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Master Thesis

Phytoremediation of Hydrological Tracers in Lab-scale Wetland Systems

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Declaration of Authorship

I, Sameera Zaman, hereby declare that the thesis *Phytoremediation of hydrological tracers in lab-scale wetland systems* is my own original work except where indicated otherwise. Any use of the works of any other authors, in any form, are properly acknowledged and referred at their point of use. This work has not been presented previously to any other examination offices.

Freiburg, 01.06.2016 Signature

Acknowledgement

This thesis would not have been as it is without the guidance and support of many people who contributed, directly and indirectly, during the whole process.

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Abstract

Recently, various studies have been conducted to understand the behavior and interaction between tracers and vegetation in constructed wetlands. Uranine (UR) and Sulforhodamine B (SRB) are being widely used to investigate natural treatment systems for pesticide pollution. Due to increase in their applicability, the complex processes influencing transformation, degradation and accumulation of the tracers need to be further investigated. This thesis is based on lab-scale wetlands with varying wetland parameters such as vegetation and saturation. It attempts to contribute to the general understanding of the tracer behaviors and interactions with specified wetland parameters. Two species, *Phalaris arundinacea* and *Typha latifolia*, were used to monitor the concentration and mass distribution pattern of the tracers under saturated and non-saturated conditions over twelve weeks. Major water quality parameters and presence of nitrates were also monitored throughout. It was found that vegetation has an impact on the behavior of the tracers, when compared to the non-vegetated methods. Possible signs of degradation and plant uptake were identified, although such processes could not be proved entirely with the results obtained. In general, more studies regarding the efficiency of pesticide removal using vegetation is necessary to contribute to the applicability and efficiency of constructed wetland systems.

Key words: Uranine, Sulforhodamine, *Phalaris arundinacea, Typha latifolia,* constructed wetlands, degradation, phytoremediation

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Abbreviations

appr = approach

pa = *Phalaris arundinacea*

tl = *Typha latifolia*

SRB/srb = Sulforhodamine B

UR/ur = Uranine

CFU = Colony-Forming Units

Rpm = rotations per minute

EDTA = Ethyldiaminetetraacetic acid

Functions	**Units**
Length	nm = nanometer
	cm = centimeter
	mm = millimeter
Mass	µg = microgram
	mg = milligram
Volume	ml = milliliter
Concentration	µg/l = micrograms per liter
	mg/l = milligrams per liter
	kg/l = kilograms per liter
Temperature	° C = degree Celsius
Light Intensity	Lx = Lux
Electrical Conductivity	µS/cm = microSiemens per centimeter
Concentration	M = Molar
Power	W = Watt

Symbols

NO_3^- = Nitrate

I_{start} = start intensity

I_{peak} = peak intensity

I_{end} = end intensity

λ_{start} = start wavelength

λ_{peak} = peak wavelength

λ_{end} = end wavelength

1. Introduction

Plants are often categorized s as a major resource for human needs and its ecological roles, but currently their value to counterbalance environmental pollution is playing a crucial role in development of green technologies (Schröder *et al.*, 2007). The emerging concept of phytoremediation to remediate contaminated soil or water has been brought to focus in the recent decades. This concept introduces the use of plants to treat a medium contaminated with organic or inorganic wastes. Phytoremediation describes the process where plants directly or indirectly absorb, store or degrade the contaminants from the medium (Cunningham and Ow, 1996). It has gained attention because the energy input is negligible compared to the conventional practices. However, phytoremediation needs more scientific research for widespread application so that it can be used effectively. The cost-efficiency and effectiveness of such processes may help us in order to naturally remediate soil and water.

This report addresses phytoremediation mainly from the aspect of pesticide pollution, which is presently one of the major threats to water quality. Due to rapid increase in global population and the necessity to meet the required food demand, pesticides have been widely used to ease the agricultural processes. However, these chemicals find a way to enter surface and groundwater through various point and non-point sources. Such contaminations are usually linked to their toxic effects on human health, as well as nature (Tilman *et al.*, 2001). Not only do such contaminations occur in real-time, some of these pesticides and their metabolites tend to persist in the nature even years after application (Gutierrez and Baran, 2009).

However, it has been widely studied that constructed wetlands as buffer zones can play out to be a good pre-treatment scheme for remediation of pesticide contaminated water. Similar to any other treatment systems, the efficiency of such processes depend highly on the hydrological characteristics of the wetlands, as well as the physical and chemical properties of the pesticides, both of which vary on a spatial and temporal scale (Passeport *et al.*, 2014). Generally, a constructed wetland can help by enabling processes such as sedimentation of suspended solids, diffusion of nutrients,

mineralization of organic products, adsorption and biological transformation through micro-organisms and vegetation (Stottmeister *et al.*, 2003, Brix, 1995).

The types of vegetation used are also very important for the efficiency of constructed wetlands as they vary depending on the target pollutant (Brisson and Chazarenc, 2008). In Europe, *Phalaris arundinacea* and *Typha latifolia* are commonly used for phytoremediation purposes, especially for mitigation of pesticide pollution (Schröder *et al.*, 2007, Vymazal and Krőpfelová, 2005). Coming from the family of Poaceae, *Phalaris arundinacea* is a tall, perennial bunchgrass. The stems can grow up to two meters in height and its thick rhizomes usually spread underground. It forms extensive single-species stands in wetlands, usually along the margins of water bodies or wet areas. It grows widely all over the world and is well known for surviving even in poor soil condition. Even though it is often categorized as invasive species in wetlands, *Phalaris arundinacea* is suggested as one of the most effective plants for phytoremediation, especially for improvement of soil quality (Lavergne and Molofsky, 2004). Similarly, *Typha latifolia* is also a wetland species growing near water all over the world. It is generally known to grow in flooded areas and is also identified to be invasive in its environment. Belonging to the family of Typhaceae, it is a perennial herbaceous wetland plants and can grow up to three meters in height, usually in submerged water. However, its rhizomes are very sensitive and the survival of the plants is very dependent on water availability and level (USDA, 2006). In this study, these two plants and their ability to act as phytoremediators are focused.

In hydrology, fluorescent dye tracers have been used in order to assess various hydrological processes due to its water-like mobility and hence, the ability to mimic inaccessible zones of wetlands. Certain fluorescent tracers are used to study hydrological processes as the fluorescence can be measured by excitation and emission wavelengths of these dyes. Uranine (UR) and Sulforhodamine (SRB) has been used in combination with *Phalaris arundinacea* and *Typha latifolia* to understand the process of solute transport, in this case, pesticide transport. For the following experiment, (UR) and Sulforhodamine B have been selected as their characteristics are suitable to mimic the behavior of certain pesticides. These tracers

act as good proxies as they do not influence the water density or flow patterns (Lange *et al.*, 2011). These two tracers are often used in combination as they can be simultaneously measured in a single scan due to having high differences in their detection wavelengths.

Many factors play role in selection of the tracers to be used such as solubility, pH dependency, ecotoxicity, adsorption and reactivity (Leibundgut *et al.*, 2009). The distribution of tracer masses, representing pesticide masses, can give an overview of where such pollutants are going. It is assumed that the pollutants are either in the soil, in the water or taken up by the plants. However, in reality, the situation is much more complex. Many physiological, chemical and biological processes can cause sorption, degradation or alteration of the product. Sorptive behavior of tracers depends on the chemical composition and interaction of the tracers with their surrounding environment (Sabatini, 2000). In this respect, it is important to know if the tracer undergoes irreversible processes, which can cause loss of the tracer, and hence, will not be completely accounted for. Another relevant reaction in this respect is photolysis of tracers. Exposure to light for a long duration can cause the tracers to be degraded or altered into different substances (Leibundgut *et al.*, 2009). The tracers can also undergo biodegradation, even though in-depth studies on this subject are lacking. However, it has been identified that biodegradation is not relevant for short term experiments as it is a long term process. Keeping these factors in mind, UR and SRB were selected. UR is a synthetic red organic compound in powder form that has no fluorescence. However, when dissolved in water, the dissociation of the compound causes lime green fluorescence. The UR molecules are negatively charged and hence do not show sorptive behavior to sand. This makes it a conservative tracer due to having low reaction and interaction with its surrounding environment. Even though UR is widely used because of its conservative behavior, drawbacks may arise due to it light and pH dependency; low pH of samples may result in less fluorescence intensity and its exposure to light can degrade it irreversibly (Gutowski *et al.*, 2015). SRB, on the other hand, is non-conservative due its electronegativity. This causes SRB to display high sorptive behavior, especially onto positively charged sand particles (Sabatini, 2000). However, when compared to UR, SRB is stable when

exposed to light and doesn't undergo photodegradation. It is also pH resistant. UR is not at all toxic to the environment, but the same cannot be said about SRB. Hence, it is important to take precautions while using these tracers in field studies. Their susceptibility to biodegradation is not entirely proven, but it could be possible. The summary of chemical and physical properties of the tracers is displayed below in table 1.1 (Leibundgut et. al, 2009). Both these tracers are cost-effective and were proven to be useful in hydrological studies.

Table 1.1: Relevant properties of UR and SRB, modified from Leibundgut et. al (2009)

	Uranine	**Sulforhodamine B**
Chemical Formula	$C_{20}H_{10}O_5Na_2$	$C_{27}H_{29}N_2NaO_7S_2$
Excitation/Emission [nm]	491/516	561/586
Relative fluorescence yield	100	7
Detection limit [mg/m^3]	0.001	0.03
Toxicity	Harmless	Sufficient
Solubility [g/l, 20° C]	300	10 (10° C)
Light sensitivity	High	Low
Sorption behavior	Very good	Insufficient

Using constructed wetlands as a treatment for pesticide pollution and water retention has proven to cost-effective and efficient. Such practices are growing, but the complexities of such procedures need to be further studied (Durst *et al.*, 2013). Global use of phytoremediation might solve the issue of water scarcity up to some extent, especially in the developing part of the world where high cost of infrastructure and maintenance plays a big role. Hence, a low cost water treatment system might be useful for closing the huge gap between economic benefit and green practices (Lishenga *et al.*, 2015). Water scarcity by itself has many different causes and effects. However, with phytoremediation, water quality can be improved by addressing both water and soil pollution. Though the contaminants can be diverse ranging from heavy metals to microbial contaminants, each of these contaminants can be treated using many different plants, but this requires further species-specific research. Small scale water treatment right at the source of pollution source is crucial to address these mentioned problems. This thesis is a

part of one such research project called *Maßnahmen für einen nachhaltigen Umgang mit Pestiziden und deren Transformationsprodukten im Regionalen Wassermanagement* (MUTReWa). This thesis examines the interaction of UR and SRB with the aforementioned wetland parameters and is an attempt to understand the behavior of the tracer under varying wetland conditions.

2. Problem and Objective

In recent management practices, the use of constructed wetlands as a water treatment system has increased. In order to improve the efficiency of such practices, the underlying processes comprising of interactions between the different components of such ecosystems need to be investigated. This research will focus on two commonly used fluorescent tracers in hydrology, UR and SRB, in order to understand how different wetland parameters might influence their behavior. Wetland conditions are imitated in this laboratory experiment to investigate if parameters, mainly water availability and vegetation, can cause degradation or transformation of these tracers through chemical, physical and biological processes.

To develop an understanding of such processes, different wetland conditions were created and the distribution of the tracers remaining in different components of the system was measured. The concentration changes of each tracer in water, sand, its pore water and gravel were measured over the span of 13 weeks. Along with that, the final mass distribution of the tracers were calculated and analyzed. This will represent the percentages of tracer in each of these components from final measurement and give an idea about the degradation or transformation processes. In addition to that, several water parameters such as pH, dissolved oxygen, electrical conductivity, temperature and presence of nitrates were also measured to understand the main factors behind such processes.

The purpose of this research is to contribute to the understanding of the behavior of UR and SRB and their interaction with other wetland components. It also aims at increasing the knowledge about its application in reality to mitigate not only pesticide pollution from agriculture, but also water pollution in general.

3. Materials and Methods

3.1 Experiment Location

The experiment was conducted at the Institute of Hydrology, Faculty of Environment and Natural Resources of Albert Ludwigs University of Freiburg (Fahnenbergplatz, 79085 Freiburg im Breisgau).

3.2 Experiment Set-up

The experiment was a continuation to a previous master thesis: "*How conservative are Fluorescent tracers?"* conducted by Schelhorn (2015). Hence, the experiment set up was in accordance to the previously conducted study.

The set up was aimed in creating laboratory scaled wetlands with varying conditions and parameters. A total of 36 black Polypropylene buckets with dimensions 335 X 404 X 539 mm and filling volume of 50 liters were set up on top of a 30 cm high wooden platform. Each bucket was identically modified for conducting the study. The schematic diagram of the buckets is displayed in figure 3.1. At the front facing side of the bucket, a plexiglass pipe with a diameter of 15 mm with a metric scale was attached for determination of the water level inside the buckets. The pipes were covered with removable plastic to reduce light exposure and the top ends were covered with paper plugs. At the bottom of the glass pipe, a chrome-plated brass tap was attached as an outlet for water sampling. The outlet was fitted with a stainless steel filter with a mesh size 0.27 mm to reduce sand infiltration. Inside the buckets were layers of gravel and sand. The gravel layer (grain size 4-8 mm, bulk density 1.56 kg/l, porosity 30%) was approximately 8cm wide at the bottom and was topped with approximately 30 cm of sand (grain size 0.01-2 mm, bulk density 1.5 kg/l, porosity 35%). Through the layers of sand and gravel, a perforated tube with a diameter of 3.5 cm and perforations of 0.1 mm was set up in the centre of the buckets. The perforations served as a filter for sand from the water in the tube. By this set-up, a closed wetland system was created.

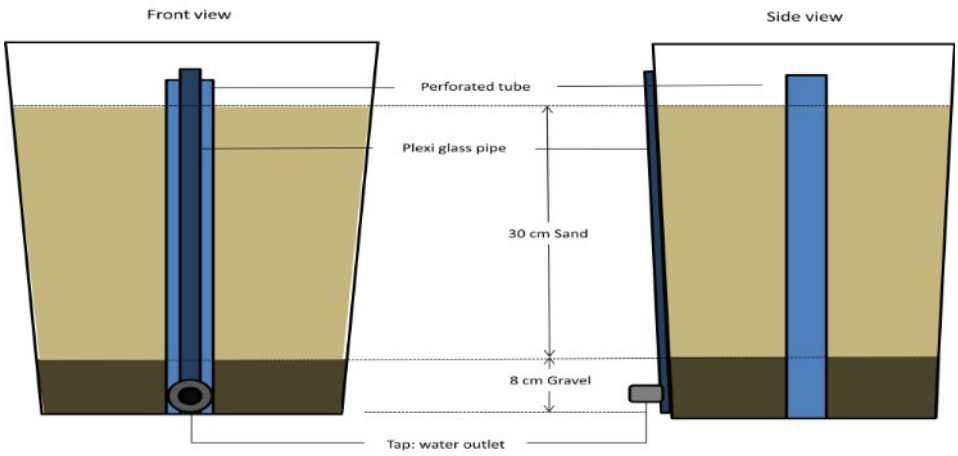

Figure 3.1: Schematic diagram of the buckets showing the front view and the side view with all the labeled components

Two wetland species, *Phalaris arundinacea* and *Typha latifolia*, were planted separately in twelve buckets each (a total of 24 buckets), and the remaining 12 buckets were left non-vegetated. *Phalaris arundinacea* was collected from the constructed wetland study site near Eichstetten (48° 05' 48" N; 07°44' 40" E), Baden-Württemberg, Germany (Schelhorn, 2015). Due to *Typha latifolia* being sensitive and non-resilient to environmental changes, extraction from the study site was not possible. They were purchased from the nursery *Kaiserstühler Staudenhof Menton* near Eichstetten. Initially, fertilizers were used for the plants to adapt better to their new artificial surroundings. However, that was 6 months before this experiment was conducted and is assumed to have no effects on the study.

The plants were provided with sufficient light despite its indoor location. Four lights (64 X 3 W with optical lenses) were installed equally spaced above the rows and a timer for 11 hours of light exposure was set (daily from 6 AM to 5 PM).

3.3 Approaches

The buckets were set up by considering a few important wetland parameters. The first parameter considered was the level and frequency of saturation. Eighteen buckets were constantly under saturation. The buckets maintained a certain level of water in

them at all times throughout the study. The other eighteen buckets were under varying saturation, identified in this report as non-saturated. In this case, the water level was allowed to drop low before re-saturating making lower amount of water available for the plants. The second parameter was the vegetation; twelve buckets were planted with *Phalaris arundinacea*, twelve buckets with *Typha latifolia*, and the remaining twelve buckets were left without vegetation. The third parameter was the tracer used, hence eighteen buckets were with the tracer UR and the other eighteen were with SRB. Table 3.1 shows the combination of all the thirty six buckets, with each approach having three replicates.

3.4 Tracer Injection

Due to a batch of Tracer being injected for the previous study, the possibility of the tracers remaining in the buckets was considered. Before the injection of the tracers for this study, the initial concentrations were measured to evaluate an accurate starting point. UR was purchased from Sigma-Alderich (CAS-no. 518-47-8) and SRB was purchased from Waldeck GmbH & Co KG (CAS-no. 3520-42-1). The tracers were re-injected on January 21, 2016. The injection was conducted according to the methods described by Schreiber (2012).

Prior to the injection, the buckets were left for approximately a week for the water levels to fall to zero in order to ensure an even distribution of tracers at all heights. The average volume of water to saturate the buckets was estimated at 800 ml of water. The target masses of the injections were 0.7 mg of UR or 1.4 mg of SRB according to the approaches. This was done by preparing 1000 ml UR solution with a concentration of 14 mg/l and another 1000ml SRB solution with a concentration of 28 mg/l. For each injection, 50 ml of the respective solutions were taken, diluted with 800 ml of tap water and poured across the surface as evenly as possible using a tin watering can (Figure 3.2). To avoid contamination, the pipettes were rinsed with tap water after each injection. In order to prevent possible photodegradation of the UR, the injection was conducted slightly after sunset in absence of light with the artificial lights turned off.

Table 3.2: List of approaches mentioning the water level, the species planted and the tracers injected in the buckets.

Approaches		**Replicates**	**Water level, plant species and tracer**
Approach 1	Appr1_Pa_UR	UR1_1	Saturated, *Phalaris arundinacea*, UR
		UR2_2	
		UR3_3	
	Appr1_Pa_SRB	SRB1_4	Saturated, *Phalaris arundinacea*, SRB
		SRB2_5	
		SRB3_6	
	Appr1_Tl_UR	UR1_7	Saturated, *Typha latifolia*, UR
		UR2_8	
		UR3_9	
	Appr1_Tl_SRB	SRB1_10	Saturated, *Typha latifolia*, SRB
		SRB2_11	
		SRB3_12	
Approach 2	Appr2_Pa_UR	UR1_13	Non-saturated, *Phalaris arundinacea*, UR
		UR2_14	
		UR3_15	
	Appr2_Pa_SRB	SRB1_16	Non-saturated, *Phalaris arundinacea*, SRB
		SRB2_17	
		SRB3_18	
	Appr2_Tl_UR	UR1_19	Non-saturated, *Typha latifolia*, UR
		UR2_20	
		UR3_21	
	Appr2_Tl_SRB	SRB1_22	Non-saturated, *Typha latifolia*, SRB
		SRB2_23	
		SRB3_24	
Approach 3	Appr3_UR	UR1_25	Saturated, Non-vegetated, UR
		UR2_26	
		UR3_27	
	Appr3_SRB	SRB1_28	Saturated, Non-vegetated, SRB
		SRB2_29	
		SRB3_30	
Approach 4	Appr4_UR	UR1_31	Non-saturated, Non-vegetated, UR
		UR2_32	
		UR3_33	
	Appr4_SRB	SRB1_34	Non-saturated, Non-vegetated, SRB
		SRB2_35	
		SRB3_36	

Figure 3.2: Preparation and injection of the tracers

3.5 Activities and Measurements

3.5.1 Boundary Conditions

Two Hobo Pendant Temperature/Light data loggers were used to measure the air temperature and the light intensity which the plants were subjected to. The measurements were made every ten minutes throughout the whole experiment.

3.5.2 Plant and Soil conditions

During the twelve weeks, the plant developments and soil conditions were recorded. This was done by basic observation, only recording the unusual occurrences in the buckets.

3.5.3 Water Level Monitoring

Each week, the buckets were watered in order to maintain the saturation level. The saturated approaches (Buckets listed under approach 1 and 3) were watered twice every week. The buckets with varying saturation (listed under approach 2 and 4) were refilled once a week until saturation and left for the water level to go down at the end of each week. For both cases, whenever a

bucket was saturated, the water level was measured before and after refilling. The volume of water used to water the buckets was also recorded.

3.5.4 Water Quality Parameter

Every week, the water quality parameters were measured which consisted of the measurements of the pH, dissolved oxygen, electrical conductivity and temperature. The measurements were conducted using a Multi 3430 device with detachable probes for each parameter. The probes were inserted into the perforated column in the centre of the bucket at a fixed height that reaches to the middle of the column. In this case, two out of three replicates for each approach were measured as the third one was left untouched for bacteria measurements.

3.5.5 Sampling, preparation and storage

Water and Sand samples were collected for background measurements before the injection of the tracers. Once the tracers were injected, water and soil sampling were conducted every alternate week. Hence, water samples were collected during the 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th}, 11^{th} and 12^{th} week. Alternately, the soil samples were collected on the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} week. During the last week, it was necessary to take an extra water sample in order to make a final calculation of the tracer mass distribution which will be explained in details under Section 4.7: Tracer Mass Balance.

Water Sampling

The water samples were collected from the tap attached to the buckets for water outlet. 100 ml of water was collected from the tap and immediately transferred to 100 ml brown glass bottles. The samples were stored in a refrigerator at 4 °C for further measurements.

Sand Sampling and preparation

The soil samples were collected by inserting a plastic pipe (inner diameter 1.3 cm and length 68.6 cm) through the sand till it reaches the gravel layer at the bottom and sucking out the whole column of sand using a detachable pump. The sampling was done each time at a random surface location in the bucket. Once the sample was pulled out, it was transferred into a beaker and the mass was recorded. It is important to note that these soil samples had significant volume of water in them. The samples were left to settle for 24 hours. During this period, the sand sediment was separated from the water. The supernatant, which is a representative of the pore water, was collected using a glass pipette and bottled in 100ml brown glass bottles similar to the water samples and stored at 4 °C in the refrigerator. The remaining wet soil samples were dried at 40 °C for 48 hours in an oven. After drying, the soil samples were weighed and the dried masses were recorded. These samples were then prepared for fluorescence measurements under two different methods.

The first methodology was derived from McMahon *et al.* (2003). Two grams of the dried soil sample were taken and mixed with 20ml of de-ionized water in a plastic centrifuging tube. The samples were then placed in a shaking machine for 1 hour at 240 rpm. Once it has been properly mixed, the samples were centrifuged at 3000 rpm for 30 minutes. The supernatant was then bottled in 100 ml brown glass bottles and stored at 4 °C in the refrigerator for measurements.

The second methodology used was the approach of Wernli (2011). Two grams of the dried soil sample was weighed in a plastic tube and 10ml of ammonia:ethanol (40:60) solution was added. The tubes were covered with aluminum foil in order to prevent exposure to light and then placed in a shaking machine for 30 minutes at 240 rpm. After mixing, they samples were stored at 4 °C in the refrigerator for at least 24 hours so that the sand particles can settle. The supernatant was measured for fluorescence.

3.5.6 Nitrate Quantification

The nitrate ions were measured using the water samples with the use of a TriBox2 measurement system attached to a TriOS ProPS sensor.

3.5.7 Tracer Analysis

The tracer analysis was conducted by measuring the fluorescence of tracers with a spectral fluorescence spectrometer (LS-50B, Perkin-Elmer) supported by the FL Winlab software. The measurements were performed on both water and soil samples taken during the span of the whole experiment, which is up to twelve weeks after the tracer injections. When scanning for tracer fluorescence in both the components, a synchronous $\partial\lambda$ scan was used ranging from 250 nm to 650 nm with a wavelength difference of 7.5 nm for both excitation and emission spectra. The synchronous $\partial\lambda$ scan has a higher degree of sensitivity due to which sharper bands are obtained compared to emission and excitation spectra. The validation function with de-ionized water sample was used every week to ensure that the device was working properly and meeting all the criteria were within functioning range. For UR, the excitation wavelength was considered at 488nm. Since fluorescence of UR is pH dependent, a drop of 2.4 M EDTA solution was added to the samples for alkalinizing them before measuring the intensity. Similarly for SRB, the excitation wavelength was 561 nm. In the case of SRB, no pH adjustment was required. All the peaks appeared at their respective wavelengths, except for the Wernli (2011) sand samples, which will be discussed later in Section 4.6. Precautions were taken while considering the peak values, as many scans gave a high background signal at the lower wavelengths. These background signals may contribute in overestimation of the peak intensities, and hence was deducted following the method mentioned in Leibundgut *et al.* (2009). Equation 3.1 was used in order to remove the background signal and calculate the peak intensity. Figure 3.3 shows the conditions under which the different components in equation 3.1 were identified (Schelhorn, 2015).

$$I_{peak\ result} = I_{peak} - I_{start} - \left(\frac{(I_{start} - I_{end})}{(\lambda_{end} - \lambda_{start})} * (\lambda_{peak} - \lambda_{start})\right) = [-] \quad \text{(Eq. 2.1)}$$

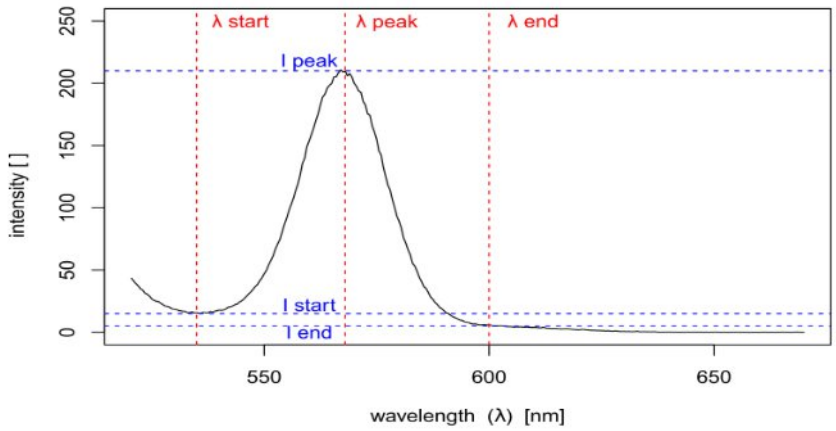

Figure 3.3: The identification of wavelength value for I_{start}, I_{peak} and I_{end} to record the intensity for the $I_{peak\ result}$ calculations.

Calibration curve

Since tracer concentrations and its resulting intensities in the fluorometer vary in terms of detectibility, purity and reactivity, a calibration was done using the batch of tracers used in this experiment. Standard tracer solutions were created and their intensities were measure. In tables 3.2 and 3.3, the known concentrations of the tracer solutions and their measured intensities are displayed respectively. Using this information, a calibration curve for each tracer was plotted (Figure 3.3 and 3.4). For zero concentration, the value of blank de-ionized water was measured and the intensity measured was subtracted from all the measurements. In all cases, the graphs showed linear correlation between the concentration and intensity of the solution. These equations were further used to calculate the concentration of the samples using their peak intensity. However, a second calibration was done with smaller concentrations to accurately determine the tracers for samples with very little tracer in them. The respective range of intensity under which each of the equation is valid is also displayed in Figure 3.4 and 3.5.

Table 3.2: Concentrations of UR and the respective intensities (without the background values) used for the calibration curve (Figure 3.4)

UR	
Original equation	
Concentration (µg/l)	Intensity
0	0
0.05	9.24
0.5	56.41
1	106.89
2	205.01
3	305.11
5	503.75
Equation for smaller concentrations	
Concentration (µg/l)	Intensity
0.00	0.00
0.05	8.23
0.25	28.91
0.50	56.41

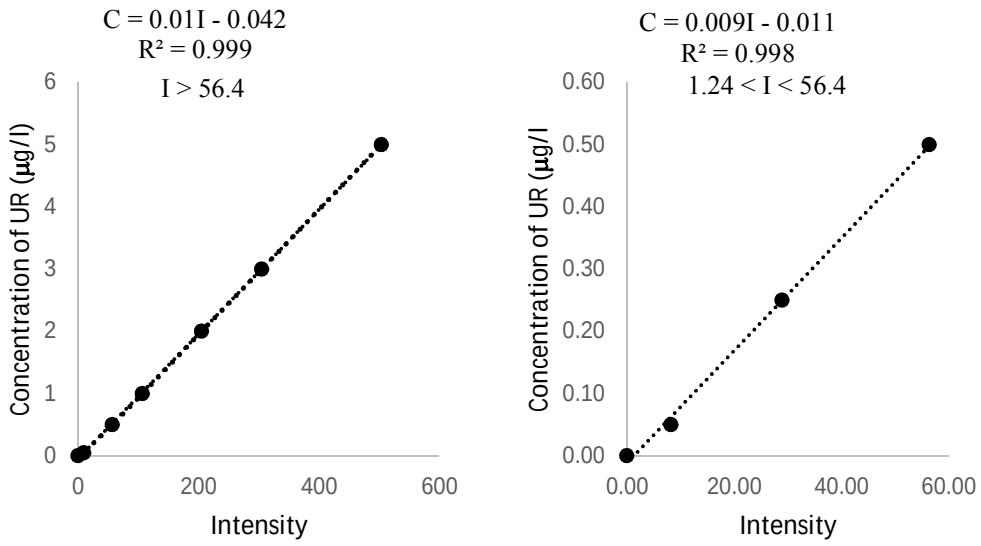

Figure 3.4: Calibration curve for UR plotted with the values of table 3.2 displaying the calibration equations, the R^2 values and the range of the intensities for which the equation is valid.

Table 3.3: Concentrations of SRB and the respective intensities (without the background values) used for the calibration curve (Figure 3.5)

SRB	
Original equation	
Concentration (µg/l)	Intensity
0	0
1	8.915
5	40.19
10	78.685
20	163.67
30	238.9
50	397.71
70	556.705
80	634.595
100	788.065
Equation for smaller concentrations	
Concentration (µg/l)	Intensity
0	0
0.01	0.78
0.1	1.18
1	8.915

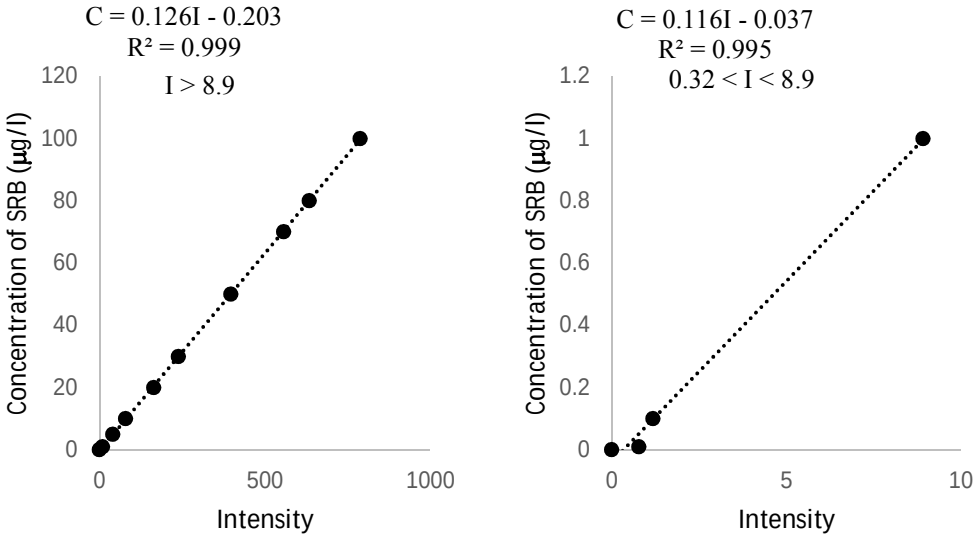

Figure 3.5: Calibration curve for SRB plotted with the values of table 3.3 displaying the calibration equations, the R^2 values and the range of the intensities for which the equation is valid.

It is important to mention that 3D scans were also conducted on one of the three replicates of each approach to get an insight of the transformation products of the tracers. The water samples and also the sand samples were scanned with a set of 35s cans from 300 nm to 650 nm. However, the scans did not display results that were significantly linked to this study. Hence, these measurements will not be discussed further in the report.

3.5.8 Bacteria Counts

At the beginning of the experiment until the first two weeks after tracer injection, the number of bacteria present in the buckets was monitored. For each bacteria count, 10ml of water in the perforated tubes in the buckets were collected using a sterilized pipette. Two different dilutions were considered to calculate the numbers of colony-forming unit (CFU), which expresses the number of bacteria or fungi in 1 ml of the water sample. Therefore, the counts were conducted with 0.1 ml and 0.5 ml of the water samples. Mentioned volumes were taken in petri dishes and mixed well with warm agar as their medium for growth. Once they were mixed well and cooled down, the dishes were placed in an incubator at 20 °C for 48 hours. After this period, the numbers of bacteria colonies were counted. This measurement was conducted in order to get an overview of the microbiological processes occurring within the buckets. However, no significant correlations were found in the previous study (Schelhorn, 2015) and the results of the first few measurements conducted were in line with the previous results. It could be due to the procedure that was being used was mainly for bacteria quantification in drinking water and does not represent relevant data linking it to biodegradation of vegetation and organic compounds in the bucket. Hence, the measurements were stopped and will not be discussed further in the report.

3.6 Software used

All data input and processing was done using Microsoft Office Excel 2007. Drawing schematic diagrams and visual modifications were conducted using Microsoft Office PowerPoint 2007 and Inkscape 0.48. The thesis was written using Microsoft Office Word 2007.

3.7 Study Period

The research was started on December 1, 2015. The first few weeks were dedicated to calculate the background conditions of the buckets due to presence of tracers from the previous study. While conducting the background measurements, various methods were applied in order to decide on the methods that will give the most reliable results during the rest of the experimental Period. The tracers were injected on January 21, 2016. From the injection date, monitoring of all the discussed parameters were conducted for the next 12 weeks until April 15, 2016.

4 Results

4.1 Boundary Conditions

Throughout the experiment duration, the air temperature and light intensity of the room were monitored using two Hobo data loggers. Figure 4.1 and Figure 4.2 displays how these conditions have varied throughout the thirteen weeks. The air temperature shows a significant rise since spring was approaching by the end of the experiment. The mean air temperature was calculated to be at 20.27 °C, with the minimum 13.65 °C of and a maximum of 24.16 °C. The light intensity fluctuation can be explained by the plant light being turned on from 6am to 5pm. Hence, during the day, the light intensity was higher and it dropped to zero at night when the plants were in the dark. The mean light intensity was 2148.5 lx, with the minimum intensity recorded at 0 lx due to night time and the maximum at 7577.8 lx.

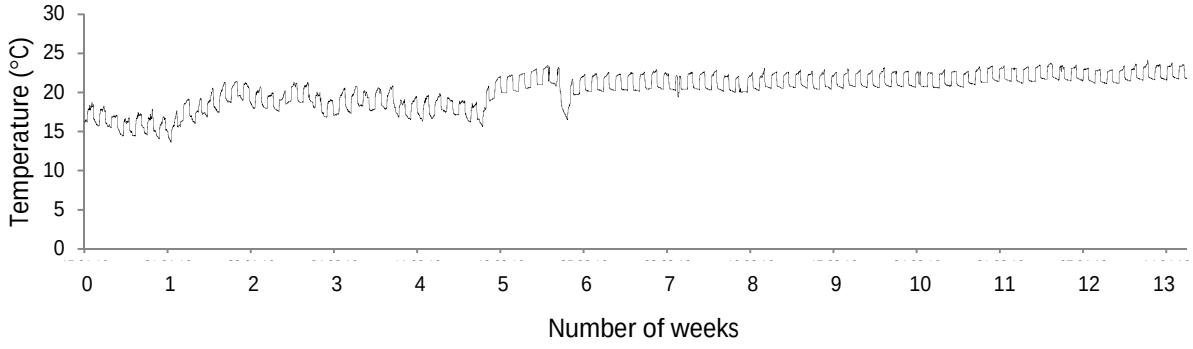

Figure 4.1: Variation of the air temperature (℃) over the data collection period

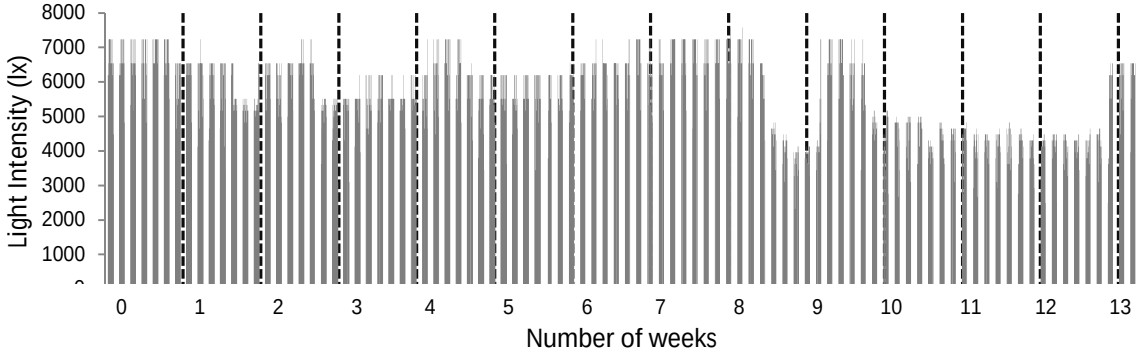

Figure 4.2: Variation of the Light Intensity (lx) over the data collection period

4.2 Water Level Fluctuation

Figure 4.3 displays the mean volume of water refilled during the twelve weeks of the study. Approaches 1 and 3 were under saturated conditions, hence it can be seen from the charts that they have been refilled twice a week in smaller volumes as compared to the non-saturated approaches. The non-saturated condition of approaches 2 and 4 were maintained by watering the buckets only once a week, but with comparatively higher volumes. The differences between the tracers showed no effect on the water required to saturate the systems. No significant differences were also found between the types of vegetation. However, it can be clearly seen that the non-vegetated buckets required little water refilling.

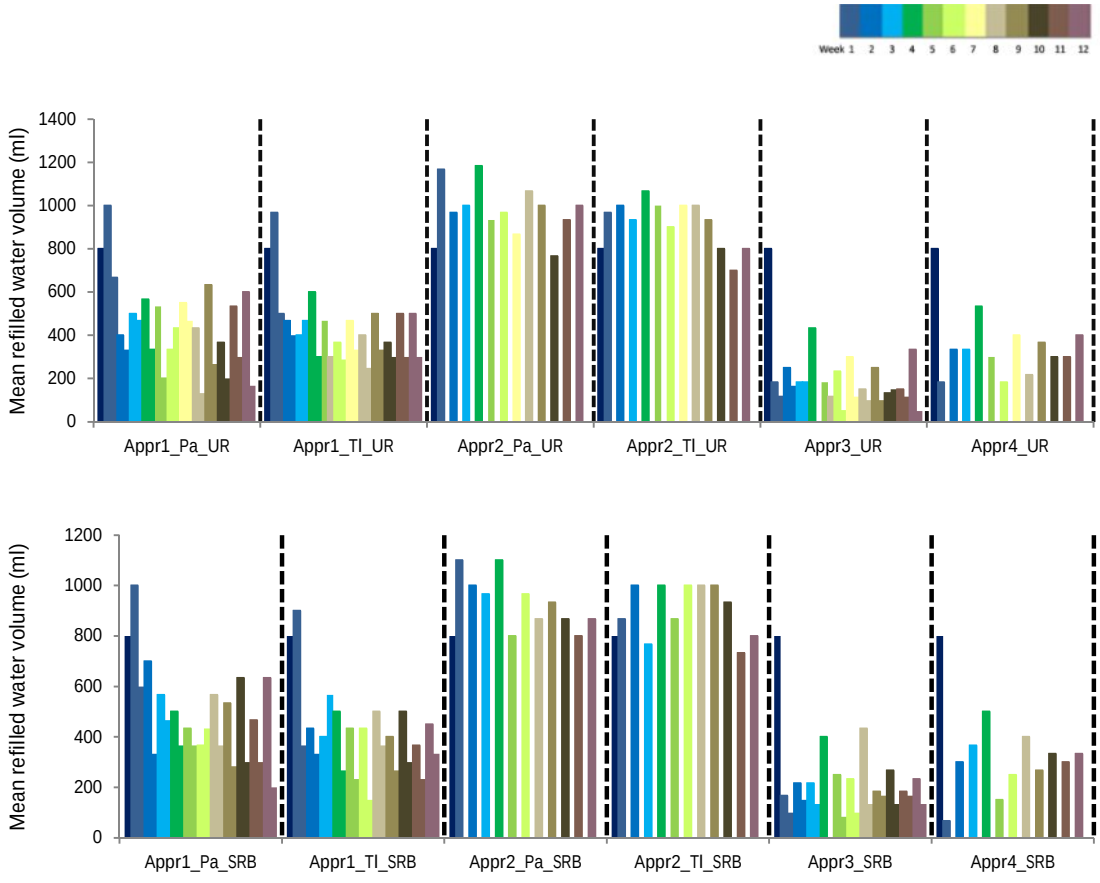

Figure 4.3: The mean volume of water (ml) refilled for each approach every week. The initial column represents the 800 ml of water refilled during the tracer injection.

4.3 Water Quality Parameters

4.3.1 pH

The mean pH values for all buckets remain close to neutral during the study period, ranging from 7.11 to 8.04 at the highest. For buckets containing UR and *Phalaris arundinacea*, the fluctuation of pH values were high, but within a very close range. The recorded values are displayed in figure 4.4.

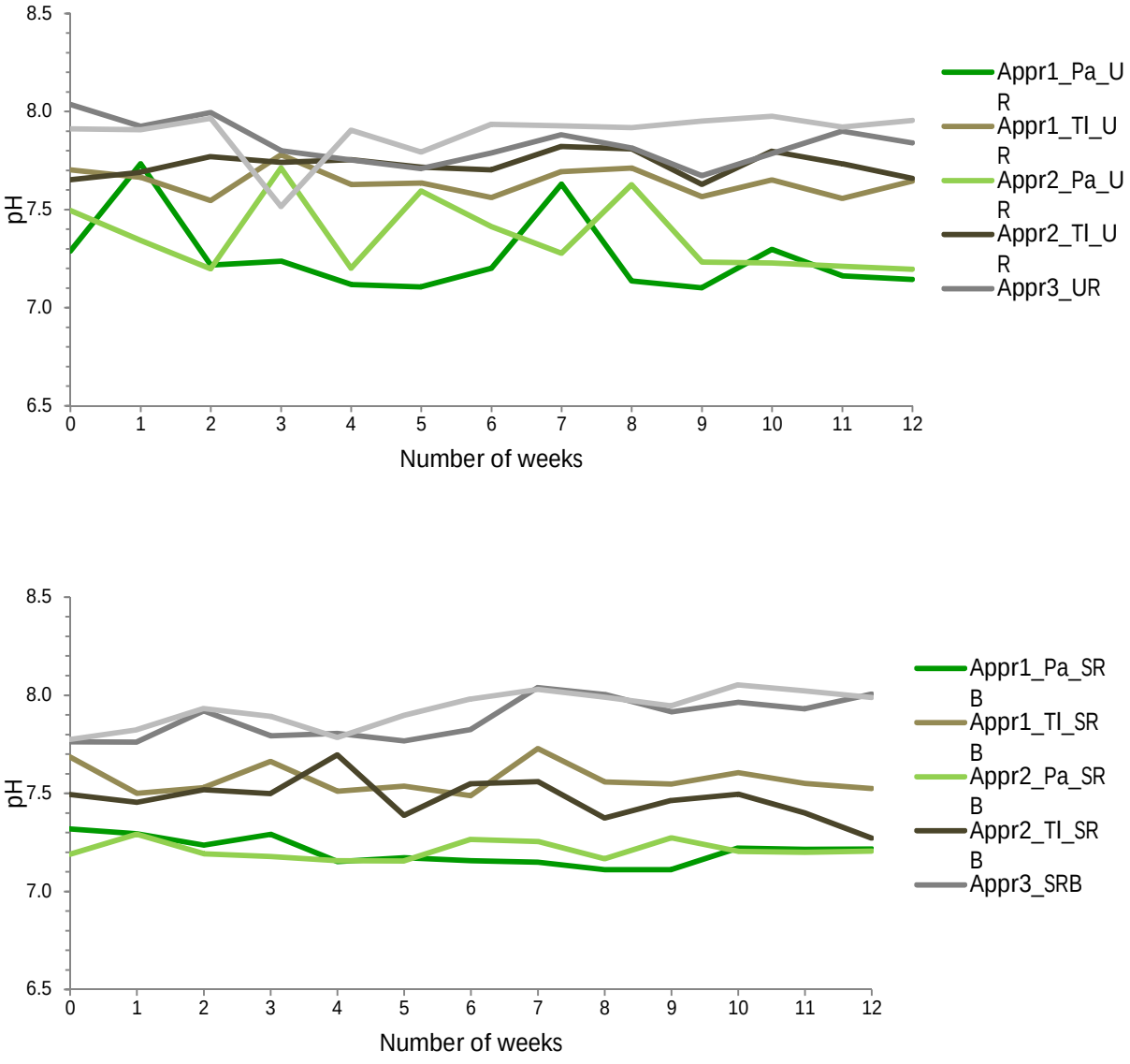

Figure 4.4: The mean change in pH values over the twelve weeks for each approach.

4.3.2 Dissolved Oxygen

The mean dissolved oxygen measured in the water during the twelve weeks is displayed in figure 4.5. In this case, clear variations can be observed as the approaches with *Phalaris arundinacea* have much lower dissolved oxygen for both UR and SRB. The non-vegetated approaches have the highest dissolved oxygen. The variation of the measurements for different approaches was high with the range from 2.2 mg/l to 8.7 mg/l. The change of tracers did not affect the general trends when compared.

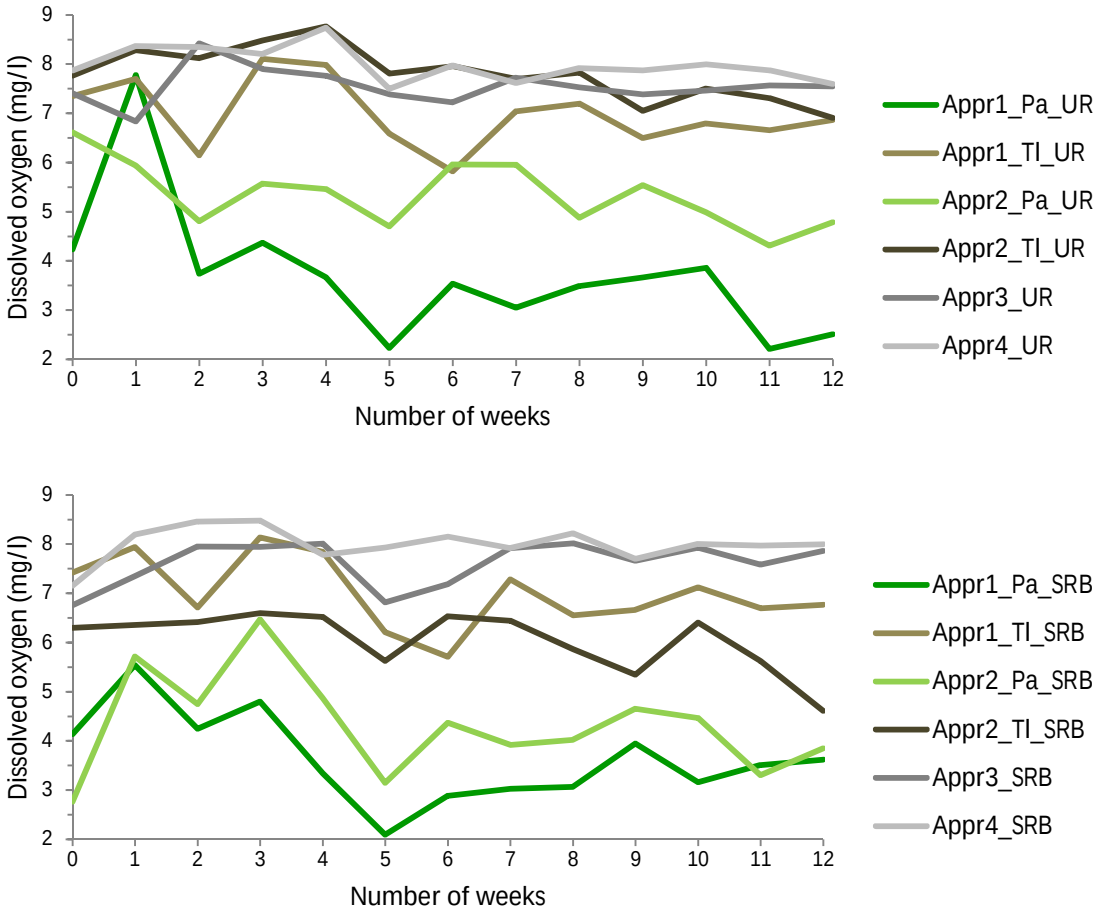

Figure 4.5: The mean changes in dissolved oxygen (mg/l) over the twelve weeks for each approach.

4.3.3 Electrical Conductivity and Water Temperature

The mean changes in the electrical conductivity of the water in the buckets are displayed in figure 4.6. The values are ranged between 752 µS/cm up to 982 µS/cm. The buckets containing *Phalaris arundinacea* displayed highest electrical conductivity ranges. A high variance can be observed between the non-vegetated buckets with saturation and non-saturation. Non-saturated and non-vegetated bucket had the water with the least electrical conductivity. Even though this parameter is temperature dependant, it can be seen in figure 4.7 that the mean water temperature did not vary significantly in the different approaches. Once again, the difference in tracers did not appear to have an impact on the trends.

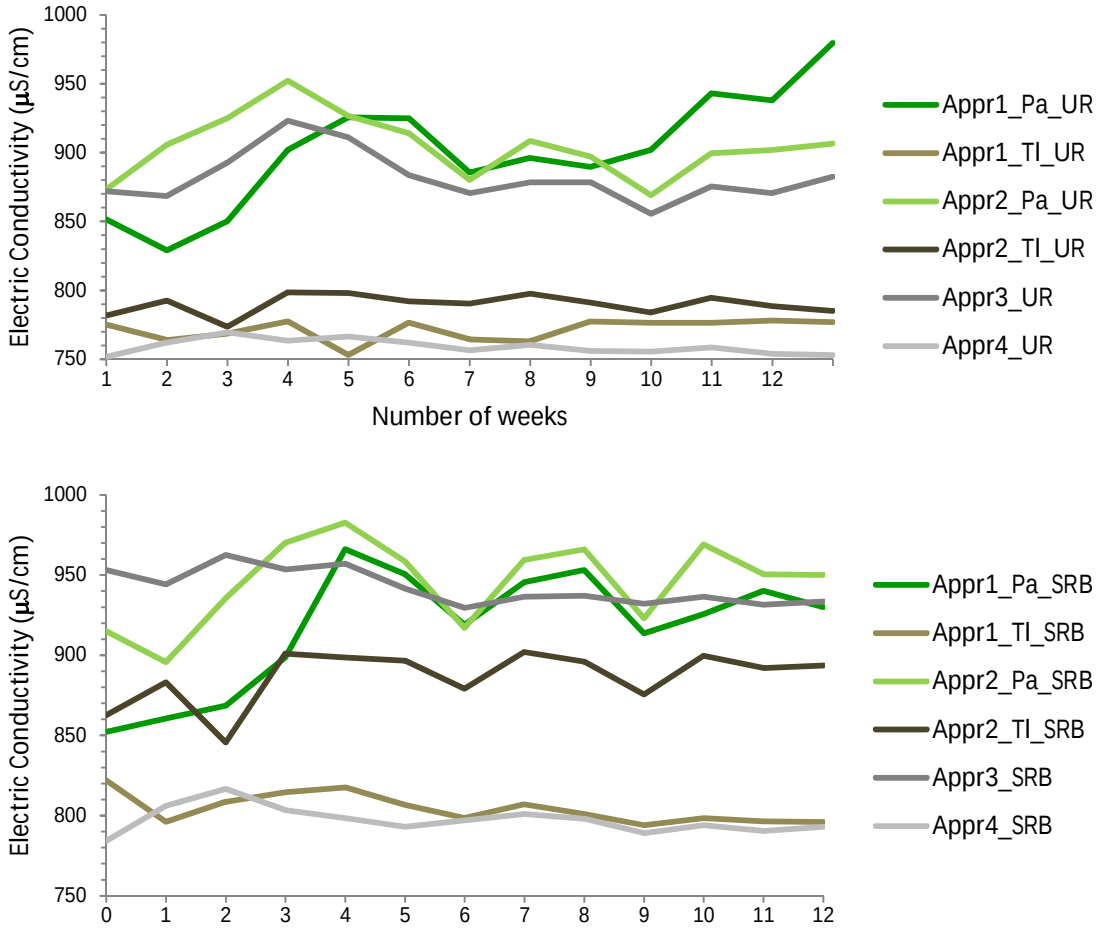

Figure 4.6: The mean changes in electrical conductivity (µS/cm) over the twelve weeks for each approach.

All water temperature measurements follow a general trend, which is most likely a result of the rise in air temperature (figure 4.1). The varying parameters did not have an impact on the temperature. The water temperature was in between 15.4 °C to 21.7 °C.

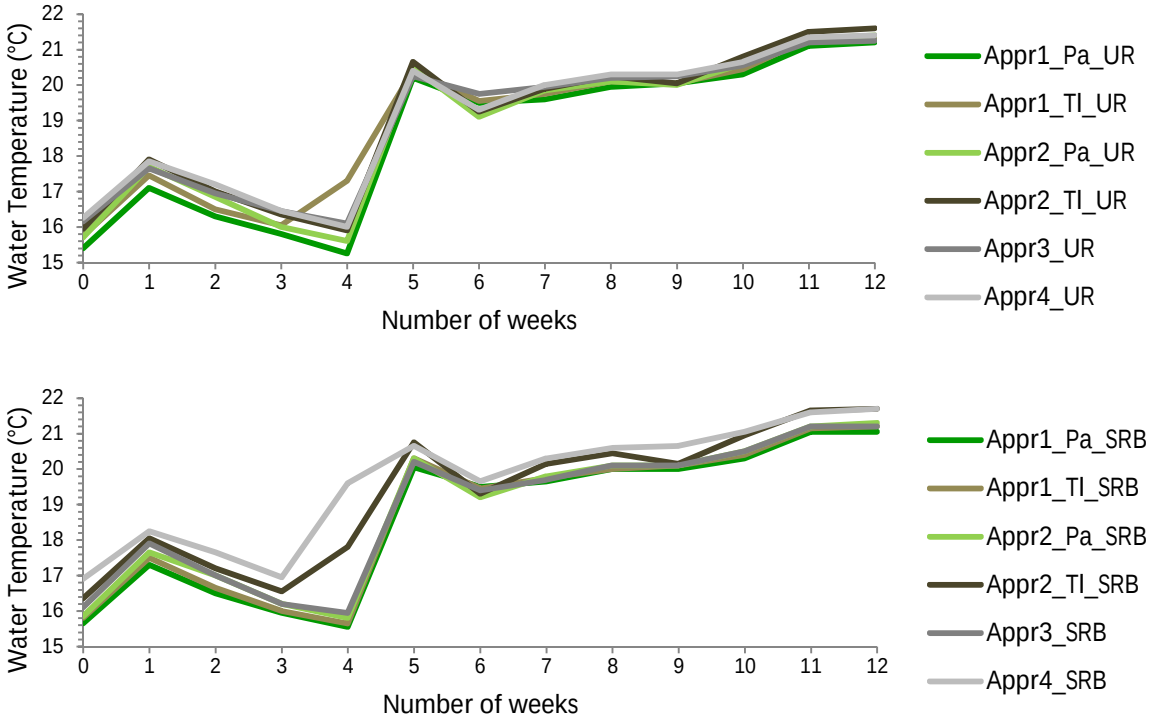

Figure 4.7: The mean changes in water temperature (°C) over the twelve weeks for each approach.

4.4 Nitrate Measurements

The mean nitrate (NO_3^-) concentration measured in the water samples for the 12 weeks is displayed in figure 4.8. Some buckets had no NO_3^- present in the water sample, while some buckets had concentrations up to 15.68 mg/l. As figure 4.8 displays, regardless of the tracer, the NO_3^- concentration was high in non-vegetated approaches. *Phalaris arundinacea* under saturated approach showed absence of NO_3^- in both cases. The general trend is more towards the concentration of NO_3^- decreasing, but in some cases the concentration has remained within the similar rages from the beginning to the end of the data collection period.

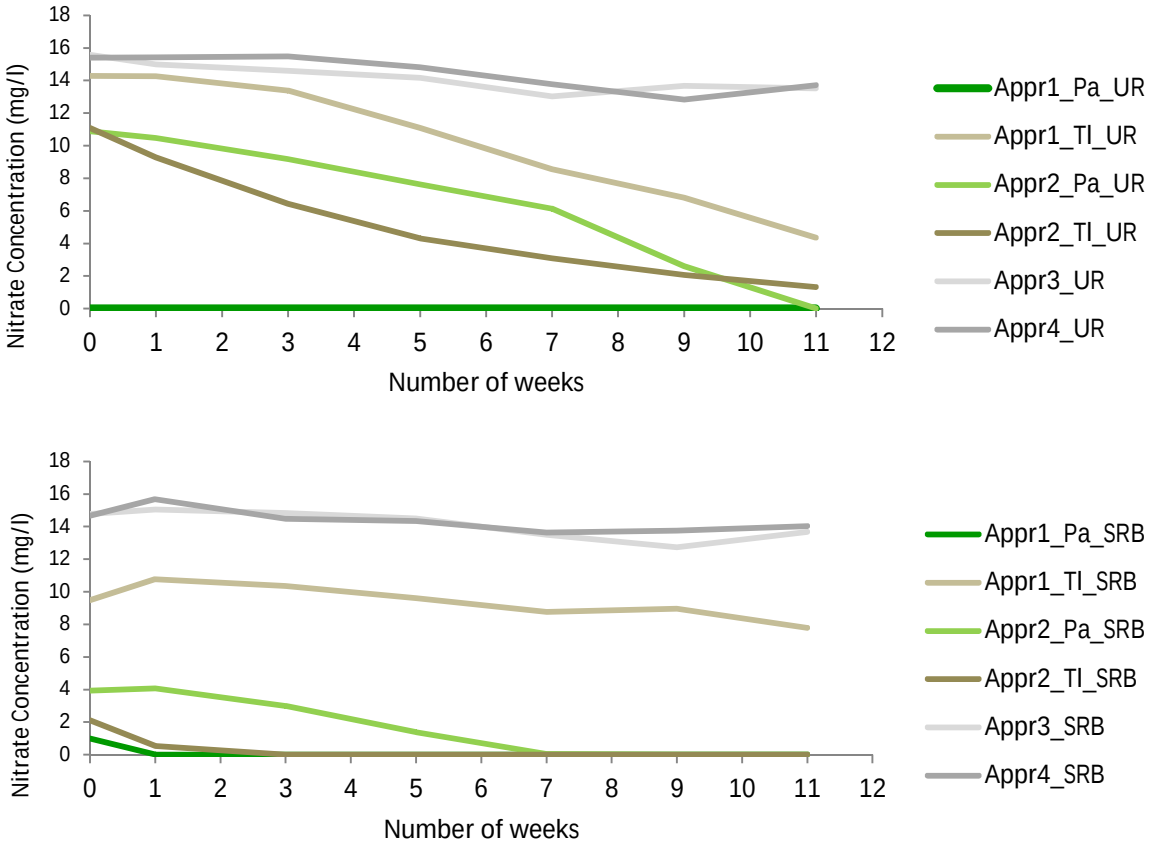

Figure 4.8: The mean changes in the concentration of NO_3^- over the twelve weeks for each approach.

4.5 Plant Conditions

The plants generally were not in perfect health conditions. The slow deterioration of the plant conditions were discussed in details by Schelhorn (2015), which suggested the main reason for this might be the inability of the plants to adapt to the environment of the lab-scale systems. During that study, *Typha latifolia* started to die out three weeks after plantation already. Since this experiment began months later, all the *Typha latifolia* was dried out. There were still remaining shoots but their activity could not be proved or neglected. Most measurements for approaches with *Typha latifolia* gave values that were somewhere between the approaches with *Phalaris arundinacea* and the non-vegetated ones. While analyzing the results, it was taken into account that the systems might be dead or undergoing different biodegradation processes when compared to *Phalaris*

arundinacea. Buckets planted with *Phalaris arundinacea* were in comparatively in better conditions. The total number of shoots declined and the plants were drying out as the experiment proceeded, but the system was considerably active as new shoots were growing in most buckets.

It is also important to mention that the buckets established a lab-scale ecosystem somehow. The plants, especially the leaves of *Phalaris arundinacea*, were infested with tiny unidentified insects. In addition to that, growths of moss, algae and some weeds were also observed. Twice during the experiment, the leaves were wiped with wet napkins and the top soil layer was mixed to ensure that the water was not blocked by the layers of growth from entering the sand. However, only the top surface was scraped off and mixed with minimum disturbance to the system.

4.6 Tracer Concentration Development

The tracer concentrations of the different components of the lab-scale wetland systems were monitored for the twelve weeks after tracer injection. As mentioned earlier, the tracer masses injected were 700 µg/l for Uranine and 1400 µg/l for Sulforhodamine B. It was predicted that once the tracers have been injected into the system, it has to be distributed in either of these following parts of the assumed closed system:

1. Water: The filtered water that is collected from the water outlet
2. Sand pore water: The water that is trapped inside the sand layers
3. Sand and gravel: Due to sorptive behavior of the tracers

Hence, the tracer concentrations of each of these components were measured in order to understand the changes or interactions occurring in the buckets. It is important to note that the graphs presented under this section displays the concentration changes in each samples over the twelve weeks, including the background measurements (at zero) and the tracer concentration development from the injection in the first week.

Water Samples

Figure 4.9 shows how the UR concentration in the water sample has changed during the twelve weeks of monitoring. Similarly, Figure 4.10 shows how the SRB concentration varied. From these figures, it is visible that the pattern varies from one approach to another. Even though the patterns could not be statistically proven for all the approaches, it can be generalized that the concentration of UR is decreasing throughout the weeks, where as the SRB concentrations are increasing. The buckets containing *Phalaris arundinacea* with UR shows that the concentration is steadily decreasing, regardless of the saturation. For buckets with SRB, the non-saturated and non-vegetated approach showed a significant increase in tracer concentrations. For all approaches containing SRB, the concentration measured during the first week was the smallest. For UR containing buckets, the weeks in between (approximately from week three to six) gave the highest concentration values before declining for the remaining weeks.

Pore Water Samples

The concentration changes in the pore water did not show any significant trend in terms of increasing or decreasing for both tracers. However, in almost all cases, the first measurement gave the highest concentrations. It is important to take into account that the pore water sampling was done during the soil sampling and hence shows similar trends to soil measurements. The mean concentration changes are represented in Figure 4.11 for the approaches with UR and Figure 4.12 with the approaches containing SRB.

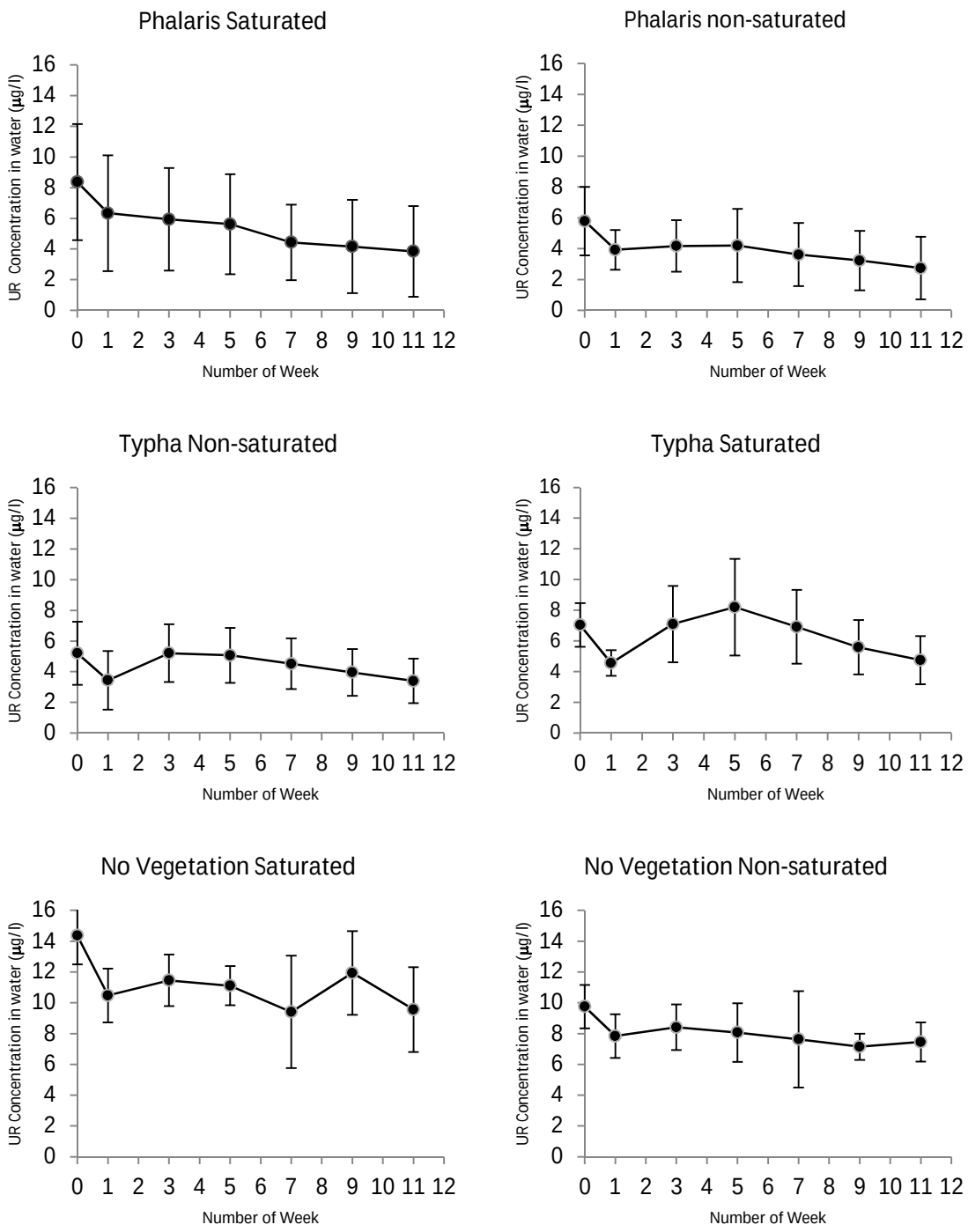

Figure 4.9: Mean UR concentration (µg/l) in water samples over the 12 weeks, represented with standard deviation error bars.

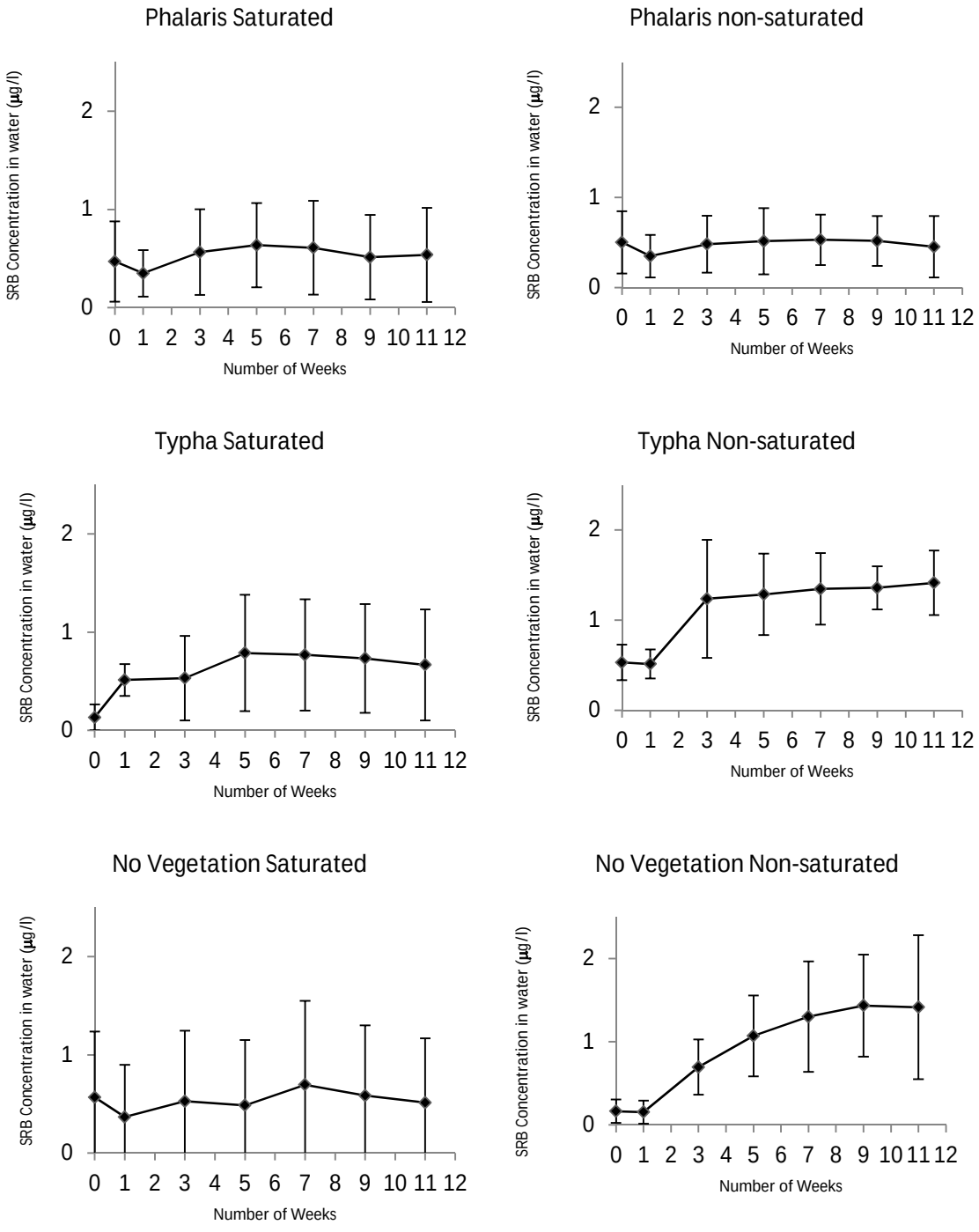

Figure 4.10: Mean SRB concentration (µg/l) in water samples over the 12 weeks, represented with standard deviation error bars.

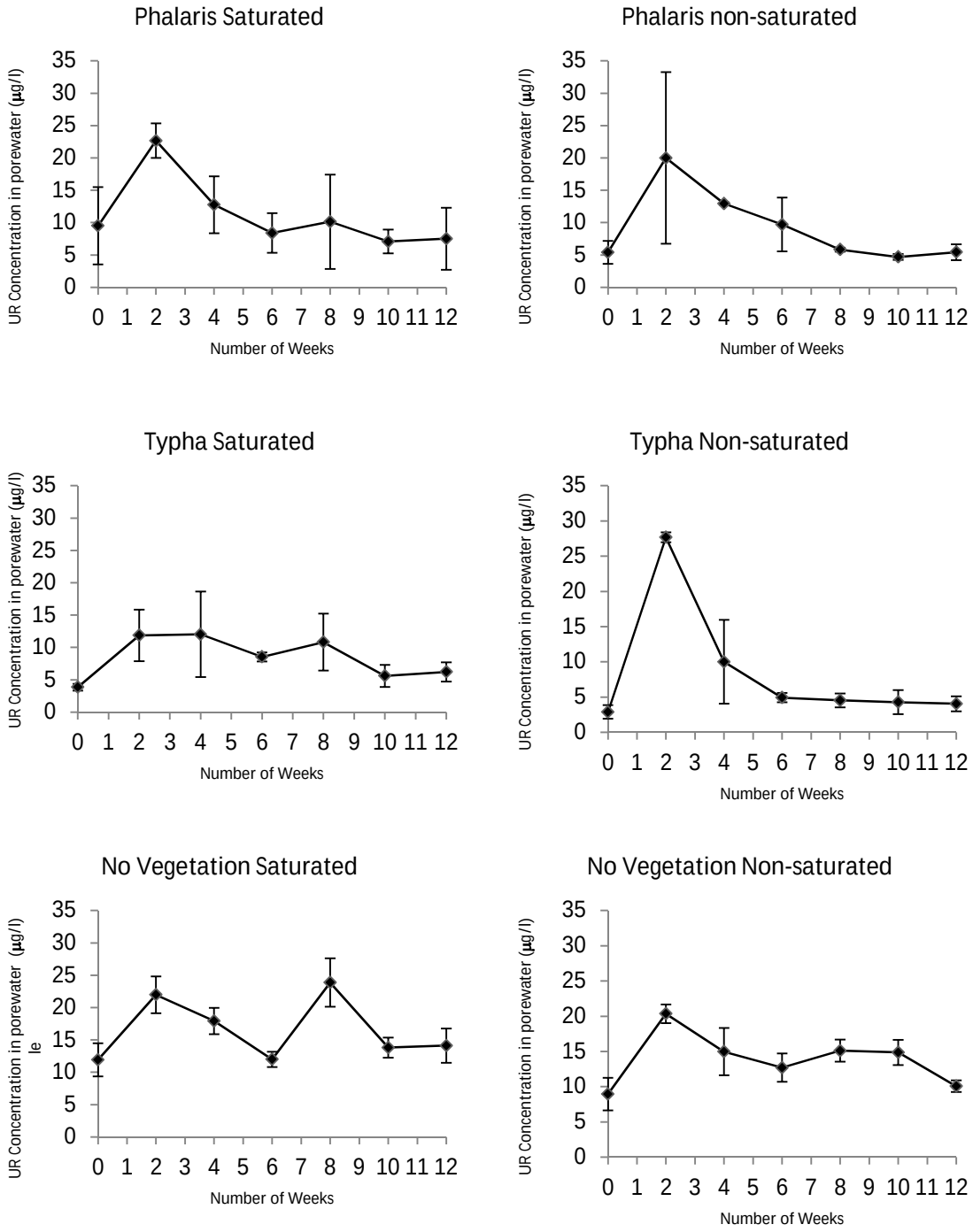

Figure 4.11: Mean UR concentration (µg/l) in pore water samples over the 12 weeks, represented with standard deviation error bars.

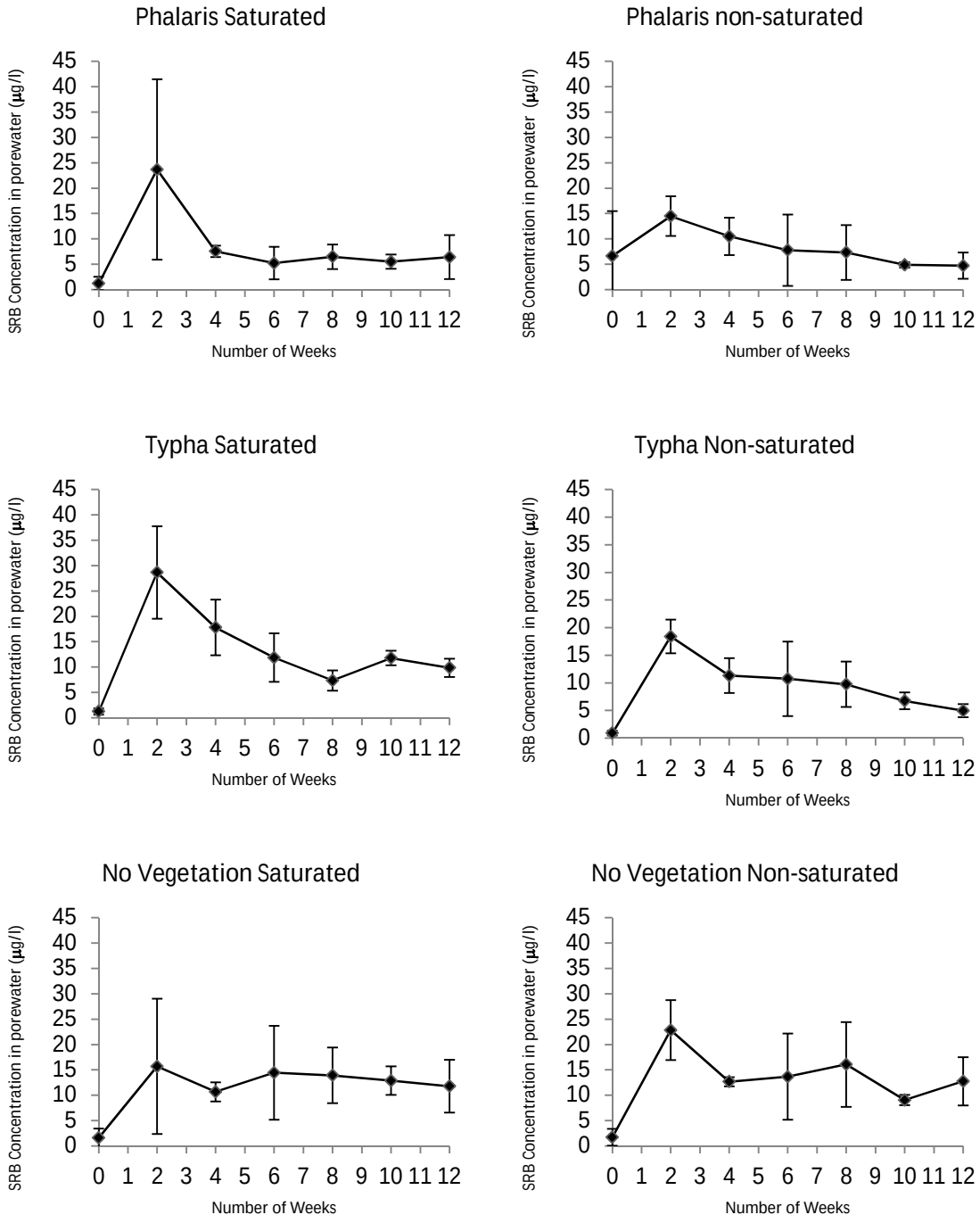

Figure 4.12: Mean SRB concentration (µg/l) in pore water samples over the 12 weeks, represented with standard deviation error bars.

Soil Samples

For soil samples, the tracer concentrations were measured using two approaches. One was modified McMahon *et al.* (2003) and the second one was followed from Wernli (2011). Figures 4.13 and 4.14 show the concentration developments in different approaches calculated from the procedure following McMahon *et al.* (2003), while Figures 4.15 and 4.16 shows the concentration changes calculated from the procedure suggested by Wernli (2011). Though the graphs from both methods show similar characteristics, the concentration of tracer calculated using the procedure of Wernli (2011) was much higher as shown in Figure 4.17 and 4.18. Regardless of the tracer, all approaches show high concentration in the second week, similar to the pore water concentrations. Due to fluctuating concentration values, no significant trend could be established.

It should also be added that the fluorescence scans for the sand samples were with high background values. Even though the background values were deducted using the removal method suggested by Leibundgut *et al.*, 2009, it may account for other substances present in the sand samples such as organic matter. For the Wernli (2011) scans, a shift in the peaks for both UR and SRB was observed. Instead of UR having the peak at the usual excitation wavelength of 488 nm, the peak appeared at 496 nm. For SRB, the peak shifted to 555 nm from its characteristic excitation wavelength of 561 nm.

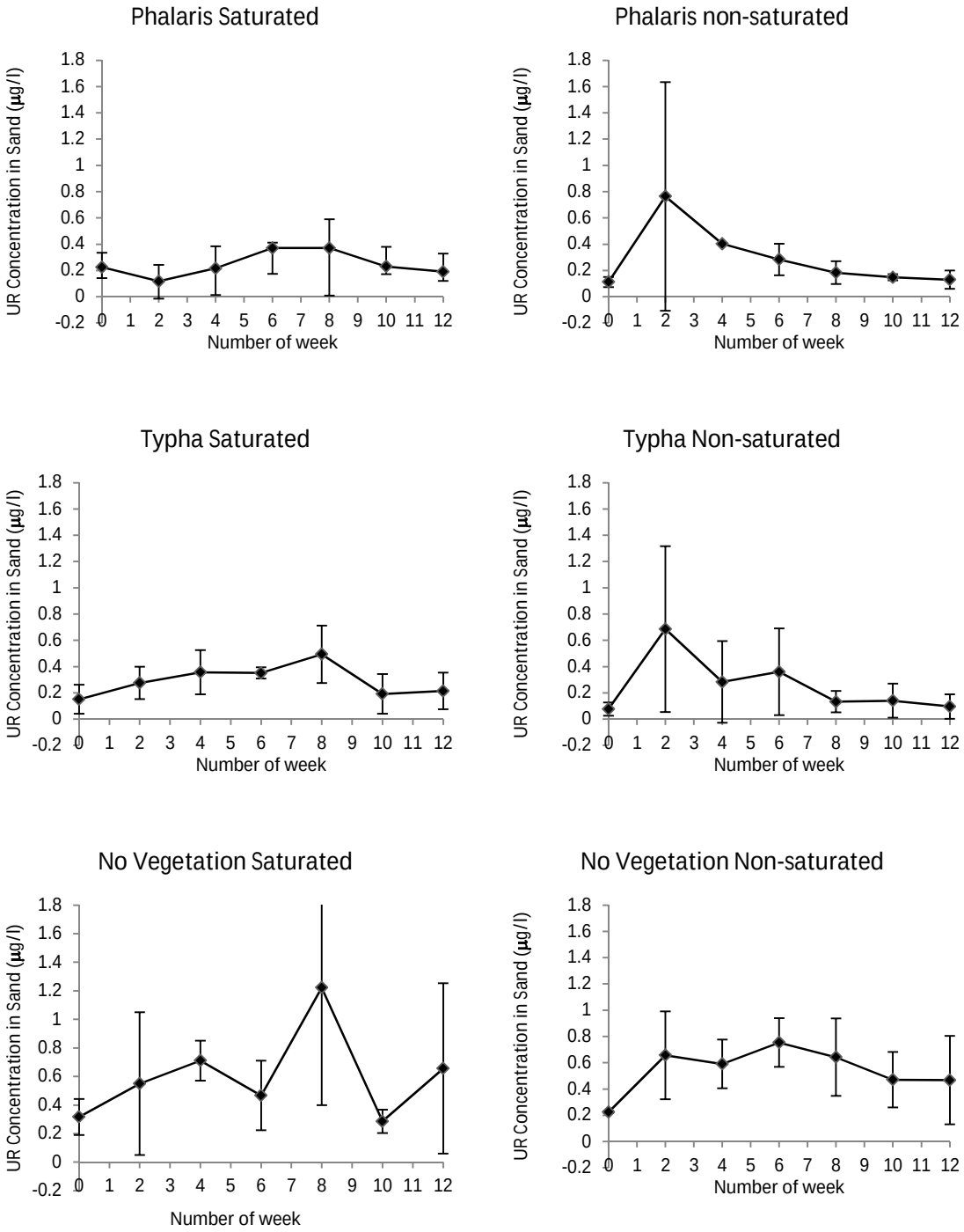

Figure 4.13: Mean UR concentration (µg/l) in sand samples over the 12 weeks calculated using the method according to McMahon et al. (2003); represented with standard deviation error bars.

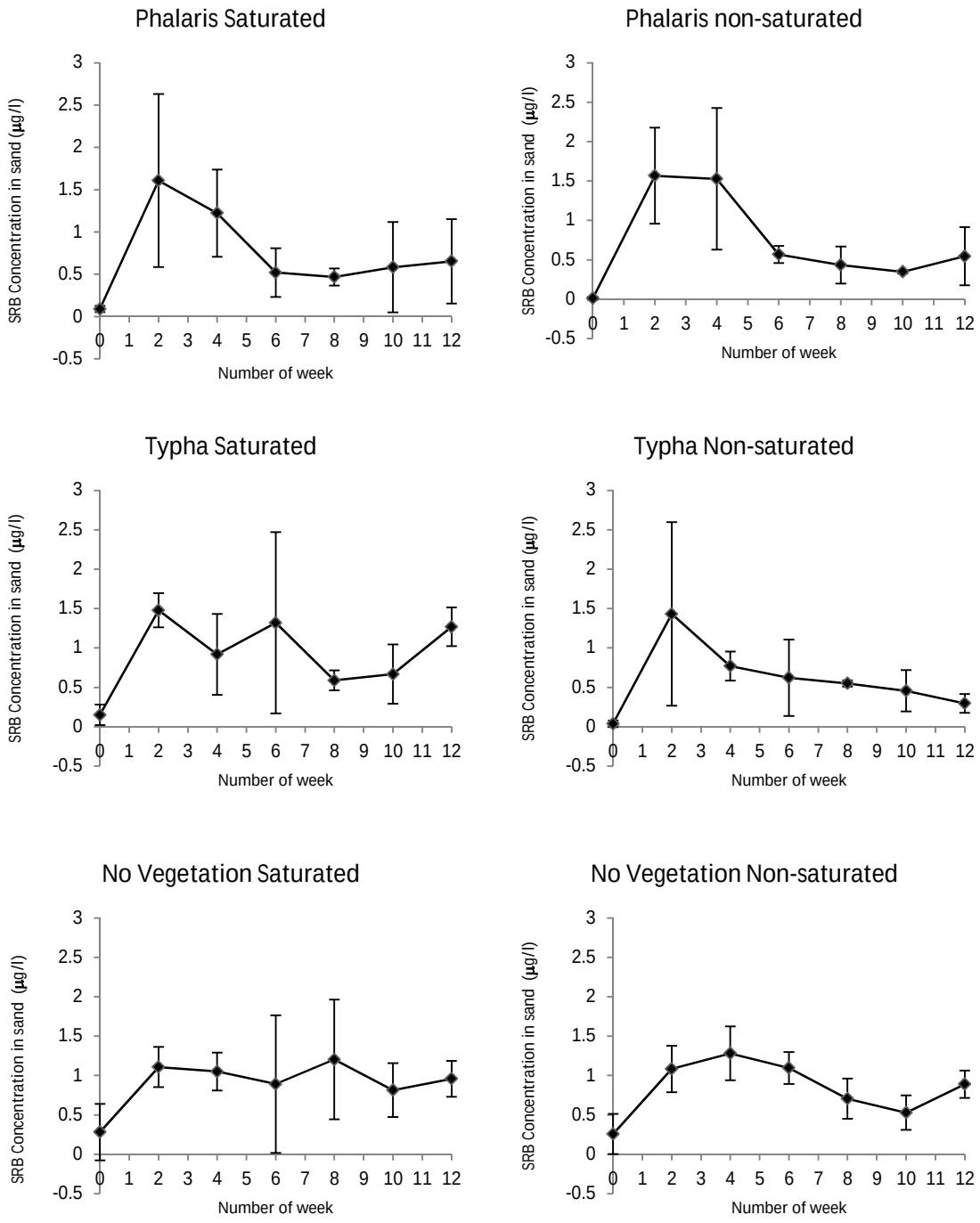

Figure 4.14: Mean SRB concentration (µg/l) in sand samples over the 12 weeks calculated using the method according to McMahon et al. (2003); represented with standard deviation error bars.

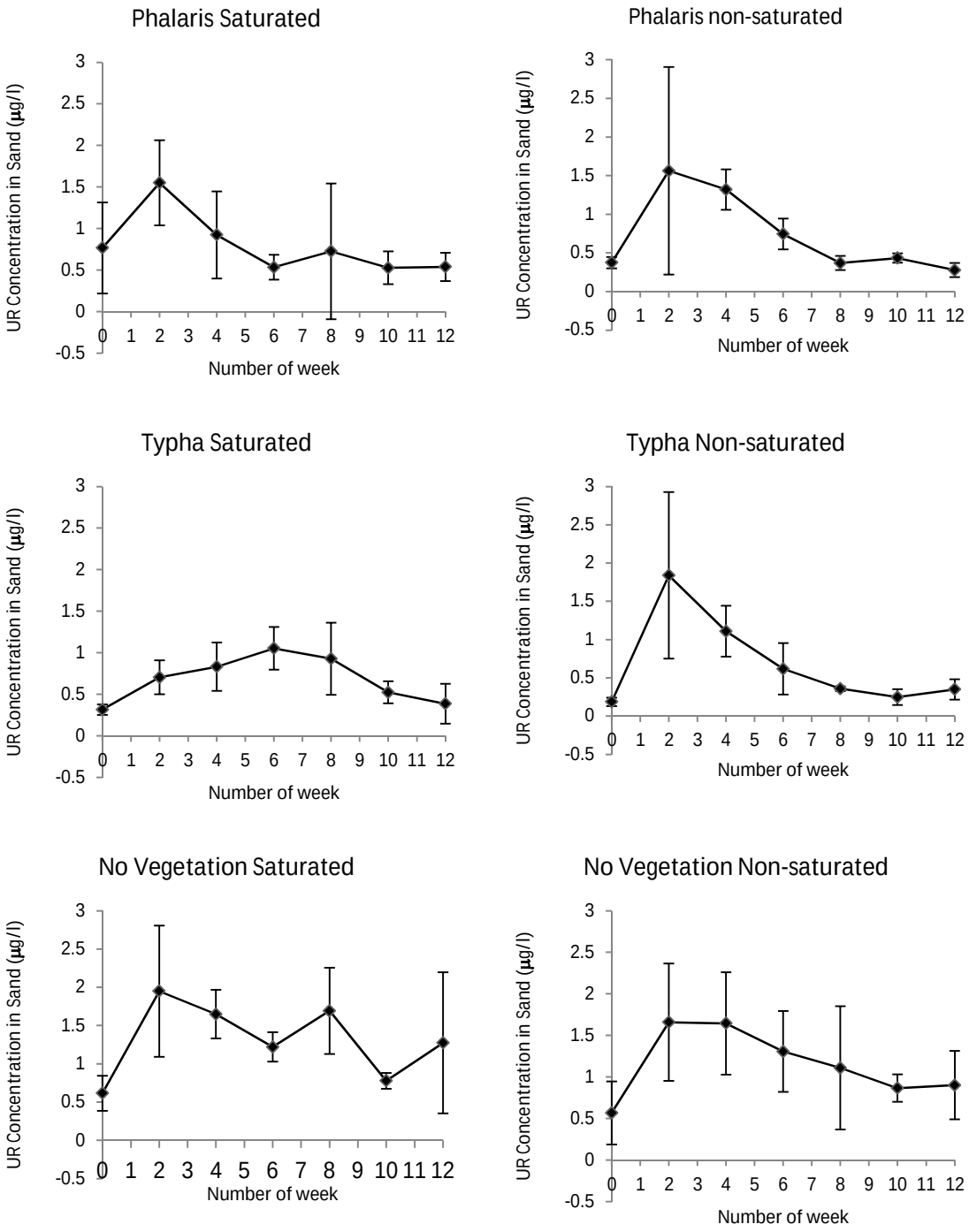

Figure 4.15: Mean UR concentration (µg/l) in sand samples over the 12 weeks using the procedure according to Wernli (2011), represented with standard deviation error bars.

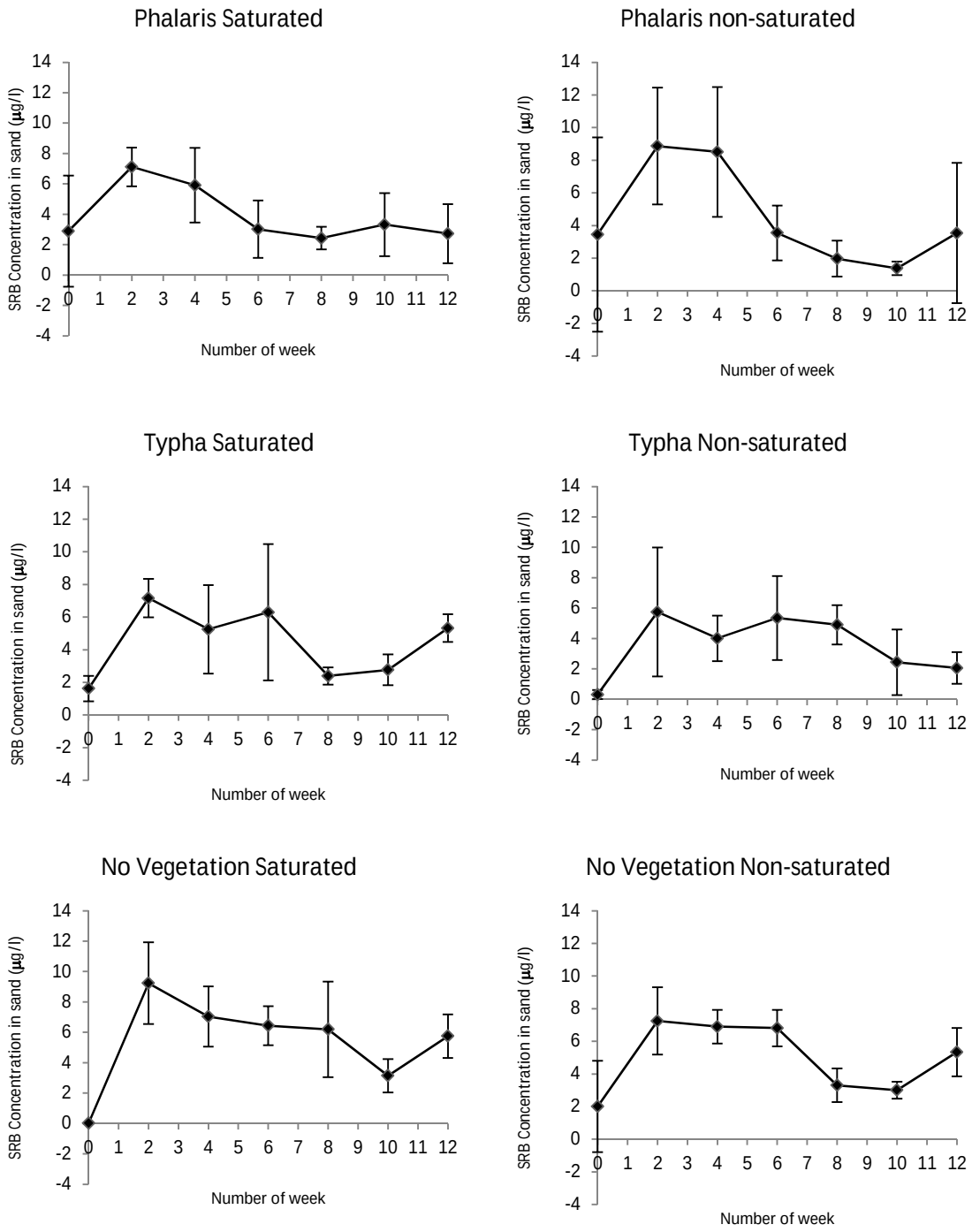

Figure 4.16: Mean SRB concentration (µg/l) in sand samples over the 12 weeks using the procedure according to Wernli (2011); represented with standard deviation error bars.

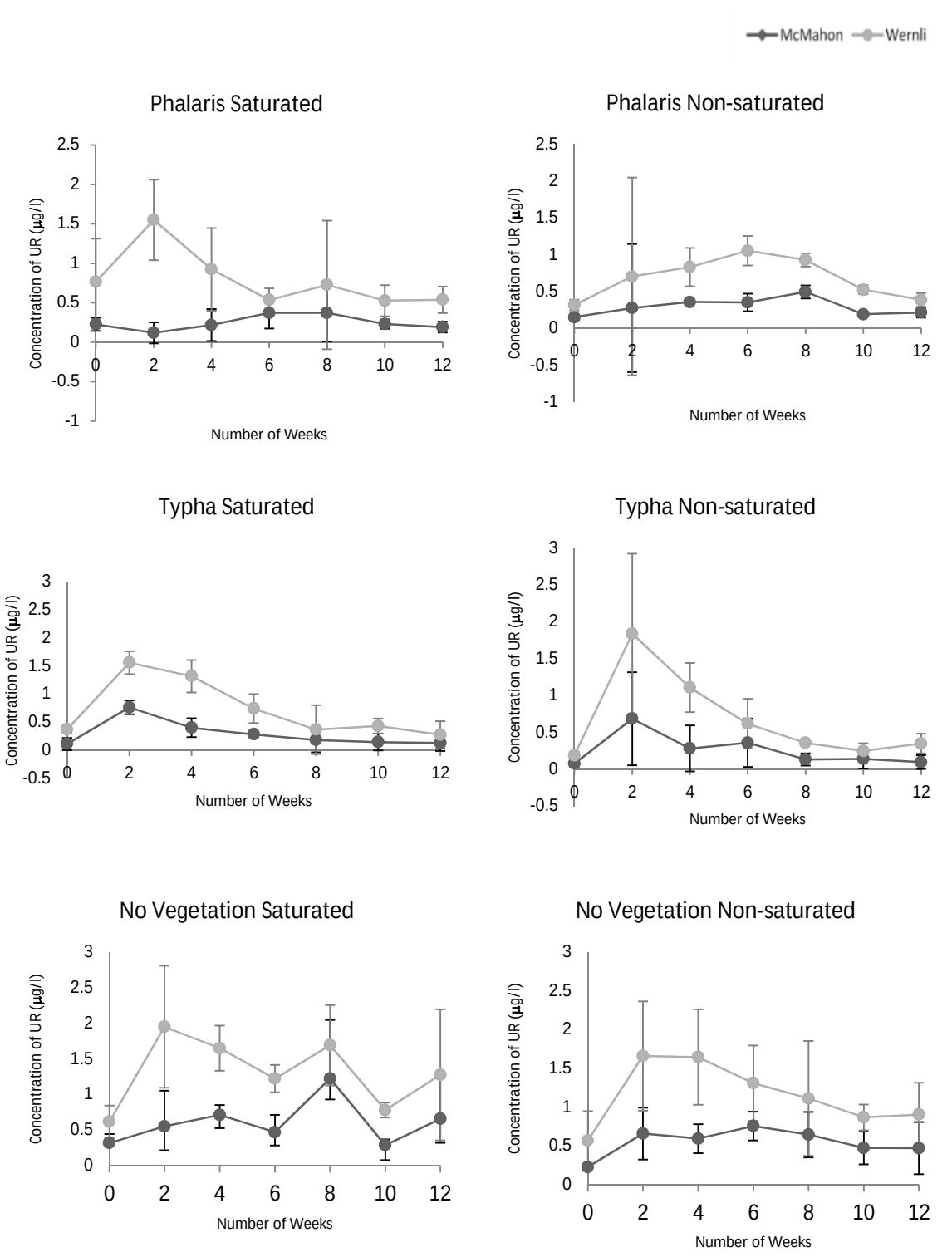

Figure 4.17: A comparison between the concentrations of UR (µg/l) in sand measured by using the procedures according to McMahon et al. (2003) and Wernli (2011).

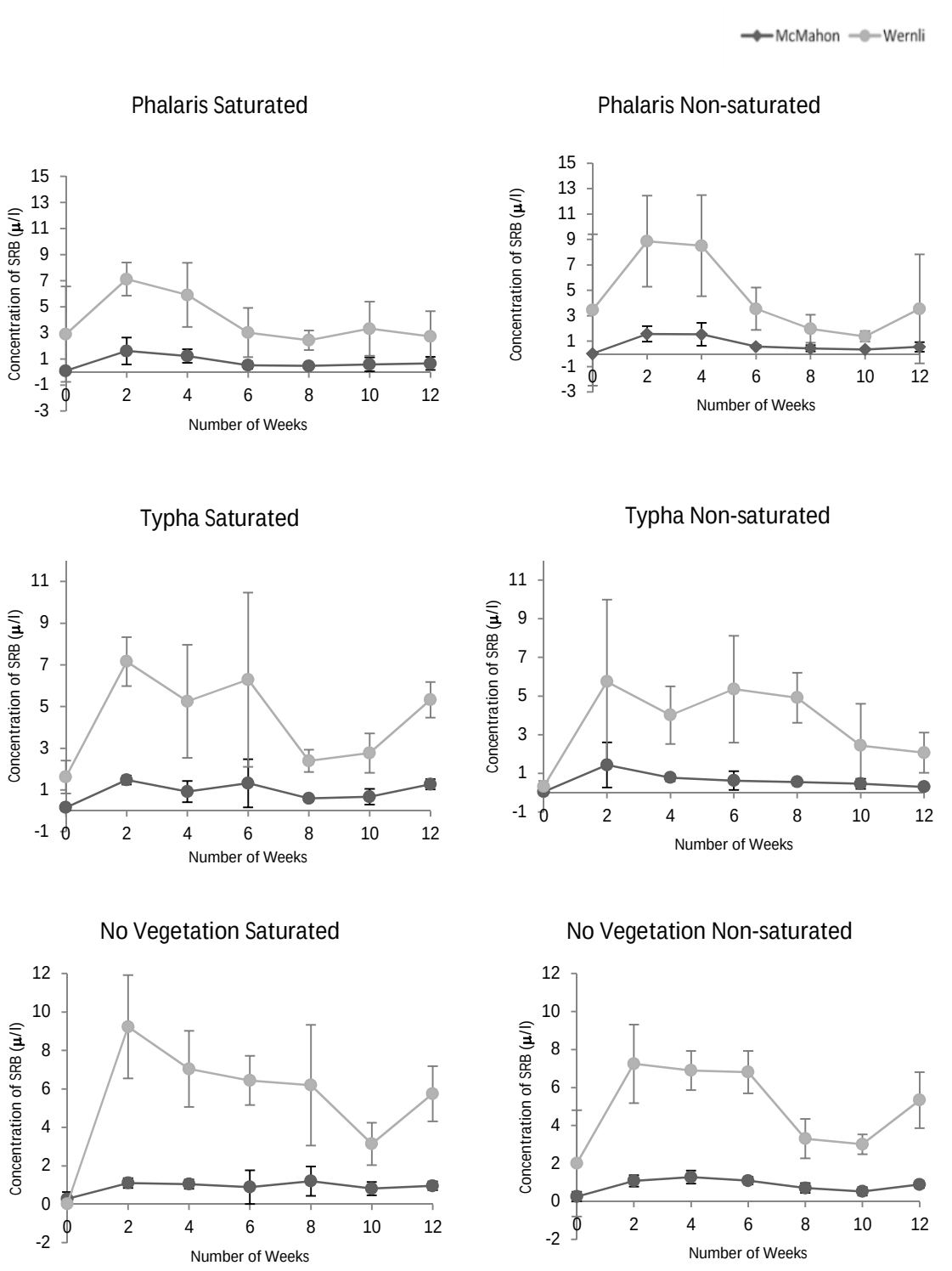

Figure 4.18: A comparison between the concentrations of SRB (µg/l) in sand measured by using the procedures according to McMahon et al. (2003) and Wernli (2011).

4.7 Tracer Mass Balance

At the end of the experiment, the final concentration values were used to calculate the final mass distribution of the tracers present in water, soil pore water, sand and gravel. The final masses were extrapolated for the whole bucket from the concentration values calculated for the last week of the experiments. The initial mass was considered a total of the background masses measured and the tracer masses injected. The final mass values calculated were subtracted from the initial masses to find out the percentage of tracer that could not be accounted for, referred to as the mass that has been dissipated. For measuring the masses of tracers in sand, the Wernli (2011) values were considered. The final distribution as a percentage of the total initial mass has been represented in Figure 4.19 for UR and Figure 4.20 for SRB. Compared to the approaches with SRB, it can be seen that the mass dissipated for UR is higher especially for vegetated approaches. For non-vegetated approaches, UR is more present in sand and its pore water compared to the ones with vegetation. It can be seen from the charts that approaches 3 and 4, which were non-vegetated, have the highest tracer recovery rates for both tracers. More UR has been found in the water, while the highest percentage of SRB was present in the sand. For the vegetated approaches, a higher percentage of UR has been recovered than SRB. No significant differences on the percentage of tracer mass recovered were found while comparing the saturated and non-saturated approaches.

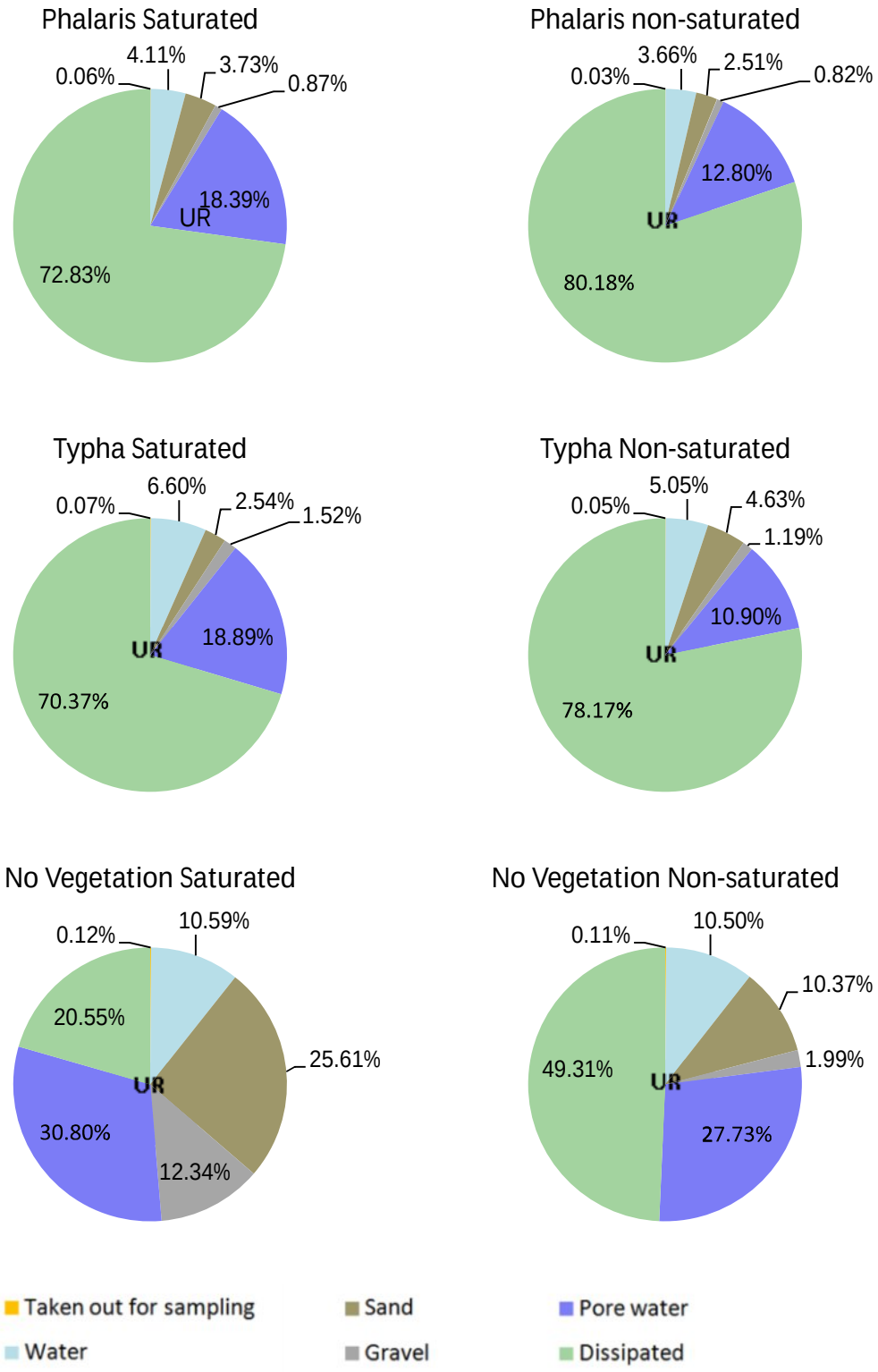

Figure 4.19: Percentage of UR distributed across different compartments at the end of the experiment.

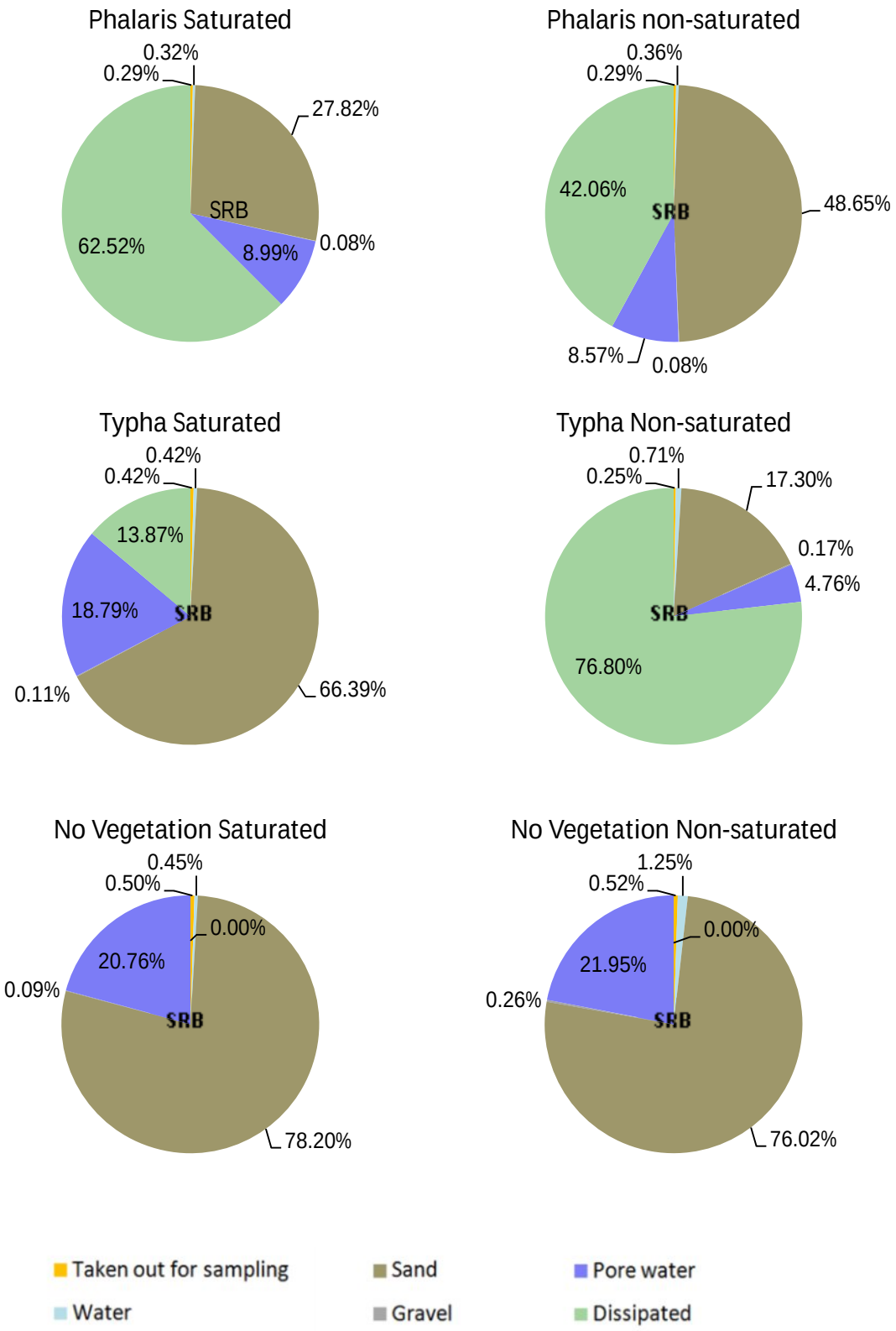

Figure 4.20: Percentage of SRB distributed across different compartments at the end of the experiment.

5 Discussion

Considering the general controlling factors of a constructed wetland, attainable steps were taken to create the artificial lab-scale wetland systems as close to natural systems as possible to understand the behavior of UR and SRB better. Underlying processes and potential correlations between different wetland parameters were attempted to be identified with the knowledge of previous studies in combination with the results of this experiment.

In general, the conditions and growth rate of the plants were poor, even though they were provided with sufficient light, water and nutrients. Both *Phalaris arundinacea* and *Typha latifolia* have been categorized as invasive species that can survive in poor soil conditions and can undergo climatic changes without being affected as much. However, this was not exactly the case for this study. *Typha latifolia* performed poorly, as most plants dried out and did not grow new shoots. Although not in great conditions, *Phalaris arundinacea* was still developing new shoots and has the ability to adapt better to its new surroundings. The potential cause for such conditions may potentially be related to the sudden change in environment, which put the plants under stress. In the field or in the nursery, the plants were mainly situated outdoors with proper air circulation and oxygen supply. The sudden shift into an underground room with heating system might have created an atmospheric shock to the plants, resulting in deprivation of the natural system they were previously in.

The injection of tracers could have affected the plants. However, UR has been proven to have no eco-toxic effects (Leibundgut and Hadi, 1997). SRB can have an impact on the plants, but the only in large concentrations (Behrens *et al.*, 2001). The tracer masses injected was too low to have any significant impact on the health of the plants. No data or previous studies were found to identify the reasons behind the lack of survivability of the plants.

5.1 Water Balance and Water Quality

In terms of water uptake, no significant difference was found between the two vegetation types, despite *Typha latifolia* being a dead system compared to *Phalaris arundinacea*. Non-vegetated approaches required less water for saturation. Plant uptake and evapo-transpiration may have led to high water requirement in the vegetated buckets. The water loss in the non-vegetated buckets is mainly due to evaporation.

No direct correlations were found between the various water quality parameters measured. The water temperature was influenced by the air temperature of the room. The water temperature throughout the weeks showed the same trend for all approaches, regardless of the vegetation, saturation and tracers.

Clear variation in dissolved oxygen in water was measured between the vegetated and non-vegetated approaches. Approaches with *Phalaris arundinacea* had the lowest oxygen content, while the dissolved oxygen in non-vegetated approaches was the highest. Oxygen uptake is not exclusively controlled by vegetation. Less oxygen might also be due to high activities of microorganisms. Presence of vegetation can enhance microbial density and activity. *Phalaris arundinacea* has a significantly higher impact on microbial activities than *Typha latifolia* (Gagnon, 2007), which can also account for the difference in dissolved oxygen between the two species. The amount of oxygen affects the process of nitrification as well, and the nitrate measurements showed some correlations although they are not statistically significant. It was found that the nitrate concentrations in *Phalaris arundinacea* were the lowest among all the approaches and the non-vegetated approaches had the highest concentration of nitrates. Once again, it could possibly be caused by the uptake of nitrates by the plants. However, when compared with the presence of oxygen, it could also be deducted that the lack of oxygen affected the nitrification processes in these buckets (Bastviken 2006). Due to less oxygen, fewer nitrates were present in buckets with *Phalaris arundinacea* and the contrary with the non-vegetated buckets.

The measured pH values indicate that all the approaches were in slight alkaline conditions with pH values close to 8. The trend in electrical conductivity was the

opposite, but also had more variations among the approaches. The approaches with *Phalaris arundinacea* had the lowest pH values but highest electrical conductivities. The non-vegetated approaches showed an opposite trend with higher pH and lower electrical conductivity. The correlation between pH and electrical conductivity has been generally proven to be inverse, meaning if the pH rises, the electrical conductivity falls. However, approach 3 (non-vegetated and saturated) has shown a different behavior for electrical conductivity as the values are relatively high for both UR and SRB containing buckets. This occurrence is only for the saturated approaches and might be linked with the frequent watering of the buckets. It could be possible that additional ions were introduced into the water while saturating, but there are no data to prove this claim.

5.2 Tracer Concentration Development

The fluorescence of the samples was measured every two weeks to understand how the concentration is changing throughout the study period. For the water samples, the measurements gave clear peaks at the usual excitation wavelengths of the tracers (488 nm for UR and 561 nm for SRB). As compared to the respective injected masses, higher UR concentrations were detected compared to SRB. However, it was difficult to statistically establish a common behavior amongst the approaches as there were fluctuations in the magnitude of the concentrations measured. Compared to the concentration in the first week and the last week, it could be generalized that UR concentrations decreased slightly whereas the SRB concentrations have increased in the water samples over the 12 weeks. The reason for UR concentration decreasing could be due the tracer being susceptible to photolytic decay. Even though precautions were taken to reduce photodegradation of UR in samples, the buckets required light exposure due to the survivability of the plants. This could have contributed to the degradation of UR on the surface of the sand although in negligible amounts. The concentrations measured for the UR containing buckets also gave higher values in between the third to sixth week. This could be due to time taken for the buckets to establish and the lag between the injection and the UR reaching the water column. Since the water samples were usually taken from the water outlet, the mixing process was not ideal. After one sampling, the buckets were saturated several times with tap water that fills up the pipe before the next sampling. This might create a time lag or

mix-up of samples in between the consecutive weeks (Schelhorn, 2015). For SRB, the fluctuations were less resulting in minimum concentration during the first week while increasing by the end of the eleventh week. This is most likely caused by the sorption behavior of the tracer which makes it accumulate more in sand due to the two being oppositely charged (Sabatini and Austin, 1991). Various factors can play a role in influencing an adsorption/desorption equilibrium, but no studies has been found to prove desorption of SRB that could lead to the increase in concentration over weeks.

Compared to the water fluorescence measurement, the peaks appearing in the sand samples displayed high background signals. However, the background signals were similar for all samples and could be a result of organic materials present in the sand. For the sand pore water measurements, the presence of colloidal materials causing turbidity might contribute to the high background signal as well (Smart and Laidlaw, 1977). Even though the samples were given 24 hours to settle, some samples had comparatively high turbidity. This background signal can cause overestimation of the tracer fluorescence measurements.

The variation and standard deviation values for both sand and its pore water were high. For this reason, a significant pattern of the concentration in terms of increasing or decreasing could not be established. For all cases, the values were significantly higher during the first sampling (on the second week). The concentration decreased in the following weeks, but had high fluctuations in the values calculated. The cause of this might have been due to the random sampling approach, where samples were not taken from a fixed point but from a random column in the bucket. In a closed system such as this, the spatial variability of tracers in sand is supposedly high. Homogenous dispersion and distribution of the tracers were only assumed. However, in reality, the vertical water flow was not ideal and the tracers might have accumulated unevenly in the sand creating difference in concentrations at different spots.

As mentioned earlier, the concentration of the sand samples were measured using two approaches. The protocol according to McMahon *et al.* (2003) follows enabling the desorption of tracers with de-ionized water, whereas the Wernli (2011), 2011 protocol uses 40:60 Ammonia Ethanol solution to achieve the desorption of the tracers from the

sand particles. The desorption efficiency of the Wernli (2011) protocol has not been evaluated (Durst *et al.*, 2013). However, results of this experiment suggests that the efficiency might be higher compared to the protocol of McMahon *et al.* (2003) since the concentrations of tracers measured was significantly higher with the Wernli (2011) protocol. This can be observed in the comparison of concentration values of both protocols, where the trend in increase or decrease was similar, but the magnitude of the changes was higher when measured with the Wernli (2011) protocol. In addition, there were visible peak shifts with the Wernli (2011) protocol. In most studies, peak shifts are associated with degradation or transformation of the tracer (Gutowski *et al.*, 2015). However, when fluorescence scans were performed on the same sand sample using the two methods, no shift in peaks occurred for the McMahon *et al.* (2003) procedure. Hence, transformation or degradation of the tracers could not be proven in this respect. The shift of the peaks could be due to the concentrated ammonia ethanol solution. When the fluorescence scans were done and the shift of peak occurred with elevated background signal, the samples were re-measured with dilution. When diluted, the peaks appeared at the usual wavelengths, but the amplitude of the peaks was very less and their clarity was disturbed. Hence, undiluted shifted peaks were considered as UR and SRB peaks, ignoring the possibility of a transformation product.

5.3 Tracer Mass balance

Various studies have suggested the suitability of *Phalaris arundinacea* and *Typha latifolia* for constructed wetlands and phytoremediation of pesticide pollution (Calheiros *et al*., 2009, Stearman *et al.*, 2003). However, the complex mechanisms of such wetland species and their tracer removal efficiency still require in-depth study in order to understand the major factors contributing to such processes (Crini, 2005).

As discussed previously in the results section, it was assumed that the tracers were in water, sand and its pore water, and in the gravel. The final masses of the tracers were calculated for the whole buckets and compared with the initial tracer masses and injection masses. The tracer masses that could not be accounted for are considered to be dissipated.

The dissipated mass of tracers could be due to degradation, transformation, uptake by plants or for general sampling and measurement errors.

In general, lower percentage of the UR mass was accounted for in comparison to SRB. Especially for vegetated buckets, the percentage of mass dissipated for UR is very high. This could lead to the speculation of vegetations favoring degradation, or even biodegradation. The loss of mass could also be assumed due to plant uptake of the tracer. Previous studies have suggested that plant uptake of the tracers are negligible. In this experiment, tracer accumulation in plants has not been measured. However, such measurement could contribute to better understanding of the tracer mass distribution in the future. In the vegetated approaches, UR showed higher occurrence in water than in sand. The data monitored in the non-vegetated approaches indicate that UR is more present in sand and its pore water compared to the vegetated approaches. Due to UR showing negligible sorption capability, it is less likely that they were accumulated in the sand. Their presence in sand pore water in high amounts could be because of spatial variability and lack of mixing process. It can be assumed that there were trapped water pockets within the sand layer resulting in such values. For all approaches, the highest percentage of SRB was found in sand, which proves the sorption behavior displayed by SRB in general. When compared between the saturation levels, no significant difference or general behavior could be established. However, the results have proven that vegetation plays a very important role in the distribution of the tracer masses. The non-vegetated approaches for both UR and SRB gave the highest tracer recovery rates. For SRB, the calculated total was even higher than the initial values and could be an overestimation of the masses calculated. It is assumed that degradation, especially biodegradation, is taking place in the vegetated buckets. However, no significant evidence could be found to prove such processes for this experiment.

In terms of the general characteristics of the tracers, most of the occurrences and behavior could be explained. The mass dissipated may be linked to interactions between the tracers and their environment. Photodegradation of UR and sorption of SRB are the two most certain reasons accounting for the loss of tracer masses. Further biodegradation or transformation processes could not be proven. Due to the high effect of vegetation on

tracer recovery, it can be deduced that vegetation is favoring transformation or uptake of the tracers. However, one of the important factors contributing to tracer losses is the experimental error. Many sampling methods, measurements and calculations were not ideal for reliable data. As pointed out earlier, the sampling methods may have overlapping of tracer masses from consecutive weeks. Spatial variability and the lack of mixing process could have contributed to over estimation of some values. With that theory, it could have also resulted in under estimation of the values. Due to this factor, the extrapolation of calculated tracer masses in the samples to the whole bucket might not be completely accurate and can contribute to errors in the calculated values. Though some of these values might have inaccuracy, the general findings should not be highly affected since all the methods and calculations were standardized for all approaches.

6 Conclusion

The study was to investigate the behaviors of two fluorescent tracers, UR and SRB, depending on different wetland conditions recreated in a lab-scale wetland system. Over the twelve weeks of the data collection period, the concentrations and mass distribution of the tracers were monitored to understand the related processes and interactions. The plants conditions were not ideal, although *Phalaris arundinacea* was developing during the study period. *Typha latifolia* was mainly acting as a dead, but an intermediate system, where there was no active vegetation but some assumed biological activities taking place. The conditions of the plants were linked to the stressful environmental change from the outdoors to an indoor underground room. The water uptake was higher in the vegetated approaches compared to the non-vegetated ones. Plant uptake and evapo-transpiration might be the reasons for this occurrence, whereas the non-vegetated buckets only lost small volumes of water due to evaporation.

The water quality parameters did not display direct influence on the processes, even though some assumptions can be made from the results. The water temperature was mainly a response to the air temperature of the room and stayed constant in terms of varying vegetation, saturation and tracer. Dissolved oxygen in water was higher for the vegetated approaches compared to the non-vegetated approaches. In this case, it could be speculated that oxygen use is not exclusive to plant uptake but might be influenced by microorganism activities. High oxygen consumption can lead to fewer nitrate availability. This can be suggested by the thesis as fewer nitrates were found in the vegetated buckets than the non-vegetated. All plants developed a slight alkaline environment. The approaches with *Phalaris arundinacea* had the lowest pH values and highest electrical conductivity, whereas the non-vegetated approaches displayed opposite behavior. Lower pH could result in higher electrical conductivity.

The fluorescence of the samples was used to monitor the concentration development for the twelve weeks. The water samples gave clear peaks at expected wavelengths for both UR and SRB. It could be generalized that UR concentrations decreased slightly whereas the SRB concentrations have increased, but there were fluctuations in the concentration

throughout the weekly measurements. For SRB, the variations were comparatively less resulting in a steady rise of concentration every week. The fluctuations could be a cause of improper dispersion and distribution of the tracer in addition to photodegradation of UR and the adsorption/desorption equilibrium of SRB.

The fluorescence in the sand samples and its pore water was more complex to measure than the water samples. These measurements displayed high background signals, which could be the result of organic materials and suspended particles in the samples. The variations within these measurements were high, for which an increase or decrease of the concentrations could not be identified. Since the sampling of sand was done at random points, the spatial variability of the tracer concentrations could be a significant cause of high variations. The two protocols used to measure fluorescence of sand, McMahon *et al.* (2003) and Wernli (2011), showed the same fluctuation but with different concentrations. From the results, it was deduced that the Wernli (2011) protocol had higher desorption efficiency since the concentration values measured were much higher compared to the McMahon *et al.* (2003) protocol for both UR and SRB. The Wernli (2011) protocol also resulted in shifted peaks for both tracers, but the presence of a transformation product could not be validated as the McMahon *et al.* (2003) protocol gave clear peaks for UR and SRB.

Finally at the end of the study period, the tracer mass balance was calculated assuming that the mass of tracers are either in water, sand, its pore water and gravel. The final mass remaining of the tracers was calculated and the percentage that could not be accounted for was considered to be dissipated. Lower mass for UR was recovered when compared to SRB. The percentage of UR mass dissipated for vegetated buckets was the highest. This was also the case for SRB, where the lowest recovery was made by the vegetated approaches. For both UR and SRB, the non-vegetated approaches had the least amount of mass dissipation. Mass dissipation could be linked to possible degradation, transformation, uptake by plants or for general sampling and measurement errors. Hence, it could be concluded that vegetation had an important impact on the tracer mass balance. The variation in saturation frequency did not seem to have any significant impact on the mass balance. In all cases, the highest percentage of SRB mass was found in sand, which

could be directly attributed to the high sorption capability of SRB on sand. UR mass was mostly distributed in water than in sand. The tracer behavior was mainly explained by their general characteristics. Photodegradation of UR and sorption of SRB are the probable reasons for the loss of tracer masses. The effect of vegetation could be assumed, but the process of biodegradation could not be confirmed from the results of this experiment.

The sampling and calculation errors should be taken into account when looking at the values. Most sampling could be affected by spatial variability, improper vertical mixing and time lags. The calculations assumed homogenous distribution of tracer in the buckets, which could lead to over or underestimation of the values. Even though *Phalaris arundinacea* and *Typha latifolia* were identified for phytoremediation of pesticide pollution, their tracer removal efficiencies and its complex mechanisms still need further understanding. The tracer accumulation in the plants could not be taken into account for this study, but such measurements could contribute to the assumption made for plant uptake of the tracers. Overall, if the mentioned errors can be addressed and avoided for future studies, a more accurate tracer distribution could be produced.

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