L]	44	52.4 ± 64.3	44	23.7 ± 7.9	44	23.7 ± 7.9	44	0.8 ± 2.1
	NO_3^--N [mg/L]	42	0.9 ± 1.2	42	0.7 ± 0.7	43	8.8 ± 6.4	43	36.7 ± 10.8
	NO_2^--N [mg/L]	44	0.1 ± 0.1	44	0.02 ± 0.0	44	1.8 ± 1.9	44	0.4 ± 0.4
	E. coli [MPN/100 mL]	40	$1.2 \times 10^7 \pm 7.1 \times 10^6$	41	$5.2 \times 10^6 \pm 3.3 \times 10^6$	40	$1.5 \times 10^6 \pm 4.4 \times 10^6$	40	$1.4 \times 10^5 \pm 4.5 \times 10^5$

E. coli concentrations are presented in geometric means.



a) b)

Figure 4-21: Influent and effluent COD and BOD_5 mean concentrations and SD (error bars) of each component in the recirculating with the JS (class A). a) COD concentrations, b) BOD_5 concentrations.

4.3.2.4 Nitrogen Transformations

Results of TN, NH_4^+-N, NO_2^--N, and NO_3^--N are shown in **Table 4-4**.

Total Nitrogen (TN)

Recirculating VFCW system combines simultaneous nitrification and denitrification process (Arias *et al.*, 2005). That TN removal was enhanced by recycling portion of nitrified effluent from VFF to the recirculation tank where denitrification occurs. TN concentrations were reduced from 107 to 55 mg/L during phase 1 and were reduced from 84 to 40 mg/L during phase 2, as shown in **Figure 4-22**. In phase 2, higher biomass was increased the TN removal rate than suspended growth, similar findings were reported by Neethling *et al.* (2010) and Al-Zreiqat (2013). Effluent TN concentration conformed to the JS (A: 45 mg/L). However, the TN effluents were statistically different ($p < 0.001$) over the study period.

Ammonium Nitrogen (NH_4^+-N)

NH_4^+-N concentrations were reduced from 61 to 2.2 mg/L during the phase 1 and were reduced from 52 to 0.8 mg/L during phase 2, **Figure 4-23.a**. During phase 2, Low NH_4^+-N concentrations entered the system, resulting with low NO_3^--N levels in the effluent, which is in agreement with Prost-Boucle and Molle (2012). NH_4^+-N was removed by high conversion of NH_4^+ to NO_3^- in the filter as a result of high DO level and nitrifying bacteria density. These findings are in agreement with Saeed and Sun (2012), Gray (2004) and Arias *et al.* (2005).

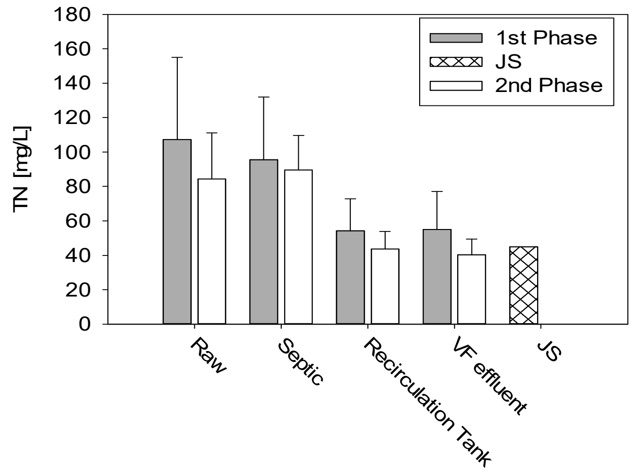
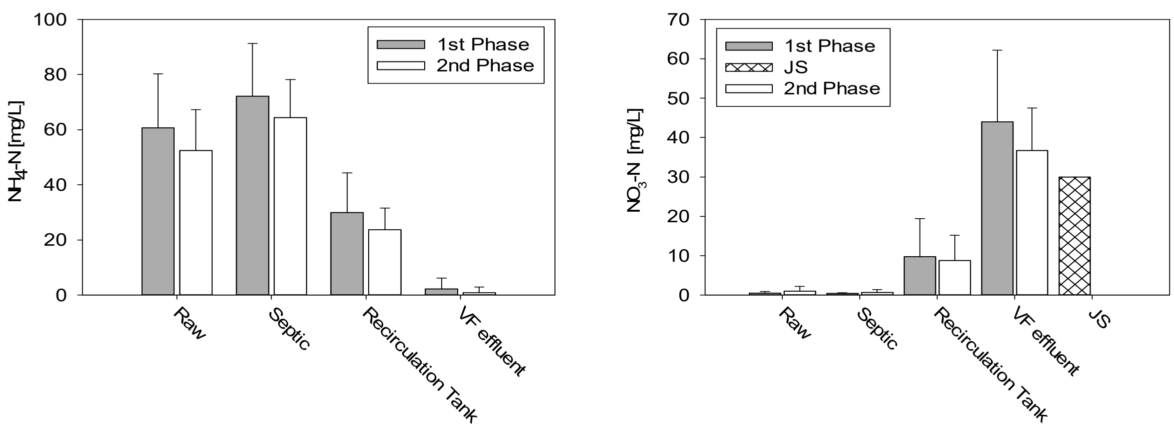


Figure 4-22: Influent and effluent TN mean concentrations and SD (error bars) during phase 1 and phase 2 with the JS (class A).

Well nitrified effluent can be shown by low NO_2^--N mean concentrations in the filter effluent. Highest NO_2^--N levels were measured in the recirculation tank of 4 and 1.8 mg/L in phase 1 and 2, respectively. The reduction in NO_2^--N values in phase 2 can be explained by enhanced denitrification capacity and low NH_4^+-N input. No significant difference in NH_4^+-N concentrations was observed during 1st and 2nd phase ($p < 0.05$).



a) b)

Figure 4-23: Influent and effluent NH_4^+-N and NO_3^--N mean concentrations and SD (error bars) of each component in the recirculating VFCW system. a) NH_4^+-N concentrations, b) NO_3^--N mean concentrations with the JS (class A) over the study period.

Nitrate Nitrogen (NO_3^--N)

During phase 1, NO_3^--N concentrations were increased sharply in the filter effluent from 9.8 mg/L in recirculation tank to 44 mg/L. NO_3^--N level was higher than the recommended levels in the JS (class A: 30 mg/L and B: 45 mg/L), as depicted by **Figure 4-23.b**. During phase 2, NO_3^--N concentrations were decreased to 37 mg/L in the VFF effluent as a result of stirring denitrification with attached biofilm. Thus, effluent NO_3^--N concentration conformed to the JS class B. Changing from suspended to attached growth increases the abundance and activity of microorganisms. Kadlec and Wallace (2008) reported that density and activity of microbes will be influenced by changing one or more factor in treatment setup. On the other hand, increasing biofilm formation and solids accumulation using plastic media can be inhibited or mitigated nitrification rate (Richards & Reinhart, 1986). NO_3^--N concentrations in the 1st and 2nd phase were statistically compared; results indicate that the effluents were significantly different ($P = 0.034$).

4.3.2.5 *E. coli* Reduction

Influent and effluent *E. coli* concentrations (geometric mean and SD) of ECO-1 are presented in **Table 4-4** and compared with the JS (class A: 100 MPN/100 mL) in **Figure 4-24**. The influent *E. coli* geometric mean was 8.5×10^6 and 1.2×10^7 MPN/100 mL during phase 1 and 2, respectively. During the 1st phase, *E. coli* concentrations were gradually decreased to 5.4×10^5 and 6.4×10^4 in the recirculation tank and filter, respectively. During the 2nd phase, *E. coli* influent concentrations increased to 1.6×10^6 and 1.4×10^5 in the recirculation tank and filter effluent, respectively.

Over the study period, the system achieved approximately 2.1 $\log_{10}$ *E. coli* reduction; 1.1 $\log_{10}$ was achieved throughout septic and recirculation tank and 1 $\log_{10}$ was achieved by the filter itself. *E. coli* is removed by sedimentation, straining and entrapment in biofilms (Stevik *et al.*, 2004, Kadlec & Knight, 1996), predation by microorganisms and natural die-off (Wand *et al.*, 2007). Effluent *E. coli* concentrations were not compatible with the JS (class A), but did conform to class C in the JS. Effluent *E. coli* concentrations were statistically similar ($p < 0.05$) over the study period. Therefore, using subsurface irrigation might be considered as alternative disinfection step for reuse in irrigation, as recommended by Gross *et al.* (2007) for decentralized recycled VFCW effluent in Israel.

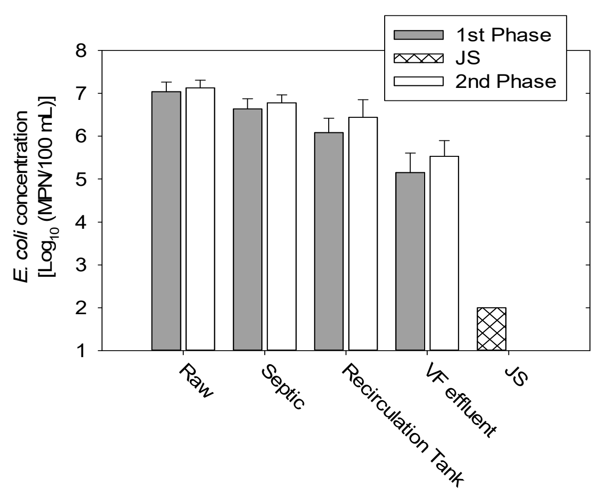


Figure 4-24: Influent and effluents *E. coli* geometric mean concentrations over the course of the study.

4.3.3 ECO-1 Pollutant Removal Evaluation and Seasonal Variability

Mass removal rate per unit area for TSS, BOD_5, COD, TN, NH_4^+-N, and *E. coli* during the study period is evaluated and compared in this part. In addition, monthly removal mean of the previous parameters is presented over the course of the study to evaluate the treatment performances with temperature variability.

4.3.3.1 TSS Removal

Figure 4-25 shows high TSS removal rate over the study period, regardless of season or temperature. Mean TSS removal rate was 54.4 g/m^2.day in phase 1 and it was 32.5 g/m^2.day in phase 2.

ECO-1 removed TSS, on average mean removal efficiencies of 97.7 % and 96.8 %, with mean mass removal rates of 53.5 g/m^2.day and 31.6 g/m^2.day in phase 1 and 2, respectively. Results did not show significant difference in TSS mass removal ($p < 0.05$) over the study period.

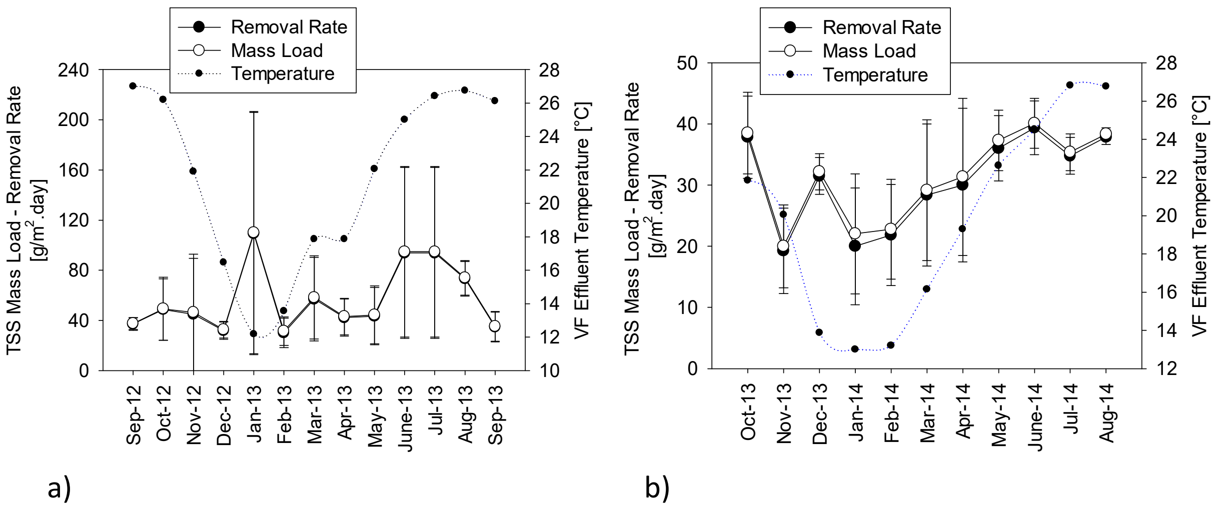
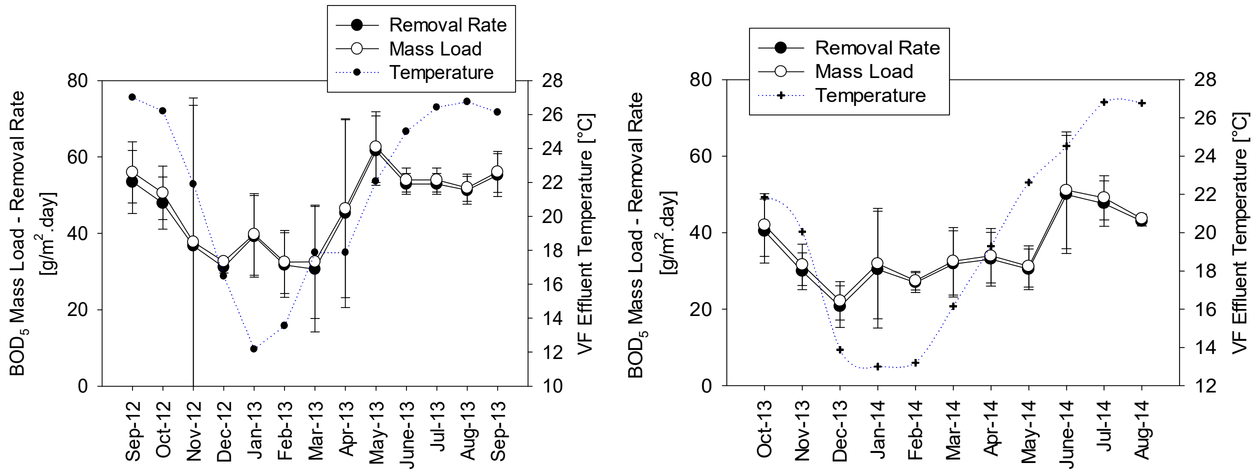


Figure 4-25: Monthly TSS removal rate and SD (error bars) over the study period, a) phase 1, b) phase 2.

4.3.3.2 Organic Matter (OM) Removal

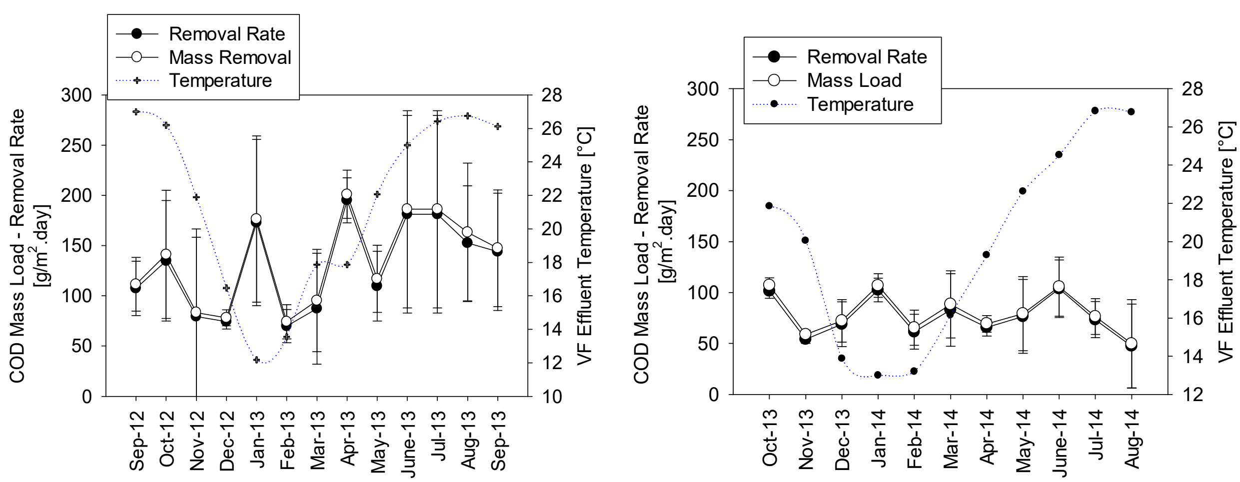
Figure 4-26 shows BOD_5 removal rate over the study period. Stable removal rates were observed, on average mean removal efficiencies were 96.8 % and 96.2 %, with mean BOD_5 removal rates of 44 g/m^2.day and 34.3 g/m^2.day during phase 1 and 2, respectively. Mean BOD_5 mass load was 45.3 g/m^2.day in phase 1 and it was 35.5 g/m^2.day in phase 2. These results are in accordance with many studies that have documented negligible temperature influence on organic matter removal in CWs (Vymazal, 1999, Wallace & Knight, 2006). Mean BOD_5 mass removal did not show significant difference ($p < 0.05$) between the two study periods.



a) b)

Figure 4-26: Monthly BOD_5 removal rate, SD (error bars) and the VF effluent temperature over the study period, a) phase 1, b) phase 2.

High COD removal rates were observed, on average removal efficiencies were 95.4 % and 94.7 %, with mean removal rates of 122.1 g/m^2.day and 76.6 g/m^2.day during phase 1 and 2, respectively. Mean COD removal rate was highly related to COD mass load, which was 127.4 g/m^2.day in phase 1 and 80.5 g/m^2.day in phase 2, **Figure 4-27**. Moreover, there was no clear trend with temperature. This finding is in agreement with Vymazal (2011). Mean COD mass removal was not significantly different ($p < 0.05$) between the two study periods.



a) b)

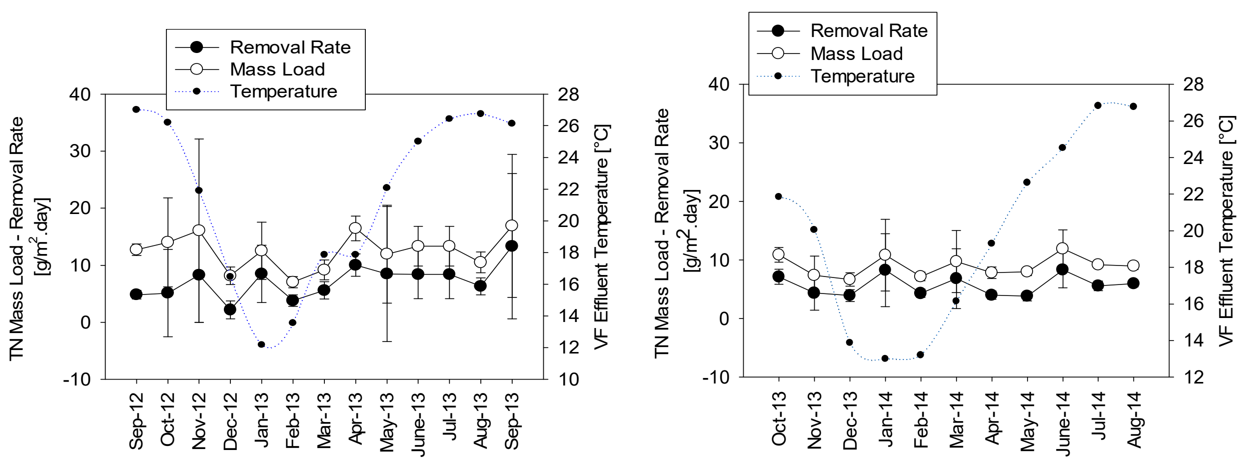
Figure 4-27: Monthly COD removal rate, SD (error bars) and the VF effluent temperature over the study period, a) phase 1, b) phase 2.

4.3.3.3 TN Removal Rate

TN removal showed differentiation ($P < 0.001$) during phase 1 and 2, indicating the operational modification improved total nitrogen removal. During the 1st phase, mean TN removal efficiency was 52.8 %, with mean mass removal rate of 7.1 g/m^2.day. Mean TN load was 12.4 g/m^2.day in phase 1 and 8.8 g/m^2.day in phase 2, **Figure 4-28**. The lower TN removal rate resulted from high nitrification rate in the filter and insufficient denitrification. While, during the 2nd phase, average TN removal efficiency was enhanced to 60 % with sufficient denitrification, with mean mass removal rate of 5.5 g/m^2.day.

As mentioned before, TN is removed via microbial processes. Adsorption, plant uptake and NH_3 volatilization process can also be involved in TN removal (Kadlec & Wallace, 2009, Vymazal, 2007). Plant uptake and NH_3 volatilization are neglected in this unplanted system in that NH_3 volatilization requires a pH of approximately 9 (Vymazal, 2007), therefore, the microbial process was the main process for TN removal. However, there was no clear trend that TN removal is influenced with temperature. Several studies have documented that CWs treatment efficiency decreased with decreasing water temperature (Allen *et al.*, 2002, Kuschk *et al.*, 2003).

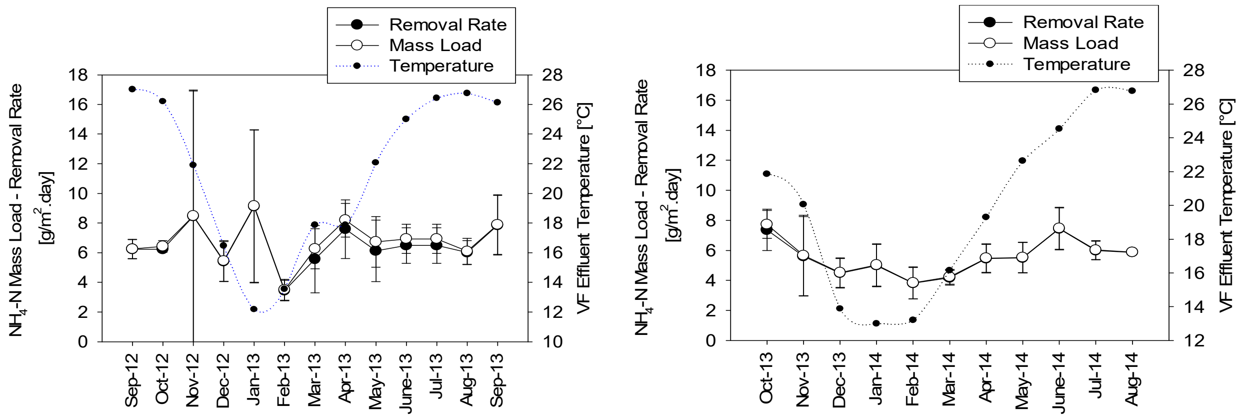
Figure 4-29 shows NH_4^+-N removal rate. The average NH_4^+-N mass load was 6.8 g/m^2.day in phase 1 and 5.5 g/m^2.day in phase 2. ECO-1 removed NH_4^+-N effectively, on average removal efficiencies of 96.5 % and 98.8 %, with mean mass removal rates of 6.6 g/m^2.day and 5.4 g/m^2.day in phase 1 and 2, respectively. There was no significant different between 1st and 2nd phase results ($p < 0.05$) over the study period.



a) b)

Figure 4-28: Monthly TN removal rate, SD (error bars) and the VF effluent temperature over the study period, a) phase 1, b) phase 2.

Ammonification occurs under anaerobic conditions (Kadlec & Wallace, 2009, Vymazal, 2007). High NH_4^+-N removal showed high nitrification rate, resulting in high NO_3^--N concentration in effluent. Similar results were documented by Von Felde and Kunst (1997) that more than 90 % NH_4^+-N removal was reported in an intermittent vertical filter with sufficient oxygen. NH_4^+-N removal rates of the system were independent on water temperature. In contrast, it was reported that temperature below 10°C could inhibit the nitrification as a result of low activity of nitrifying bacteria (Xie *et al.*, 2003).

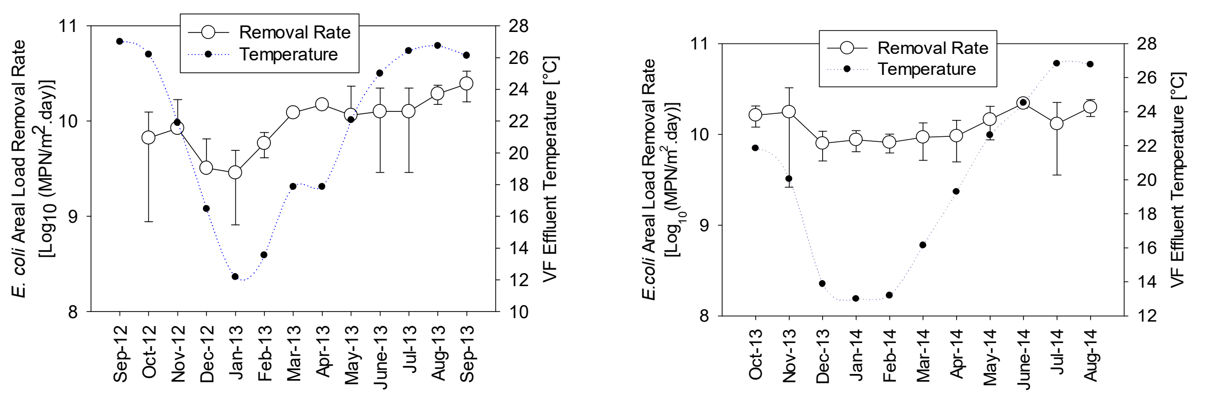


a) b)

Figure 4-29: Monthly NH_4^+-N removal rate, SD (error bars), and the VF effluent temperature over the study period, a) phase 1, b) phase 2.

4.3.3.4 *E. coli* Areal Load Removal

E. coli areal removal rates over the course of the study are shown in **Figure 4-30**. No apparent effect of seasonal variability on *E. coli* reduction over the study course. A total of 2 log reductions were removed from the system, but the *E. coli* concentrations were still higher than the national JS. Similar results were obtained by Sklarz et al. (2009) that 2 log reductions were achieved by a recirculating VFCW and further 1 log was achieved using a UV irradiation unit. In this study, further disinfection was provided using subsurface irrigation system.



a) b)

Figure 4-30: Monthly $\log_{10}$ *E. coli* areal removal rates with SD (error bars) of the recirculating system over the study period, a) phase 1, b) phase 2.

4.3.4 ECO-2 Treatment Performance

The treatment performance of the two-stage VFCW system was evaluated by the conformity of effluent to the JS. The system was monitored in two phases: phase 1; monitoring the system in a steady state (February 2012 to September 2013), and phase 2; monitoring of altered system via raw step-feeding application (October 2013 to August 2014). The results of 1st and 2nd phases were statistically compared (ANOVA) to examine the optimization of TN removal with associated parameters.

4.3.4.1 Physico-chemical Parameters

Results of pH, EC , DO, redox potential, turbidity and TSS were summarized (Mean and SD) in **Table 4-5**. The mean values of the previous parameters were statistically similar ($p < 0.05$) during phase 1 and 2.

The mean pH values ranged from 7.3 to 7.7, which conform to the JS (6 - 9). In the 1st stage, the pH decreased due to OM decomposition and nitrification. However, there was no significant change in pH values during treatment process.

The effluent EC was reduced gradually until 1st stage VF (unplanted bed), on average 1682 µS/cm in the 1st phase and 1527 µS/cm in the 2nd phase due to high settlement of suspended particles and elements (Bitton, 1994, Kadlec *et al.*, 2000). Subsequently, the effluent EC increased through filtration in the 2nd stage (planted bed), on average 1969 µS/cm and 1854 µS/cm in the 1st and 2nd phase, respectively. The EC increment can be explained by increasing salts concentrations due to water loss via evapotranspiration. Furthermore, plant root exudates stimulate solubility of some salts and elements from the VF bed matrix. High EC variation (SD) was observed over the study course related to the varying received water quantity and quality. The EC results conformed to the JS (0.7 - 3000 µS/cm) over the study period.

During phase 1, effluent DO concentration rose substantially through 1st and 2nd stage of 5.6 and 7 mg/L, respectively, compared to the raw wastewater of 0.7 mg/L. High DO effluent is a result of intermittent load and plants oxygenation. Vymazal et al. (1998) reported that *Phragmites australis* species has a transfer potential of 2 $gO_2/m^2.d$ to the root zone. During step-feeding application, mean DO content in the 2nd stage was diminished to 4.8 mg/L due to mixing with un-oxygenated wastewater. Effluent DO content conformed to the JS (higher than 2 mg/L) over the study period.

Results of redox potential over the course of the study are shown in **Figure 4-31**. During phase 2, redox measurements increased to 148 and 177 mV in the 1st and 2nd stage, respectively. In step-feeding (mixing) tank, low redox level was measured, on average of 28 mV, providing anoxic condition for denitrification.

Table 4-5: Influent and effluent physico-chemical parameters (means ± SD) and number of samples (N) of each component in the two-stage VFCW during the course of study (phase 1 and 2).

		pH		EC [µS/cm]		DO [mg/L]		Redox Potential [mV]		TSS [mg/L]		Turbidity [NTU]	
	Parameter	N		N		N		N		N		N	
1st Phase	**Raw**	72	7.4 ± 0.2	72	1808.5 ± 218.9	70	0.7 ± 0.7	70	-249.2 ± 126.8	62	465.5 ± 379	62	402.2 ± 216.6
	Septic Tank	70	7.5 ± 0.2	70	1918.5 ± 212.7	70	1.8 ± 1.5	70	-262.4 ± 83.3	62	122.6 ± 77.5	62	134.8 ± 77.7
	1st stage VF	70	7.4 ± 0.2	70	1681.9 ± 172.9	70	5.6 ± 0.9	70	77.8 ± 125.7	62	3.8 ± 2.8	62	5.5 ± 5.7
	2nd stage VF	69	7.7 ± 0.2	70	1968.8 ± 372.5	69	7.0 ± 1.1	69	116.3 ± 120.2	62	1.6 ± 1.6	62	1.3 ± 1.7
2nd Phase	**Raw**	44	7.6 ± 0.3	44	1617.7 ± 179.7	44	0.6 ± 0.5	40	-231.6 ± 58.8	41	311 ± 92.5	44	434.8 ± 236.9
	Septic Tank	44	7.5 ± 0.2	44	1743.2 ± 181.8	44	1.5 ± 0.9	42	-235.9 ± 48.9	41	106.9 ± 56.4	44	230.3 ± 116.5
	1st stage VF	44	7.6 ± 0.2	44	1527.4 ± 143.3	44	6.0 ± 0.8	42	147.5 ± 84.6	41	3.9 ± 1.9	44	11.4 ± 10.8
	Step-feeding Tank	35	7.7 ± 0.2	35	1525.5 ± 148.4	35	1.5 ± 1.2	33	28.4 ± 141.1	33	84.8 ± 56.6	35	129.9 ± 84.9
	2nd stage VF	44	7.3 ± 0.2	44	1853.5 ± 166.3	44	4.8 ± 0.8	44	176.9 ± 118.9	41	3.6 ± 1.8	44	10.5 ± 11.2

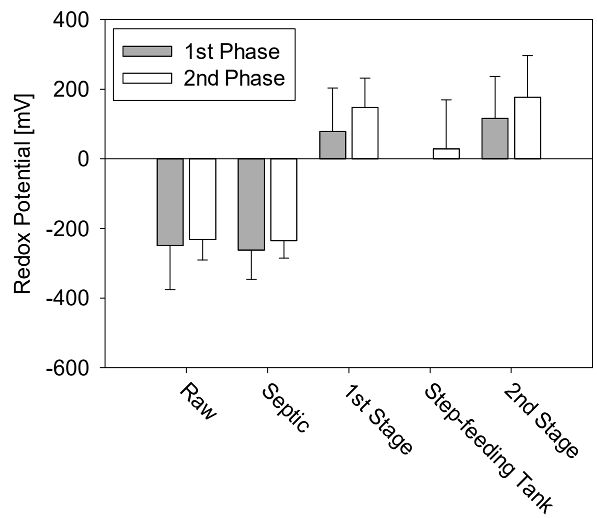


Figure 4-31: Influent and effluent redox mean values and SD (error bars) over the course of the study (phase 1 and 2).

4.3.4.2 Total Suspended Solids (TSS) and Turbidity

Figure 4-32.a shows the mean TSS concentrations of each component in the system compared to the JS over the study period. During phase 1, high variation (SD) in TSS influent was observed due to the changes in the quantity of water usage. TSS concentrations were reduced to 3.8 mg/L in the 1st stage and reduced to 1.6 mg/L in the 2nd stage, indicating high TSS removal via sedimentation and filtrating capacity. During phase 2, the mean TSS effluent of 2nd stage was measured of 3.6 mg/L due to additional TSS from raw step-feeding. Effluent TSS concentrations were highly compatible with the JS (class A: less than 50 mg/L).

Figure 4-32.b presents the turbidity values of each component in the system with the JS during the study period. During phase 1, mean turbidity values were reduced from 403 to 5.5 NTU in the 1st stage and were reduced continuously through the 2nd stage to 1.3 NTU. During phase 2, turbidity levels were reduced from 435 to 11.4 NTU in the 1st stage and were reduced by the 2nd stage to 10.5 NTU. Both beds show lower turbidity removal in the 2nd phase compared with the 1st phase, as observed in the recirculating system. Therefore, during phase 2, turbidity removal was reduced substantially in the VFCW beds as a result of low filtrating capacity and low adsorption process over time. It is also correlated with hydraulic overloading, which reduce the hydraulic residence time and filtering time during phase 2. Furthermore, high turbidity in the 2nd stage effluent could be caused by raw step-feeding application. However, effluents turbidity conformed to the JS (less than 10 NTU). Comparing 1st and 2nd phase effluents, TSS concentrations were statically similar ($p < 0.05$) and turbidity of vertical filter effluents was statically different at $p < 0.01$.

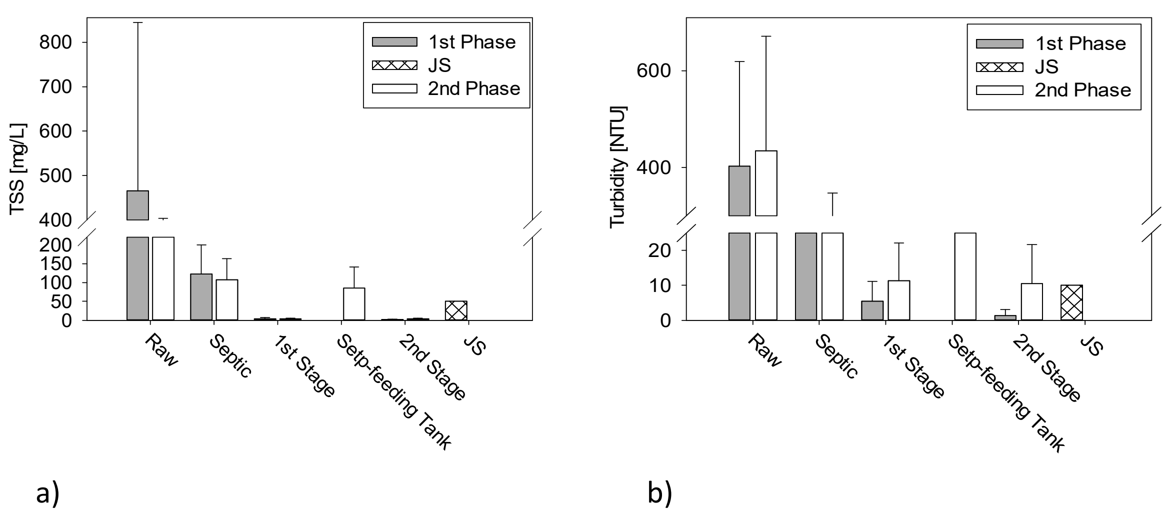


Figure 4-32: Influent and effluent TSS and Turbidity mean concentrations and SD of each component in the two-stage VFCW with the JS -class A-, a) TSS concentrations during phase 1 and 2, b) Turbidity concentrations during phase 1 and 2.

4.3.4.3 Organic Matter (OM)

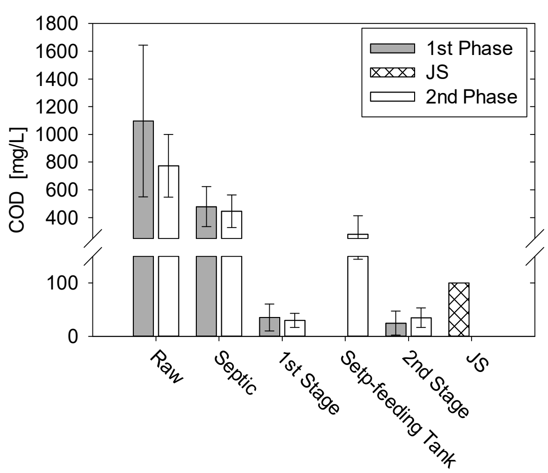
Table 4-6 summarizes the COD and BOD_5 results of each component in the system.

COD

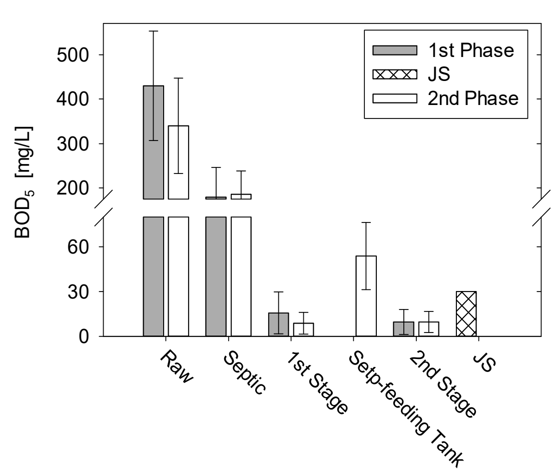
Results of influent and effluent COD concentrations conformed to the JS, **Figure 4-33.a**. The COD concentrations reduced from 1096 to 25 mg/L during the 1st phase and reduced from 774 to 35 mg/L during the 2nd phase, indicating the effectiveness of VFCWs in eliminating OM even with raw step feeding application. Similar effluent COD concentrations were reported by Langergraber *et al.* (2009) in planted two-stage VFCW with *Phragmites*, in Vienna. High COD variation caused high SD on a few occasions over the study period. The highest COD removal was observed in the septic tank which was in accordance with other VFCW studies (Stefanakis & Tsihrintzis, 2009). In other studies, step-feeing application was reduced the COD removal as reported by Stefanakis *et al.* (2011), who compared a HFCW with and without step-feeding.

BOD_5

Results of influent and effluent BOD_5 concentrations conformed to the JS, **Figure 4-33.b**. Mean BOD_5 concentration was reduced from 430 to 15.7 mg/L in the 1st stage and 9.7 mg/L in the 2nd stage filter. OM is removed through aerobic and anaerobic bacterial activity (Vymazal, 2002). Moreover, results indicated fast organic degradation rate in the 1st stage filter via by physical and biological mechanisms, which is in agreement with other VFCW studies (Stefanakis & Tsihrintzis, 2009). During phase 2, BOD_5 concentrations were reduced from 340 to 8.8 mg/L in the 1st stage filter. Mean BOD_5 effluent of step-feeding tank was found to be 54 mg/L and it was reduced to 7.1 in the 2nd stage filter. The results indicated high effectiveness in eliminating OM over the study period. Similar results were documented by Langergraber *et al.* (2009). Comparing the 1st and 2nd phase effluents, COD and BOD_5 concentrations were statically similar ($p < 0.05$).



a)



b)

Figure 4-33: Influent and effluent COD and BOD_5 mean concentrations and SD (error bars) of each component in ECO-2 system with the JS (class A) , a) COD concentrations during the 1st and 2nd phase of the study, b) BOD_5 concentrations during the 1st and 2nd phase of the study.

Table 4-6: Two-stage VF system influent and effluent water quality (means ± SD) and number of samples (N) of each component during the experimental study (1st and 2nd phase).

			BOD_5 [mg/L]		COD [mg/L]		TN [mg/L]		NH_4^+-N [mg/L]		NO_3^--N [mg/L]		NO_2^--N [mg/L]
	Parameter	N		N		N		N		N		N	
1st Phase	**Raw**	72	429.9 ± 123	69	1095.9 ± 547.8	71	107.3 ± 47.7	70	60.8 ± 19	71	0.5 ± 0.6	63	0.06 ± 0.1
	Septic Tank	63	179.8 ± 67	70	478.5 ± 144.3	68	99.8 ± 27	70	74.4 ± 15.9	64	0.4 ± 0.2	61	0.02 ± 0.02
	1st stage VF	63	15.7 ± 14.1	70	35.6 ± 25.5	68	74 ± 23.2	69	0.5 ± 0.44	65	65.7 ± 18.5	61	0.32 ± 0.01
	2nd stage VF	62	9.7 ± 8.4	70	24.9 ± 22.6	62	76.5 ± 24.7	66	0.02 ± 0.02	64	76.0 ± 28.9	59	0.03 ± 0.03
2nd Phase	**Raw**	44	340.1 ± 108	44	773.8 ± 225.8	44	84 ± 17	44	52.4 ± 14.8	43	0.9 ± 1.2	44	1.1 ± 0.1
	Septic Tank	43	185.9 ± 52.2	42	445.7 ± 117.8	41	84 ± 17	44	66.1 ± 16.9	43	1.2 ± 1.4	44	0.05 ± 0.1
	1st stage VF	43	8.8 ± 7.3	42	30.2 ± 13.3	43	70.3 ± 15.4	44	147.5 ± 84.6	40	53.5 ± 12.7	44	0.35 ± 0.7
	Step-feeding Tank	34	53.9 ± 22.5	32	279.2 ± 134.9	34	64 ± 16.2	35	23.9 ± 28.4	34	35.7 ± 15.3	34	1.7 ± 1.1
	2nd stage VF	43	9.7 ± 7.7	42	34.9 ± 18.4	37	52.4 ± 14.9	42	0.2 ± 0.3	43	50.2 ± 21.9	44	0.3 ± 0.3

4.3.4.4 Nitrogen Transformations

Results of TN, NH_4^+-N, NO_2^--N, and NO_3^--N are shown in **Table 4-6**.

Total Nitrogen (TN)

Results of influent and effluent TN concentrations during the investigation period with the JS are depicted in **Figure 4-34**. Mean TN concentration was reduced from 107 to 77 mg/L through ECO-2 system. The TN elimination in the system limited due to carbon deficiency that struggled denitrification. Thus, combination of nitrification and denitrification processes is necessary to achieve higher TN treatment efficiency (Vymazal, 2007, Langergraber *et al.*, 2009). Plant uptake is a minor nitrogen removal mechanism, while microbial transformations provide the majority of TN removal (Kadlec & Knight, 1996). Gersberg *et al.* (1983) reported that the role of plants was minimal in TN removal in constructed wetlands.

Step feeding can effectively solve the insufficiency of carbon source to promote denitrification (Stefanakis *et al.*, 2011). Many studies reported the improvement of TN removal in VF and HF CW by adopting a step-feeding strategy (Stefanakis *et al.*, 2011, Li *et al.*, 2014). External carbon source actuates denitrifies activity, therefore, high TN elimination rate will be achieved. Mean TN concentrations were reduced from 84 to 70 mg/L in the 1st stage and were reduced to 52 mg/L in the 2nd stage.

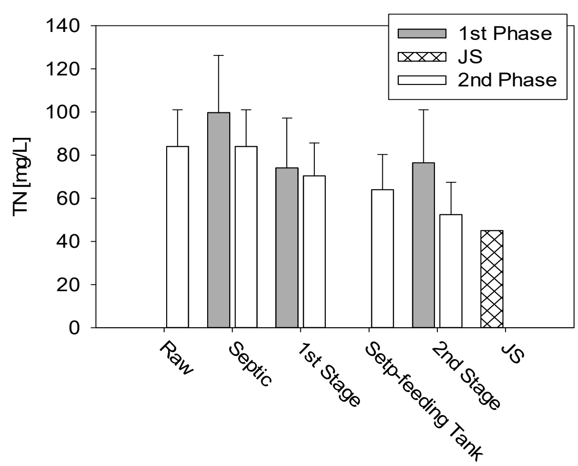


Figure 4-34: Influent and effluent TN mean concentrations, SD (error bars) and the JS (class A) over the course of the study.

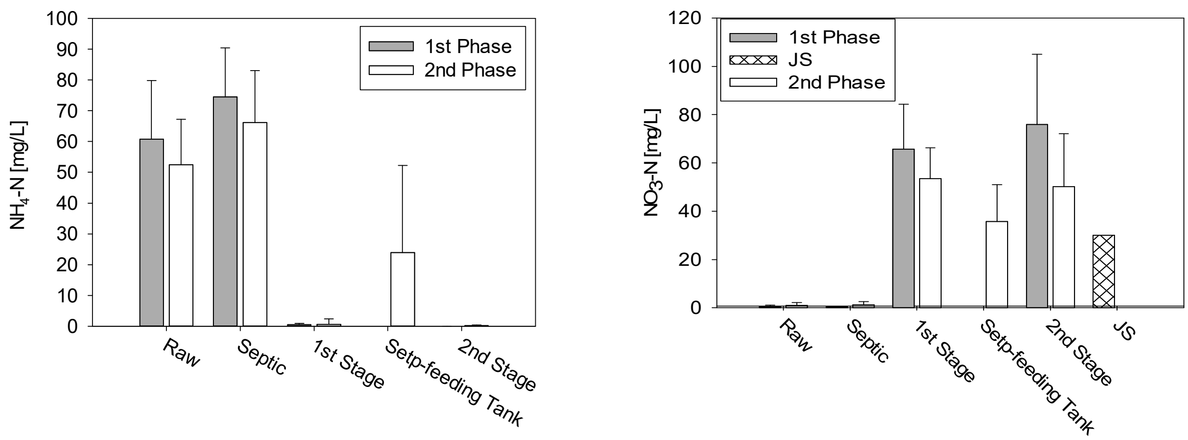
The TN removal for phase 1 and phase 2 was statistically different ($p < 0.001$). The phase 2 effluent TN concentration conformed to the JS (class B: 70 mg/L).

Ammonium Nitrogen (NH_4^+-N)

Mean NH_4-N concentrations were reduced from 61 to 0.02 mg/L during the 1st phase and were reduced from 52 to 0.2 mg/L during the 2nd phase, **Figure 4-35.a**. No significant difference in NH_4-N concentrations were observed during 1st and 2nd phase ($p < 0.05$), indicating high conversion of NH_4 to NO_3 in the VF bed, which revealed high DO level and nitrifying bacteria density, achieving a nitrified effluent, which is in agreement with Saeed and Sun (2012), Gray (2004) and Arias *et al.* (2005).

Nitrate Nitrogen (NO_3^--N)

During the 1st phase, mean NO_3^--N effluent concentrations were increased throughout 1st and 2nd stage, with average effluent concentrations of 66 and 76 mg/L, respectively. NO_3^--N level was higher than the recommended levels in the JS (30 mg/L), **Figure 4-35.b**. During the 2nd phase, mean effluent NO_3^--N concentration was decreased in the 2nd stage to 50.2 mg/L as a result of stirring denitrification in the step-feeding tank. Availability of carbon source combined with anoxic condition and sufficient retention time increased denitrification in the system. However, NO_3^--N concentration in the second phase was still higher than class A in the JS, but it conformed to the JS class B. Mean NO_3^--N concentrations in the 1st and 2nd phase were statistically compared; results indicated that effluents were significantly different ($P < 0.001$).



a) b)

Figure 4-35: Influent and effluent NH_4^+-N and NO_3^--N mean concentrations and SD (error bars) of each component in the two-stage VFCW system, a) NH_4^+-N concentrations during 1st and 2nd phase, b) NO_3^--N mean concentrations over the study period with the JS (class A).

4.3.4.5 *E. coli* Reduction

E. coli concentrations (geometric means and SD) of each component in the system are presented in **Table 4-7** and compared with the JS in **Figure 4-36**. The influent *E. coli* geometric mean was 8.7×10^6 and 1.2×10^7 MPN/100 mL during 1st and 2nd phase, respectively. In phase 1, *E. coli* removal conformed to the JS, class B. *E. coli* concentrations were gradually decreased of 4×10^5 and 3.4×10^2 in the 1st and 2nd stage filter, respectively. *E. coli* removal was higher in planted 2nd stage, which is in agreement with other studies (Decamp & Warren, 2000b, Karathanasis *et al.*, 2003) that report planted filters improve pathogen removal. The plants improve the filtration capacity and provide higher surface area for microorganisms (Brix, 1994a).

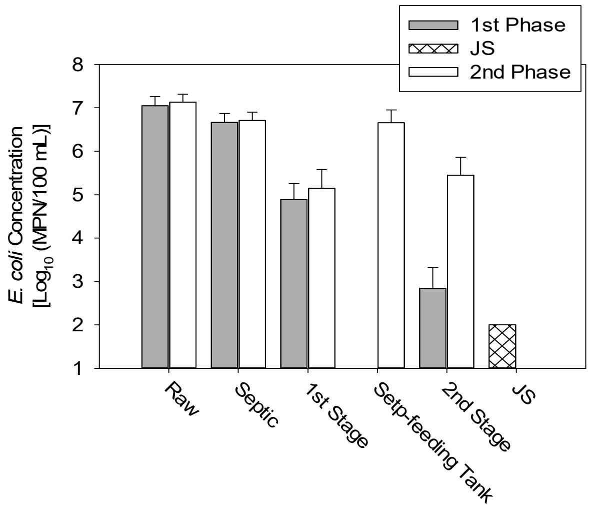


Figure 4-36: *E. coli* geometric means with SD (error bars) over the course of the study.

During the 2nd phase, *E. coli* removal was influenced by *E. coli* from raw wastewater step-feeding, therefore, the removal rate was decreased. *E. coli* concentrations were ranged of 5.2×10^4 and 5.5×10^4 in the 1st and 2nd stage filter, respectively. 3.5 log reductions were achieved during step-feeding application; however, the effluent was conformed to the JS (class C). Additionally, hydraulic overloading issue increased the pathogen input in the 2nd stage. Geometric means *E. coli* in the 1st and 2nd phase were significantly different ($P < 0.001$).

Table 4-7: *E. coli* geometric mean concentrations of each component in the two-stage system.

			***E. coli* [MPN/100 mL]**
	Parameter	N	
1st Phase	**Raw**	53	$8.7 \times 10^6 \pm 7.0 \times 10^6$
	Septic Tank	59	$3.8 \times 10^6 \pm 2.8 \times 10^6$
	1st stage VF	59	$4.0 \times 10^4 \pm 1.0 \times 10^5$
	2nd stage VF	58	$3.4 \times 10^2 \pm 1.4 \times 10^3$
2nd Phase	**Raw**	40	$1.2 \times 10^7 \pm 7.1 \times 10^6$
	Septic Tank	40	$4.4 \times 10^6 \pm 2.8 \times 10^6$
	1st stage VF	40	$5.2 \times 10^4 \pm 2.4 \times 10^5$
	Step-feeding Tank	39	$7.7 \times 10^5 \pm 4.4 \times 10^6$
	2nd stage VF	40	$5.5 \times 10^4 \pm 4.4 \times 10^5$

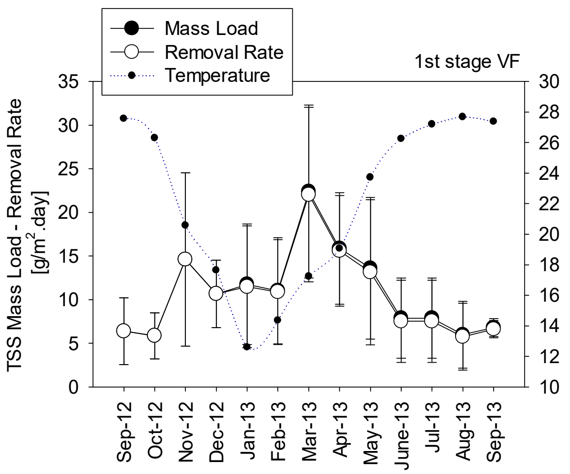
4.3.5 ECO-2 Pollutant Removal Evaluation and Seasonal Variability

Mass removal rate per unit area of TSS, BOD_5, COD, TN, NH_4^+-N, and *E. coli* during the 1st and 2nd phase are evaluated and compared in this part. In addition, monthly removal mean of the previous parameters are presented over the course of the study to assess the treatment performances under temperature variability.

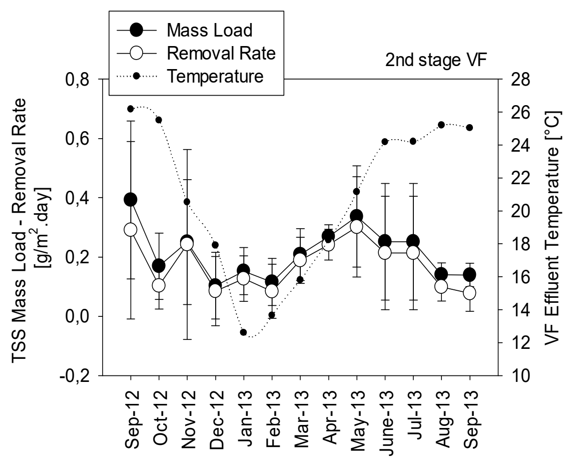
4.3.5.1 TSS Removal

During the 1st phase, TSS highly removed through two-stage VF system, on average removal efficiencies of 99.1 and 99.8 % in the 1st and 2nd stage, respectively. **Figure 4-37** shows high TSS removal rate over the study period. Despite season and temperature, TSS was greatly removed via higher filtering capacity. The majority of TSS was removed throughout 1st stage. The TSS mean mass removal rates were 10.7 g/m^2.day in the 1st stage and 0.2 g/m^2.day in the 2nd stage, which was highly related to mean mass load of 11.0 and 0.2 g/m^2.day in the 1st and 2nd stage, respectively.

During step-feeding application, TSS removal was effective and stable with average efficiencies of 99 and 97.8 % in the 1st and 2nd stage, respectively. The TSS mean mass removal rates were found to be 7.9 g/m^2.day in 1st stage and 0.4 g/m^2.day in 2nd stage, which was highly related to mean mass load of 8.1 and 0.7 g/m^2.day in the 1st and 2nd stage, respectively. In comparison, there was no significant difference on TSS removal ($p < 0.05$) over the study period, indicating high TSS treatment performance with step-feeding operating.



a)



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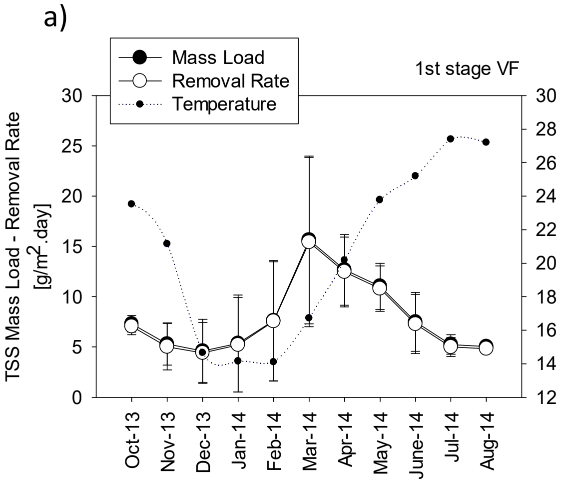

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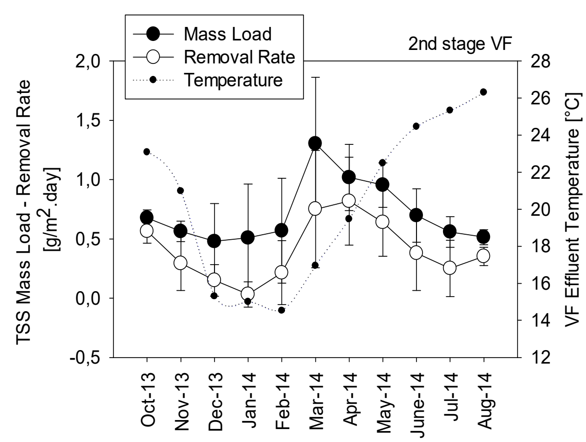
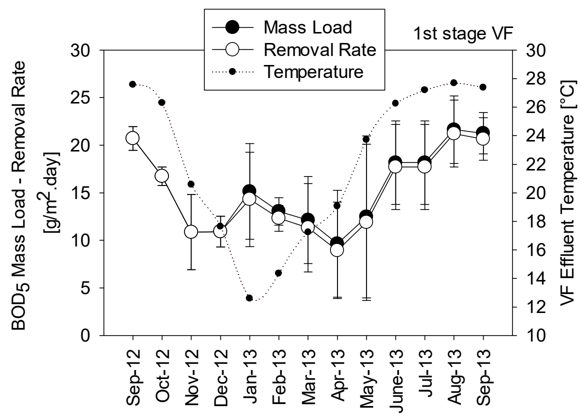


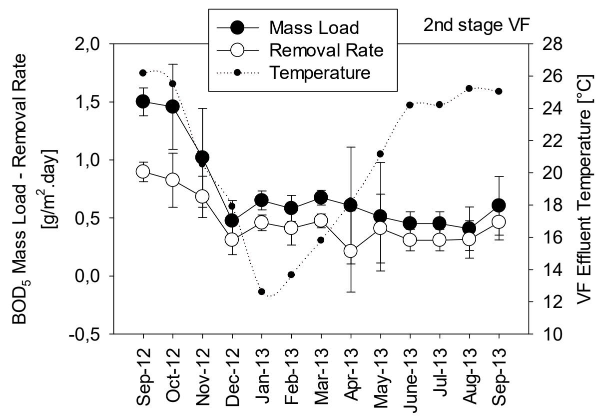
d)

Figure 4-37: Monthly TSS load, removal rate, SD (error bars), and effluents temperature, a) and b) during phase 1, c) and d) phase 2.

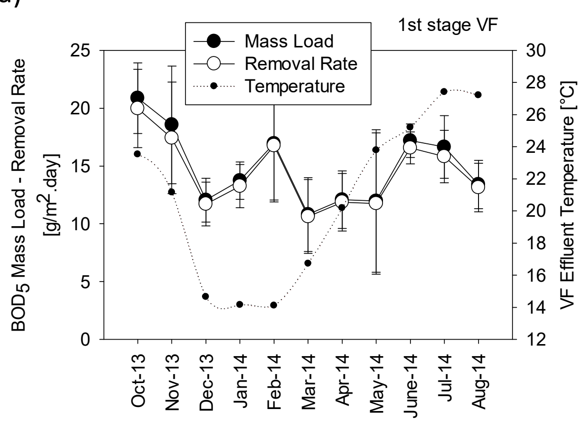
4.3.5.2 Organic Matter Removal

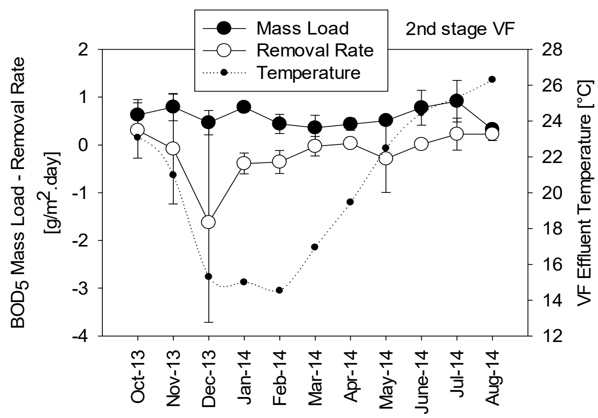
BOD_5 removal was independent on water temperature over the study period, **Figure 4-38**. During phase 1, effective and stable removal rates were observed, on average removal efficiencies of 98.2 and 99 % in the 1st and 2nd stage, respectively. OM was removed via sedimentation and microbial decomposition (Kadlec, 1999b). On the other hand, Herouvim *et al.* (2011) reported that higher OM was removed by planted VFCWs due to high oxygen capacity, microbes and bacteria activity in the root zone. The BOD_5 mean mass removal rates were 14.7 g/m^2.day in the 1st stage and 0.2 g/m^2.day in the 2nd stage, which was highly related to mean mass load of 15.2 and 0.7 g/m^2.day in the 1st and 2nd stage, respectively. Most of the BOD_5 was already removed in the 1st stage.





a) b)



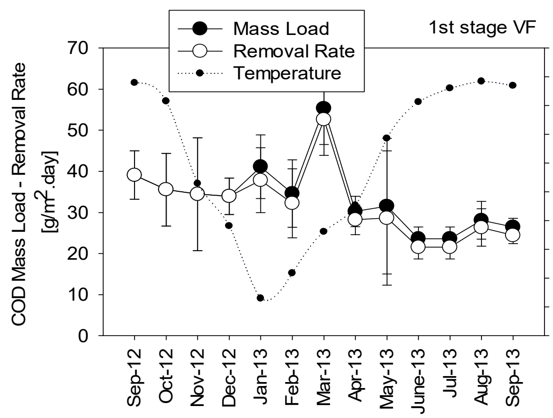


c) d)

Figure 4-38: Monthly BOD_5 load, removal rate, SD (error bars), and effluents temperature, a) and b) phase 1, c) and d) phase 2.

During phase 2, BOD_5 was highly removed even with step-feeding application, on average removal efficiencies of 98.1 and 93.9 % in the 1st and 2nd stage, respectively. The BOD_5 mean removal rate was 14.5 g/m^2.day in the 1st stage and -0.2 g/m^2.day in the 2nd stage, and mean mass load of 15 and 0.6 g/m^2.day in the 1st and 2nd stage, respectively. These differentiations also could be appeared as a result of hydraulic overloading in the 2nd stage that it was increased the concentrations of pollutants. BOD_5 removal rate did not respond to seasonal changes over study period. In accordance to many studies have documented negligible temperature influence on organic matter removal in CWs (Vymazal, 1999, Wallace & Knight, 2006). There was no significant difference on BOD_5 removal rate ($p < 0.05$) over the study period.

Figure 4-39 shows COD removal over the course of the study period. During phase 1, COD mean removal efficiencies were 97.1 and 98.7 % in the 1st and 2nd stage, respectively. The COD mean removal rate was 29.0 g/m^2.day in the 1st stage and 1.4 g/m^2.day in the 2nd stage, which was highly compatible with mean mass load of 31.4 and 2.1 g/m^2.day in the 1st and 2nd stage, respectively. On the other hand, COD was highly removed in the 1st stage and was slightly reduced during step-feeding adoption in the 2nd stage that could ascribe to the enhanced denitrification efficiency (Fan, Liang, *et al.*, 2013). The COD mean removal efficiencies were 97.1 and 90.8 % in the 1st and 2nd stage, respectively. The COD mean removal rate was 22.0 g/m^2.day in the 1st stage and 2.5 g/m^2.day in the 2nd stage, with mean mass load of 23.6 and 2.8 g/m^2.day in the 1st and 2nd stage, respectively.



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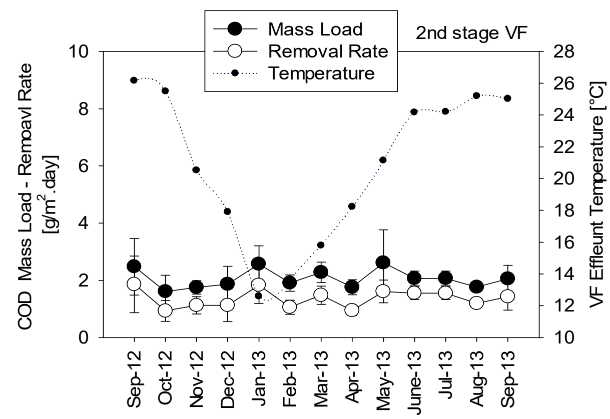

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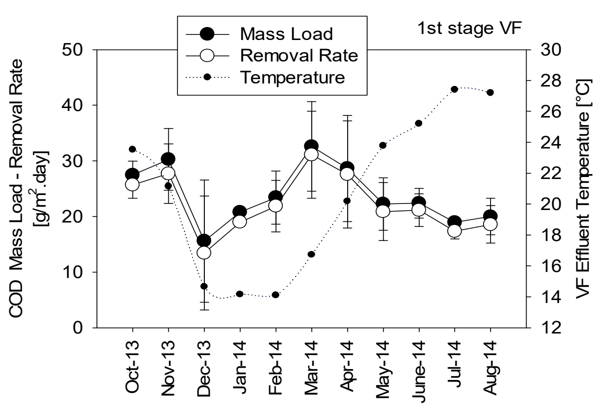

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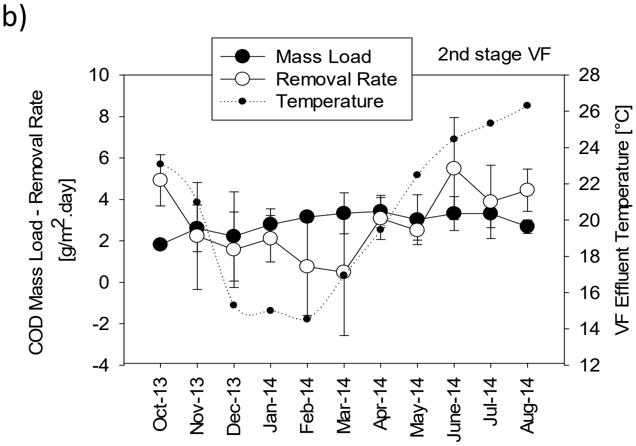


d)

Figure 4-39: Monthly COD load, removal rate, SD (error bars), and effluents temperature, a) and b) phase 1, c) and d) phase 2.

Overall, this system functioned well with step-feeding despite the hydraulic overloading issues, which showed a slight reduction in COD treatment performance. The COD removal rates in the current study are in agreement with the 91 % documented by Fan, Liang, *et al.* (2013) which also used step-feeding in an intermittently dosed planted VF wetland. COD removal did not show clear impact of low temperature, which was agreed by Vymazal (2011). The COD removal rate was significantly similar ($p < 0.05$) over the study period.

4.3.5.3 TN Removal Rate

Figure 4-40 shows TN mass removal over the study period. During phase 1, TN mean removal efficiencies were 44 and 54.6 % in the 1st and 2nd stage, respectively. The TN mean removal rate was 5.2 g/m^2.day in the 1st stage and 1.9 g/m^2.day in the 2nd stage, with mean mass load of 9.7 and 4.2 g/m^2.day in the 1st and 2nd stage, respectively.

TN was removed in the 1st stage due to effective nitrification under aerobic conditions. NO_3^--N was not removed in the 2nd stage as a result of lack denitrification capacity. Denitrifying bacteria use organic compounds as electron donors and a source of cellular carbon and using nitrate as an electron acceptors (Vymazal, 2007).

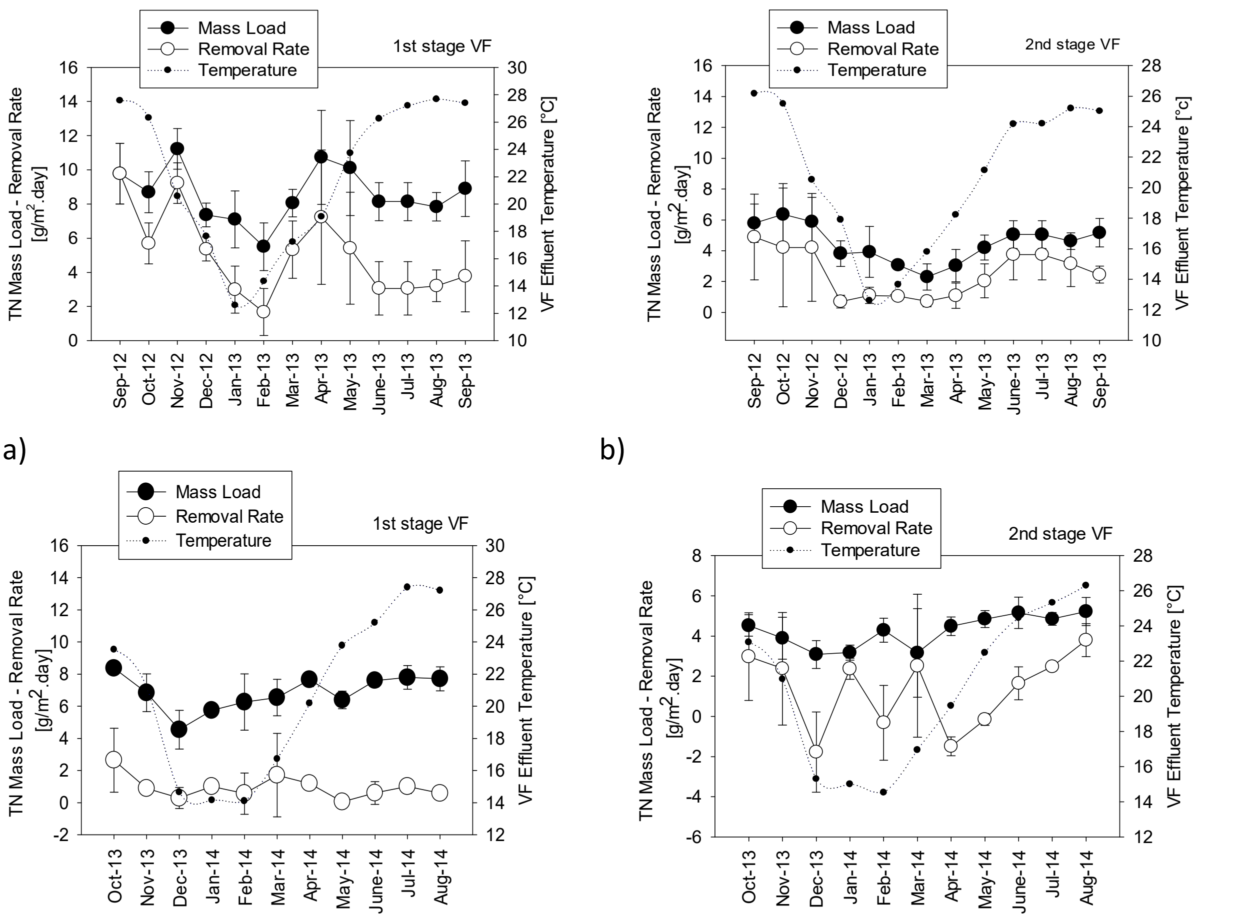


Figure 4-40: Monthly TN load, removal rate, SD (error bars), and effluents temperature, a) and b) phase 1, c) and d) phase 2.

On the other hand, many studies documented that microbial density and activity are enhanced in the plant rhizosphere (Brix & Arias, 2005a, Kadlec & Wallace, 2009). Lin *et al.* (2002) reported 4 - 11 % of TN was removed by plant uptake in a planted wetland. Nevertheless, the results indicated that TN removal by plant uptake is small, which was also reported by Keffala and Ghrabi (2005).

The operational modification enhanced the TN removal, even it was overloaded two times, which reduced the residence time and increased the pollutant mass load. Mean TN removal efficiencies were 40.7 and 45.7 % in the 1st and 2nd stage, respectively. Similar results obtained by Wang *et al.* (2010) that TN removal was enhanced form 55 to 65 % by providing carbon source for denitrification using a shunt distributing wastewater in a VFCW. Lin *et al.* (2002) reported the effective NO_3 removal efficiency (89 - 90 %) using fructose as a carbon source (3.5 of COD: N ratio). The TN mean mass removal rate was 2.5 and 1.6 g/m^2.day in the 1st and 2nd stage, with mean mass load of 6.8 and 4.2 g/m^2.day in the 1st and 2nd stage, respectively. There was a significant difference in TN removal ($p > 0.001$) over the study period

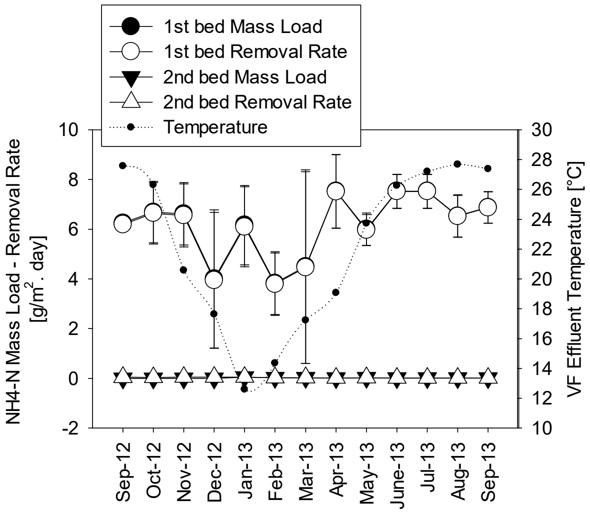
NH_4^+-N removal was high over the study period, as depicted in **Figure 4-41**. During the 1st phase, NH_4^+-N average removal efficiencies were 99.3 in the 1st stage that all NH_4^+-N removed through 1st stage. Intermittent dosing has been shown to improve NH_4^+-N removal by adding more oxygen the filter, increasing the nitrifying bacteria density and activity (Fan *et al.*, 2013). Jia *et al.* (2010) observed that NH_4^+-N removal was 93.9 % in an intermittently dosed VFCW microcosm, while TN removal was 46.86 % due to carbon deficiency. NH_4^+-N mean removal rates were 6.3 g/m^2.day in 1st stage and 0.0 g/m^2.day in 2nd stage, which showed high nitrification capacity in 1st bed.

During the step-feeding phase, NH_4^+-N removal was effective with average efficiencies of 99 % in the 1st stage. The NH_4^+-N mean removal rates were 5.3 g/m^2.day in the 1st stage and 0.1 g/m^2.day in the 2nd stage. NH_4-N removal did not appear to be temperature dependent. While, a reduction in nitrifiers activity could be observed in temperature below 10 °C, similar trend with denitrifiers that its activity increased in higher temperature and decreased in low temperature (Faulwetter *et al.*, 2009). In comparison, there was no significant difference on NH_4^+-N removal ($p < 0.05$) over the study period, indicating high nitrification rate in the VF bed over the entire study period.

4.3.5.4 *E. coli* Areal Load Removal Rate

Figure 4-42 shows *E. coli* areal removal rates over the course of the study. During the 1st phase, *E. coli* removal was relatively high and the effluent conformed to the JS class A. *E. coli* removal for the two-stage system was 4.4 $\log_{10}$ reduction.

During the 2nd phase, *E. coli* removal was not as good due to step-feeding of raw wastewater to the second. In addition, accidental hydraulic overloading increased the pathogen input in the 2nd stage. Nonetheless, 3.5 log reduction was still achieved with step-feeding application, which conformed to the JS class C. The *E. coli* removal rates were statically significantly different at $p < 0.001$. *E. coli* removal did not appear to be temperature dependent.



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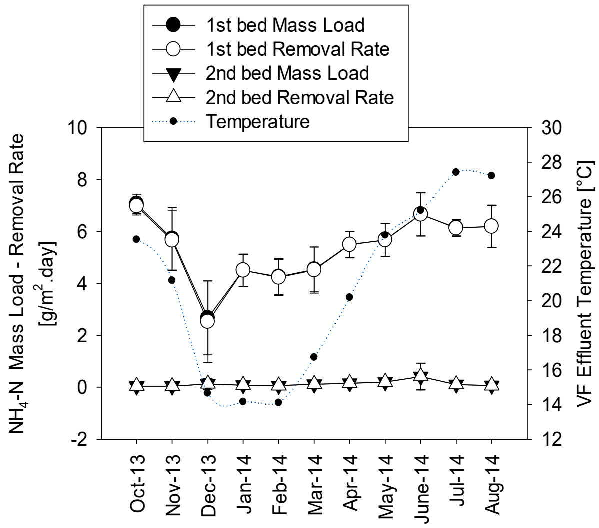
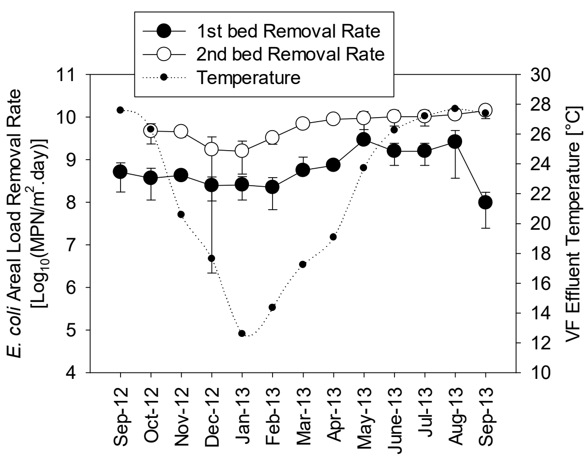


b)

Figure 4-41: Monthly NH_4^+-N mass load, removal rate, SD (error bars) and effluents temperature, a) phase 1 and b) phase 2.



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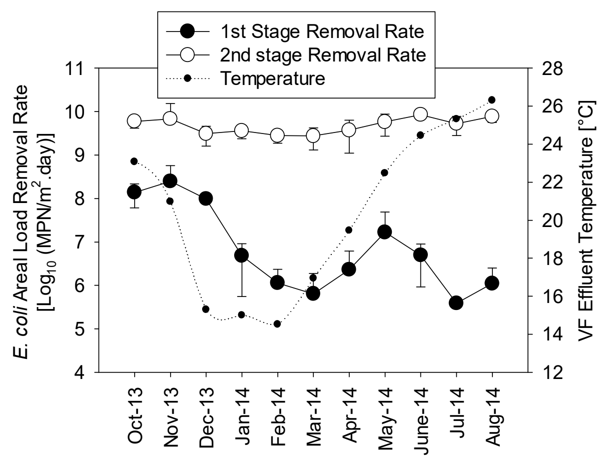


b)

Figure 4-42: Monthly $\log_{10}$ *E. coli* areal load removal rates with SD (error bars) and effluent temperature over the study period, a) phase 1, b) phase 2.

5. Reuse in Irrigation Field

5.1 Field Description and Methodology

The experimental reuse field at Fuhais facility is comprised of three plots, with total area of 330 m^2. The field was modified and leveled to accommodate the parallel plots. The plots were divided by terraces (50 - 70 cm height) that provided a surface area of 110 m^2 (10 m width and 11 m length) for each plot. The soils in all plots have been classified as clay loam based on the texture triangle of the US Department of Agriculture. The original soils were alkaline with high pH (7.7 - 8.4). The irrigation water was supplied by subsurface irrigation system from tap water, ECO-1 and ECO-2 effluent. The irrigation schedule was controlled by the PLC in the control room. The plots have been cultivated with lemon trees as a common cultivated plant in Jordan, which shows high adaptation with climatic conditions and it is economically essential fruit crop grown in the region. The lemon trees were 4 - 5 years old in the beginning of the study and were planted between irrigation lines with plant spacing 1.8 m, as shown in **Figure 5-1**. The plots are:

1. Plot (P1) (Control Unit), was irrigated by tap-water from tap tank.
2. Plot (P2), was irrigated by the ECO-2 effluent irrigation tank.
3. Plot (P3), was irrigated by the ECO-1 effluent irrigation tank.

Each plot had two parallel rows of five trees, while extra four trees have been planted in the control plot in case one of the trees died during the study. Furthermore, each plot was divided into two parts (A and B) related to supplied water quantities; subplot A received approximately 11 mm/day and subplot B received around 6 mm/day.

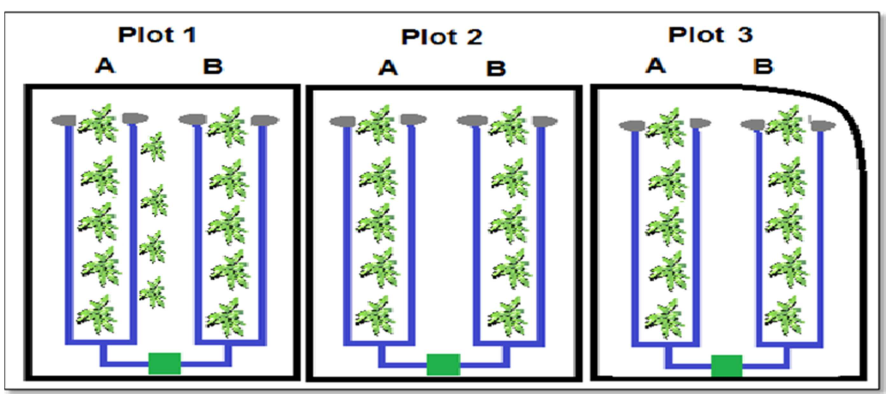


Figure 5-1: The experimental reuse plots layout.

According to the FAO guidelines (FAO, 2003), tap water is suitable for irrigation without any restriction, thus, it was used as a control in our research. In contrast, using treated wastewater should be controlled by guidelines in irrigation related to its potential impacts on soil and crops. During the spring, the control plot was fertilized one time using complex of N, P_2O_5, and K_2O (1 mg/L), when a yellowing pattern was significantly noticed in leaves, **Figure 5-2**. To avoid any leaching of fertilizer to the soils, the solution was sprayed to the leaves directly. Trees in the P2 and P3 did not show the yellowing pattern due to the self-nutrition by treated wastewater. While, trees in the P2 and P3 did not show the yellowing pattern due to the self-nutrition by treated wastewater.

Figure 5-2: The experimental irrigation plots with lemon trees in 2014.

5.1.1 Subsurface Irrigation System

The main objective of using subsurface irrigation system was to supply sufficient water to trees and prevented any contact between the effluents and workers or plants above the soil. Lamm and Camp (2007) reported that such systems enhanced nutrient usage efficiency when nutrients can be supplied through treated wastewater. Moreover, it minimizes the potential risks of human contact with treated wastewater and reduces the growing of undesired weeds when the water applied near the trees roots.

In Jordan, different forms of agricultural activities are used such as tillage and plow soil to prepare field for planting and removed the weeds, which would contest water and nutrients. Hence, the perforated irrigation pipes were installed in trenches at depth of 60 cm, the orifices were drilled every 0.5 m along pipes and its direction was upward, **Figure 5-3**. The excavated trenches have been filled with sand beneath distribution pipes, then the pipes covered by half cut big pipes (15 cm diameter) in order to boost the dispersion of effluent and expose water to air. In addition, sand increases the capillary rate and wetting pattern in the soil. The system has protected against root intrusion and biofilm development by installing a geotextile layers then filled with thin soil layer. Over the study period, weeds in the irrigation plots were removed by manual eradication of the roots. According to a maintenance program, the irrigation pipelines were designed to end with valve risers, which used to regular flushing in order to increase the longevity of the system and avoid emitter clogging by accumulated solids.

The irrigation was scheduled three times daily since February 2012 (at 8:00, 16:00, and 20:00). The water split for the subplots via a distribution valve. The water amount was measured by a flowmeter in each part. **Table 5-1** shows the approximate water quantity in each plot.

Table 5-1: The measured water quantities in A and B parts in each reuse plot, in April 2014.

Plot 1 (control / tap-water)		**Plot 2 (ECO-2 effluent)**		**Plot 3 (ECO-1 effluent)**	
A (L)	B (L)	A (L)	B (L)	A (L)	B (L)
135.0	68.0	119.4	50.2	111.4	64.2

Figure 5-3: The subsurface irrigation system under construction.

5.2 Experimental Data

Irrigation water analysis used for water modeling (Saturation Indices) in order to predict the water-soil interaction. The JS and the FAO guidelines (Ayers & Westcot, 1985) for treated wastewater in irrigation were used to evaluate the other measured parameters in the irrigation water.

5.2.1 Saturation Indices (SI)

The potential for chemical reaction during irrigation (dissolve or precipitate) can be determined by the chemical equilibrium of the water with the mineral phases. SI is one of the widely used methods to determine the activities of water with a mineral phase. SI is defined in **Equation 5-1**, as pointed out by Drever (1997):

$$\mathrm{SI} = \log(\mathrm{IAP}/\mathrm{K_{eq}}) \qquad \text{Equation 5-1}$$

Where:

IAP: Ionic Activity Product.

K_{eq}: mineral equilibrium constant at a given temperature.

If SI is equal zero (IAP = K_{eq}), the water is in equilibrium (saturated) with the mineral phase. If SI is negative (SI < 0, IAP < K_{eq}) the water is under-saturated with the mineral and it will dissolve the mineral from soil to reach the equilibrium state (dissolution reaction). If SI is positive (SI> 0, IAP > K_{eq}) the water is over saturated and mineral will precipitate in soil to reach the equilibrium state (precipitation reaction).

The SI for water samples (tap water and the effluents before and after modifications) were calculated using the laboratory and field measurements by Geochemical Modeling with PHREEQC (Parkhurst & Appelo, 1999) in AquaChem 2012.1 software. Calcite, halite, aragonite, gypsum, anhydrite, and dolomite were calculated for each sample as the most common minerals phases.

5.2.2 Soil Sampling and Analysis

Soil property changes in the soil of the reuse plots were assessed by investigating some soil physical, chemical, and biological properties over the study period. However, reuse is a long-term research so these results are considered as short-term investigation.

Before irrigation application, virgin soil samples were collected from each subplot at 0 - 20, 20 - 40, and 40 - 60 cm depths. Subsequently, a regular soil samples (every four months approximately) were collected at the same depths in each subplot to identify the impact of different water qualities and quantities on soil properties. Soil sample from each subplot was collected as a composite sample from three soil samples at the same depth. A screw auger and small handling spade (for shallow samples) were used to collect the soil samples in plastic bags. The equipment were disinfected and cleaned for microbiological analysis after each sample. In total, 18 soil samples were collected in each sampling event and analyzed for the same parameters in the soil laboratory in Al-Balqa Applied University.

5.2.2.1 Soil Analytical Methods

Soil samples were prepared and analyzed according to standards methods in the International Center for Agricultural Research in the Dry Areas (ICARDA, 2001, 2013). Samples were placed in a room for one day (air-drying), then samples were crushed and sieved using a 2 mm sieve for physical and chemical analyses.

Soil Physical Properties

Soil moisture (SM) content was determined in soil samples in oven-dry, using an electric oven with thermostat. 10 g of air-dried and sieved soil was dried at 105°C for 24 hour and reweighed after cooling down in a desiccator for 30 minutes. Soil moisture was calculated using **Equation 5-2**.

$$SM(\%) = \frac{[\text{wet soil(g)} - \text{dry soil(g)}]}{\text{dry soil(g)}} \qquad \text{Equation 5-2}$$

Particle size distribution was determined using Hydrometer method. 40 g of air-dried and sieved soil was reacted with 10 mL H_2O_2 and 60-mL dispersing solution in 500 L beaker. After 24 hour, the mixture transferred and stirred in distilled water (1 L), 40 seconds later silt and clay reading was taken by a hydrometer (sand 0.05 - 2 mm, silt 0.002 - 0.05 mm, and clay < 0.002 mm). The solution mixed again and after four hours, the clay reading was taken. Sand was calculated online using the Soil Texture Calculator-United States Department of Agriculture (USDA) from silt and clay results. The soil texture was classified using the USDA textural triangle.

Soil structure was determined two times during the study by dry aggregate method, using a set of sieves with different diameters (2.0, 1.4, 1.0, 0.7, 0.5, 0.35, 0.25, and 0.125 mm). Samples were collected and prepared without the sieving step. After shaking, weight of retained soil in each sieve was measured and calculated as a percent of each sieve size.

Infiltration tests were conducted in the plots at the beginning and end of the experiment. According to the FAO (1988) standard methods, the infiltration capacity was measured using a double ring infiltrometer. The outer ring prevents the lateral movement of water in the soil from the inner ring. Hereby, the vertical movement of water was measured in the inner ring by reporting the water level drop over time.

Soil chemical analysis
Soil organic matter (SOM) was determined using Weight Loss-on-Ignition (LOI 360 °C). 5 g of soil sample was dried at 105 °C to remove the SM. Sample was weighed and heated at 360 °C for two hours and re-weighted again after cooling. The SOM was calculated by **Equation 5-3**.

$$SOM(\%) = \frac{[W105°C - W360°C]}{W105°C} \times 100 \qquad \text{Equation 5-3}$$

Where:
W105 °C = weight the dry soil at 105 °C
W 360 °C = weight the soil after 360 °C

Soil pH and EC were measured in 1:1 distilled water to soil extract. pH was measured using calibrated probe. The EC was measured from suspension after filtering using Whatman 42 filter paper.

Soluble Na^+ and soluble K^+ were measured using the EC extract via flame photometer, as documented previously in water sample. Soluble Ca^{2+}, Mg^{2+}, Cl^-, CO_3^{2-}, and HCO_3^- were measured by titration as mentioned in water sample methods.

NO_3^--N was measured by a spectrophotometric method using chromotropic acid. 10 g of dried and sieved soil was reacted with 50 mL 0.02 N copper sulfate solution ($CuSO_4.5H_2O$). Suspension was filtered after 15 minutes of shaking and mixed with 1 mL chromotropic acid solution (0.1 %). 6 mL of concentrated H_2SO_4 was added to sample after cooling down. Sample was measured with standards at 430 nm wavelength after 45 minutes when yellow color was developed. The concentration was calculated by **Equation 5-4**.

$$\mathrm{NO_3(ppm)} = \mathrm{NO_3}_{\text{(from calibration curve)}} \times \left(\frac{\mathrm{V}}{\mathrm{Wt}}\right) \times \left(\frac{\mathrm{V_2}}{\mathrm{V_1}}\right) \qquad \text{Equation 5-4}$$

Where:
V = total volume of extract (mL)
Wt = weight of air dry (g)
V_1 = volume of soil extract (mL)
V_2 = total volume of sample for measurement (mL)

Extractable Phosphorus (Av. PO_4^{3-}) was measured using the sodium bicarbonate ($NaHCO_3$) as suitable procedure for alkaline soil (Olsen, 1954). 5 g dried and sieved soil was reacted with ammonium molybdate solution, ascorbic acid and a small amount of antimony was used for color development in the soil extracts (from yellow to colorless). Then, Av. PO_4^{3-} was measured by Spectrophotometer at 882 nm wavelength and calculated from standards curve equation.

Soluble SO_4^{2-} was measured in a water extract from EC using Turbidimetric method. Extract water was analyzed as normal water samples using barium chloride solution.

Microbiological Analysis
Soil samples were collected once (September 2013) and transported within 24 hour by ice-box to a laboratory in the National Center for Agricultural Research and Extension (NCARE) in Baqaa, Amman. Total and fecal coliform, and *E.coli* were measured in number of colony forming units per 100 milliliters (CFU/100 mL) using the multiple fermentation tube methods according to the Standard Methods (APHA, 1995).

Soil samples were sterilized and diluted. 1 ml of each sample was used for dilutions to be tested in triplicate tubes. The tubes were incubated for 24 and 48 hours at 35 °C. *E.coli*, total and fecal coliform bacteria were measured and counted by gas production in lactose liquid. Tube was considered a positive tube if showed gas bubbles inside inverted glass. The number of positive tubes was used to estimate the number of colonies.

Calculation

Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage (ESP) were calculated as indicators of salinity. They are defined in **Equation 5-5** and **5-6** (Sumner, 1993):

$$\mathrm{SAR} = \frac{\mathrm{Na^+}}{\sqrt[2]{(\mathrm{Ca^{2+}}+\mathrm{Mg^{2+}})/2}}$$ Equation 5-5

Where: Na^+, Ca^{2+}, and Mg^{2+} concentrations in (meq/L).

Many researchers reported the relationship between soil ESP and SAR (Levy & Hillel, 1968, Richards, 1954a). ESP was calculated from SAR as proposed from the United States Salinity Laboratory (USSL).

$$\mathrm{ESP} = \frac{[100\times(-0.0126+0.01475\times\mathrm{SAR})]}{[1+(-0.0126+0.01475\times\mathrm{SAR})]}$$ Equation 5-6

5.2.3 Tree Visual Assessment

The relationship between plants and water was investigated based on a visual appearance of plants (Sumner, 1993). Thus, the effects of reuse on fruit production and tree growth were observed in the experimental plots over the study period. Some parameters such as height (cm), number of twigs, foliage, fruit, and leaves color were checked for each tree on regular basis. During the study, a regular pruning was done to improve the shape of trees by removing over crowd branches to keep the trees open to the sunlight. However, the observation test considered these changes as a baseline for growth yield.

5.2.4 Statistical Analysis

The irrigation water were statistically compared (one way ANOVA) to determine the difference between irrigation water. Additionally, the trees measured parameters were compared in Paired *t*-test to determine the effect of water quantity on growth rate. The soil samples are presented without any statistical analysis.

5.3 Results and Discussion of Irrigation Water Qualities

Results of investigated parameters in the irrigation water are presented in this part with its potential in irrigation. In addition, the equated SI is presented as indicator for soil-water interaction (precipitation or dissolution reaction).

5.3.1 Irrigation Water Qualities

Table 5-3 shows the physicochemical results of irrigation water with the JS for irrigation (JISM, 2006). The minimum water temperature was recorded during winter of 10.2 ^oC, while the maximum was reported in the summer of 29.7 ^oC. Based on the JS, irrigation water temperature conformed to the JS (4 - 30 ^oC).

The pH average values were slightly alkaline, and there was no statistically significant difference in pH between tap-water and the VFCWs effluent. The average value of tap-water was 7.5, while it was ranged of 7.3 - 7.7 in the VFCWs effluent. According to the JS and Ayers and Westcot (1985), the pH values are within the recommended range of 6 - 9. Thus, the risks of pH in the applied water are insignificant, as agreed by Ayers and Westcot (1985). The one concern, that a slightly high pH in soil and water tends to increase the magnesic and calcic precipitation (Pitts, 1996). The precipitation may cause potential issues related to the irrigation system when drip irrigation is used.

EC_w indicates the soluble salts content in water. The water samples were of the following ionic ratio: $Na^+ > Ca^{2+} > Mg^{2+}$; $HCO_3^- > SO_4^{2-} > Cl^-$ and $Na^+ > Ca^{2+} > Mg^{2+}$; $Cl^- > HCO_3^- > SO_4^{2-}$, as the abundant ions in samples. The effluent EC_w was within the acceptable ranges for water suitability for irrigation (especially for citrus trees) according to the national guidelines. Tap-water EC_w was statistically different ($p > 0.05$) compared to the treated wastewater from ECO-1 and ECO-2 over the study period. Ayers and Westcot (1985) reported that EC_w higher than 0.7 dS/cm on long-term caused soil salinity issues. Therefore, USEPA (2004) guidelines recommended excess irrigation dosed for leaching in case of high EC_w.

GTZ (2006) documented that citrus fruits are sensitive to water salinity that it should be less than 1.7 dS/m. **Table 5-2** presents the varying crops sensitivity to water salinity as experienced in the Jordan Valley by GTZ. Plants are classified into four categories: sensitive, salt moderately sensitive, salt tolerant plants, and salt highly tolerant plants.

Table 5-2: Salt tolerance of plants in the Jordan Valley (after GTZ, 2006)

EC (dS/m)	**Crops**
< 1.7	Citrus, carrot, strawberry, and onion
1.7 - 3.0	Olive, pepper, cucumber, cauliflower, lettuce, watermelon, cabbage, and grapes
> 3.0	Asparagus, date palms, barley, wheat, tomato, squash, eggplants, sweet corn, potato, alfalfa, rocket, and parsley

dS/m: decisiemens per meter = 1000 µS/cm.

Table 5-3: Chemical Characteristic of the irrigation water applied in the experimental plots during 2012 to 2014 (mean ± SD) and irrigation quality standards in the JS 893/2006 (after JISM, 2006).

Parameter	Tap water	ECO-1	ECO-1M	ECO-2	ECO-2M	JS 893/2006
EC [µS/cm]	855 ± 133.5*	1534 ± 166.3	1365 ± 372.5	1969 ± 293.7	1854 ± 136.1	0.7 - 3000
pH	7.5 ± 0.3	7.3 ± 0.3	7.4 ± 0.2	7.7 ± 0.2	7.3 ± 0.3	6 - 9
TSS [mg/L]	ND	10.1 ± 8.0*	10.8 ± 6.0*	1.6 ± 1.6*	3.6 ± 1.8*	15 - 50
Turbidity [NTU]	0.47 ± 1.1*	12.0 ± 9.4	21.8 ± 12.5*	1.3 ± 1.7*	10.5 ± 11.2	5 -10
Na^+ [mg/L]	89.7 ± 6.4*	162.0 ± 45.2	146.6 ± 6.9	218.4 ± 38.3	204.6 ± 14.7	< 230
K^+ [mg/L]	10.6 ± 2.2*	14.2 ± 3.4	13.5 ± 4.1	23.4 ± 4.6*	19.6 ± 2.7	**
Mg^{2+} [mg/L]	23.1 ± 2.1*	34.0 ± 4.7	29.9 ± 3.0*	37.7 ± 5.8	37.0 ± 2.8	< 100
Ca^{2+} [mg/L]	48.1 ± 4.9*	96.2 ± 10.1	88.2 ± 6.5*	126.2 ± 14.5	123.2 ± 8.4	< 230
Total-PO_4 [mg/L]	ND	8.4 ± 2.0	6.8 ± 0.89	3.2 ± 1.6	2.9 ± 0.57	< 30
PO4-P^{3-} [mg/L]	ND	6.9 ± 1.4	6.0 ± 1.2	2.5 ± 1.1	2.7 ± 0.5	**
Cl^- [mg/L]	216.3 ± 16.9	191.4 ± 3.3	202.1 ± 8.2	198.5 ± 27.1	205.6 ± 13.8	< 400
HCO_3^- [mg/L]	128.1 ± 5.1*	256.3 ± 46.4	195.3 ± 13.9*	317.3 ± 31.7	311.2 ± 20.1	< 400
SO_4^{2-} [mg/L]	3.84 ± 0.6	192.0 ± 20.7	161.7 ± 22.9	264.0 ± 17.8	218.7 ± 20.6	< 500
Ca/Mg	1.2	1.7	1.8	2.0	2.0	> 1.0
SAR	2.7	3.6	3.4	4.4	4.2	6 - 9
ESP	2.6	3.9	3.7	4.9	4.7	**

SAR: sodium adsorption ratio, unitless parameter. ESP: exchangeable sodium percentage. ND: not detected. * Statistically significant (P > 0.05).
** Not mentioned in the JS 893/2006 standard. Ca/ Mg ratio was calculated from meq/L concentrations.

5.3.1.1 Sodium (Na^+) and Chloride (Cl^-)

Na^+ and Cl^- are the main salinity parameters in water. In phase 1, mean Na^+ concentrations were 89.7, 162.0, and 218.4 mg/L in the tap-water, ECO-1 and ECO-2, respectively. In the ECO-1M and ECO-2M effluents, a slight reduction was observed to 146.6 mg/L and 204.6 mg/L, respectively (**Figure 5-4**). Over the study period, Na^+ concentrations in the irrigation water conformed to the JS and Ayers and Westcot (1985), less than 230 mg/L.

Cl^- concentrations in the irrigation water conformed to the JS and Ayers and Westcot (1985), less than 400 mg/L. There was no significant difference in Cl^- concentrations between irrigation water over the study period. Pettygrove and Asano (1984) reported that high Cl^- content (more than 600 mg/L) in irrigation water caused leaf injury for citrus trees, which also agreed by Walker *et al.* (1982) that high Cl^- can cause degradation in citrus trees growth rate and reduce the leaf gas exchange.

Ca^{2+} concentration mitigates the negative impacts of Na^+, which addressed by SAR. The calculated SAR values were 2.66, 3.62 and 4.38 in the tap-water, ECO-1, and ECO-2 effluents, respectively. While, SAR values reduced slightly to 3.4 and 4.2 in the ECO-1M and ECO-2M effluents, respectively. SAR values of the irrigation water were highly compliance with the JS, Ayers and Westcot (1985) guidelines (6 - 9) and GTZ (2006) guidelines (less than 6).

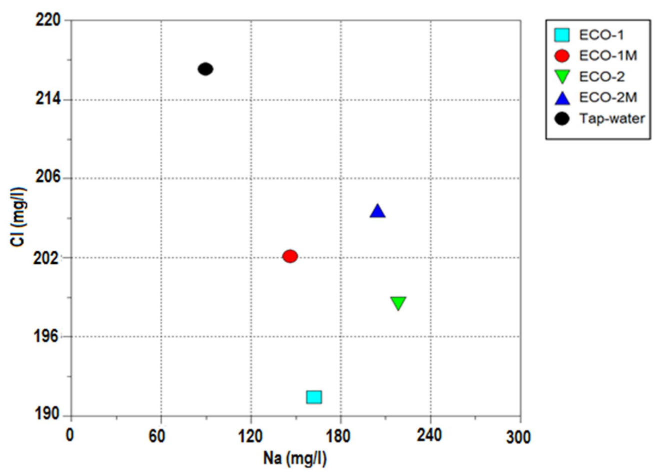


Figure 5-4: The Na and Cl concentrations in the irrigation water at Fuhais.

SAR values can be used in integration with the EC_W values, using the Wilcox's graphic in the United States Salinity Laboratory (USSL) diagram (Richards, 1954b) in order to classify the irrigation water. According to USSL diagram, tap-water, ECO-1, ECO-1M and ECO-2M effluents were belonged to C3 - S1 class, as shown in **Figure 5-5**. While, the ECO-2 effluent was belonged to C3 - S2 class, denoting high salinity and medium sodicity hazards. High SAR causes dispersion and swelling of clay minerals, hereby, reducing soil permeability and infiltration rates, and increasing the formation of hard clay crusts (Rhoades et al., 1992).

Furthermore, SAR - ESP relationship can be used as indicator for Na toxicity that high Na^+ concentration causes dispersing soils by replacing the Ca^{2+} and Mg^{2+} from soil. The calculated ESP values were ranged of 2.6 - 4.9 in the irrigation water. The irrigation water ESP values conformed to the recommended level (less than 5) (Al-Shammiri *et al.*, 2005).

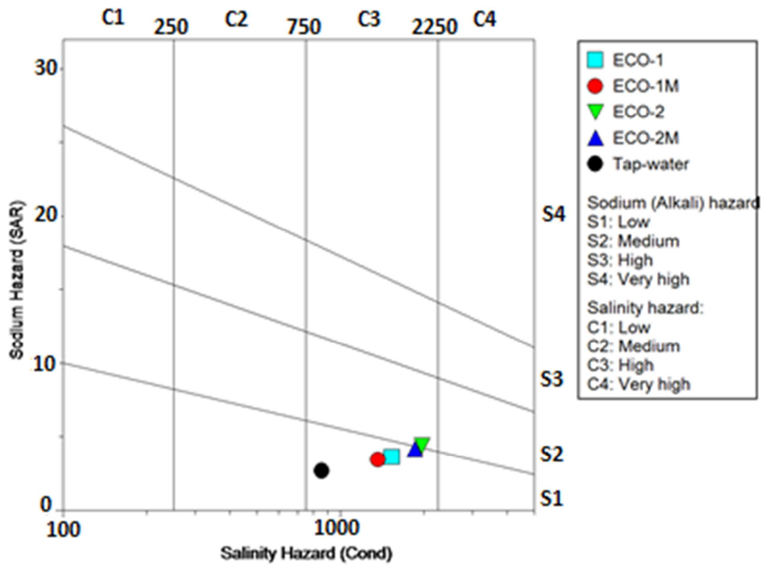


Figure 5-5: The Wilcox's diagram of the irrigation water within C3 - S1 and C3 - S2 classes.

5.3.1.2 Calcium (Ca^{2+}) and Magnesium (Mg^{2+})

Ca^{2+} and Mg^{2+} concentrations were significantly below the JS maximum limits of 230 and 100 mg/L, respectively, **Figure 5-6**. Ca^{2+} concentrations were 96.2 and 88.2 mg/L in the ECO-1 and ECO-1M effluents, respectively. While, it was 126.2 mg/L in the ECO-2 and 123.2 mg/L in the ECO-2M effluents. Mg^{2+} concentrations were measured of 34.0 and 29.9 mg/L in the ECO-1 and ECO-1M effluents, respectively. Whereas, it was 37.7 mg/L in the ECO-2 and 37.0 mg/L in the ECO-2M effluents.

Ca^{2+} and Mg^{2+} are essential for soil friability and plant nutrition. According to USEPA (2004) and Ayers and Westcot (1985), Ca^{2+} and Mg^{2+} maximum limits are 400 and 60 mg/L, respectively. Therefore, exceeding these concentration in irrigation water leads to adverse effects such as increasing soil pH and reducing of the availability of essential nutrients in soil (Al-Shammiri *et al.*, 2005). Richards (1954b) reported that Ca^{2+} and Mg^{2+} are useful for soil structure. On the other hand, researchers found that high Mg^{2+} has negative effects on soils. McNeal *et al.* (1968) presented that blended Na-Mg soils showed lower hydraulic conductivities than Na-Ca soils under same conditions. Thus, a higher ESP value in soils will observed when a Ca/Mg ratio is less than one in the irrigation water (Rahman & Rowell, 1979). If the ratio near or less than one, Ca^{2+} plant uptake from soil-water will be minified due to impacts of high Mg^{2+}. The ratio of Ca/Mg in irrigation water can be applied to predict a potential of Ca disablement. The calculated Ca/Mg ratio in the irrigation water was higher than 1 over the study period, **Table 5-3**. Thus, there is no possibility of adverse effects such as decline of the infiltration rate by continuous irrigation due to increasing Mg^{2+} in soils.

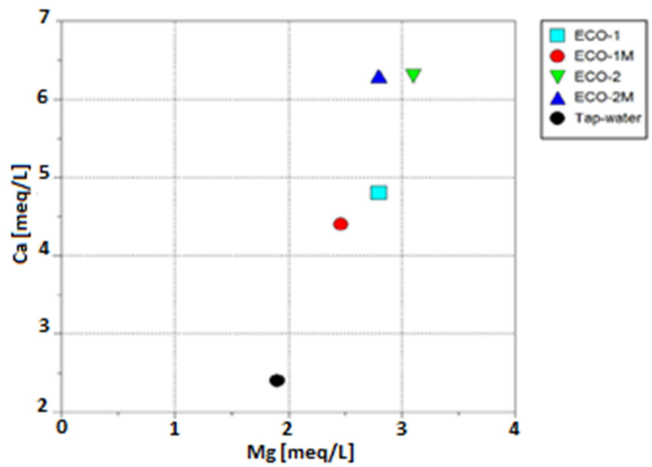


Figure 5-6: Ca^{2+} and Mg^{2+} concentrations in the irrigation water.

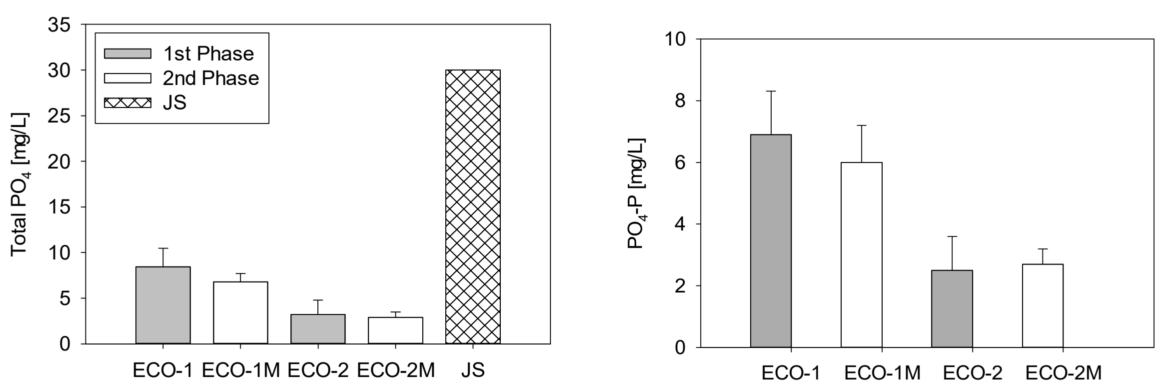
5.3.1.3 Macronutrients

***Potassium* (K^+)**

K^+ in irrigation water is usually used as fertilizer, thus there is no specific recommended level in the JS JISM (2006). However, less than 80 mg/L is the recommended level in irrigation water as reported by GTZ (2006). The average K^+ concentrations were measured in the tap-water, ECO-1 and ECO-2 effluents of 10.6, 14.2, and 23.4 mg/L, respectively. Furthermore, a slight reduction was observed in the K^+ concentrations in the 2^{nd} phase of 13.5 mg/L in ECO-1M and 19.6 mg/L in ECO-2M effluents.

Phosphorous (Total- PO_4) and Orthophosphate (PO_4^{3-}-P)

Phosphate is an essential nutrient for plants, usually added to soils in fertilizers. Nevertheless, Total-PO_4 concentrations were below the maximum limits of 30 mg/L as recommended in the JS, **Figure 5-7**. Most of phosphate compounds have low solubility in water; therefore, using treated wastewater provides it in soluble phase, reducing artificial fertilizers usage.



a) b)

Figure 5-7: Total-PO_4 and PO_4^{3-}-P concentrations in the effluents over the study period, a) Total-PO_4 concentrations over the study period with the JS, b) PO_4^{3-}-P concentrations in the effluents over the study period.

Total Nitrogen (TN) and Nitrate (NO_3^--N)

TN and NO_3^--N concentrations were reduced to achieve the regulatory targets in the JS. Based on Ayers and Westcot (1985) and Pettygrove and Asano (1984), excessive TN spurs vegetative growth or decreases crop quality and maturity. NO_3^--N values were reduced and conformed to the limit assigned by the JS (class B) and the EPA (2003) guidelines (less than 50 mg/L).

5.3.1.4 Bicarbonate (HCO_3^-) and Sulfate (SO_4^{2-})

HCO_3^- and SO_4^{2-} concentrations in irrigation water were in compliance with the JS and Ayers and Westcot (1985) guidelines, less than 400 and 500 mg/L, respectively. **Figure 5-8** shows the average HCO_3^- and SO_4^{2-} concentrations in the irrigation water. High HCO_3^- concentration removes Ca^{2+} and Mg^{2+} from soil, therefore, increasing the soil pH (Al-Shammiri *et al.*, 2005) and sodium carbonate formation. In this study, sum of Ca^{2+} and Mg^{2+} was greater than HCO_3^- concentrations, indicating limited formation of sodium carbonate.

SO_4^{2-} does not cause any toxicity to plants that its solubility is relatively low and it precipitates in soils. Modaihsh *et al.* (1994) reported that irrigation water rich with SO_4^{2-} affects the pH, EC_w and increases the content of nutrients in soil.

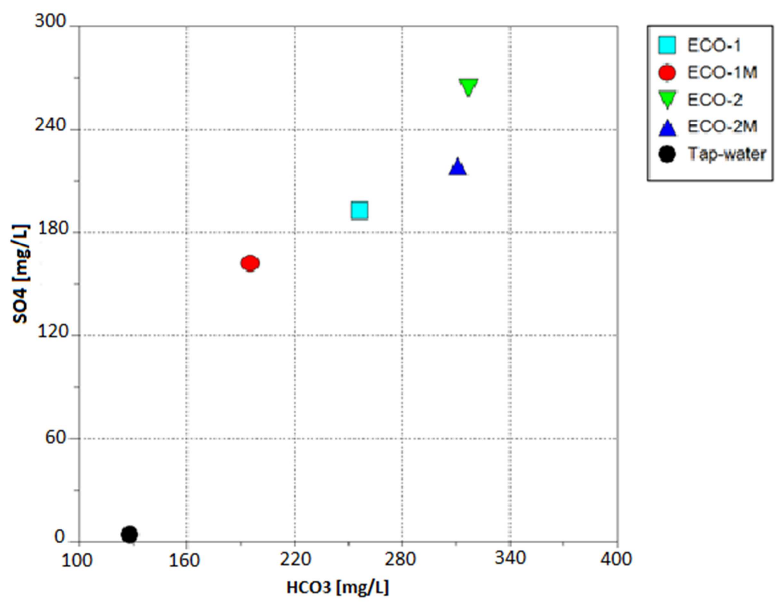


Figure 5-8: The concentrations of HCO_3^- and SO_4^{2-} in the irrigation water.

5.3.1.5 Heavy Metals

In irrigation water samples during 1st and 2nd phase, some heavy metals in very low concentrations were detectable, and they conformed to the JS and Ayers and Westcot (1985) guidelines in agriculture (**Table 5-4**). Similar results of As-Samra effluent were reported by (Al-Nakshabandi *et al.*, 1997). Revealed that treated wastewater in Jordan contains insignificant amounts of heavy metals, lower than the JS guidelines. In this study, the concentrations of these elements were low and should not have any influence on reuse.

Table 5-4: Concentration of some heavy metals in the irrigation water over the study period.

Parameter	Tap-water	ECO-1	ECO-1M	ECO-2	ECO-2M	JS 893/2006
Cu [mg/L]	ND	0.005	0.005	0.004	0.005	< 0.2
Fe [mg/L]	ND	0.007	0.006	0.005	0.006	< 5.0
Pb [mg/L]	ND	< 0.001	< 0.001	< 0.001	< 0.001	< 0.2
Mn [mg/L]	ND	0.005	0.004	0.005	0.006	< 0.2
Zn [mg/L]	ND	0.108	0.143	0.141	0.242	< 5.0

ND: not detected.

5.3.2 Saturation Indices (SI)

Calcite, halite, aragonite, gypsum, anhydrite, and dolomite SI of irrigation water were calculated using the water quality measurements in **Table 5-1**. Inaccuracy degree of 0.1 for calcite and gypsum and 0.2 for dolomite are recommended on the calculations (Lee, 1993 , Al-Suhail, 1999).

5.3.2.1 Calcite ($CaCO_3$)

The distribution of calcite SI is shown in **Figure 5-9. a**. Tap-water was under saturated with respect to calcite that water has the potential to dissolve calcite from the soil and increase the pH value due to calcite dissolution (APHA, 1995). That can be explained by the low SO_4^{2-} concentrations in tap-water. Similar results reported by Al-Suhail *et al.* (2005), that calcite and dolomite SI were exceeded the equilibrium limit when the SO_4^{2-} concentration increased in water.

The effluents of ECO-1 and ECO-1M showed SI values near equilibrium level (SI = 0). While, the ECO-2 and ECO-2M effluents were over-saturated that tend to precipitate calcite on soils. In this study, a tendency of calcite is relatively high due to lower Mg/Ca ratio in waters. Furthermore, the precipitation of calcite increases the soil salinity when a diminish of Ca/SO_4 occurred (Lorite-Herrera *et al.*, 2008). Nevertheless, Bower *et al.* (1965) reported that $CaCO_3$ precipitation causes a decrease in soil salinity and it is useful indicator for irrigation water quality. However, such precipitation processes take a long time and the equilibrium sate becomes reversed upon re-irrigation.

5.3.2.2 Aragonite ($CaCO_3$)

Aragonite is another form from carbonate minerals. Tap-water, ECO-1, and ECO-1M effluents were under-saturated with respect to the mineral that aragonite will be dissolved from soils (**Figure 5-9. b**). Aragonite SI of the ECO-2 and ECO-2M effluents were super-saturated, tending to precipitate this mineral on soils in order to reach the equilibrium state.

5.3.2.3 Halite (NaCl)

The main source of Na^+ and Cl^- in water or soil samples is halite dissolution. The irrigation water qualities showed negative values of SI (under-saturated), as shown in **Figure 5-9. c**. Giving a strong indicator that dissolution of soil halides will be taken place in order to reach a saturation state.

5.3.2.4 Gypsum ($CaSO_4.2H_2O$)

The irrigation water qualities were under-saturated with respect to $CaSO_4.2H_2O$, hence a dissolution of soil gypsum is possible, **Figure 5-9. d**. This mineral is moderately soluble in water (Bock, 1961) indicating low abundance of gypsum in irrigation water. The presence of gypsum is useful for soil that it is used in ameliorating high Na^+, Cl^- and other salts concentrations from soils.

5.3.2.5 Anhydrite ($CaSO_4$)

The irrigation water qualities were under-saturated with $CaSO_4$ and dissolution of soil anhydrite is possible, **Figure 5-9. e**. $CaSO_4$ is a product of gypsum transformation, which associated with high salinity and temperature.

5.3.2.6 Dolomite ($MgCa(CO_3)_2$)

The saturation state of dolomite is similar to that of calcite and aragonite. Dolomite SI were over-saturation in ECO-2 and ECO-2M effluents. Whereas, it were under-saturated in the other irrigation water, which means higher tendency to dissolve $MgCa(CO_3)_2$ from soils. **Figure 5-9. f** shows the spatial variation of dolomite SI. This mineral can be crystalized in soil if the Mg/Ca ratio in water exceeds 5 - 10 and it probably forms in lower salinity conditions.

Overall, the irrigation water were under-saturated with respect to anhydrite, gypsum, and halite, indicating no deposition of these minerals in soils and there are no adverse effects such as clogging and reduction in soil permeability. Calcite, dolomite, and aragonite were super-saturated in the ECO-2 effluents, hereby, its progressive mineralization increases slightly in P2.

5.3.3 Suitability of these effluents for irrigation

Results of irrigation water parameters of the effluents were indicated the subsequent restrictions to effluent usage.

- Slight to moderate restriction of irrigation water use on citrus crops, especially for ECO-2 effluents.

- During phase 2, there was a need to consider the potential of clogging in the irrigation system especially for the ECO-2M effluent, which is correlated with higher turbidity in effluent.

- SAR values were lower than the threshold of toxicity (6 - 9) that requires a slight to moderate restriction on reuse in subsurface irrigation.

- The NO_3^--N concentrations conformed to the JS (category B: 45 - 70 mg/L), which requires a slight to moderate restriction on reuse in subsurface irrigation.

- The heavy metals concentrations were lower than the JS range, thus, it should not have any adverse impacts on effluents reuse.

- *E. coli* numerations were high in the effluents but using subsurface irrigation system provides disinfection step during transportation through soil.

- The ionic relationships and the tendency of precipitation or dissolution minerals showed undergoing a process of dissolution of anhydrite, gypsum, and halite from the soil content. In the ECO-2 effluents, calcite, dolomite, and aragonite are going to precipitate in soils in the 2[nd] reuse plot.

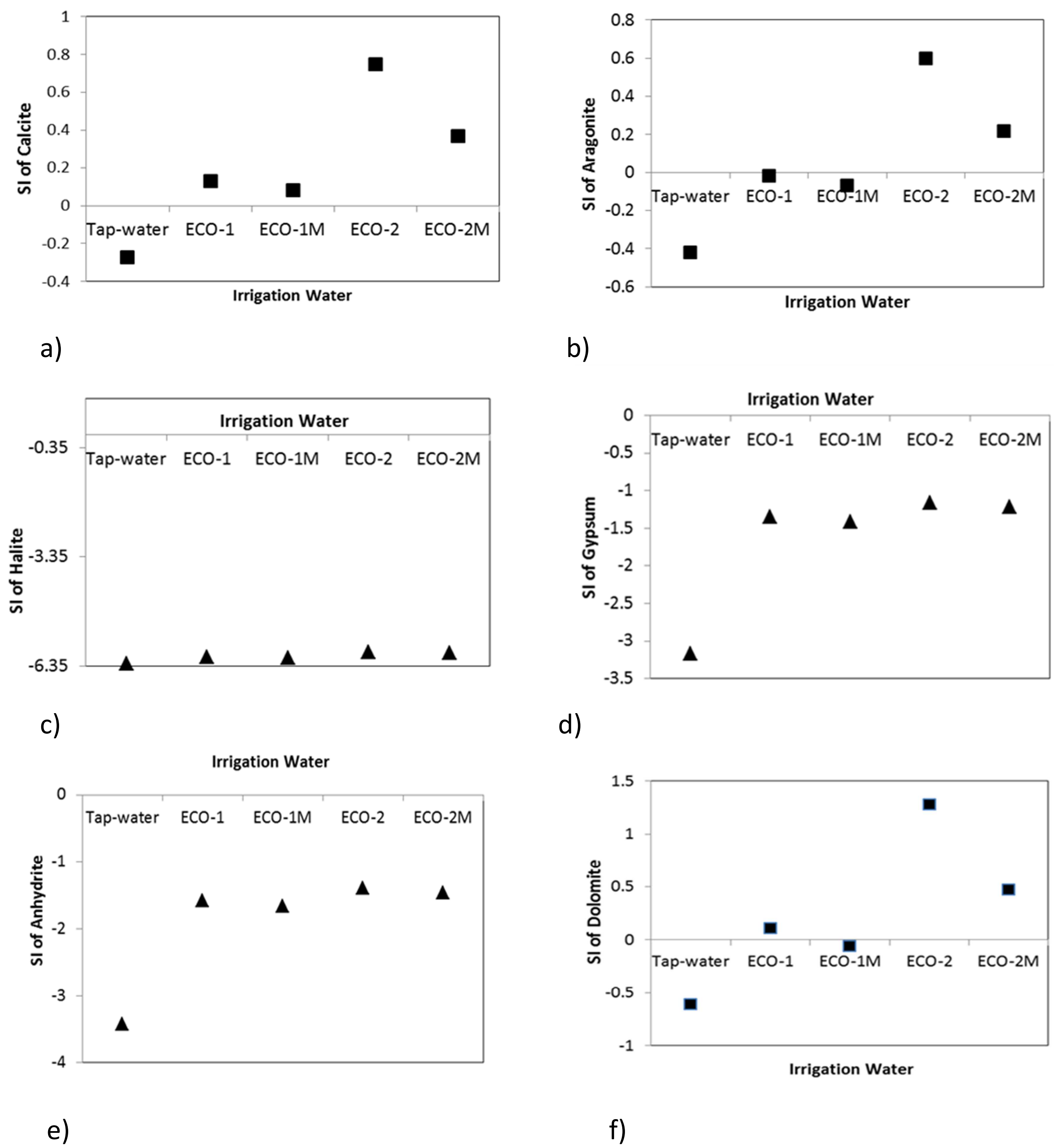


Figure 5-9: SI of calcite, aragonite, halite, anhydrite, gypsum, and dolomite of the irrigation water.

5.4 Results and Discussion of Soil Properties

5.4.1 Soil Physical Properties

Soil texture, structure, and infiltration rate are important soil properties that addresses the impacts of treated wastewater on soils. These properties influence the soil permeability, reflecting the suitability of soil for irrigation.

5.4.1.1 Soil Texture

A soil texture analysis was measured once every year. No changes were observed in soil texture among the reuse plots during irrigation application. Results of soil texture, in samples at 0 - 20, 20 - 40, and 40 - 60 cm depths, were classified as clay loam (medium textured soils) according to the USDA soil texture classification (**Figure 5-10**). The clay fraction was ranged of 30.8 - 33.8 %; the silt fraction was ranged of 29.8 - 35.0 % and the sand fraction was ranged of 32.2 - 37.4 %, as shown in **Table 5-5**. A slight increase was observed in silt and clay fractions, whereas, sand was decreased slightly. However, the results on the textural triangle showed that the soils were stable and balanced texture over the study period.

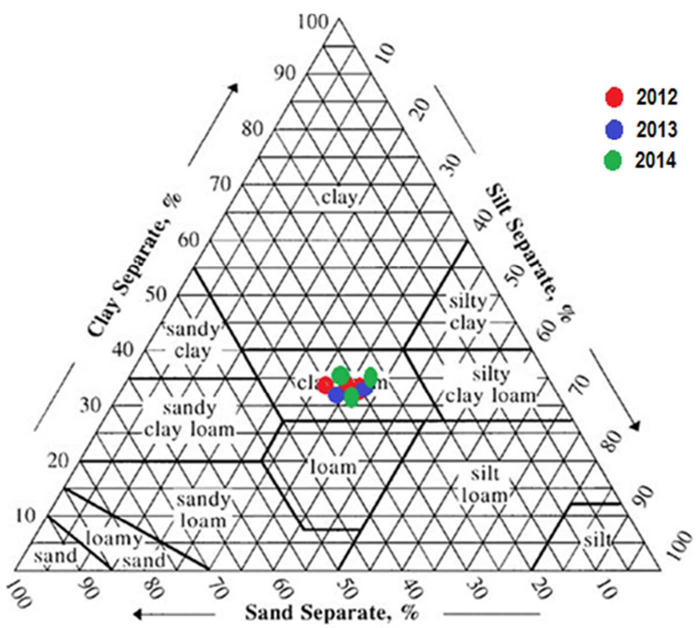


Figure 5-10: Clay loam soil classification in all plots over the study period.

The texture of soils is almost stable and unable to alter directly from irrigation water (FAO, 1988). Soil texture influences the amount of water and air, and infiltration rate. In clay loam classification, clay and silts are predominant that soils have small pores between moderate and small particles. Thus, water will be retained into the soils longer than other coarser soil texture. In addition, GTZ (2000) reported that a high clay percentage affected and incremented the soil moisture capacity. Abedi-Koupai *et al.* (2006) reported that a high percentage of clay increased pollutants adsorption via soil.

Table 5-5: Soil texture and infiltration rates during reuse application in the experimental irrigation plots.

Plot (irrigation water)	Year	Sand [%]	Silt [%]	Clay [%]	Texture	Infiltration rate [mm/hr]
P 1 (control/ tap-water)	2012	37.4	29.8	32.6	Clay loam	79.1
	2013	35.9	32.1	31.9		-
	2014	35.4	33.8	30.8		93.6
P 2 (ECO-2 effluent)	2012	35.1	33.9	31.0	Clay loam	78.0
	2013	34.1	34.8	31.1		-
	2014	34.5	32.2	33.4		65.8
P 3 (ECO-1 effluent)	2012	32.3	34.3	33.4	Clay loam	86.8
	2013	33.8	34.3	33.8		-
	2014	32.2	35.0	32.8		98.3

5.4.1.2 Soil Structure

The physical structure of soil reflects the arrangement of soil particles, the water and air availability in soil (Lal R, 1991). It is also described by soil aggregate, which is influenced by water content, soil texture, organic matter, mineralization process, and pH. It was measured twice over the study period in 2013 and 2014. In 2013, the results of soil aggregates were well graded with 1.7, 1.4, 1.3 coefficient of gradation in P1, P2 and P3, respectively, **Figure 5-11**. In 2014, the soil aggregates were slightly enhanced of 2.1, 1.9, 1.6 coefficient of gradation in the P1, P2 and P3, respectively. In the P2 and P3, the gradation coefficient enhanced as a results of continuous input of dissolved organic from effluents. A and B parts were similar over the study period without any significant difference.
The changes in soil structure can be observed by long-term irrigation with effluents. Juan and Blanca (2014) reported a positive influence of treated wastewater irrigation, improving the soil aggregate and porosity. Therefore, it affects the plant growth, increasing the ability of roots to distribute and take up water and nutrients easily from soils (Pardo *et al.*, 2000). In contrast, Misra and Sivongxay (2009) showed a degradation in soil structure after using treated wastewater as a results of Na^{+} accumulation. A few studies showed a reduction in soil porosity due to high suspended solids in effluents (Cox et al., 1997, Coppola et al., 2004).

Agricultural activities such as tillage may contribute in soils structure improvement. Furthermore, types of crops enhance this merit by its roots, which characterized by high thickness and long length (Czarnes *et al.*, 2000). Raimbault and Vyn (1991) showed that soil aggregates was high for continuous cultivation of alfalfa (*Medicago sativa*), whereas adverse effect observed form soybean in the same study.

The results from soil samples at 60 cm (near the irrigation pipelines) showed similar aggregates percentage over the study period. Whilst, Coppola *et al.* (2004) observed that using flooding and spray irrigation methods increased soil compaction and erosion (soil aggregates deterioration).

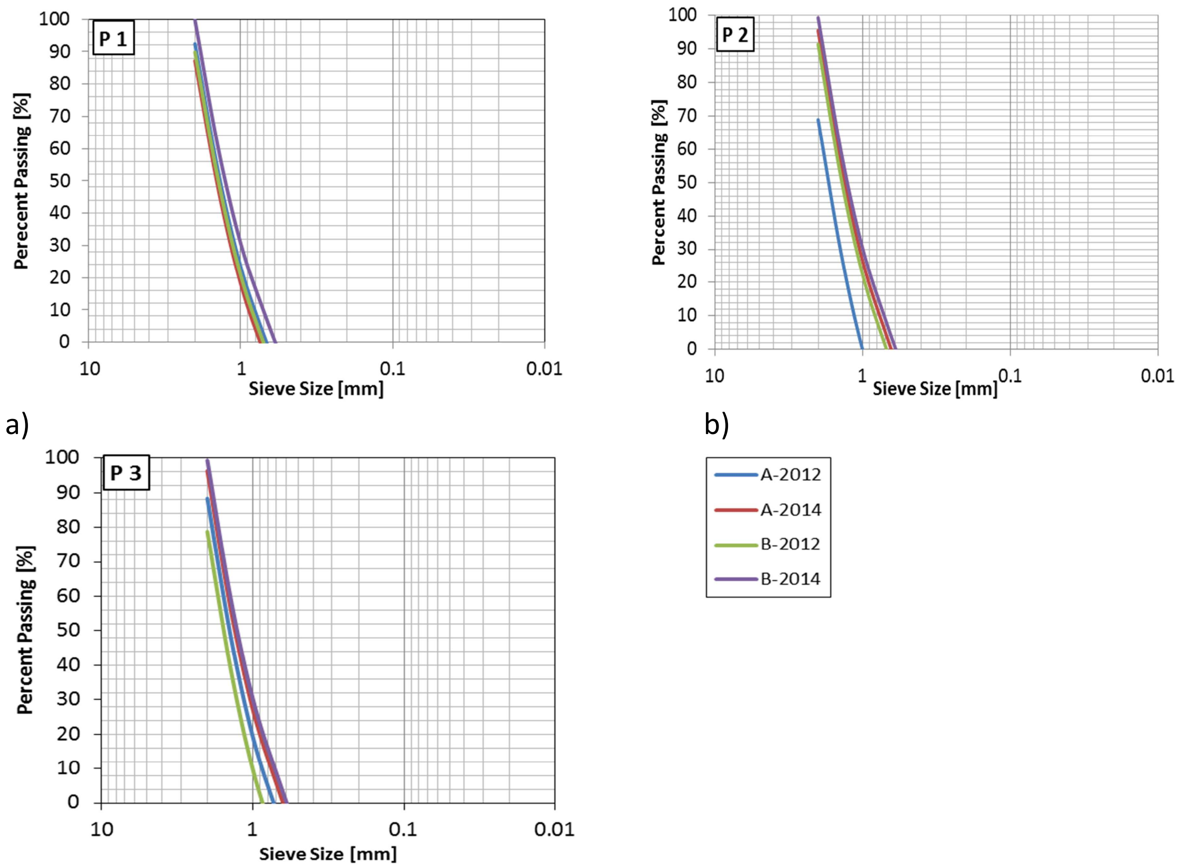


Figure 5-11: Soil aggregates in the experimental reuse plots over the study period, a) control plot, b) 2nd plot, c) 3rd plot.

5.4.1.3 Soil Moisture (SM)

Higher SM values were measured in the winter (January) as a result of water ingress via participation. Highest SM percentage was reported at 40 - 60 cm close to the irrigation pipelines in all plots, **Figure 5-12**. However, A part in all plots showed higher SM content compared to B part as a results of doubled irrigation water volume. P2 and P3 showed higher SM content compared to P1, which is referred to the higher TSS and OM in the effluent that increased soils holding capacity. In addition, SM in P3 could be correlated with higher clay content, which is relatively higher than other plots.

The gradual increase in SM from the bottom to the top of soils indicated the high efficiency of upward movement of water (capillary rise) through the soils. Water can be sucked upward and covered a long distance (more than 80 cm), however, fine soil texture (clay) needs long time for capillary raise process (FAO, 1988). The maximum capacity of SM capacity was not significantly different among the virgin and cultivated soils. SM capacity is considered as the most effective factor in pathogens removal. Oron *et al*. (2001) reported that SM on a soil was closed to the field capacity (around 20 %); a significant elimination in all pathogens was recorded. Based on this finding, the soils in the plots are able to reduce the pathogens since the SM around 20 % at 40 - 60 cm depth.

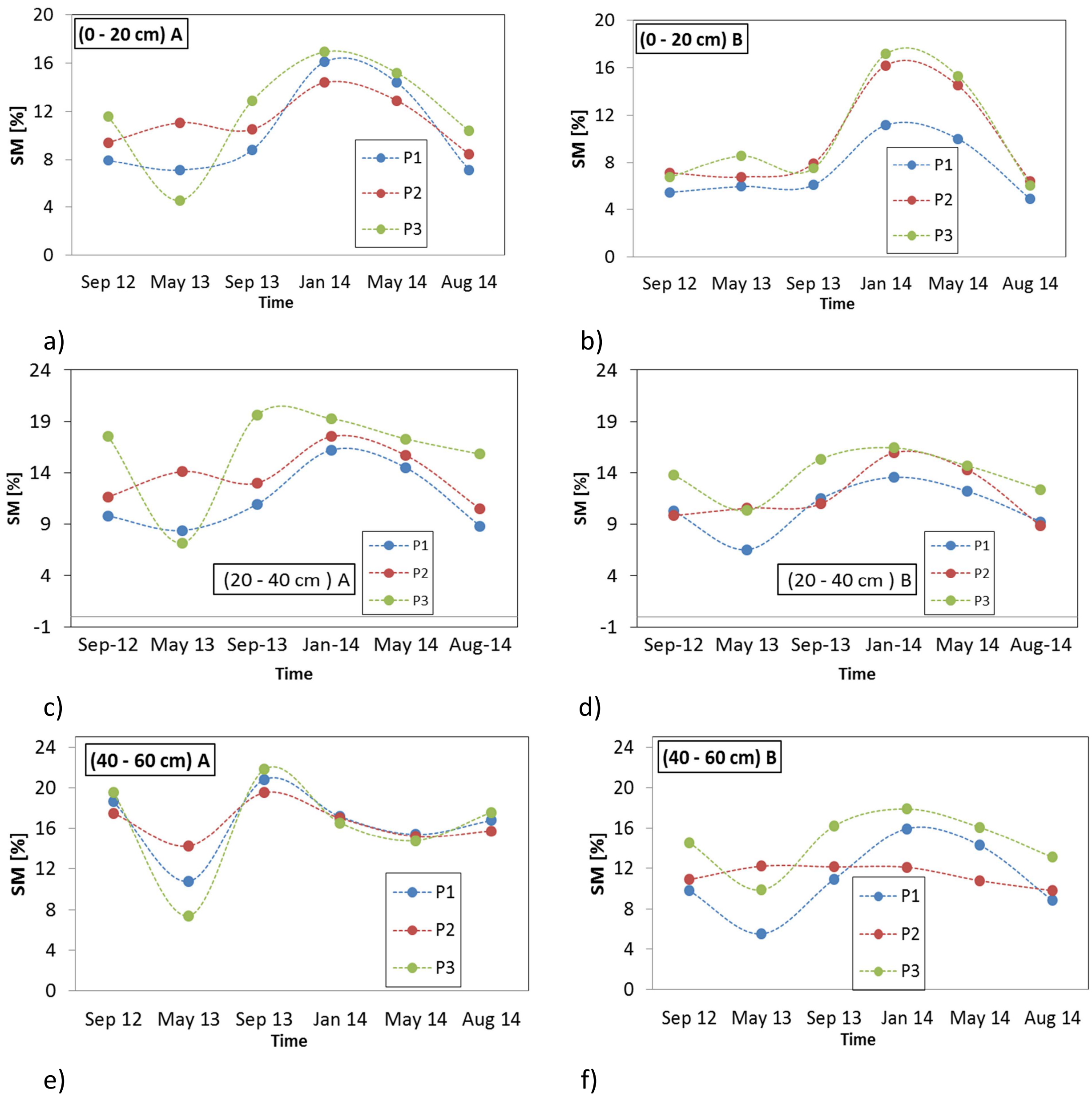


Figure 5-12: SM percentage in the irrigated plots at various depths over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

5.4.1.4 Soil Infiltration Rate

This test measures the velocity of water or rain to seep into the soils and determines the potential of runoff (Lado & Ben-Hur, 2009). Infiltration rate was measured two times during the study (April 2012 and May 2014), **Table 5-5**. No significant variation was observed among the experimental plots in the infiltration rate measurements (**Figure 5-13**). The mean values of infiltration rate in the P1 and P2 were 86.1 and 92.6 mm/hour, respectively. Similar results were reported during short-term investigation by Attaallah (2013) that using treated wastewater did not cause a considerable change in infiltration capacity.

On the other hand, there was a noticeable reduction from 78.0 to 65.8 mm/hour in the P2. That can be caused by high Na^+ concentration, which reduced the rate of infiltration in the soils. Many studies showed that reduction in soils infiltration rate (Oster & Schroer, 1979) and hydraulic conductivity (McNeal *et al.*, 1968) was compatible with soil salinity reduction and sodicity increasing. Lado and Ben-Hur (2009) showed that the infiltration rate was diminished in an effluent irrigated soil for long-term (more than ten years). This reduction was mainly a result of seal permeable formation in irrigated soils. Based on the classification for infiltration rates in FAO (1988), the infiltrability in these plots is relatively high (more than 50 mm/hour).

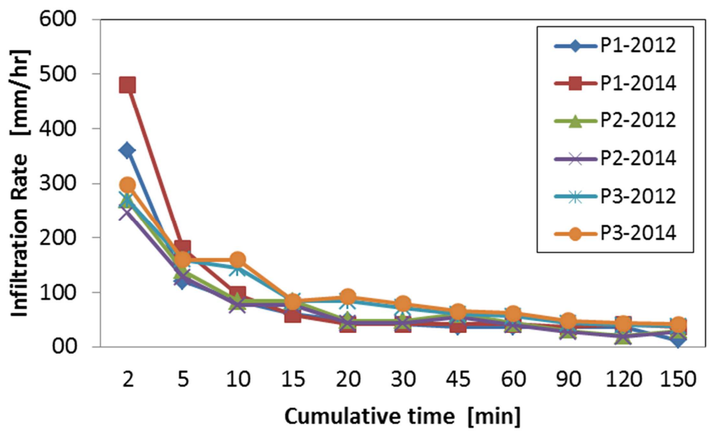


Figure 5-13: Infiltration rate in the reuse plots in 2012 and 2014.

Soil physical properties could be affected after long-term by irrigation with treated wastewater (indirect impact), while direct effects on soil chemistry such as pH, incrementing salinity or organic matter could be noticed in short-term reuse application (Roesner, 2007).

5.4.2 Results and Discussion of Soil Chemical Properties

Results of chemical soil properties are presented and discussed in this part. The pH, ECs, SAR, organic matter, microbial and other cations and anions of the soils at different depth have been compared between the experimental plots and subparts.

Results prior reuse application

Results of chemical analysis of virgin soil prior irrigation application are shown in **Table 5-6**. ECs was relatively low (non-saline) and ranged from 210 - 235, 250 – 285, and 205 - 230 µS/cm in P1, P2, and P3, respectively. Alkaline pH values ranged from 7.7 in the upper layer to 8.4 at 40 - 60 cm depths. SAR and ESP were in the normal range for soil samples. Concentrations of available cations and anions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, CHO_3^-, NO_3^-, SO_4^{2-}, and Av.P) were within the normal range for soils in the area. Na^+ and Cl^- were the dominant ions after CHO_3^-. The soil organic matter (SOM) ranged from 0.7 - 1.3 %, which is normal for soils in semi-arid region (normally less than 1.5 %) (ICARDA, 2013).

Table 5-6: Initial physicochemical properties of soils in the experimental reuse plots.

Parameters	0 - 20 cm			20 - 40 cm			40 - 60 cm		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
pH	7.7	7.9	8.2	8.1	7.9	8.3	7.9	8.2	8.4
ECs [µS/cm]	215	250	210	210	265	205	235	285	230
Ca^{2+} [mg/L]	21.8	19.9	19.2	23.9	20.0	13.8	21.8	22.0	16.8
Mg^{2+} [mg/L]	9.5	7.5	6.9	9.8	6.0	3.4	9.7	9.6	8.1
Na^{+} [mg/L]	27.4	23.7	23.4	24.4	24.7	23.2	23.7	23.2	21.5
K^{+} [mg/L]	11.0	2.0	2.5	2.4	2.1	2.5	2.3	2.1	2.3
Cl^{-} [mg/L]	75.7	83.7	117.6	56.7	74.6	120.3	59.1	61.7	104.6
CHO_3^{-} [mg/L]	196.1	172.6	208.2	225.1	238.8	259.3	272.9	243.1	260.2
NO_3^{-} [mg/L]	1.1	2.14	1.3	1.1	2.9	7.1	1.3	4.1	3.8
SO_4^{2-} [mg/L]	2.1	4.4	1.4	1.4	4.1	1.6	1.7	2.4	2.3
Av. P [mg/L]	0.3	0.1	0.27	0.3	0.3	0.5	0.4	0.3	0.3
SOM [%]	1.3	0.7	1.0	0.8	1.3	1.1	1.1	1.3	0.9
SAR	1.2	1.2	1.2	1.1	1.2	1.5	1.1	1.0	1.1
ESP	0.55	0.30	0.33	0.43	0.55	0.27	0.46	0.87	0.32

P1: Control plot (tap-water); P2: Plot irrigated with the ECO-2 effluent; P3: Plot irrigated with the ECO-1 effluent, Av. P: Available phosphate. SOM: Soil organic matter.

Soil Chemical Properties during Irrigation Application

5.4.2.1 Soil pH

As prospective for soils in semi-arid countries, calcareous soils, alkaline pH values observed in all plots layers (parts A and B) over the study period. Soil pH values were ranged of 7.2 to 8.5, which were within the desired range in agricultural soils. Soil pH showed a slight decline in both A and B parts in the three plots. There was no different in the pH values between the control and other plots.

After implementing the modification options, slight reductions were recorded in soils pH due to pH reduction in the effluents. Same finding was reported by Kunhikrishnan *et al.* (2013), that soil pH values decreased in reuse application as a result of OM mineralization in the irrigated soil. Although, other researchers found that pH reduction comes due to nitrification of NH_4^+-N from the effluent (Stamatiadis *et al.*, 1999, Hussein, 2009). In our study, this explanation is neglected (low NH_4^+-N concentrations in the irrigation water). Nevertheless, there was no clear evident that using subsurface irrigation system affected the pH values according to results at different depth. On the other hand, other studies (Rusan *et al.*, 2007, Rattan *et al.*, 2005) reported that soil pH values increased over a long-term of irrigation with sewage and wastewater effluents, related to the continuous salts input.

5.4.2.2 Soil Salinity (ECs)

ECs reflects the soluble salts concentration in soil and suitability for cultivation. The ECs values were relatively low in all plots. The highest ECs values reported in the summer (September 2013 and August 2014) due to high evaporation, while ECs dropped in January 2013 and 2014 as a result of leaching by rain.

In phase 1, the ECs of the top soil layers showed a slight increase in all plots and subparts from 250 to 480 µS/cm (**Figure 5-14**).

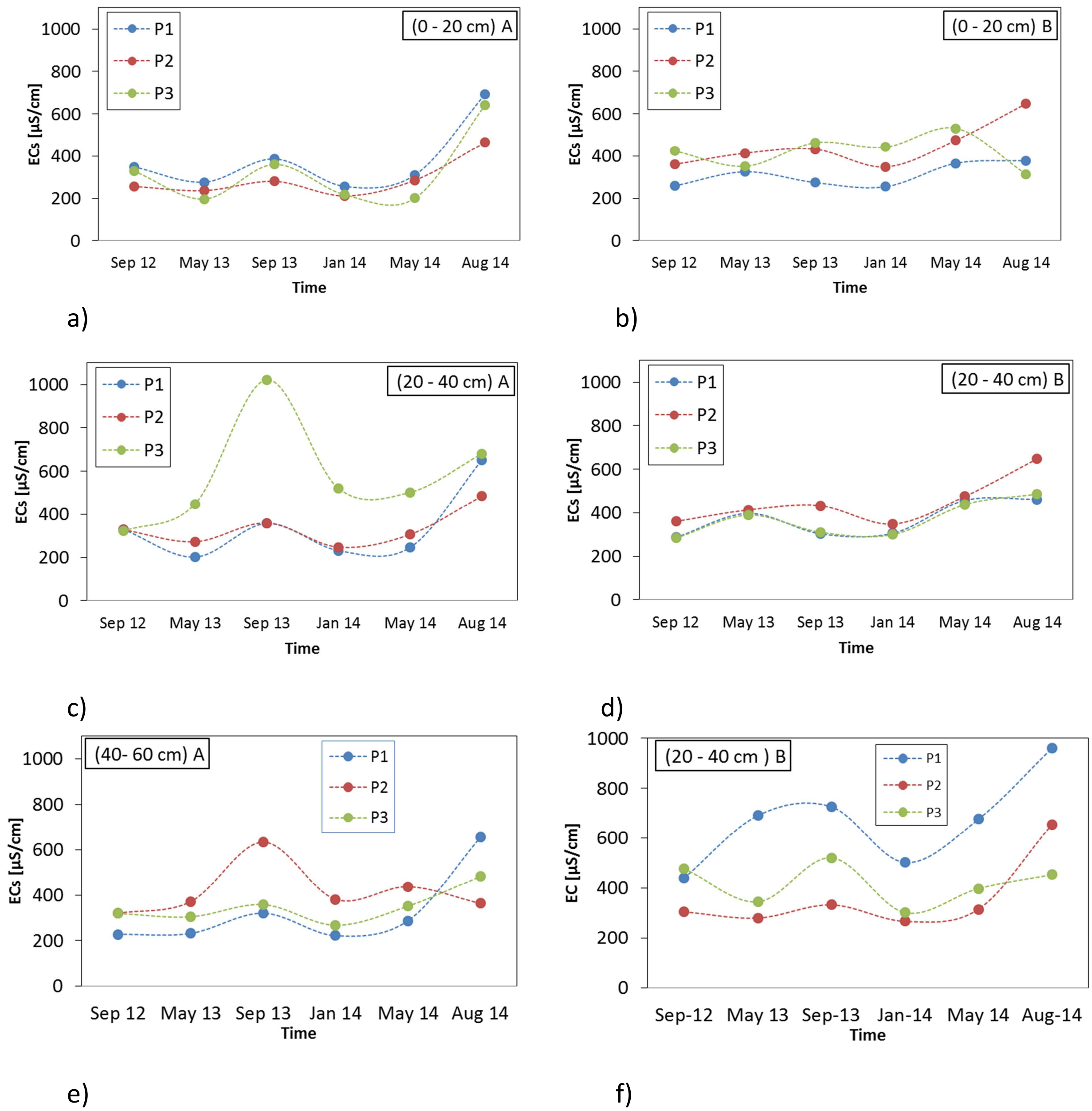


Figure 5-14: ECs among the reuse plots and its parts A and B over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

In phase 2 (September 2013 - August 2014), ECs values increased in A parts, on average 363 to 693 µS/cm. Similar results showed an increment in the ECs in the topsoil using subsurface irrigation systems due to salts accumulation (Oron *et al.*, 1995, Oron *et al.*, 1999, Ayers & Westcot, 1985, Oron & DeMalach, 1987, DeMalach & Pasternak, 1993). In B part in the P2 and P3, higher ECs values were observed that indicates using more water in subsurface irrigation system in case of saline water reducing salts accumulation in the top soil, in accordance with Heidarpour *et al.* (2007a).

ECs values of subsoil at 20 - 40 cm were increased dramatically in A part in P3, whereas it showed a slight increase in the other plots. Increasing EC_s in this wet zone was connected to continuous transmission of salts upward through soil matrix. Many studies showed similar tendency of increasing ECs with distance from water source (Al-Nakshabandi *et al.*, 1997, Judah, 1985, Singh *et al.*, 1978). However, there was a doubt that EC_s value of 1022 µS/cm in P3 in the September of 2013 related to mole activity that dug the soils during that time.

The salinity of the subsoil at 40 - 60 cm decreased slightly in A parts during phase 1 due to continuous leaching. The highest ECs values observed in the P2 in A part, which was compatible with high effluent EC. Furthermore, it was correlated with SI in the ECO-2 effluents that calcite, dolomite, and aragonite could be precipitated in soils. These accumulated salts in soils affect water movement through soil matrix (Heidarpour *et al.*, 2007b). On the other hand, a significant increase in ECs observed in B part in P1 as a direct result of dissolution of soil minerals. Soil salinity at 60 cm was increased with distance upward from the emission point of water.

In the end of the study, ECs was increased in the reuse plots, but it is still (non-saline) below the recommended limit to be a saline soil (greater than 4 dS/m) (Richards, 1954a). Nevertheless, using treated effluents and tap-water showed the same trend of incrementing the ECs. In contrast, findings by Al-Shdiefat *et al.* (2009) showed that fresh water does not affect ECs effluents do.

5.4.2.3 Cations (Na^+, Ca^{2+}, and Mg^{2+})

Na^+, Ca^{2+}, and Mg^{2+} concentrations in irrigated soils were similar to the virgin soils concentrations. In August 2014, the concentration almost doubled due to high evaporation rate that increased concentration of ions. The concentrations of Na^+, Ca^{2+}, and Mg^{2+} were within the normal range for agricultural soils in Jordan. However, the variations of these cations with time can be obviously affected by capillary rise, leaching after heavy rains and plants uptake as documented by Tarchouna *et al.* (2010).

High Na^+ concentrations were observed in all plots at 40 - 60 cm in A part and 20 – 60 cm in B part (**Figure 5-15)**. The impact of high Na^+ on soil matrix depends on other cations concentrations in the soils (Heidarpour *et al.*, 2007a). Na^+, undesirable cation, causes a reduction in soil permeability, therefore, reduction in infiltration rate and water storage capacity, and incrementing soil crusting and erosion (Shainberg, 1984, Hardy *et al.*, 1983). Additionally, high concentrations of Na decline plant uptake of nutrients such as K^+ and Ca^{2+}, therefore, reduce plants growth.

SAR showed the same trend of Na^+ concentration among plots and subparts. In A part at 20 cm, the highest SAR values were calculated of 2.3, 2.5, and 2.4 for P1, P2 and P3, respectively, as a result of increasing Na^+ adsorption from irrigation water in soils (Lado & Ben-Hur, 2009). There was no variation between reuse plots and subparts, **Figure 5-16**.

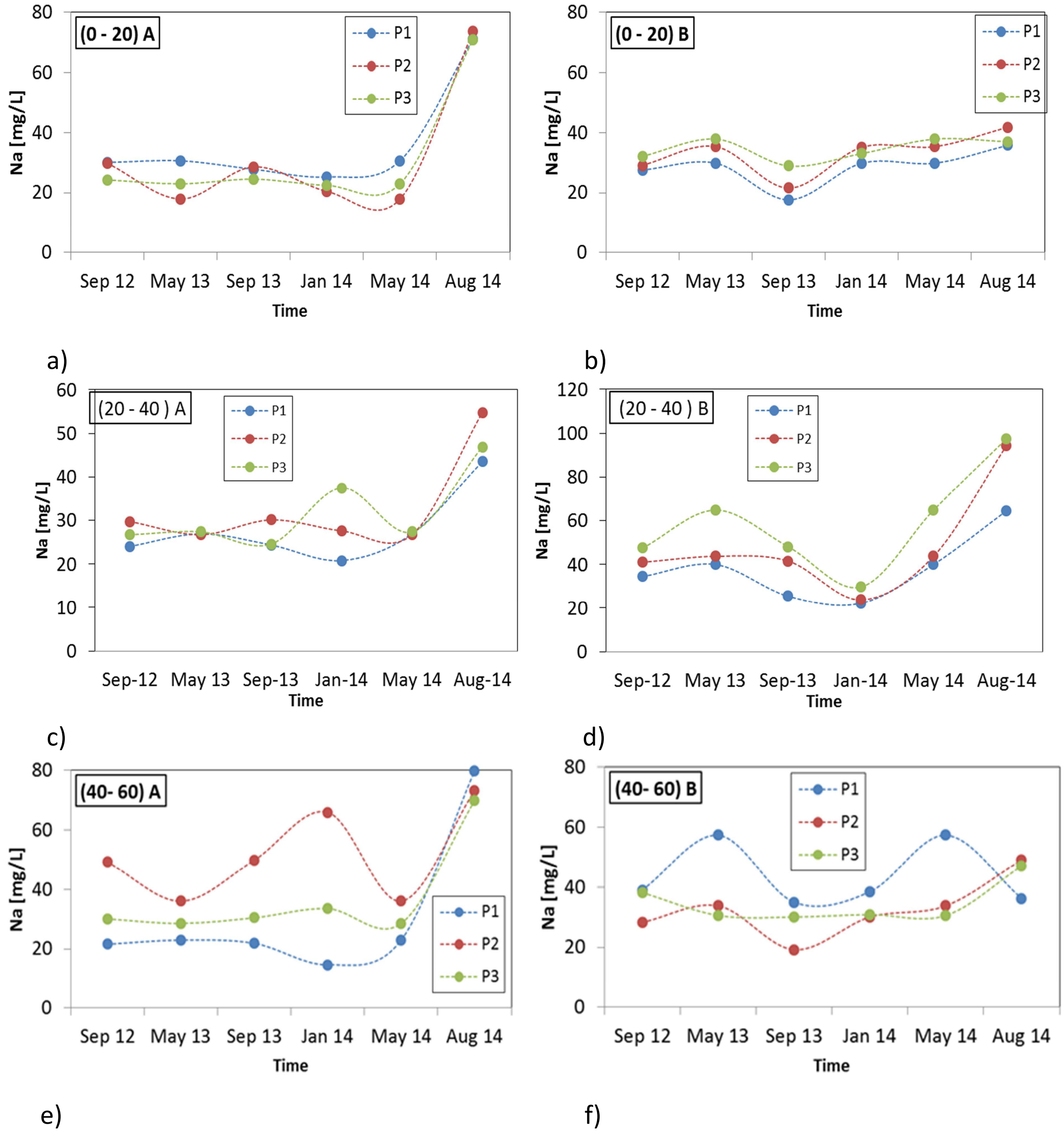


Figure 5-15: Na^+ concentrations in the reuse plots and subparts (A and B) over the study period. a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

The Ca^{2+} and Mg^{2+} levels were increased in the topsoil layers in all plots and subparts. As showed in the SI results, the soil-water interaction tends to precipitate calcite, aragonite, and dolomite in the P2 that means increasing the Ca^{2+} concentrations. Ca^{2+} and Mg^{2+} cations improve the soil aggregation by increasing the aggregation between clay and OM. Moreover, high pH causes precipitation of Ca^{2+} and Mg^{2+} from water, which increases SAR and increments the exchangeable Na^+ level in the soils (Western Fertilizer Handbook, 1995). LaHaye and Epstein (1971) reported the role of Ca^{2+} for salt tolerance for plants. In particular, in citrus plants salt tolerance can be increased by high Ca^{2+} concentration in soils, which suppress undesired salts (Na^+, Cl^-) transmission to the leaves (Banuls *et al.*, 1991).

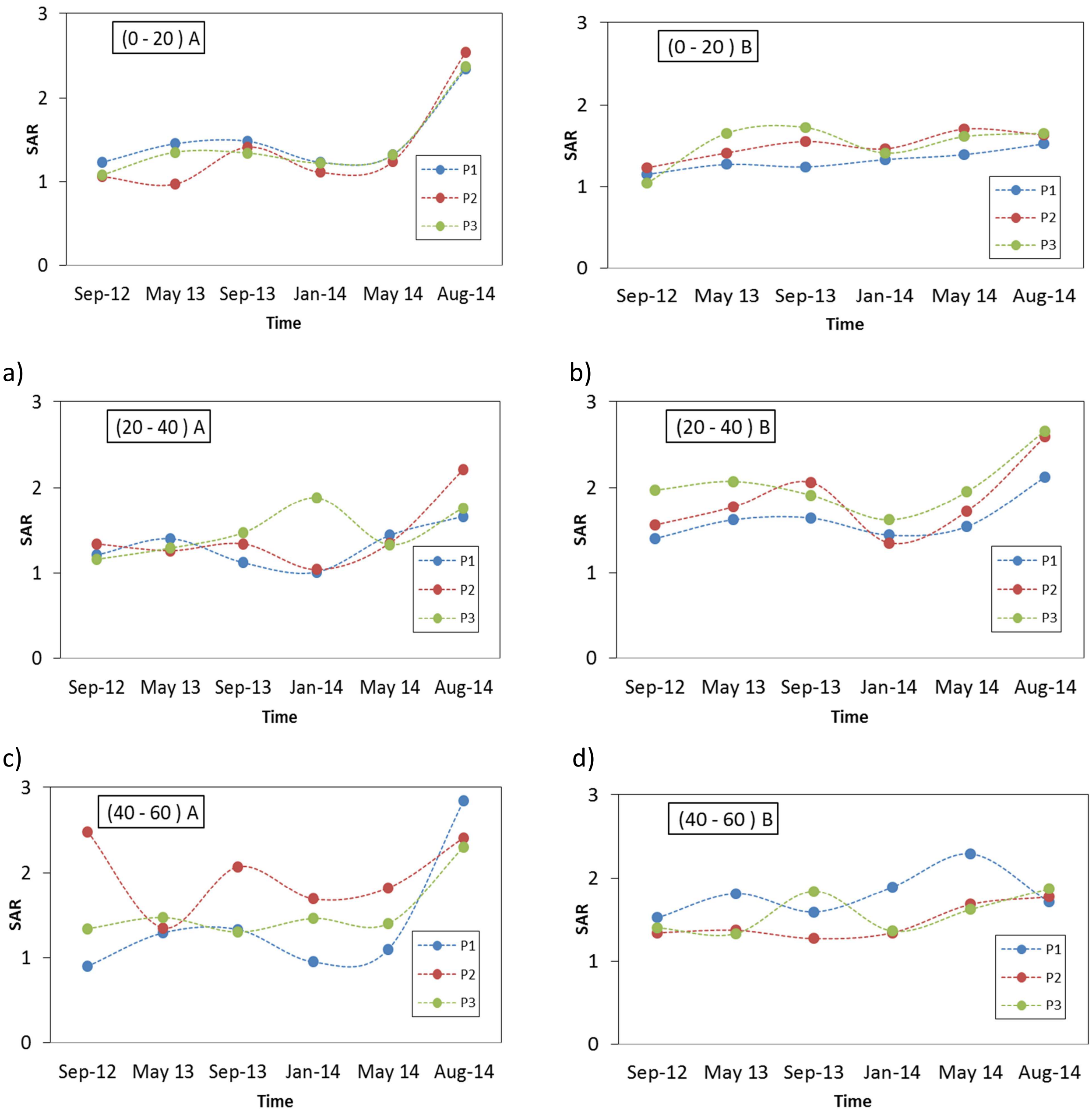
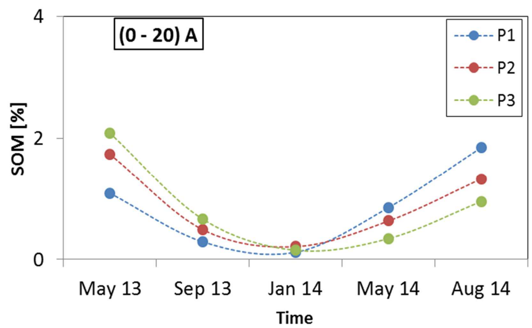


Figure 5-16: SAR values in the reuse plots and subparts (A and B) over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm depth.

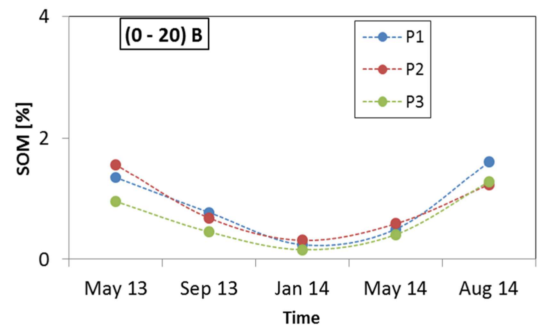
Ca^{2+} can be removed from soils by percolation of water, plant uptake, and mineralization in the form of calcite in alkaline soil (Pettygrove & Asano, 1984). During evaporation, Na^+ can remain in water and soil, while Ca^{2+} and Mg^{2+} precipitate as a mineral (calcite, carbonates, or magnesite (Tarchouna *et al.*, 2010). In arid and semi-arid areas, Ca^{2+}, Mg^{2+}, and Na^+ are the dominant cations in soils. However, these cations are contributing to increase soil pH by increasing the OH concentration in soil solution (Buckman & Brady, 1960, Miller & Donahue, 1995).

5.4.2.4 Soil Organic Matter (SOM)

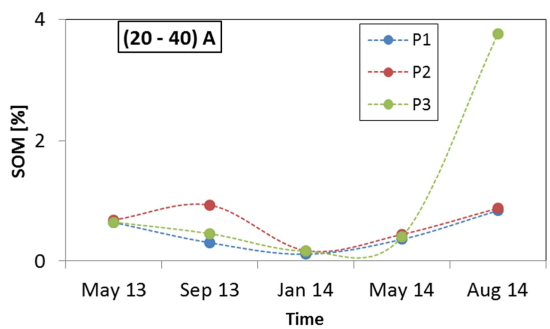
In arid and semi-arid regions, soils have low OM contents (Bronick & Lal, 2005). SOM content increased during summer and decreased during winter. Plant and weed residues from spring until summer increases the SOM at the topsoil layers. SOM also varied due to the amount of OM in water, climatic conditions, and tillage method. In comparison to P1, SOM increased in the soil profile in P2 and P3 related to the continuous supply of OM by effluents, **Figure 5-17**.



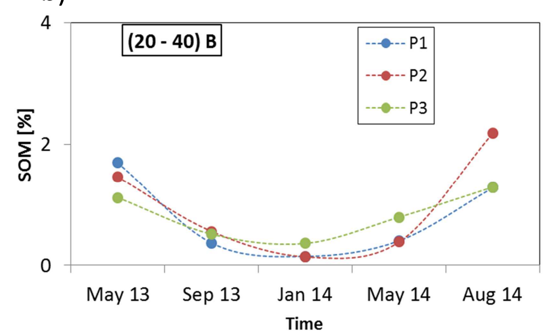
a)



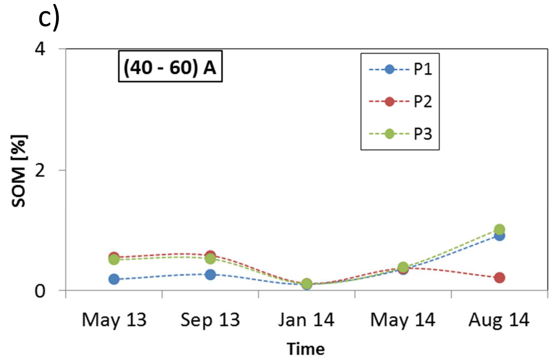
b)



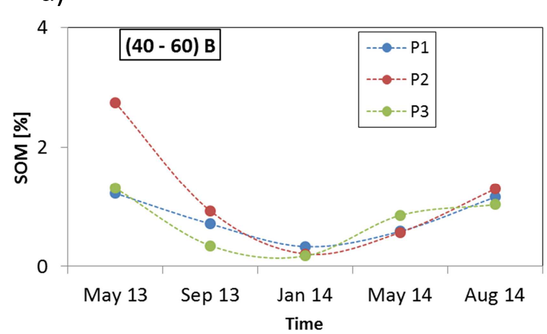
c)



d)



e)



f)

Figure 5-17: SOM values in the reuse plots and its parts A and B over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

Highest SOM during the study period was measured at 20 - 40 cm of 3.77 % in P3, due to the higher OM content of the ECO-1 effluent. In accordance with Kiziloglu *et al.* (2008), Lado and

Ben-Hur (2009), Mojiri (2011). In the end of monitoring, SOM was increased in irrigated soils in both A and B parts along soil profile.

Many Farmer in the region apply the animal manure as a method to increase the SOM, therefore, improving the plant growth. The accumulation of SOM in irrigated fields improves soil structural, reduce soil compaction and opposes salinity hazards (Ayers & Westcot, 1985). Thus, long term of reuse application increases the SOM in soils, which may improve the soil quality particularly in arid and semi-arid areas.

5.4.2.5 Macronutrient (K^+, NO_3^- , and PO_4^{3-})

Potassium, nitrogen, and phosphate are essential elements for plants growth. Results of these primary elements in soils were relatively low, despite of high nitrate concentrations in the irrigation effluents.

Potassium (K^+)

K^+ concentrations were increased in the reuse plots, especially in B subparts, as shown in **Figure 5-18**. According to Cottenie (1980), the K^+ levels in the plots were considered very low (less than 15 mg/L) to satisfy plant needs and reach the optimum range. Over the study period, the lowest K^+ concentrations were measured on the topsoil related to plant uptake or cation exchange (Heidarpour *et al.*, 2007a). In particular, K^+ is a vital salt that used widely in fertilizers to enhance the plant growth and soil fertility. Thus, the increase of K^+ concentration in the soil could cover the crop requirements.

K^+ reached the highest value of 10.6 mg/L at the depth of 20 - 40 cm, in P3 part B during summer. That showed the effects of applied irrigation water amount and weather conditions (evaporation factors). While, in A part a slight reduction in K^+ concentration observed at 40 - 60 cm. This is due to leaching by excess water.

Nitrate (NO_3^-)

NO_3^- concentrations were increased in the soil horizons after reuse application compared to the virgin soil. However, at 20 cm depths, there was inconsiderable variation in NO_3^- concentrations between the experimental plots, **Figure 5-19**. While, in the end of monitoring (August 2014), NO_3^- concentrations in P2 and P3 were sharply increased in B parts. This incrementing probably related to high nitrogen content in soils greater than crop needs. Similar results have been reported by Xu *et al.* (2010), Heidarpour *et al.* (2007a).

The level of NO_3^- also decreased in A part after implementing the modification, whereas this trend did not observe in B part. The highest level of NO_3^- concentrations was measured of 35.5 mg/L in P3 at 20 - 40 cm depth. However, a reduction in NO_3^- concentrations were observed during winter seasons as a result of washing out by rain. NO_3^- does not held by soil, but it moves easily through soil downward (Juan & Blanca, 2014). Thus, the concentrations of NO_3^- were low at 40 – 60 cm (near the irrigation pipes) that could accelerate NO_3^- percolation instead of moving upward. Moreover, excess moisture near irrigation pipelines (wet zone) reduces the availability of NO_3^- in soils and it could be denitrified. In agreement with Russell *et al.* (1993) reported that irrigated soil with treated wastewater produced a peak in N_2O followed by an instant drop in gas production. The results showed very low NO_3^- among the reuse plots; even it decreased in the 2nd phase of monitoring as a result of TN and NO_3^- reduction in the applied effluents. In the end of monitoring, the highest concentrations were reported of 18.1 and 17.3 mg/L in B part of the P2 and P3, respectively.

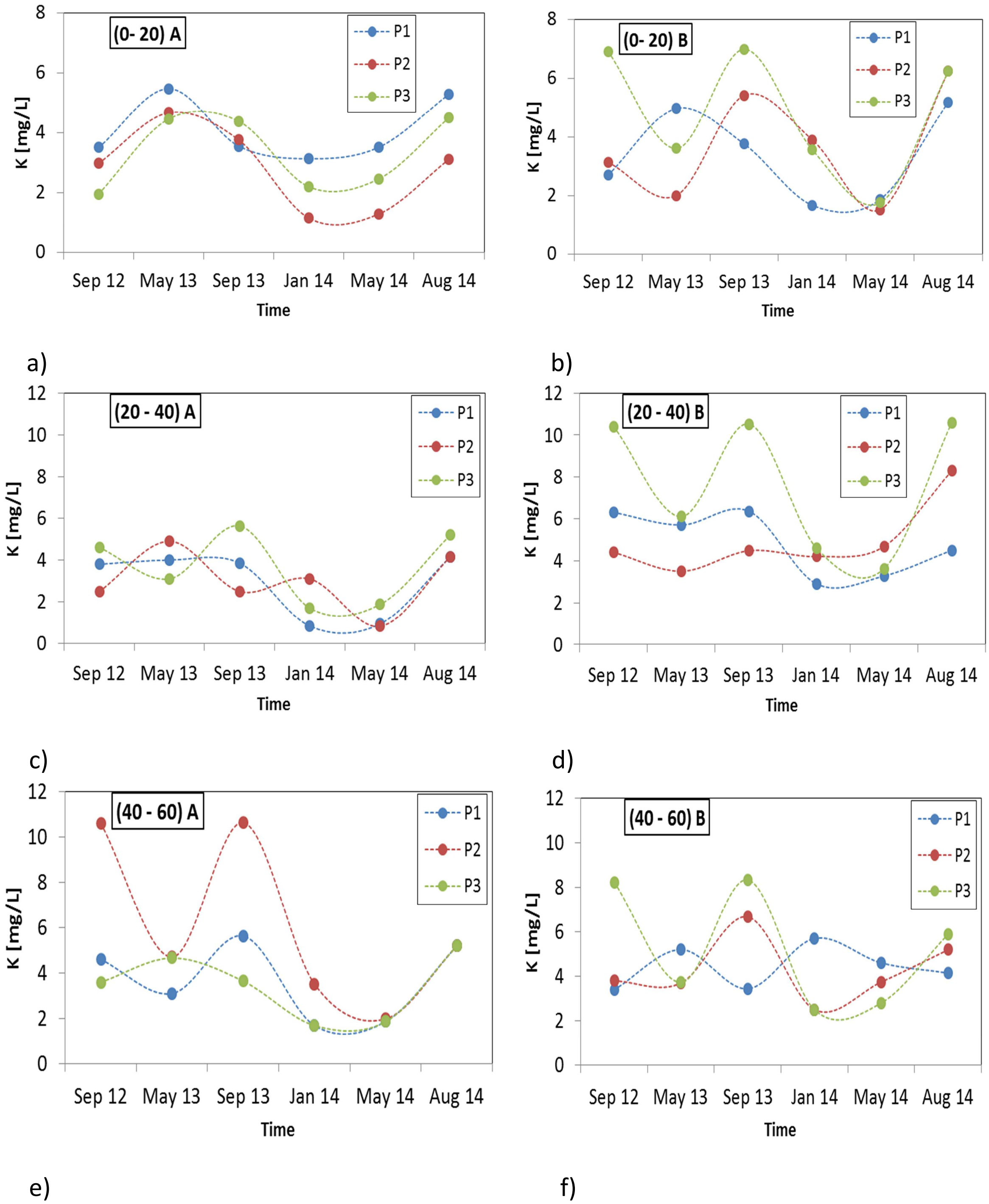


Figure 5-18: K^+ concentrations in the reuse plots and subparts over the study period. a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

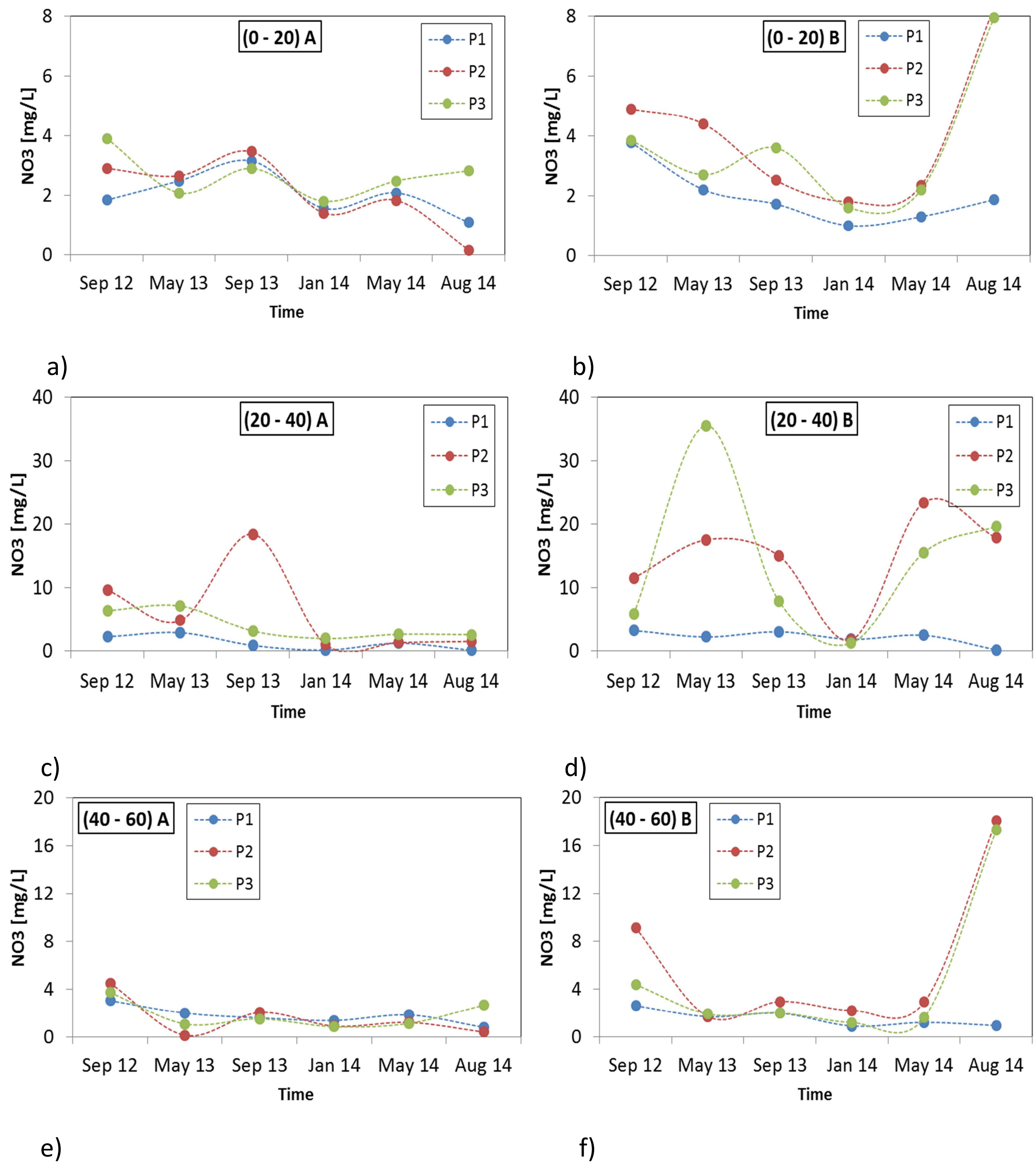


Figure 5-19: NO_3^- concentrations in the reuse plots and subparts over the study period. a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

Extracted Phosphate (Available P)

Phosphate is an essential plant macronutrient, which is very rare in soil and usually provided by fertilizers. Furthermore, in alkaline soils, PO_4^{3-} precipitates with Ca^{2+} then few remains available for plants uptake. In our study, the results showed the extract PO_4^{3-} from soils (Av. P).

The PO_4^{3-} concentration increased in all plots compared to the virgin soil, however, it was below the critical level less than 18 - 25 mg/L (Olsen, 1954). Results showed low concentrations of

PO_4^{3-} in topsoil layers in all plots due to continuous plant uptake, **Figure 5-20**. Therefore, there was no difference between reuse plots over the study period. In the end of the study, PO_4^{3-} concentration was increased to 68.4 mg/L in B part of P2. This is probably related to decomposition of OM and plant residue that increased the Av. P in the top of soil.

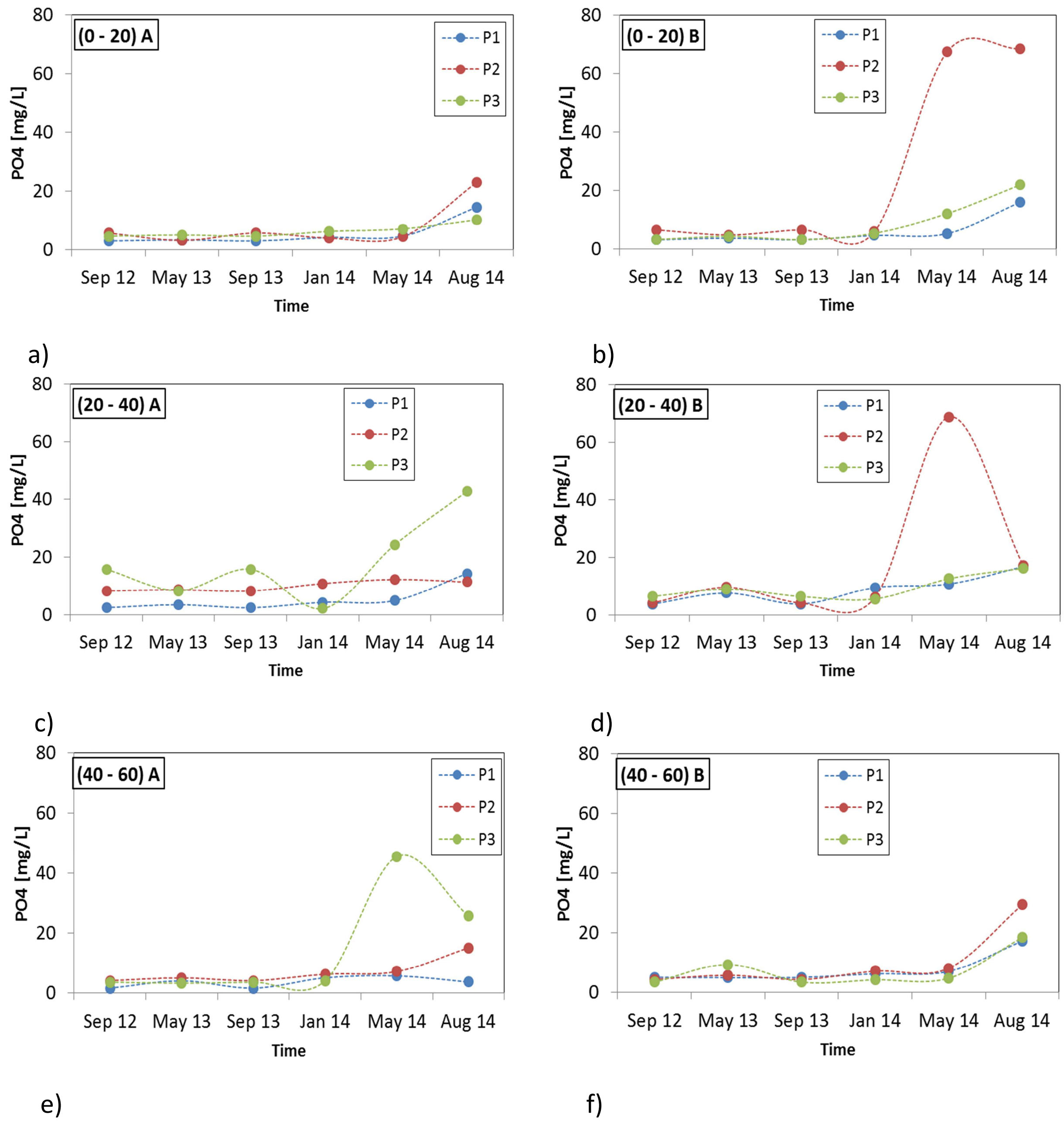


Figure 5-20: Av. P concentrations in the reuse plots and subparts over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

At 20 - 40 cm depth, PO_4^{3-} concentrations increased in A parts of P2 and P3. P3 showed the higher phosphate concentration of 42.7 mg/L, which was compatible with PO_4^{3-} concentration in the effluent. On the other hand, a stable content of PO_4^{3-} was observed in B parts until it increased to 68.8 mg/L. At 40 - 60 cm depth, PO_4^{3-} concentrations demonstrated similar trends at 20 – 40 cm. PO_4^{3-} concentrations increased in P3 (A part) to 25.6 mg/L. In general, the effluents contain small amounts of phosphorous ranged from 2.9 -8.4 mg/L. Thus, reuse for irrigation is valuable to plants without negative impacts in environment, even for long-term of reuse (Girovich, 1996, Degens *et al.*, 2000).

5.4.2.6 Anions (Cl^-, CHO_3^-, and SO_4^{2-})

The concentrations of chloride, bicarbonate, and sulfate were increased in irrigated soil compared to the virgin soil. Cl^- concentrations slightly increased on the topsoil layers in A and B parts at 20 cm depth over the study period, **Figure 5-21**. However, in the end of monitoring, Cl^- concentrations increased merely in A part as a results of continuous chloride supply with high evaporation rate.

At 20 - 40 cm, Cl^- concentrations in both A and B parts were increased in summer and slightly declined in winter over the study period. Guohua *et al.* (2000) highlighted that Cl^- concentration in soil is not fixed according to its mobility in soil. That illustrates the fluctuation of Cl^- concentrations in this layer (wet zone).

On the other hand, the Cl^- concentrations at 40 - 60 cm depth were stable in all plots and ranged from 40 to 80 mg/L. In B part, Cl^- concentrations increased in the end of monitoring in P2, while it decreased in P3. This incrementing is probably related to dissolution of Cl^- from sodium chloride (NaCl) and magnesium chloride ($MgCl_2$) from soils. The concern, Cl^- is a toxic anion for plants that moves easily through soil-water and it can be taken up by plants (Bohn et al., 2002). Thus, the toxicity concentration is ranged of 4 - 7 mg/L for sensitive plants and 15 - 50 mg/g for tolerant plants (Ayers & Westcot, 1985). However, if Cl^- exceeds the optimal concentration, injury symptoms will be appeared such as leaf burn or dry tissue of the crop (Ayers & Westcot, 1985).

The concentrations of HCO_3^- at the soil surface were constant over the study period, **Figure 5-22**. However, HCO_3^- levels were increased merely in the control plot (A and B parts), which was associated with higher Na^+ in soils (Thompson *et al.*, 2001). Thus, it considered as indicator for sodicity hazards. Precipitation of Ca and Mg carbonate from water reduces the level of dissolved Ca and Mg, therefore, increases the SAR (Bohn *et al.*, 2002).

Bicarbonate concentrations at 20 - 40 and 40 - 60 cm were slightly decreased compared to the virgin soil. This is due to leaching by continuous water. In contrast, at 60 cm in the B part of P2, the level of bicarbonate was increased to 300 mg/L that might come from soils.

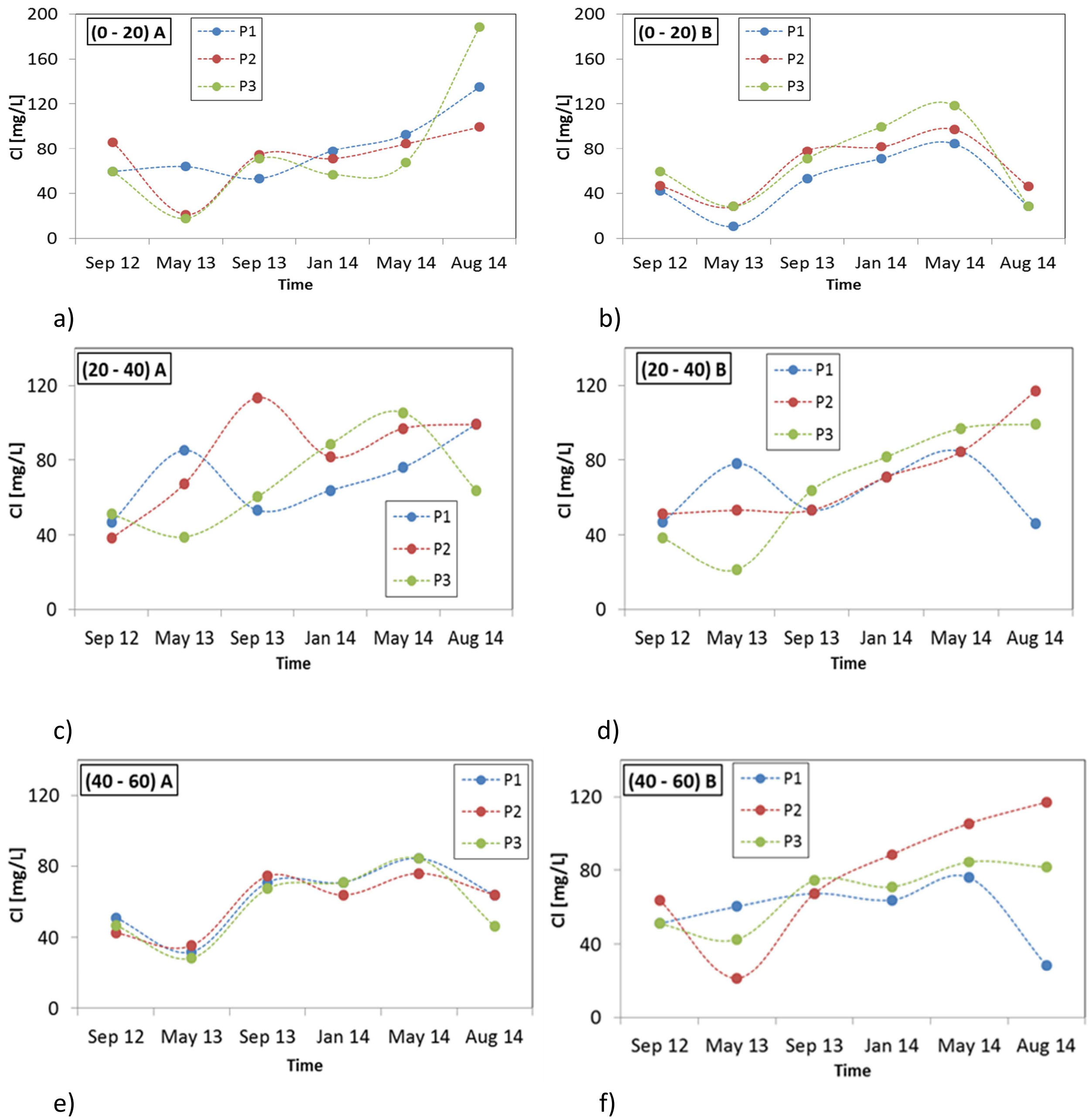


Figure 5-21: Cl^- concentrations in the reuse plots and subparts (A and B) over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

SO_4^{2-} concentrations were slightly increased on the topsoil layers in all A and B parts during the study period (**Figure 5-23**). There was no significant difference between irrigation plots, indicating high sulfate concentrations in the effluents did affect soil SO_4^{2-} concentrations. In the end of monitoring, SO_4^{2-} concentrations were increased merely in B part of P2.

At 20 - 40 cm depth, SO_4^{2-} concentrations were similar in the plots and subparts. On the other hand, the concentration of SO_4^{2-} increased at 40 - 60 cm depth in all plots and subparts. This incrementing was probably related to dissolution of sulfate from soils. A study by Modaihsh *et* *al.* (1994) reported that irrigation water rich with SO_4^{2-} affected the pH, EC_w and increased the content of nutrients in soil.

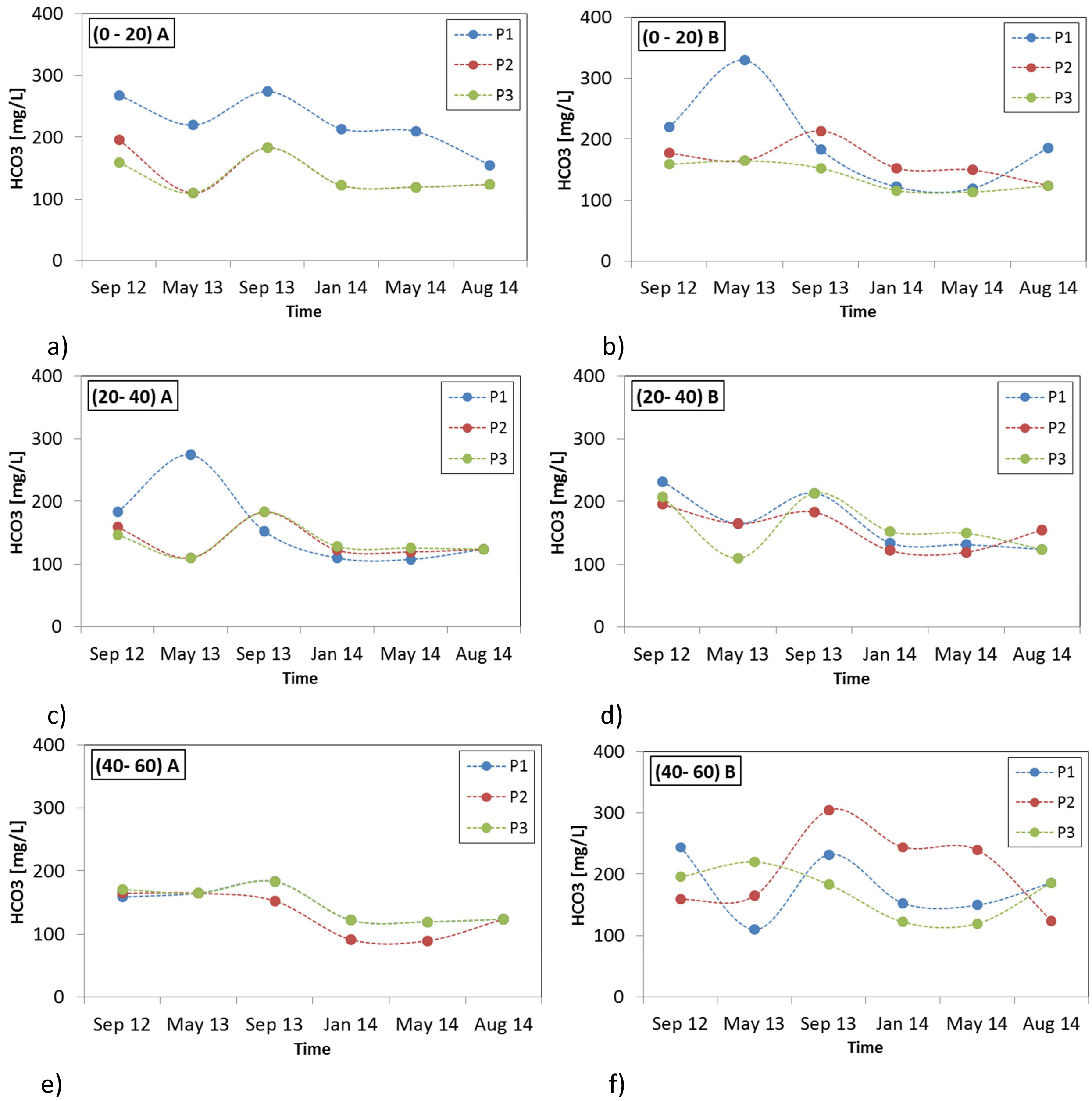


Figure 5-22: HCO_3^- concentrations in the reuse plots and subparts over the study period. a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

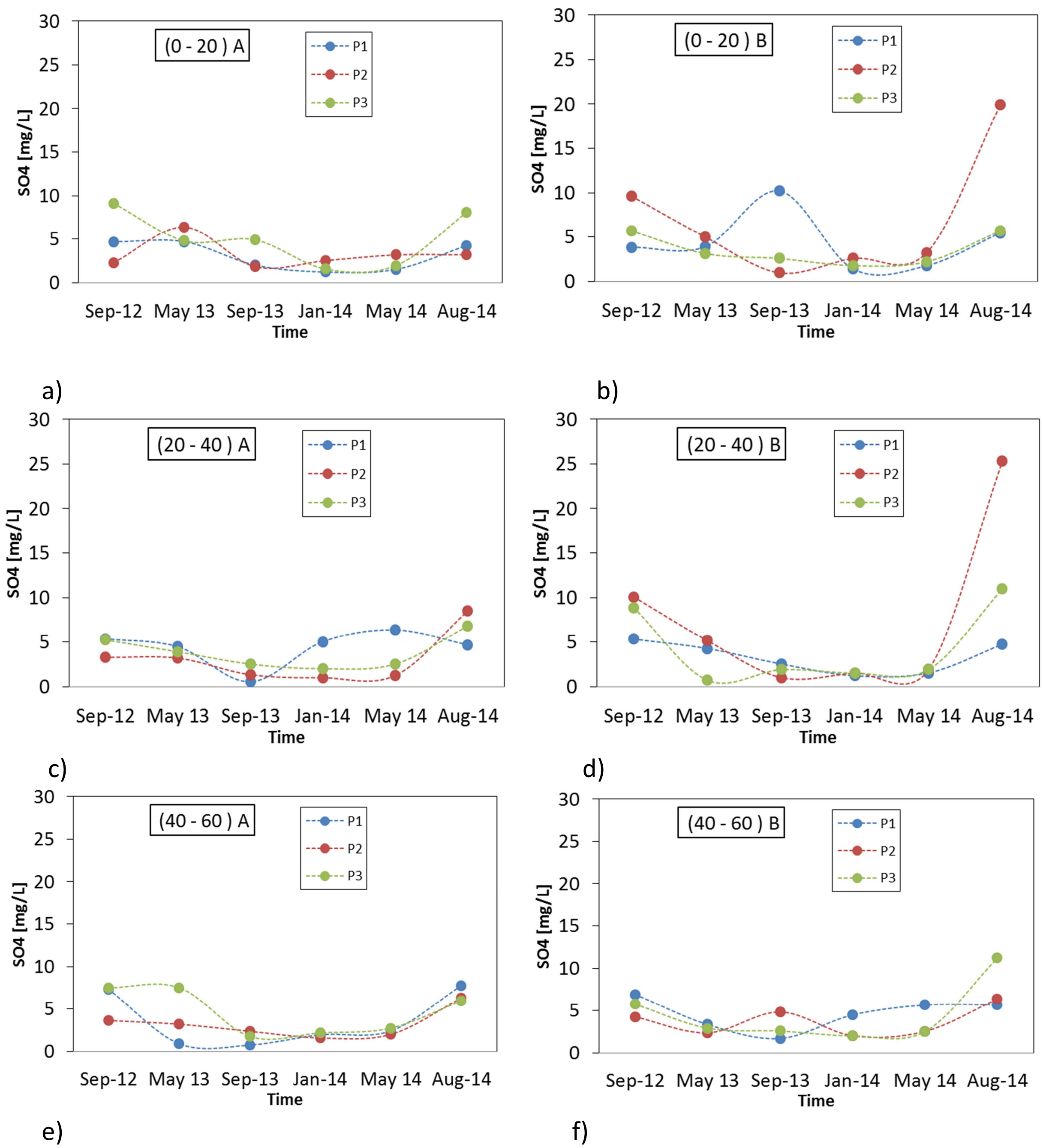


Figure 5-23: SO_4^{2-} concentrations in the reuse plots and subparts over the study period, a) and b) 0 - 20 cm, c) and d) 20 - 40 cm, e) and f) 40 - 60 cm.

5.4.2.7 Bacteria (Total Coliform, Fecal Coliform and *E. coli*)

The results revealed an absence of total coliform, fecal coliform, and *E. coli* in the irrigated soils, indicating the effectiveness of using subsurface irrigation with reuse application. In accordance with Oron *et al.* (2001).

5.5 Visual Lemon Trees Assessment

The effect of applying different treated wastewater qualities and quantities was evaluated on cultivated 34 Lemon trees. Plants growth, productivity, and wellness were determined based on the visual appearance of trees through the measurement of the trees height (cm), number of twigs, flowers and fruit and described the foliage color during the experiments.

5.5.1 Effects on Height Growth

The mean height increment increased from the beginning of measurement in 2012, as shown in **Figure 5-24**. The effects of using different water qualities and quantities during the experimental period on the trees height were statistically not significant ($p < 0.05$).

In the end of experiment, in 2014, the average height was similar among lemon trees in the reuse plots and subparts. Additionally, higher height growth ratings have been correlated with high irrigation regime (A parts) merely in the reclaimed water plots. While, there was no difference between trees height in P1 under different irrigation regime. That is agreed by Parsons *et al.* (2001) observed high rates of reclaimed water irrigation enhanced growth and production of citrus trees. Based on their results, a significant difference in trees growth rate was observed in the 4th year between trees irrigated from well water and treated water. Many results have been reported by Parsons and Wheaton (1992) that tree height was significantly lower for orchards irrigated with fresh water compared with other irrigated by reclaimed wastewater.

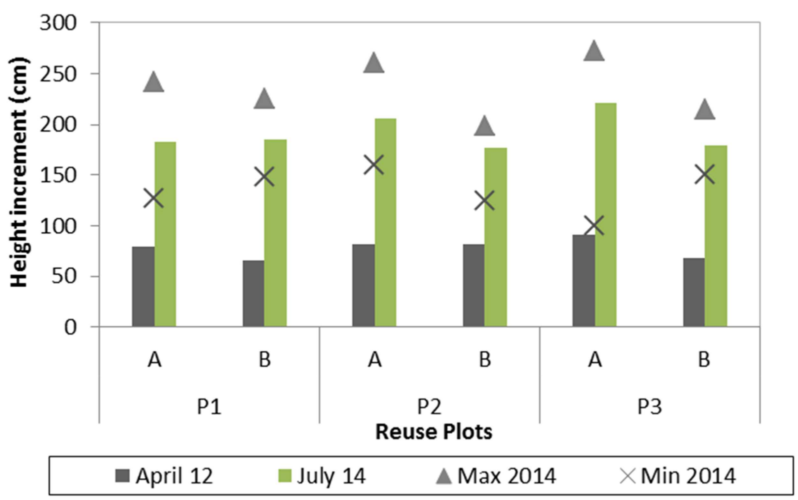


Figure 5-24: Average of trees height (cm) in the reuse plots and subparts after plantation (April 2012) and in the 3rd year with maximum and minimum measurements.

5.5.2 Effects on wellness (foliage color)

Based on the visual assessment, leaf toxicity symptoms did not observe despite of high Cl^- concentration in the irrigation water. This was probably related to using subsurface irrigation, which provides extra purification for water through soils before uptake by roots. On the other hand, based on leaves analysis by Zekri and Koo (1994) they measured higher Na^+ and Cl^- concentrations in leaf samples from orchards irrigated with reclaimed water under higher irrigation applications.

A symptomatic of deficient nitrogen was observed mainly on the leaves in the control plot such as pale, narrow, and slightly rolled foliage, **Figure 5-25**. In addition, yellowish leaves appeared highly in the P1 as a result of insufficient macronutrient elements. Many researchers confessed that reclaimed wastewater is an important source of nitrogen for citrus trees (Zekri & Koo, 1994, Parsons & Wheaton, 1992). In our experiment, nitrate level in the effluents was higher than the recommended level in the JS, but it was within the optimum range in soils for citrus trees growth.

Figure 5-25: Yellowish and slightly rolled leaves in the control (1st) plot as symptomatic of insufficient macronutrients during the experimental period.

5.5.3 Effects on Growth Density (twigs)

Number of twigs increased from the beginning of measurement in 2012 in all plots and subplots, as shown in **Figure 5-25**. The higher number of twigs formed during spring period (mainly March) after clipping. In the end of experiment (August of 2014), the average number of twigs was significantly different among trees, plots and sub-parts.

P1 and P2 did not show a significant difference under different irrigation regime (A and B) ($p < 0.5$). The mean values were marked difference between trees in the same row that some trees had several new twigs and other had none. In P3, higher growth density has been observed under high irrigation regime (in A part).

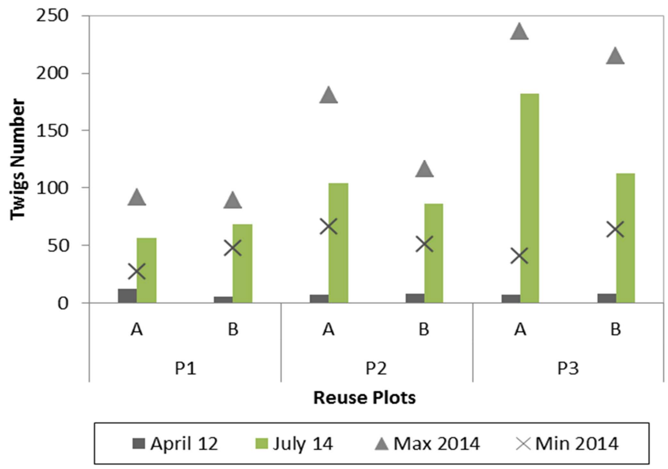


Figure 5-26: Twigs number in each subplot in the reuse plots after plantation (April 2012) and on the 3rd year with maximum and minimum measurements.

5.5.4 Effects on Productivity (Flower and fruit)

After the 2nd year of plantation, the number of flowers and fruit increased. The higher number of flowers and fruit were observed during production period (December to April). However, highest number of flowers and fruit were measured in the March and April 2014. **Table 5-7** shows the highest averages and SD of flowers and fruit in the experimental irrigation plots in the 3rd year. Highest fruit production and size were observed under high irrigation rate (A part) with reclaimed water. However, fruit peel was coarser than fruit peel under lower irrigation regime (B part).

Table 5-7: Averages and standard deviations of flowers and fruit in the three irrigation plots (P1, P2 and P3), during the 3rd year after plantation.

	P1		**P2**		**P3**	
Parameter	A	B	A	B	A	B
Flowers [a]	35 ± 23.1	41 ± 14.8	48 ± 17.9	21.4 ± 13.4	71.6 ± 35.6	55 ± 43.1
Fruit [b]	22 ± 21.3	11 ± 2.8	39. ± 40.2	17 ± 12.5	45 ± 50.3	41 ± 0*

a: highest number of flower in the March 2014. b: greater number of fruit in the April 2014. * Fruit number from one tree.

Fruit juice content was usually reduced by highest irrigation rate, related to the higher fruit production. This agreed by Parsons *et al.* (2001) found high reduction in juice content with higher irrigation rate, but it is still higher than fresh water. On the other hand, Koo and Zekri (1989) observed that reclaimed water utilization reduced soluble solids and acid concentration in the juice due to higher soil water content in the irrigated field.

6. Conclusions and Recommendations

The study was set out to explore the suitability of various VFCW designs, in Germany and Jordan, to produce high quality effluents that conform to the legal requirements and health standards (objective 1). The research has also sought to optimize the VFCWs treatment performance in order to conform to the JS for reuse in irrigation, particularly using several costless and simple operational modifications (objective 2). Additionally, the research has addressed a short-term evaluation of VFCW effluent reuse in irrigation with a subsurface irrigation system in Jordan (objective 3). The results showed a number of important conclusions.

6.1 Conclusions

1. In Germany, at LRB, pair of two-stage system (planted and unplanted) showed high removal efficiency of TOC, BOD_5, and TSS over the study period. Generally, there was no significant role of plants *(Phragmites australis*) on the treatment performance. TN and *E. coli* reduction were optimized by implementing a 30 cm saturated layer in the first stage of the two-stage wetland system.

2. In Germany and Jordan, the impact of temperature was negligible in the VFCWs treatment performance over the study period. Thus, these robust designs are appropriate for decentralized wastewater treatment under different climatic conditions.

3. In Jordan, Both ECO-1 and ECO-2 designs have shown high removal efficiency of COD, TSS, and BOD_5 over the study period, conforming to the JS category-A.

- In ECO-1, TN and NO_3^- reduction were optimized, by converting the recirculation tank into attach growth tank (using electric conduit pipes as plastic media), conforming to the JS category A and B, respectively. Over the study period, *E. coli* reduction were constant, 2 $\log_{10}$ reduction, conforming to the JS category C (more than 1000 MPN/100 mL).

- In ECO-2, TN and NO_3^- reduction were optimized by raw wastewater step-feeding application, conforming to the JS category B. On the other hand, *E. coli* reduction influenced negatively by step-feeding modification, conforming to the JS category C. That *E. coli* reduction can not be optimized using raw wastewater step-feeding method.

4. Evapotranspiration was relatively high in ECO-2 (2nd stage planted with *Phragmites australis*) during summer, which increased the salinity of the effluent.

5. Tap-water, ECO-1, and ECO-2 irrigation water denoted high salinity and low to medium SAR values that requires a slight to moderate restriction on reuse in irrigation. Thus, a proper periodic monitoring of soil fertility and quality parameters are required to ensure successful and safe reuse of wastewater for irrigation.

6. Tap-water, ECO-1, and ECO-2 irrigation water denoted high salinity and low to medium SAR values that would require a slight to moderate restriction on reuse in irrigation. Thus, a proper periodic monitoring of soil fertility and quality parameters is recommended in order to ensure sustainable and safe reuse of wastewater for irrigation.

- Soil results revealed an absence of total coliform, fecal coliform, and *E. coli* in the irrigated soils, indicating the effectiveness of using subsurface irrigation as a disinfection step.

- Using treated effluents and tap-water showed the same trend of increasing soil salinity compared to the virgin soil salinity. However, higher soil salinity was observed at the top soil layer (0 - 20 cm) in B part (less amount of water) due to high evaporation and capillary rise that increased salts accumulation in top soil zone.

7. Lemon trees irrigated with reclaimed water showed higher trees height, fruit production, and healthy leaf rather than control plot (P1). Thus, reclaimed wastewater in irrigation provides a gainful alternative to tap-water and fertilizers application, according to the plant growth, productivity, and wellness as a sustainable source.

6.2 Recommendations for Future Work

- An appropriate local standards are required for decentralized treatment plants.

- Rigorous operational mentoring and flowmeters are recommended to ensure high treatment efficiency in the treatment designs.

- More studies are needed to improve the *E. coli* removal and salinity reduction in ECO-1 and ECO-2 designs.

- Partial saturated bed as a single VFCW is recommended to apply on large scale. Furthermore, it will be suitable design in Jordan due to high TN and NO_3^- removal, conforming to the JS category A.

- Unplanted constructed wetlands are recommended for arid and semi-arid areas, which reduce the water loss via evapotranspiration and reduce the effluent salinity compared to planted system.

- The effect of effluents reuse using subsurface irrigation system over long-term should be the focus of future research, considering the impact on soils, plants, and ground water.

- In Jordan, it is important to define the suitable agricultural areas for reuse application considering type of soils and trees, and the ground water level and quality.

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