



# Wetland based buffer zone technology

## Generic guideline

Target audience: Scientists, Consultancies, contractors, authorities, feasibility test labs

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## 1 INTRODUCTION

The term wetlands refers to “areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions” (USA EPA Clean Water Act). This document is related to wetlands implemented as a buffer zone between agricultural land and water bodies like river water to intercept pollutants flowing towards surface water. More specifically, generic guidelines are described for evaluating the feasibility and impact of wetland based buffer zones to reduce fluxes of nitrate and pesticides towards surface water likes rivers and lakes. The guidelines aim to describe (i) means to investigate the capacity of wetlands to mitigate nitrate and pesticide pollution of nearby surface water bodies, (ii) possible means to design/manage wetland for maximal protection of surface waters from pollution by agricultural pollutants focusing again on nitrate and pesticides and (iii) monitoring approaches to evaluate the impact of the buffer zone on the surface water body. This information is especially useful for scientists, consultants and authorities who are considering wetlands to mitigating diffuse pollution.

This document was composed in the frame of the FP7 project AQUAREHAB (GA 226565), and comprises outcomes and lessons learned during this project. For nitrate focus is on the subsurface compartment of the wetland while for pesticides it is on the surficial compartment.

DISCLAIMER: Although the information described in this document is believed to be reliable and accurate, the guideline does not offer warranties of any kind.

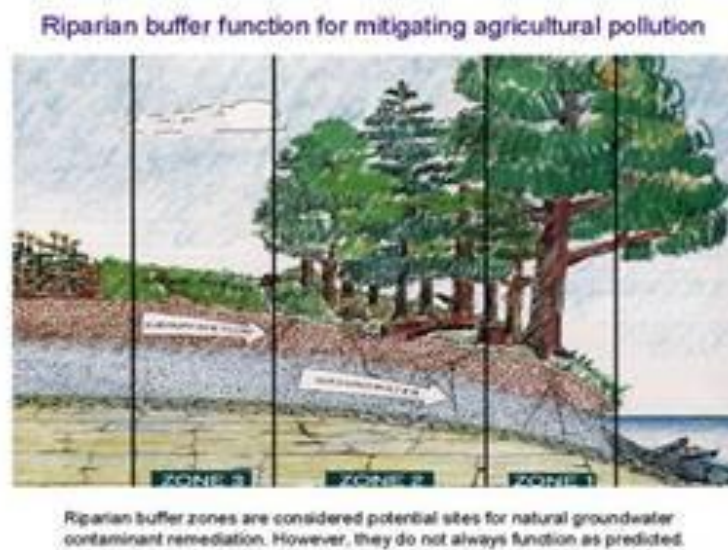
## 2 BACKGROUND INFORMATION ON THE WETLAND BASED BUFFER ZONE TECHNOLOGY

### 2.1 GENERAL EXPLANATION OF THE TECHNOLOGY PRINCIPLE

**Riparian buffer zones** can be defined as permanently vegetated buffer zones between land and a water body, which are separately managed from the rest of a field or catchment with the aim to mitigate the runoff and drainage of various agricultural pollutants (nitrates and pesticides) or entry of such pollutants in surface water via upwelling groundwater. Such riparian buffer zones can take the form of small forests, grass land and wetlands (Figure 1).



A.



B.

**Figure 1: Representation of a wetland (A) and riparian buffer zone (B) for coping with diffuse pollution (pesticides, nitrate) in agricultural areas.**

Roughly, for activity understanding and because of reasons of simplicity, a wetland zone can be divided in two major compartments, i.e., the “above surface compartment” (also designated as “surficial” compartment) and the “subsurface compartment”. The surficial compartment receives nitrate and pesticides from agricultural land through drainage, erosion and run-off but during flooding also from the river itself. The subsurface compartment receives pollutants from the agricultural land through leaching. Nitrate is removed by plant-uptake and biological conversions while pesticide concentrations will be reduced by retention on soil particles, sorption and microbial degradation. In the “above surface compartment” also photodegradation can contribute to pesticide removal.

Transformation/restoration of agricultural land into a wetland system will supply a buffer zone between the land and the surface water body resulting into a longer flow path of pollutants to enter the river in which biotic and abiotic processes contribute to pollution mitigation. AQUAREHAB results show that this flow path will also depend on the extent of flooding in case of subsurface contamination. Flooding will force the groundwater to well up through the peat layer

(in case hydraulic conductivity of the peat layer is sufficiently high) and as such brings it in contact with organic carbon rich materials that stimulate denitrification and hence nitrate removal.

Wetlands are further often characterized by an organic layer (peat layer, plant material etc.) as interphase between the surficial compartment and the subsurface compartment. This peat layer developed from degraded plant material and is characterized by a high organic carbon content. The peat layer plays probably an important role in pollutant mitigation since it has a high sorbing capacity for pesticides and supplies dissolved organic carbon for denitrification. In addition, it will specify the redox conditions in the interphase between above surface compartment and the subsurface compartment since it is expected that any oxygen entering the water in the peat layer of the surficial compartment will be rapidly depleted over a few millimeters. Experiments from AQUAREHAB suggest that pesticides entering and draining through the above surface compartment will be degraded but will be also partially sorbed to the peat layer. Desorption steered by degradation and degradation rates/population size of degraders will then determine effective pesticide degradation rates.

## 2.2 TARGETED SUBSTANCES

The substances targeted in this guidelines are pesticides and nitrate (Table 1). The guidelines are directed towards agricultural landscapes.

**Table 1: Overview of substances that are targeted by the “wetland based buffer zone” technology.**

Targeted substances		Emission sources
Class	Specific substance	
Nutrients	Nitrate	Agriculture
Pesticides	Atrazine <sup>1,6</sup>	Agriculture
	Diuron <sup>2</sup>	Agriculture (vineyard)
	3,4-DCA <sup>2</sup>	Agriculture (vineyard)
	Glyphosate <sup>2</sup>	Agriculture (vineyard)
	Chlorpyrifos <sup>3</sup>	Agriculture
	Mecoprop <sup>4</sup>	Agriculture
	Metsulfuron-methyl <sup>4</sup>	Agriculture
	Isoproturon <sup>4</sup>	Agriculture
	Deltamethrin <sup>5</sup>	Agriculture
	Fluometuron <sup>6</sup>	Agriculture

Ref.: <sup>1</sup>(Anderson *et al.*, 2002), <sup>2</sup>(Bois *et al.*, 2013), <sup>3</sup>(Karpuzcu *et al.*, 2013), <sup>4</sup>(Larsen *et al.*, 2001), <sup>5</sup>(Muñoz-Leoz *et al.*, 2009), <sup>6</sup>(Weaver *et al.*, 2004)

## 2.3 REACTION MECHANISMS

### 2.3.1 Nitrate:

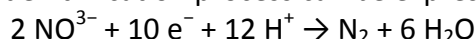
Nitrate removal in a wetland is commonly attributed to denitrification and plant uptake (Mayer *et al.* 2006).

**Denitrification** implies the reduction of nitrate to nitrogen gas and is performed by denitrifying microorganisms. These organisms are ubiquitous in the natural environment and use nitrate for respiration thereby consuming/oxidizing an electron donor for the nitrate reduction.

Denitrification generally proceeds through some combination of the following intermediate forms:



The complete denitrification process can be expressed as a redox reaction:



The electron donor can consist of organic carbon, but also pyrite. Pyrite is used as electron donor for autotrophic nitrate reduction (Hayakawa et al. 2013). Both processes occur under anoxic conditions and are inhibited in the presence of oxygen.

Denitrification in both compartments (subsurface & above surface) will mainly depend on the supply of electron donors, and absence of oxygen. In the subsurface, often anoxic conditions predominate which is of interest for denitrification.

**Plant uptake** could be seasonally important but microbial denitrification is dominant against plant uptake (Buss et al. 2005).

### 2.3.2 Pesticides

Although anaerobic degradation of pesticides has been described, pesticide biodegradation mainly occurs under aerobic conditions. Several reports describe accelerated biodegradation of pesticides in soils treated with those pesticides implying an adaptation of specific microbial populations to degrade that pesticide. Several bacterial strains have been described which use specific pesticides as sole source of carbon and energy (Table 2). Depending on the pesticide, this capacity is not or well-widespread between bacteria.

**Table 2: Selected list of bacterial strains that use specific pesticides as sole source of carbon and energy.**

Bacterial strain	Pesticide catabolic capacity	Reference
<i>Variovorax</i> sp. SRS16	Linuron	Sorensen et al., 2005
<i>Sphingomonas</i> sp. SRS2	Isoproturon	Sorensen et al. 2001
<i>Pseudomonas</i> sp. ADP1	Atrazine	Mandelbaum et al. 1995
<i>Cupriavidus pinatubonensis</i> JMP134	2,4-D; MCPA	Don and Pemberton, 1981
<i>Sphingomonas paucimobilis</i> UT26	Lindane	Nagata et al., 1999
<i>Sphingomonas herbicidovorans</i> MH	Mecoprop	Muller et al., 2004
<i>Sphingomonas</i> sp. KN65.2	Carbofuran	Nguyen et al., 2014
<i>Aminobacter</i> sp. MSH1	Dichlobenil	Sorensen et al., 2007

Bioavailability and hence the sorption capacity of the environment will/can influence pesticide biodegradation. Sorption of pesticides is mainly related with the organic carbon content of the soil matrix.

## 2.4 DEVELOPMENT STAGE OF THE TECHNOLOGY

Wetland restoration is often performed as a combined re-meandering of the river slopes and raise of the river bed to obtain hydroperiods (flooding) of the surrounding wetlands/riparian areas in wet periods. The technology of wetland restoration as such can be classified as a (commercially) available technology and has been used in full scale river restoration projects for instance in Denmark in the Odense river basin. Although the technology is well-known around Europe, the documentation of its contribution to pollutant mass removal and how this is translated into the “best” wetland restoration approach for pollutant mitigation, is limited. Therefore, the technology of wetland restoration directed towards pollutant mitigation can be considered as “emerging”. The application of the models described in this guideline aims at directing wetland implementation and managing towards improved pollutant mitigation.

## 2.5 APPLICABILITY AND BOUNDARY CONDITIONS OF THE TECHNOLOGY

Different factors will influence the efficiency of pollutant removal activities in wetlands. In the “surficial compartment” and “subsurface compartment”

- Width of the wetland (distance between “producing” agricultural land and “receiving” surface water body)
- Vegetation and depth of the root zone where plants can take up nitrogen (Asmussen et al., 1979; Cooper, 1990). Vegetation can also supply oxygen for pesticide degradation but also DOC for denitrification.
- Hydrological flow paths that can for instance favor microbial denitrification (i.e., saturated anaerobic soils, adequate carbon supplies, floodplain connections)
- Existence of a peat layer, organic carbon content and hydraulic conductive of the peat layer
- Geohydrology of the subsurface: Hydrological flow paths, structure, organic carbon content, water chemistry (redox, oxygen concentrations, DOC concentrations, ...).
- Redox conditions

Factors that are especially of importance for pesticide degradation in the surficial compartment are oxygen content in surficial water, TOC content in soil, number of pesticide degraders in soil. Factors that are important for the model of nitrate fate in the subsurface are zonation of redox, zonation of TOC content, hydraulic conductivity including of peat layer, flooding level, and geohydrology.

## 2.6 LONGEVITY OF THE TECHNOLOGY

After installation, the wetland based buffer zones needs regular maintenance to maintain the “above surface compartment” wetland functionalities and ensure longevity. These maintenance measures and their frequency of application include activities related to vegetation control (mowing, planting of species, grazing), control of pests and dredging (as sedimentation may fill up a controlled flooding basin and compromise water storage in the long run). Some researchers



postulate that “above surface compartment” wetlands nutrient removal efficiency declines with wetland age with nutrient reduction functioning becoming reduced with 50% after 50 years.

## 2.7 COST OF THE TECHNOLOGY

Investment and operating costs will depend on the size of the area and site-specificities (hydrogeology) of the area which has to be restored/was restored and local labour costs. As a lead, the investment cost of the re-meandering of a 10 km stretch of the Odense river and concomitant re-establishment of 350 ha wetland was 0.8 MEURO (2003-prices (exclusive VAT)). Other numbers of installation costs found in literature are \$3,500 to \$80,000 per acre in the US. Additional costs might include costs related to the landowner compensation (if applicable). Annual operating costs will be minimal and will include costs related to control/maintenance measures and monitoring. Costs are available for a controlled flooding basin in Bernissem (Sint-Truiden, Belgium) along the Melsterbeek that was installed in 2009. The total cost of purchasing the area of 14 ha was 0.6 Meuro. The additional total cost of construction was estimated to be 184.249 euro. However, for this specific project, (pristine) soil from sewage construction works was used to build the levees, reducing the actual construction including labour cost, to 0 euro (Watering van Sint-Truiden, personal communication, April 25, 2012).

## 2.8 PERFORMANCE OF THE TECHNOLOGY

Numbers regarding performance of pollutant removal are only primarily available regarding N-removal in the above surface (surficial) compartment.

### 2.8.1 Nitrate removal

The efficiency of nutrient removal in the above surface compartment of natural wetlands seems to be negatively correlated with the nutrient loading (Fisher and Acreman, 2004). Examples of numbers for N-removal vary from 90% with a nutrient load of 10 kg/ha/year and 40-50% in case of 1000 kg/ha/year. Other reported denitrification rates are reported per liter and range from 0.004 to 26.5 mg nitrate/l-day (Gibert et al., 2008) or first order constants in the range  $3 \cdot 10^{-5}$  to  $1.4 \text{ day}^{-1}$  (Leverentz et al. 2010) for different types of vegetal-based materials and under different conditions. As much as 2,000 to 3,000 kg of nitrate-nitrogen can be denitrified per hectare of wetlands per year, depending on the hydraulic conditions. In the AQUAREHAB project, experiments performed with material of top layer (1 m) of Brynemade wetland gave a range of first order constants between 0.15 and  $1.5 \text{ day}^{-1}$  that corresponds to an initial denitrification rate from 3.7 to 37.5 mg nitrate/l-day. Assuming a porosity of 0.15 the initial rates will be equivalent to values that range from 460 to 4600 kg of nitrate-nitrogen that can be denitrified per hectare of wetland per year.

In the subsurface compartment, reported denitrification rates are 30 to  $120 \mu\text{g N kg}^{-1} \text{ d}^{-1}$  within 10 m versus  $<1$  to  $40 \mu\text{g N kg}^{-1} \text{ d}^{-1}$  at  $>30$  m from the stream (Kellogg et al., 2005). Other studies report  $210 \mu\text{g N kg}^{-1} \text{ h}^{-1}$  (Flite et al., 2001) and  $1.8 \text{ kg N m}^{-2} \text{ yr}^{-1}$  (Maitre et al., 2005). In the AQUAREHAB project the typical denitrification rates below 1 m in Brynemade could be around  $2 \cdot 10^{-2} \text{ day}^{-1}$  that in an aquifer of porosity 0.15 and soil density of  $1500 \text{ Kg/m}^3$  correspond to initial velocities below  $11 \mu\text{g N kg}^{-1} \text{ d}^{-1}$

## 2.8.2 Pesticide removal

Pesticide degradation rates in wetland systems have hardly been studied. In AQUAREHAB, mineralization of the herbicides MCPA, isoproturon and bromoxynil octanoate ( $60 \mu\text{g kg}^{-1}$ ) in subsurface wetland samples, was tested with oxygen, nitrate or ferrihydrite as terminal electron acceptors at  $8^\circ \text{C}$ . The mineralization pattern was highly variable. MCPA was mineralized under oxic conditions by all top samples (0-1,2m), and to a variable degree in deeper samples. MCPA was also mineralized in some samples with nitrate as the electron acceptor, whereas mineralization was not detectable with ferrihydrite. Under oxic conditions, bromoxynil octanoate was mineralized by all top samples (0-1m), but was not mineralized in most of the deeper samples. Under anoxic conditions, bromoxynil octanoate was mineralized only in samples from a depth of 1,2 m at the edge of the wetland, and mostly with ferrihydrite. Isoproturon was the least degradable of the pesticides showing only oxic mineralization and only by the surface layers. Effective mineralization rates were not calculated.

MCPA and isoproturon mineralization rates under aerobic conditions were however determined for surficial compartment samples taken during 2 years from a wetland in Sint-Truiden, Belgium, using the logistic character of the mineralization curves and the modified Gompertz model (Zwietering *et al.*, 1990)). The maximal rate of mineralization of MCPA ranged between 0.2 and 22.2 %/day and the mean and median were 8.3 and 7.3 %/day, respectively. The maximal rate of mineralization of isoproturon ranged between 0.0 and 20.3 %/day and the mean and median were 1.2 and 0.5 %/day, respectively. Other authors also studied the mineralization of pesticides in sediment from the surficial compartment of wetlands and rate constants and half lives are summarized in Table 3.

**Table 3 First order rate constants and half lives of pesticides under aerobic conditions in the surficial compartment of wetlands**

Compound	First order rate constant ( $\text{d}^{-1}$ )	Half lives (d)
MCPA	$0.44^1$	1.6
Isoproturon	$0.26^1$	2.7
Atrazine	$0.023 - 0.15^{2,5}$	30.1 – 4.6
Chlorpyrifos	$0.02 - 0.69^3$	34.7 – 1.0
Deltamethrin	$0.007 - 0.026^4$	96 – 27
Fluometuron	$0.026 - 0.027^5$	27 – 25

Ref.: <sup>1</sup>AQUAREHAB-project, averages over a two year period, <sup>2</sup>(Anderson *et al.*, 2002), <sup>3</sup>(Karpuzcu *et al.*, 2013),

<sup>4</sup>(Muñoz-Leoz *et al.*, 2009), <sup>5</sup>(Weaver *et al.*, 2004)

## 2.9 CO-EFFECTS

### 2.9.1 Positive co-effects

Positive co-effects of the wetland based buffer zone technology comprise protection of nature and preservation of biodiversity, provision of products (timber, fish, reeds, ...), flood control, groundwater recharge, microclimate stabilization and surface water/sediment storage. Actually, currently, these are often the main drivers for installing wetlands. Costs that were saved by installing a wetland in Sint-Truiden (Belgium) that aims primarily at flood control are available. Due to flooding, citizens requested claims of in total 2,557,749 euro from 1998 until 2002, of which an estimated 60 %, *i.e.* 1,534,650 euro, was paid. Together with the intervention costs of the fire brigade after intense flooding in 2002, the total cost of flooding was 1,559,650 euro during the 5

years from 1998 until 2002, which yields an average of 311,930 euro per year. Much of these costs could be reduced by the installation of a controlled flooding basin in 2009 along the Melsterbeek in Bernissem (Sint-Truiden, Belgium)(Watering van Sint-Truiden, personal communication, April 25, 2012).

Brander *et al.* (2013) reviewed the economic valuation of regulating services provided by wetlands in agricultural landscapes and found that mean (median) values for flood control, water supply and nutrient recycling were 6923 (427), 3389 (57) and 5788 (243) USD/ha/yr, respectively (Brander *et al.*, 2013).

### **2.9.2 Negative co-effects**

In some cases, wetlands used to reduce nutrient loadings have been reported to become degraded (in case of overtaxing). Incomplete denitrification can release the greenhouse and ozone depleting gas, nitrous oxide. Methanogenic activities can release the greenhouse gas methane (Verhoeven *et al.*, 2006). If plants are harvested, they will rerelease nitrogen in the soil.

### 3 GENERIC APPROACH TO DETERMINE APPLICABILITY OF THE WETLAND BASED BUFFER ZONE TECHNOLOGY FOR NITRATE REMOVAL

#### 3.1 OVERVIEW OF DIFFERENT STEPS IN THE APPROACH

The purpose of this guideline is to describe what to consider when evaluating the applicability of a wetland as activated riparian zone for mitigating nitrate pollution migrating towards freshwater bodies via subsurface transport. More specifically, it describes (a) types of parameters to be considered, (b) methods for deriving these parameters, and (c) a model which integrates the determined parameters and allows predicting nitrate fate in the **subsurface** in a wetland system under different flooding conditions. The models can be used; (i) to predict the reactive transport behaviour in the subsurface in an existing wetland in order to estimate nitrate reduction fluxes in that wetland or (ii) to predict the effects of specific manipulations in a wetland system on the reactive transport of nitrate and nitrate reduction fluxes in the wetland.

The steps and methodologies are;

- STEP 1: Hydrological connection between wetland and catchment
- STEP 2: Characterization of hydrogeology and hydrochemistry
- STEP 3: Characterization of soil and organic sources
- STEP 4: Anoxic laboratory batch denitrification experiments

The rationale is to start by evaluating how a specific wetland is connected to a contributing catchment (step 1) and then go through a series of steps (2-4) where the hydrogeology, hydraulic functioning and potential for nitrate removal are assessed.

#### 3.2 STEP 1: HYDROLOGICAL CONNECTION BETWEEN WETLAND AND CATCHMENT

The first step is to characterize the hydrogeological setting of the wetland in the contributing catchment (Hill, 1996). Figure 2 shows a wetland that is connected to the upland or contributing catchment in different ways leading to different wetland hydrology.

- Figure 2a illustrates a **wetland bounded by semi-permeable aquitard**. The thickness of the wetland aquifer is small therefore restricting inflow and the contributing catchment is small (local flow system). In such a system groundwater discharge to the wetland from the catchment is low, but, on the other hand, any changes in e.g. net infiltration or recharge in the catchment will immediately lead to large water table fluctuations. Groundwater flow in the wetland is mainly horizontal towards the river.
- Figure 2b shows the same situation, but **without the aquitard**. The thicker wetland aquifer (or flow area) causes less resistance to inflow, so discharge to the wetland is higher, but more stable. The higher discharge may lead to the formations of upwelling (seeps) near the hill slope and subsequently overland flow plus deeper and longer subsurface flow paths.
- In Figure 2c a similar situation is displayed, but now the **wetland is connected to a hilly catchment**. Local flow systems will develop in the catchment itself and recharge water is thus diverted away (to other rivers) and the wetland will now receive groundwater of various ages; young from nearby local flow systems and old from more regional flow systems. The

groundwater quality of the two types of water can be different. In this case the wetland may receive a significant amount of inflow causing upwelling in several parts of the wetland followed by overland flow.

- Figure 2d shows the situation during **over-bank flooding of the wetland**. The shallow horizontal flow paths now changes completely; groundwater discharge now takes place right at the maximum flood inundation and flow is more or less stagnant below the flooded part of the wetland (Jensen et al., 2013a).

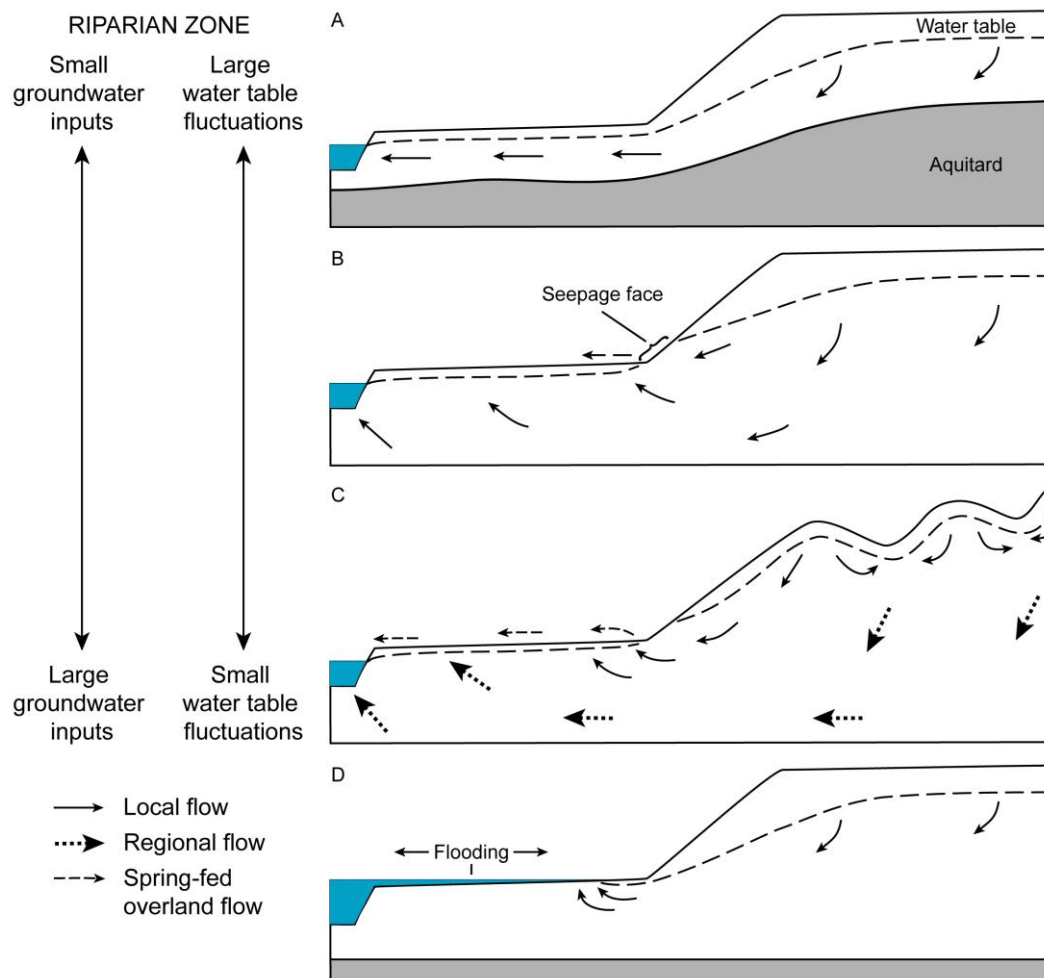


Figure 2: Wetlands connected to the contributing catchment in three different ways. (adapted from Hill, 1996).

The following characteristics are needed to understand the general wetland hydrology (for more specifics, see below);

- 3D physiography of the wetland-catchment continuum, i.e., topography, catchment size, geology. This information can be gathered from digital elevation models and existing or new well bores.
- Estimates of recharge (e.g. from climate stations recording precipitation and net evapotranspiration)
- River stage to record in- and out-of-bank floods

The type of wetland and how it connects to the catchment will help to estimate the amount of groundwater inflow to the wetland and decide how often the position of the water table needs to be measured depending on how stable the input is.

### 3.3 STEP 2: CHARACTERIZATION OF HYDROGEOLOGY AND HYDROCHEMISTRY

Wetland hydrogeology and hydrochemistry are notoriously heterogeneous and optimally a suite of methods are needed to characterize the wetland. Figure 3 shows a classical wetland with a top peat layer capping a sandy aquifer with shallow horizontal flow to the river.

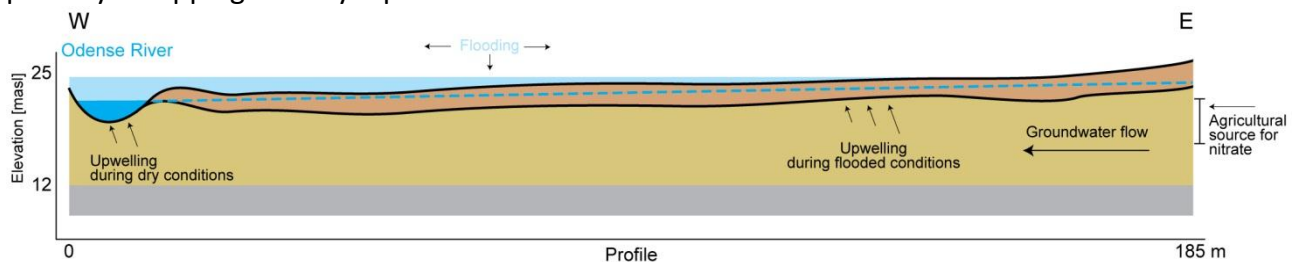


Figure 3: As in Figure 2a, but now illustrating the flow field in a flooded wetland.

Several field-investigative methods can be applied to develop a conceptual model of the wetland hydrogeology (Jensen et al., 2013a):

1. **Dimensions and topography of wetland**, e.g., width and slope. *Slope could be a substitute for a hydraulic gradient if wells are not present. Width directly affects the flow residence time.*
2. **Geophysics**: e.g. electrical resistivity tomography. The resistivity of the subsurface can help explain the stratigraphy, e.g. clay has a much lower resistivity than sand. A challenge is though that the resistivity ranges of different materials overlap, so ground-truth data in terms of borehole information is desirable. *This will feed a model with the needed geometry of the wetland and internal architecture of sedimentary structures.*
3. **Deep boreholes**: map the stratigraphy of the wetland and get sediments for carrying out grain size analysis (to obtain estimates of hydraulic conductivity) or measuring reduction potential. *This is needed as ground-truth data for constraining the geophysical surveys and for building the conceptual model and/or provide hydraulic and hydrochemical parameters.*
4. **Hand-drillings**: map the thickness of a peat layer. *Needed in the model, especially because a peat layer can both be more and less permeable and is known to be a highly reactive zone and thus a sink of nitrate.*
5. **Installation of a network of nests (or multi-level) of piezometers**: measure hydraulic heads. Depending on the type of wetland and the stability of the inflow, hydraulic heads can be measured manually (stable inflow) or using pressure transducers with frequent sampling of data. *Needed for understanding the flow system and eventually for calibrating the model.*
6. **Hydraulic tests**: Slug-tests in piezometers (aquifer or river bed) to assess the average horizontal hydraulic conductivity and the heterogeneity. Infiltration tests at various depths in the peat layer to estimate infiltration capacity and/or saturated vertical hydraulic conductivity of the peat. Falling head tests in stand pipes in the river bed to estimate the vertical hydraulic conductivity of the river bed. *Needed for estimating Darcy fluxes and eventually as input to the model.*
7. **Discharge measurements**: Use seepage meter or temperature profiling for estimating the flux to the river. *Can be used to compare with the estimates from step 1 and with the simulation output from the model.*

Methods used to develop a conceptual model of the wetland hydrochemistry are:

- Strategy for sampling of groundwater and river water: Analysis of nitrates, nitrites, ammonia, oxygen, temperature, DOC and other anions several times during a season. *Needed to map redox zones, indicate denitrification areas, and used in the model to calibrate reactive parameters*
- Groundwater age tracers. *Can be used to estimate denitrification rates without a numerical model.*
- Collection of soil cores for laboratory experiments (see below).

It is crucial to understand the wetland hydrology in order to design a network of wells for, not only measuring water table, but also water quality and subsequently the interpretation. In Figure 4a the flow in the wetland system is rather straight forward. Because of the shallow uniform flow from the wetland margin to the river any disappearance of nitrate is most likely related to nitrate removal (denitrification). If groundwater ages are available (see e.g. Tesoriero and Puckett, 2011, where Chloro-Fluoro-Carbons, CFCs, were used) then decrease in concentration can be computed with a simple plug-flow model (see also an example below).

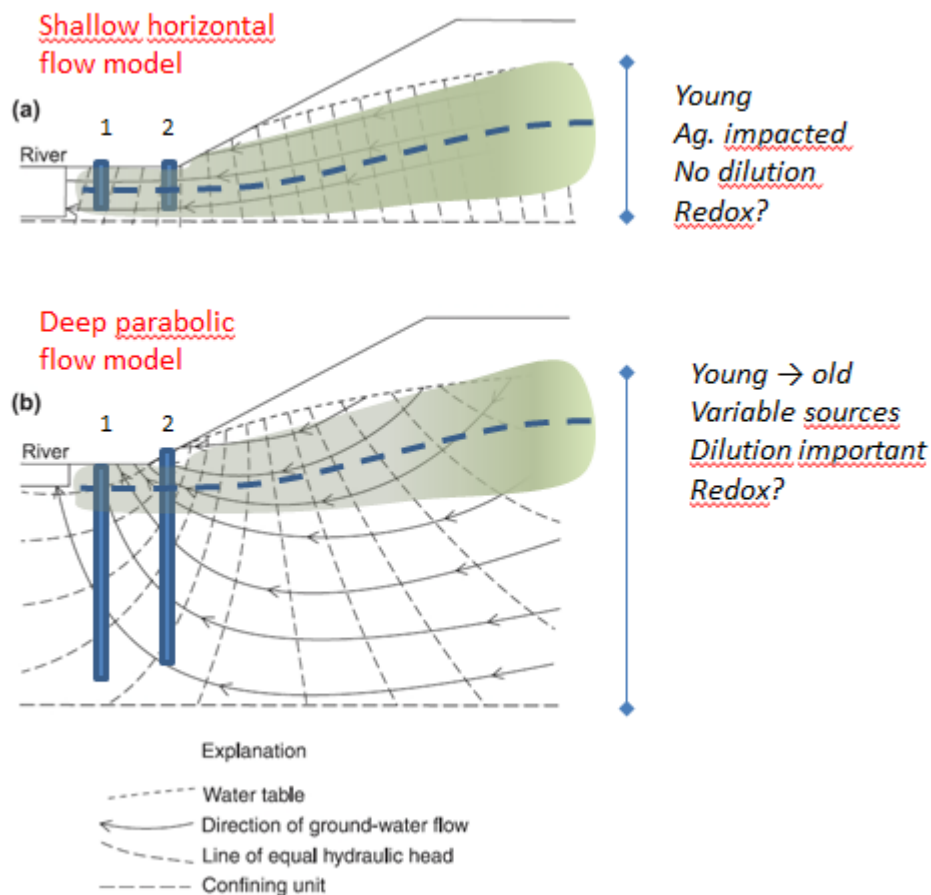


Figure 4: (a) Wetland system with a shallow aquifer and uniform groundwater flow to the river. Nitrate removal from well 2 to 1 is due to denitrification. (b) Wetland system with deep aquifer and significant mixing of young (shallow) and old (deep) groundwater. Nitrate removal from well 2 to 1 can be due to both denitrification and dilution. Adapted from Puckett et al. (2002).



However, if the situation is as shown in Figure 4b, then disappearance of nitrate can be due to both denitrification and dilution by older groundwater from below. This is a consequence of wetland hydrogeology and its connection to the upland (Figure 2).

From the measurement of these groundwater quality parameters, the possibilities (A to H) listed in Table 4 could be obtained.

**Table 4 Possible combinations of groundwater measurements of nitrate, oxygen and DOC**

Case	A	B	C	D	E	F	G	H
NO <sub>3</sub>	Low	Low	Low	Low	High	High	High	High
O <sub>2</sub>	Low	Low	High	High	Low	Low	High	High
DOC	Low	High	Low	High	Low	High	Low	High
<b>Denitrification potential</b>	<b>++</b>	<b>+++</b>	<b>-</b>	<b>+</b>	<b>+</b>	<b>++</b>	<b>-</b>	<b>-</b>

In cases A to D the lack of nitrate could indicate different degrees of potential denitrification. Case B indicates that the conditions of oxygen and DOC are the best for denitrification. In cases C and D the presence of oxygen will stop denitrification and the lack of nitrate could indicate absence of this contaminant. The combination in D is rare under equilibrium conditions (unstable) as excess of DOC and presence of aerobic microorganisms would decrease oxygen and would evolve to cases A or B. Cases E to H indicate lack of denitrification because of the high nitrate concentration, however, case F points to an excess of nitrate that exceeds the denitrification capacity. Case E would need DOC from another source as is not sufficient for denitrification. Cases G and H have a presence of oxygen that halts denitrification

This discussion indicates that denitrification tests with surrounding soil or with leachates rich in DOC from source layers (peat) need to be performed to finally elucidate the potential of denitrification in critical zones of the aquifer. In step 4, such denitrification test are described.

### 3.4 STEP 3: CHARACTERIZATION OF SOIL AND ORGANIC SOURCES IN WETLANDS

The objective of this step is to explore (a) the amount and type of organic carbon in the soils and (b) the feasibility to release DOC that could stimulate denitrification. Soil samples collected during step 2 activities can be used for characterisation of the organic carbon.

A good approach to explore the availability of total organic matter in the soil includes soil leaching tests followed by DOC-measurements on the leachate. The amount of DOC will make it feasible to estimate the maximum nitrate elimination from literature studies.

### 3.5 STEP 4: ANOXIC LABORATORY BATCH DENITRIFICATION TESTS

The objective of this step is to explore directly via lab scale experiments the capacity of denitrification in soil, which can be performed as a function of depth, under anoxic conditions (nitrogen bubbling) and thermostatic temperature control (representative temperature of



groundwater). Addition of extra carbon source to some test conditions allows to evaluate potential limitation of the denitrification by the DOC content.

Several types of tests can be performed, depending on site observations made in steps 2 and 3:

- **Anoxic tests assessing denitrification in a soil suspension with known nitrate concentrations** The setup of this type of experiments is based on (1) contacting the soil and nitrate solution in sealed bottles/tubes, (2) incubating them during an experimental time covering the residence times at the field site, (3) opening and filtering the solution to quantify the nitrogen concentration species and DOC. If excess of organic matter is present in the soil continuously denitrification will proceed in a short time (1 week). If denitrification does not occur, it means that the soil itself does not have the capacity to release DOC and leachates from upper layers (e.g. peat) could be used as source of DOC for denitrification.
- **Anoxic denitrification tests in soil suspension with soil leachates and nitrate.** The aim is to assess if denitrification is feasible using DOC from the upper layers as electron source. The first step is to acquire the soil leachate rich in DOC by means of a normalized 24 h leaching test. Samples from the upper layer at the studied site are preferred to be used. The leachate is characterized and nitrate and soil are added and put in sealed bottles/tubes in a similar way as in the assay described above. Similar assays using with other materials in the field (e. g. degraded vegetation) could also be performed.

In general, batch denitrification processes can be described with a first order kinetics

$$C(t)=C_0 \cdot \exp(-\lambda \cdot t)$$

where  $C(t)$  is the concentration of nitrate in water over time,  $C_0$  the initial concentration of nitrate,  $\lambda$  is the first-order constant and  $t$  is the elapsed time. This expression could be linearized as follows;

$$\ln [C(t)]=\ln [C_0]-\lambda \cdot t$$

In this way by linearly correlating  $\ln (C(t))$  versus  $t$ , it is possible to obtain  $\lambda$ . This procedure could be applied to small portions of samples taken at several depths. The kinetics is dependent on the concentration of available dissolved organic matter for denitrification (Calderer, 2010), as the denitrification is an electron demanding process. Thus the term  $\lambda$  depends on the presence of this organic matter. The lack of organic matter could limit denitrification. If the denitrification rate is low and denitrification is not complete at the end of the experimental period, there could be limitations of organic matter. This aspect is not included in the general model and has to be tested for each site. The denitrification kinetic values derived from the above described assays, superimposed to the dissolved oxygen content in groundwater and the temperature, will allow calculating an average denitrification capacity of the site studied and will identify the zones where denitrification potential is present and/or could be enhanced.

## 4 GENERIC APPROACH TO DETERMINE APPLICABILITY OF WETLAND BASED BUFFER ZONES FOR PESTICIDE REMOVAL

### 4.1 OVERVIEW OF DIFFERENT STEPS IN THE APPROACH

This part of the guidelines will describe which steps are needed to evaluate the pesticide removal capacity of wetlands by either sorption and biodegradation.

The steps and methodologies are:

- STEP 1: Assess sorption of pesticides to wetland sediment
- STEP 2: Assess pesticide biodegradation and mineralization capacity/rates

### 4.2 STEP 1: SORPTION

Adsorption of pesticides to soil largely determines the fate of these compounds in wetlands and depends on both the compound itself and soil characteristics. Strong sorbing compounds interact strongly with soil and therefore can be retained easily within wetlands, whereas these compounds are rather not bio-available, leading to lower degradation.

The distribution coefficient of adsorption  $K_d$  (l/kg) is the ratio of the concentration of the compound sorbed to the soil and the concentration of the compound in solution at equilibrium. For most pesticides, sorption is largely due to organic carbon, due to which the  $K_d$  can be normalized for organic matter, yielding the  $K_{oc}$  (l/kg), which is compound-specific. As a rule of thumb, pesticides with a  $K_{oc} < 100$  l/kg are considered weakly sorbing, those with a  $K_{oc} > 1000$  l/kg strongly sorbing and those in between moderately sorbing. Weakly sorbing pesticides have a tendency to leach to groundwater, whereas strongly sorbing pesticides rather sorb to the soil and may reach surface water through erosion (Reichenberger *et al.*, 2007).

The best approach to determine sorption ( $K_d$ ) is to directly measure in a laboratory batch setup, for which a standardized assay is described in the Commission Directive 2001/59/EC (European Commission, 2001).

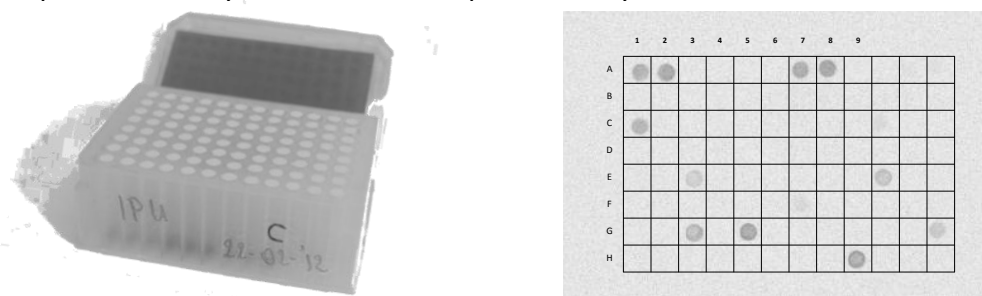
For each pesticide, a series of 3 different soil:solution (10 mM  $\text{CaCl}_2$ ) ratios can be used: *e.g.* 1:1 (*e.g.* 10 g dry soil, 10 ml water), 1:5 and 1:25. Within AQUAREHAB the following test procedure was followed: The soil:water suspensions were shaken head-over-end and after 4 h, 24 h and 48 h the suspensions were centrifuged and 500  $\mu\text{l}$  samples were taken from the water phase to determine the residual concentration of pesticide in the solution. The most convenient soil:solution ratio for calculating  $K_d$ -values is the one for which the percentage adsorbed after 48 h is higher than 20 % and lower than 50 %.

### 4.3 STEP 2: PESTICIDE DEGRADATION & MINERALIZATION ASSAY

The microbial degradation of pesticides depends on a number of biotic and abiotic constraints. In the first place, micro-organisms that are able to degrade (mineralize) the pesticide of interest, have to be present. This can be tested in a laboratory setup with idealized conditions referred to

below as ‘pesticide mineralization assay’ (1). When micro-organisms are present, physicochemical conditions of the microenvironment should be suitable for degradation of the compound. Pesticide degradation is often, but not always, an aerobic process, and oxygen is mostly limited in wetland sediment or in subsoil. Therefore, the mineralization of pesticides should be tested in different redox conditions (2). Next, sorption and diffusion of the pesticide in wetland sediment may reduce the bioavailability and concentration of the pesticide, leading to lower mineralization of the compound in wetlands. Accordingly, the mineralization of the pesticide should be tested in realistic conditions, which can be achieved in microcosm systems (4). Sorption and diffusion obscure the actual mineralization kinetics of a pesticide, consequently, kinetics of degradation (mineralization) can only be determined (3) from experiments in idealized conditions.

- (1) Pesticide mineralization assay** Mineralization of pesticides can be tested in the laboratory with  $^{14}\text{C}$ -labelled compounds. The compound of interest is added to a soil sample and the production of  $^{14}\text{CO}_2$  is monitored. The high-throughput setup, developed by Johnsen *et al.* (Johnsen *et al.*, 2009) proved very convenient (Figure 5, left). With this method,  $\text{CO}_2$  can be captured on  $\text{Ca}(\text{OH})_2$  crystals that are coated on a sealing film (Seal Plate-PCR-SP, Axygen Inc., Union City, CA, USA), which can be exchanged on a regular basis and wrapped in cling film. A storage phosphor screen (Agfa HealthCare, Belgium) should be exposed to the samples for 48 h and scanned with a Typhoon scanner (GE Healthcare). An example of the image that is produced is shown in Figure 5 (right). When a significant amount of  $^{14}\text{CO}_2$  is produced (10 – 60 % of the amount of  $^{14}\text{C}$ -pesticide that was initially added) within a reasonable timespan (few weeks to few months), the pesticide is said to be mineralized by the microorganisms that are present in the soil sample. This method was used in the AQUAREHAB project to study the mineralization kinetics of MCPA and IPU in sediment from a recently reconstructed wetland in Bernissem (Sint-Truiden, Belgium). We observed differences in mineralization potential within the wetland and also between the seasons over a period of two years. Data will be published by Vandermeeren *et al.*



**Figure 5: mineralization experiment in a deep-well microplate as described by (Johnsen *et al.*, 2009)(left) and example of an image after scanning (right)**

- (2) Pesticide mineralization assay in different redox conditions** The pesticide mineralization assay that was mentioned above can be performed in the absence of  $\text{O}_2$ , a condition that usually prevails in wetland sediments. A study by Holliger *et al.* (Holliger *et al.*, 1993) is an example of how  $\text{Mn}^{4+}$ ,  $\text{Fe}^{3+}$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  are used as electron acceptor in anaerobic conditions. Generally, anaerobic mineralization of pesticides is a much slower process (months to years) than aerobic mineralization.
- (3) Determination of degradation kinetics** Most models that describe the fate of a pesticide in the soil or on a catchment scale, use first order kinetics of pesticide degradation.

Fomsgaard reviewed models for the mineralization kinetics for low concentrations of pesticides in surface and subsurface soil (Fomsgaard, 1997). However, pesticide degrading biomass (PDB) may either grow when the pesticide is abundant – resulting in logistic mineralization curves – or decay when the pesticide is absent or when environmental conditions are not favorable (*e.g.* freezing in winter or dessication in summer). Models that take growth and decay of PDB into account may be more realistic (Cheyins *et al.*, 2010; Sniegowski *et al.*, 2009).

**(4) Microcosm wetland mineralization assay** During the AQUAREHAB project, a wetland was simulated on a small scale, in order to investigate the mineralization of MCPA and isoproturon in wetland conditions. The experiment was set up in Erlenmeyer flasks provided with a NaOH trap (to trap  $^{14}\text{CO}_2$  produced from  $^{14}\text{C}$ -MCPA and  $^{14}\text{C}$ -isoproturon (IPU) as shown in Figure 6. 50 g of soil were inundated by 50 ml of artificial surface water and the pesticide was added to the surface water layer. The produced  $^{14}\text{CO}_2$  was captured in the NaOH traps and sampled regularly. Incubation was done at 20 °C in the dark.

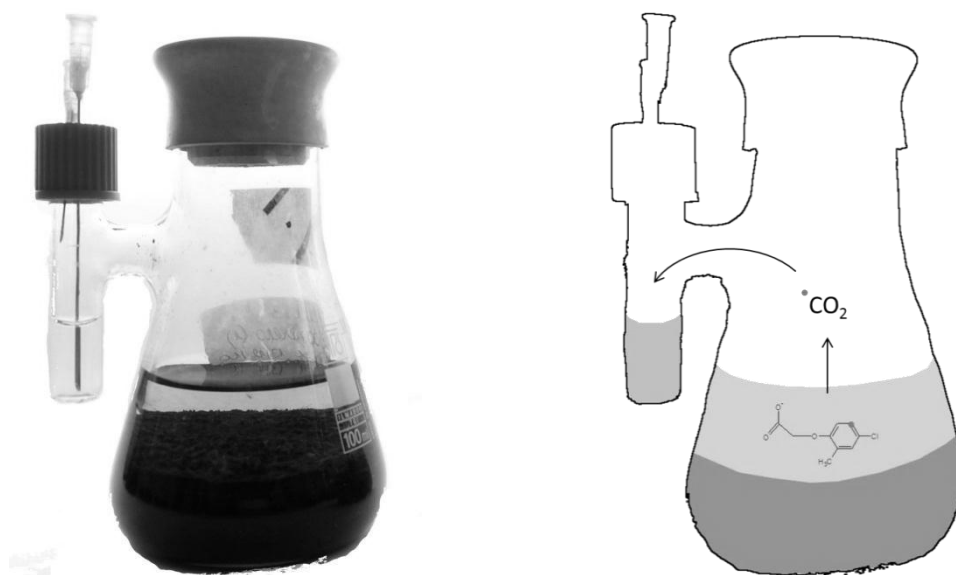


Figure 6: Microcosm wetland mineralization assay: experiment with sediment from Brynemade (Denmark)(left) and conceptual design (right)

## 5 APPROACHES TO DESIGN WETLAND BASED BUFFER ZONES FOR MITIGATING AGRICULTURAL POLLUTION FLOWING TOWARDS SURFACE WATERS

This part addresses measures that can be used to improve nitrate and pesticide removal activities in wetlands and as such improve their agricultural pollutant mitigation activities. As in section 4, it addresses nitrate removal in the subsurface compartment on the one hand and pesticide biodegradation in the surficial zone of a wetland on the other hand.

### 5.1 NITRATE REMOVAL IN THE SUBSURFACE COMPARTMENT

As explained in 2.5, the various parameters affecting for nitrate removal are (Jensen et al. ,2013b):

- Hydraulic residence time ( $\tau_G$ ), i.e., how quickly will nitrate move from the wetland margin to the river
- Denitrification rate. If this is assumed as a first-order process, then the reaction can be characterized with a reaction time scale, or half-life ( $\tau_R$ )
- Permeability contrasts between a surficial peat layer and the more permeable wetland aquifer
- Flooding frequency and length of flooding (hydroperiods)

A low hydraulic residence time and a high denitrification rate enhance nitrate removal. Likewise, a low-permeable peat layer will cause groundwater to by-pass this layer and upwelling is less likely. These characteristics are intrinsic properties of a wetland and not easy to manipulate or activate.

#### ***Hydraulic manipulations***

Flooding is used as a mechanism for storage of organic matter and nutrients from rivers on the flood plain (Poulsen et al., 2012). Re-meandering of former channelized rivers and narrowing the river width and depth can be used to increase and control flooding. The key point here is that these will also under given circumstances have a positive effect on nitrate removal in groundwater.

Jensen et al. (2013b) found that flooding enhances nitrate removal because of two mechanisms:

- (1) During flooding groundwater will discharge upward towards the flood plain and thus get into contact with the typically anoxic surficial organic layers (as shown in Figure 3). During this process part of the nitrate is quickly removed. The location of upward flow moves forth and back with the maximum flooding inundation. The amount of flow seeping through the peat layers was found to vary from 25-37% depending on the hydraulic conductivity of the peat, i.e., the higher the hydraulic conductivity, the greater the discharge and nitrate removal.
- (2) During flooding, a stagnant zone develops below the flooded part of the wetland. Any nitrate that moved into this zone before flooding is trapped and there is now more time for denitrification to complete, even in cases with low rates. In the most favourable cases of those studied by Jensen et al. (2013b) they find that nitrate removal increases by 112% compared to the same situation with no flooding. This system is characterized by a small contrast in permeability between the peat layer and the sand, a low denitrification rate in the sand and forcing the wetland to be flooded 75% of the year.

### ***Chemical manipulations***

From previous steps, bottlenecks for denitrification will be determined and several strategies for activation based on chemistry could be outlined, based on presence of organic matter and oxygen.

If the problem of denitrification is a lack of dissolved organic matter, organic matter (electron donor) can be added. This situation may occur when soils poor in organic matter (e.g. sandy soil) are present in upper or deep layers or with soils that release only recalcitrant DOC. Providing organic matter in a wetland can be done by promoting the formation of rich organic matter layers (e.g. peat) in the upper part of the aquifer that ensure a vegetal growing cycle or adding some sources of organic matter present in the fields. This last strategy has been proposed in several references (Gibert et al., 2008) in the form of horizontal Permeable Reactive Barrier (PRB). This organic matter rich top layer will leach DOC to deeper layers, providing the proper quantity and quality of DOC.

If the problem of denitrification is the excess of oxygen, the addition of sources of DOC in the upper layers could make a depletion of oxygen due to aerobic microorganism activity. Other ways to avoid the oxygen to enter in some aquifer points will include flooding (see hydraulic manipulations) or compaction. These strategies could be antagonistic with pesticides elimination, where aerobic environment is needed.

Finally, a possible combination of several previous strategies (hydraulic and chemical) could be needed to increase denitrification in an easier way. Part of these strategies could be tested before its implementation at lab or pilot scale in order to check synergistic or antagonistic processes. Small continuous box-type setups with the proposed approaches could be used at lab scale in order to demonstrate the feasibility of the approaches suggested. Indicators as the comparison of denitrification of batch of step 4 and denitrification of continuous setups, could be used to show the success of the activation mechanisms to implement.

### ***Modelling considerations***

In some cases a simple analytical solution can be used to approximate mass reduction given a few wetland characteristics. The analytical solution for the steady-state relative concentration ( $C_r$ ) of a plug flow reactor with a flow residence time of  $\tau_G$  and nitrate undergoing first-order decay with a rate of  $\lambda$  is;

$$C_r = \frac{C}{C_0} = e^{-\lambda\tau_G}$$

where  $C$  is nitrate concentration of water flowing out of the reactor (here groundwater discharge from the riparian zone to the river) and  $C_0$  is the concentration of water entering the reactor (here groundwater inflow to the riparian zone). A similar model was used by Tesoriero and Puckett (2011). Mass reduction is thus;

$$M = 1 - C_r$$

It is convenient to express mass reduction ( $M$ ) according to the Damkohler number ( $D$ ) defined as;

$$D = \frac{\tau_G}{\tau_R}$$

where  $\tau_R$  is reaction time expressed as;

$$\tau_R = \frac{\ln(2)}{\lambda}$$

Thus, D is given by the ratio of flow residence time and reaction time here characterized by the half-life of the reaction. When D is large (slow groundwater flow) mass reduction is high and vice versa. Using Darcy's law the Damkohler number may also be written as;

$$D = \frac{L/v}{\tau_R} = \frac{L \times n / (K \times i)}{\tau_R}$$

Thus, riparian zone characteristics such as; width (L), hydraulic head gradient (i), bulk hydraulic conductivity (K), and porosity (n) all are embedded in this non-dimensional number. The width may be reduced by the length of an aerobic zone at the wetland margin (where no nitrate removal will take place).

Despite its simplicity, Jensen et al. (2013b) demonstrates that this simple model expressing M as function of wetland characteristics gives a conservative estimate of mass reduction. Flooding, the presence of a higher-reactive peat layer, dispersion, etc. all will increase mass reduction.

## 5.2 PESTICIDE REMOVAL IN THE SURFICIAL COMPARTMENT

Measures to increase the pesticide degrading activity/performance of a wetland have been hardly studied.

**Bioaugmentation:** In case the pesticide degrading capacity for the pesticide that is targeted is not present, addition of specialised bacteria able to degrade this compound can be added. This process is called bioaugmentation. (for instance Cycon et al., 2009) and for water treatment systems for mitigating direct pesticide pollution (Sniegowski et al., 2011) but they do not look feasible taking into account the relatively large surface areas of wetlands. On the other hand, we do not know whether such introduced specific degraders easily spread and colonize a wetland ecosystem when for example introduced at a specific location, an approach that is feasible.

Experiments performed in wetland microcosms in the AQUAREHAB project have shown that specific degraders can colonize the sediment of wetlands and remain active for a long period (Vandermeeren et al., unpublished) while Runes et al. (2001) showed the successful introduction of atrazine degraders through addition of atrazine-primed soil in a constructed wetland. An interesting application of bioaugmentation though was recently reported by Takagi et al. (2011). These authors developed charcoal-pesticide degrader formulations for creating barriers with increased removal efficiency to prevent dissipation of the pesticides to non-target environmental compartments. A similar approach was studied in WP3 of AQUAREHAB but using a synthetic carrier.

**Biostimulation via nutrient addition.** In case a pesticide biodegrading potential is present at a site, it can be stimulated by providing nutrients (organic and/or inorganic), but this has not been studied as such in wetlands. The biodiversity of larger animals in wetlands as providers of organic nutrients might contribute as such to improved pesticide biodegradation but again this has not been studied.

**Phytoremediation.** Also the potential phytoremediation character due to the presence of macrophytes in wetlands should be considered as previously shown in soil in which microbial pesticide biodegradation is stimulated in the rhizosphere (Lin et al. 2011). Stearman et al. (2003) showed improved simazine and metalochlor degradation in constructed wetlands vegetated with bulrush compared to non-vegetated wetlands indicating that hypophytes can improve pesticide biodegradation in wetlands. Similarly, degradation rates of butachlor in rhizosphere soil from a riparian wetland planted with *Phragmites australis* and *Acorus calamus* were significantly higher than rates in non-rizosphere soil (Yang et al. 2011). The precise mechanism how macrophytes stimulate pesticide biodegradation in wetlands, however, was not studied. Possible reasons might be the local availability of oxygen at the roots in the sediment and/or increased microbial activity due to plant exudates.

**Hydraulic retention times.** Other measures to improve pesticide biodegradation in wetlands that can be considered are related to the scale of the wetland. Enlargement of the flow path or reduction of the water flow velocity do increase the residence time of the pesticide in the wetland system. Transport models that integrate a proper description of the wetland hydrology to accurately describe the transport of a pesticide through a wetland can help in that.



## 6 GENERIC APPROACH TO MONITOR EFFECTS OF WETLAND RESTORATION ON SURFACE WATER STATUS

### 6.1 INTRODUCTION

The construction of wetlands in the riparian zone is considered a promising approach to mitigate the diffuse pollution of agricultural pollutants. This part of the guideline is focused on the assessment of effects of wetland construction on the overall environmental status of the impacted stretch of the river. A specific attention is given to nutrients and pesticides.

### 6.2 EXPERIMENTAL APPROACH

Invariably, a comprehensive study must be carried out according to provisions of the EU Water Framework Directive (WFD, 2000/60/EC) on the changes in the **ecological status** of the investigated water body as a response to wetland construction in the riparian zone. The presence of the WFD priority substances (PS) listed in the Environmental Quality Standards Directive (EQS Directive, 2008/105/EC and its recent upgrade 2013/39/EU) has to be investigated as well in order to obtain an indication of the **chemical status** of the investigated water body. The worst of the two then determines the overall status of the impacted water body.

The ecological status of a water body should be based on a survey results of **five Biological Quality Elements** (BQEs; macrozoobenthos, phytobenthos, phytoplankton, macrophytes, fish), including their **supporting general physico-chemical (including nutrients) and hydromorphological parameters**.

A general **target** (mainly WFD PS) and **non-target screening** (suspect or previously unknown chemicals which may enter the environment) of chemical pollutants in the surface river water and sediment samples and their **prioritisation** should be performed to identify potential **river basin specific pollutants**. This relates to Annex V of the WFD, which states that EU Member States are obligated to include in the classification of overall ecological status “other pollutants” discharged in significant quantities into a water body (specific to an individual river basin). Once identified, these pollutants must be included in monitoring schemes and their environmental quality standards (EQS) should be derived. Here, the inclusion of additional, mainly organic, pollutants in the list is often hampered by the lack and quality of available data because in a typical monitoring effort the possible presence of thousands of other substances (including wide range of new types of pesticides and their degradation products) that could potentially enter the environment is overlooked.

A possible way out (not currently included in the legislation) might be **ecotoxicological screening** of the samples with a battery of bioassays to find out sites signalling pronounced toxic effects, fractionate the samples and find out with state-of-the-art analytical technologies which toxicants are responsible for the effects. This approach is often termed as **Effect Directed Analysis** (EDA, Brack et al. Environmental Sciences Europe 2012, 24:29 ).

## 6.3 DEFINITIONS

Ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems. Good ecological status is the status of a surface water body classified in accordance with Annex V of the WFD. Ecological status classification has some basic principles:

- Type-specific classification;
- Selected quality elements should reflect the anthropogenic stress/pressure;
- Classification by used quality elements should fulfil normative definitions;
- The assessment procedure is based on the comparison to reference conditions.

The target for monitoring and the basic unit for classification is the water body. A 'surface water body' is a discrete and significant element of surface water such as a lake, a reservoir, a stream, river or canal, part of a stream, river or canal, a transitional water or a stretch of coastal water.

Ecological classification consists of a number of quality elements:

- Biological quality elements;
- Physico-chemical quality elements;
- Hydromorphological quality elements;
- River basin specific pollutants (other pollutants or specific synthetic or non-synthetic pollutants).

### 6.3.1 Normative definitions for biological quality

WFD sets the normative definitions for individual biological quality elements (phytoplankton, phytobenthos and macrophytes, benthic invertebrates, fish), for each category (e.g. rivers, lakes) and for high, good and moderate status.

The normative definitions provide a basis for classifying surface waters according to their ecological status. Biological as well as supporting hydromorphological and physico-chemical elements are to be used in the assessment of ecological status. **Ecological status classification** should be made, based on relevant biological and physico-chemical monitoring results. The ecological status is represented by the lower of the value of the biological and physico-chemical monitoring results for the relevant quality element. Normative definitions should express the taxonomic composition and abundance; the disturbance ratio of sensitive taxa to insensitive taxa and the level of diversity. These are expressed using **metrics** and/or **indices**. The observed value of metric and/or index is divided by the reference value of the metric and/or index. Results of the assessment thus vary between 0 and 1, with high ecological status represented by values close to one and bad ecological status by values close to zero (cf. Figure 7).

The ecological quality ratio scale should be divided into five classes for each surface water category, ranging from high to bad ecological status, by assigning a numerical value to each of the boundaries between the classes. The value for the boundary between the classes of high and good status, and the value for the boundary between good and moderate status should be established through an intercalibration exercise carried out at the national level in each Member State.

Generally for **high status** - There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water

body type from those normally associated with that type under undisturbed conditions. The values of the BQEs for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion. These are the type specific conditions and communities.

Generally for **good status** - The values of the BQEs for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.

Generally for **moderate status** - The values of the BQEs for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.

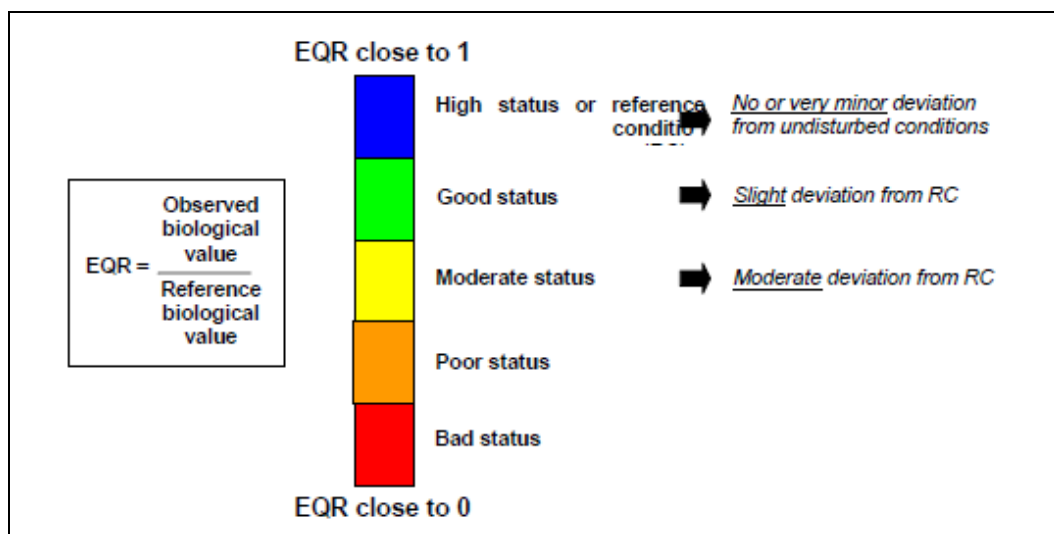


Figure 7: Basic principles for classification of ecological status based on Ecological Quality Ratio (EQR).

It should be stressed here that limit values for nutrients may vary for different types of water bodies, i.e. as an example, if the nitrate concentration puts the water body into worse pollution class category than the results of the worst performing BQE then its limit value must be adjusted.

### 6.3.2 River basin specific (other) pollutants

For specific synthetic and non-synthetic (river basin specific) pollutants national environmental quality standards have to be developed. Pollutants that are toxic, persistent and likely to bioaccumulate, are relevant for each individual country. An Environmental Quality Standard (EQS) indicates the concentration of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment. For specific non-synthetic pollutants (heavy metals) natural background concentration values should be identified for individual water bodies.

### 6.3.3 Chemical status assessment

According to the WFD, 'good' surface water chemical status means the chemical status required to meet the environmental objectives for surface waters established in Article 4(1)(a), that is the

chemical status achieved by a surface water body in which concentrations of pollutants do not exceed the EQSs established in Annex IX and under Article 16(7), and under other relevant Community legislation setting environmental quality standards at Community level. The EQS Directive aims to ensure a high level of protection against risks to or via the aquatic environment stemming from the 45 priority substances and certain other pollutants, by setting EQS.

As regards presentation of monitoring results and classification of chemical status, WFD states that where a body of water achieves compliance with all EQS established in Annex IX, Article 16 and under other relevant Community legislation setting EQS, it shall be recorded as achieving 'good' chemical status. If not, the body shall be recorded as failing to achieve good chemical status. Member states must provide a map for each river basin district illustrating chemical status for each body of water, colour-coded (in accordance with the second column of the Table 5) to reflect the chemical status classification of the body of water.

**Table 5: Colour codes for chemical status classification**

Chemical status classifications	Colour code
Good	Blue
Failing to achieve good	Red

## 6.4 DATA INTERPRETATION

### 6.4.1 Indication of the ecological status of the investigated technology sites

For the indication of the ecological status, various classification schemes should be selected for individual BQEs taking into consideration local conditions and legislation already in place. For more details on the individual assessment schemes, see the AQUAREHAB Deliverable D1.1. The overall ecological status has to be determined by the lowest score for any of the BQEs.

It is recommended to use at least five physico-chemical quality elements for the assessment: pH, oxygen, conductivity, total nitrogen and total phosphorus. The parameters should be well within the range ensuring good functioning of ecosystem (national/WFD implied limit values) and adjusted appropriately based on the results of biological monitoring.

The list of river basin specific pollutants contributing to the overall ecological status evaluation and their national EQS values should be available at the national level for each river basin. If not, an attempt should be made to suggest a list of candidate compounds to be considered for future monitoring and their limit values (Predicted No-effect Concentrations (PNEC) as a basis for derivation of legally binding EQS values). Here, an advanced approach for derivation of lowest PNEC values and prioritisation developed within the NORMAN network is recommended (NORMAN Prioritisation framework for emerging substances, April 2013, <http://www.norman-network.net/?q=node/126>).

### 6.4.2 Indication of the chemical status of the investigated technology sites

According to Annex 4 of the WFD, surveillance monitoring of priority substances required for determination of legally requested chemical status assessment must be done on a monthly basis for the period of one year. The EQS Directive defines the application of EQS in the following way:

- “For any given surface water body, applying the EQS-AA (“EQS-annual average”) means that, for each representative monitoring point within the water body, the arithmetic mean of the concentrations measured at different times during the year does not exceed the standard”;
- “For any given surface water body, applying the EQS-MAC (“EQS-maximum allowable concentration”) means that the measured concentration at any representative monitoring point within the water body does not exceed the standard”.

When the assessment of the chemical status cannot be done in-line with the WFD requirements, which is a usual case at wetland restoration projects, the following procedure is suggested to be applied:

- An indication of the chemical status to be given for the sampling site under investigation (not a water body);
- For compliance checking, the proposed EQS-AA for inland waters should be used, as the concentration value of a substance recorded during the surveys can be considered as an approximate representative of commonly occurring values rather than the maximum value of the year. This assumption is naturally not valid for seasonally affected occurrence of substances such as pesticides and their degradation products.

Determination of the chemical status is necessary to understand the impacts which may cause degradation of the ecological status. The results obtained within the ‘technology checking’ sampling campaigns should provide a comprehensive snapshot on the pollution by WFD PS of the investigated water body and provide an indication of the chemical status at each sampling site.

### 6.4.3 Overall classification

Classification of the ecological and chemical status is made, based on the following scheme (Figure 8).

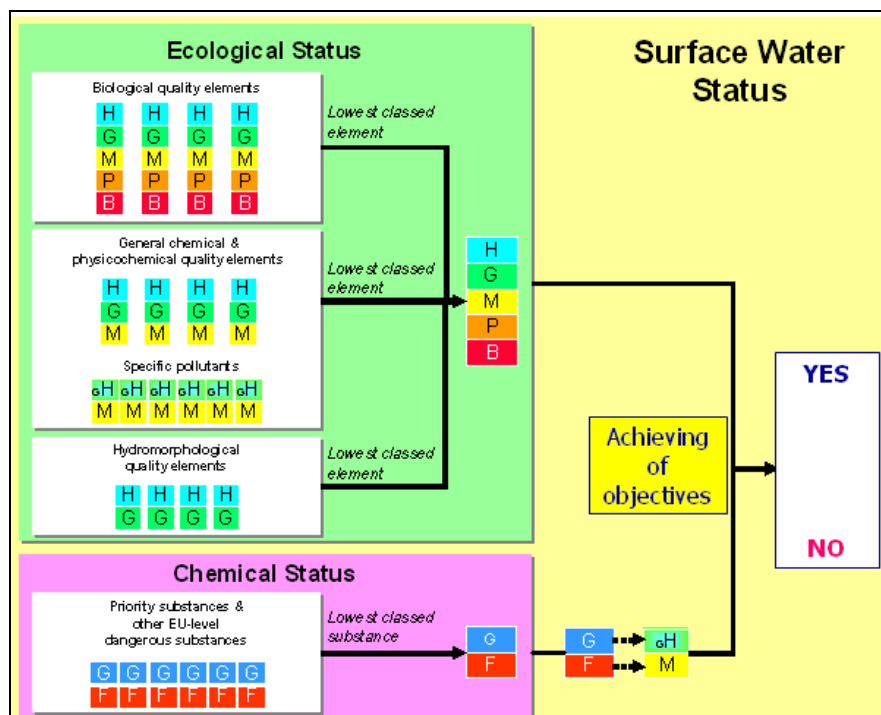


Figure 8: Basic scheme of ecological and chemical status assessment including quality elements. Ecological status is classified according to 5 categories: H – High; G – Good; M – moderate; P – Poor and B – Bad.

#### 6.4.4 Frequency of monitoring

For operational monitoring, which relates i.a. to monitoring of performance of environmental technologies, the frequency of monitoring required for any parameter must be determined by country so as to provide sufficient data for a reliable assessment of the status of the relevant quality element. As a guideline, monitoring should take place at intervals shown in the Table 6 unless greater intervals would be justified on the basis of technical knowledge and expert judgement.

Table 6: Frequencies of the monitoring

Quality element	Rivers	Lakes
<b>Biological</b>		
Phytoplankton	6 month	6 month
Other aquatic flora	3 years	3 years
Macro invertebrates	3 years	3 years
Fish	3 years	3 years
<b>Hydromorphological</b>		
Continuity	6 years	
Hydrology	continuous	
Morphology	6 years	6 years
<b>Physico-chemical</b>		
Thermal conditions	3 months	3 months

Quality element	Rivers	Lakes
Oxygenation	3 months	3 months
Salinity	3 months	3 months
Nutrient status	3 months	3 months
Acidification status	3 months	3 months
Other pollutants	3 months	3 months
Priority pollutants	1 month	1 month

In the ideal case, the requirements of the WFD should be followed as closely as possible. This would require the monitoring of WFD PS at least 12 times per year (once a month) and monitoring most of the BQEs and river basin specific pollutants four times per year. Lesser frequencies might be acceptable for evaluation of performance of wetland restoration involving field surveys twice a year but for a longer period of time (3 – 4 years) to observe the trends in changing environmental status after installing the technology. The surveys of BQEs should preferably be performed in different periods of year including spring and autumn. The ecological and chemical status of a water body determined in a way not fully compliant with the legislation should be termed as 'indicative'.

In the final effort only a limited set of relevant BQEs, supporting general physico-chemical parameters and chemical pollutants should be monitored to estimate the performance of the technology, complying with the definition of so-called 'operational monitoring' according to the WFD.

#### **Important note:**

At the evaluation of the performance of a technology, for each site and each year an indication of ecological and chemical status should be determined, following the WFD procedures as closely as possible, however, not necessarily assessing the entire water body or river (basin). The terms ecological status and chemical status refer to the *indication* of ecological/chemical status per sampling site and per year. The chosen sites might not be necessarily representative of the river under investigation, and therefore no general conclusions about the ecological/chemical status of the water body or river (basin) as a whole could be drawn on the basis of the particular assessment.

## **6.5 CONCLUSION**

The guidelines for monitoring of activated riparian zones provide an overview of the related relevant EU legislation and a set of practical recommendations how to carry out such monitoring in situations when the full compliance with the legal requirements is too costly or not possible.

The focus is on the assessment of processes of nitrate and pesticide removal in field wetland systems. Nitrates, belonging to the group of general physico-chemical quality elements and chemical pollutants (including pesticides) not regulated at the EU level contribute to the assessment of the *ecological status*, which must be determined for each water body in every river basin in the EU. Similarly, occurrence of chemical pollutants (including pesticides) regulated at the

EU level (WFD priority substances; Annex I of the EQS Directive) is used for assessment of the *chemical status* of a water body impacted by the construction of the technology.

The proposed methodology has been successfully tested within the AQUAREHAB project (cf. Deliverable D1.1) and the recorded trends of biology quality elements clearly indicate that the life (water fauna and flora) is spreading quickly in the newly formed river bed after construction of a wetland and the ecological status improves. A pollution by metals and various non-regulated chemical pollutants from diffuse pollution sources remains a problem to be addressed.

## 7 GENERAL CONCLUSIONS

This guideline instructs or advices on several aspects regarding the use/feasibility/improvement and monitoring of wetlands for their use as a buffer strip/riparian zone for mitigating diffuse agricultural pollution of agricultural pollutants, i.e., nitrate and pesticides, of surface water bodies. For nitrate, pollution through subsurface migration of nitrates is considered and its mitigation by subsurface denitrification activities and methods were discussed to determine denitrification rates and to predict nitrate removal/transport in the subsurface and eventual improvements. For pesticides, pollution through run-off and drainage and methods to assess pesticide removal capacities in the surficial compartment of the wetland is considered. Monitoring focused on methodologies for assessing and evaluating the ecological and chemical status of the receiving surface waters.



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## 9 REFERENCES

- ANDERSON, K.L., WHEELEN, K.A., ROBINSON, J.B., TUOVINEN O.H. 2002. Atrazine mineralization potential in two wetlands. *Water Research*, 36: 4785–4794
- ASMUSSEN, L.E., SHERIDAN, J.M., BOORAM, C.V. JR. 1979. Nutrient movement in stream flow from agricultural watersheds in the Georgia Coastal Plain. *Trans. ASAE* 22:809–815, 821.
- BOIS, P., HUGUENOT, D., JÉZÉQUEL, K., LOLLIER, M., CORNU, J.Y., LEBEAU, T. 2013. Herbicide mitigation in microcosms simulating stormwater basins subject to polluted water inputs. *Water Research*, 47: 1123–1135.
- BRANDER, L.M., BROUWER, R., AND WAGTENDONK, A. (2013). Economic valuation of regulating services provided by wetlands in agricultural landscapes: a meta-analysis. *Ecological Engineering*. 56: 89– 96.
- BUSS, S.R., RIVETT, M.O., MORGAN, P., BEMMENT, C.D., Attenuation of nitrate in the sub-surface environment, U.K. Environment Agency, Report SC030155/SR2, 2005.
- CALDERER, M., *Study of Denitrification and Reductive Dechlorination Processes Applied to Groundwater Bioremediation*. Doctoral Thesis. UPC and CTM. Manresa, 2010.
- CHEN D.J.Z and MACQUARRIE, K.T.B., Numerical simulation of organic carbon, nitrate and nitrogen isotope behaviour during the denitrification in a riparian zone, *Journal of Hydrology* 293 2004, 235-254.

- CHEYNS, K., MERTENS, J., DIELS, J., SMOLDERS, E., SPRINGAEL, D. (2010). Monod kinetics rather than a first-order degradation model explains atrazine fate in soil mini-columns: Implications for pesticide fate modelling. *Environmental Pollution*, 158 (5), 1405-1411.
- COOPER, A.B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. *Hydrobiologia* 202:13–26.
- CYCON, M., WOJCIK, M. PIOTROWSKA-SEGET Z. (2009). Biodegradation of the organophosphorus insecticide diazinon by *Serratia* sp and *Pseudomonas* sp and their use in bioremediation of contaminated soil. *Chemosphere* 76, 494-501.
- DODLA, S.K., WANG, J.J., DELAUNE, R.D. and COOK, R.L. Denitrification Potential and its Relation to Organic Carbon Quality in Three Coastal Wetland Soils. *Science of the Total Environment*, 12/15, 2008, vol. 407, no. 1. pp. 471-480.
- DON RH & PEMBERTON JM (1981) Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. *J. Bacteriol.* 145: 681-686.
- FISHER, J., ACREMAN, M.C. 2004. Wetland nutrient removal: a review of evidence. *Hydrology and Earth System Sciences*. 8: 673-685
- GIBERT, O., POMIERNY, S., ROWE, I. and KALIN, R.M. Selection of Organic Substrates as Potential Reactive Materials for use in a Denitrification Permeable Reactive Barrier (PRB). *Bioresource Technology*, 11, 2008, vol. 99, no. 16. pp. 7587-7596
- HAYAKAWA, M., HATAKEYAMA, R. ASANO, Y. ISHIKAWA AND S. HIDAKA. 2013. Nitrate reduction coupled with pyrite oxidation in the surface sediments of a sulfide-rich ecosystem. *Journal of Geophysical Research: Biogeosciences*. 118: 639–649.
- HAYCOCK, N.E. and MUSCUTT, A.D. Landscape Management Strategies for the Control of Diffuse Pollution. *Landscape and Urban Planning*, 2, 1995, vol. 31, no. 1–3. pp. 313-321.
- HERNÁNDEZ M. E., MITSCH, W. J., Denitrification Potential and Organic Matter as affected by Vegetation Community, wetland age and plant introduction in created wetlands, *J: Environ. Qual.*, 2007, Vol. 36, p. 333-342.
- HILL, A.R., 1996. Nitrate removal in stream riparian zones. *J. Environ. Qual.* 25, 743–755.
- Hoffmann, C.C. and A. Baatrup-pedersen. (2007). Re-establishing freshwater wetlands in Denmark. *Ecol.Engineer.* 30: 157-166.
- JENSEN, J. K., P. ENGESGAARD, AND B. NILSSON (2013a), Hydrology of seasonal flooded riparian zones, *submitted to Water Resources Research*, December 2013.
- JENSEN, J. K., P. ENGESGAARD, AND B. NILSSON (2013b), Simulation of flow and nitrate transport in seasonal flooded riparian zones, *submitted to Advances in Water Resources*, December 2013.
- JOHNSEN A.R., HYBHOLT T.K., JACOBSEN O.S., AAMAND J. (2009) A radiorespirometric method for measuring mineralization of [14C]-compounds in a 96-well microplate format. *J. Microbiol. Meth.* 79:114-116.
- JØRGENSEN, P.R., URUP, J., HELSTRUP, T., JENSEN, M.B., EILAND, F., VINTHER, F.P. "Transport and reduction of nitrate in clayey till underneath forest and arable land." *Journal of Contaminant Hydrology* ,2004,. 73(14): 207-226.
- KARPUZCU, M.E., SEDLAK, D.L., STRINGFELLOW, W.T. (2013) Biotransformation of chlorpyrifos in riparian wetlands in agricultural watersheds: Implications for wetland management. *J. Hazard Materials* 244: 111-120.
- KELLOGG, D.Q., GOLD, A.J., GROFFMAN, P.M., ADDY, K., STOLT, M.H., BLAZEJEWSKI. G. 2005. In situ ground water denitrification in stratified, permeable soils underlying riparian wetlands. *J. Environ. Qual.* 34:524-533.
- KINZELBACH, W., SCHÄFER, W., HERZER, J., *Numerical Modeling of Natural and Enhanced*

- Denitrification Processes in Aquifers*, Water Resources Research, 27, 1991, 1123-1135,
- LARSEN, L., JØRGENSEN, C., AAMAND, J. 2001. Potential mineralization of four herbicides in a ground water--fed wetland area. *Journal of Environmental Quality* 30:24-30.
- LEVERENZ, H. L., HAUNSCHILD, K., HOPES, G., Tchobanoglous, G., Darby, J. L., Anoxic treatment wetlands for denitrification, *Ecological Engineering* 36, 2010, 1544-1551.
- LIN, C-H, LERCH, RN, KREMER, RJ, GARRETT, HE. 2011. Stimulated rhizodegradation of atrazine by selected plant species. *J Environ Qual* 40, 1113-1121.
- MACQUARRIE, K., T., B., SUDICKY, E., A., *Multicomponent Simulation of Wastewater-Derived Nitrogen and Carbon in Shallow Unconfined Aquifers I. Model Formulation and Performance*, *Journal of Hydrology*, 2001, 47, 125-143
- MAÎTRE, M., COSANDEY, A.C., PARRIAUX, A., GUENAT. C. 2005. A Methodology to Estimate the Denitrifying Capacity of a Riparian Wetland. *J. Environ. Qual.* 34: 707-716
- MANDELBAUM, R.T., ALLAN, D.L., AND WACKETT, L.P. (1996). Isolation and characterization of a *Pseudomonas* sp. that mineralizes the s-triazine herbicide atrazine. *Appl. Environ. Microbiol.* 1995, 61(4):1451
- MAYER, P.M.; S.K. REYNOLDS; M.D. MCCUTCHEN; and T.J. CANFIELD. 2007. Meta-analysis of nitrogen removal in riparian buffers. *Journal of environmental quality*, vol. 36, no. 4. pp 1172-1180.
- MAYER, P.M., REYNOLDS, S.K. JR., MCCUTCHEN, M.D., CANFIELD, T.J. 2007. Meta-analysis of nitrogen removal in riparian buffers. *J. Environ. Qual.* 36: 1172–1180.
- MAYER, P.M.; S.K. REYNOLDS; M.D. MCCUTCHEN; and T.J. CANFIELD. 2006. Riparian Buffer Width, Vegetative Cover, and Nitrogen Removal Effectiveness: A Review of Current Science and Regulations. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-05/118, 2006.
- MISITI, T.M., HAJAYA, M. G., PABLOSTATHIS, 2011. Nitrate reduction in a simulated free-water surface wetland system, *Water research* 45, 5587-5598.
- MONOD, J., *The Growth of Bacterial Cultures*, annual review of microbiology, 3, pg. 371-394, 1949
- MÜLLER, T. A., BYRDE, S. M., WERLEN, C., MEER, R. VAN DER, KOHLER, H. E., MU, T. A., & MEER, J. R. VAN DER. (2004). Genetic Analysis of Phenoxyalkanoic Acid Degradation in *Sphingomonas herbicidovorans* MH. *Applied & Environmental Microbiology*, 70(10), 6066–75.
- MUÑOZ-LEOZ, B., GARBISU, C., ANTIGÜEDAD, I., ALONSO, M.L., ALONSO, R.M., RUIZ-ROMERA, E., 2009. Deltamethrin Degradation and Soil Microbial Activity in a Riparian Wetland Soil. *Soil Sci.* 174, 220–228.
- NAGATA, Y., ENDO, R., ITO, M., OHTSUBO, Y., TSUDA, M. 2007. Aerobic degradation of lindane (γ-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. *Applied Microbiology and Biotechnology*. 76:741-752.
- OANH NGUYEN, T.P., HELBLING, D.E., BERS, K., FIDA, T.T., WATTIEZ, R., KOHLER, H.P.E., SPRINGAEL, D., DE MOT, R. (2014). Genetic and metabolic analysis of THE carbofuran catabolic pathway in *Novosphingobium* sp. KN65.2. *Applied Microbiology and Biotechnology*, accepted.
- POULSEN, J.B., HANSEN, F., OVESEN, N.B., LARSEN, S.E., KRONVANG, B., 2013. Linking floodplain hydraulics and sedimentation patterns along a restored river channel: River Odense, Denmark. *Ecol. Eng.*
- PUCKETT, L.J., COWDERY, T.K., MCMAHON, P.B., TORNES, L.H., STONER, J.D., 2002. Using chemical, hydrologic, and age dating analysis to delineate redox processes and flow paths in the riparian zone of a glacial outwash aquifer-stream system. *Water Resour. Res.* 38.
- RITTMANN B.E. and MCCARTY P.L. (2001). *Environmental Biotechnology: Principles and Applications*. New York, McGraw-Hill.

- RUNES, H., JENKINS, J., BOTTOMLEY, P., 2001. Atrazine degradation by bioaugmented sediment from constructed wetland. *Applied Microbiology and Biotechnology*. Vol. 57, 427-432
- SAEED, T. and GUANGZHI, S., Kinetic modelling of nitrogen and organics removal in vertical and horizontal flow wetlands, *Water Research*, 45 (2011), vol 45 , 3137-3152.
- SCHÄFER, D., SCHÄFER, W., KINZELBACH, W., *Simulation of Reactive Processes Related to Biodegradation in Aquifers-2. Model Application to a Column Study on Organic Carbon Degradation*, *Journal of Contaminant Hydrology*, 1998, 31, 187-209.
- SIRIVEDHIN, T. and GRAY, K.A.. Factors affecting denitrification rates in experimental wetlands: Field and laboratory studies. *Ecological Engineering*, 26, 2006: 167–181.
- SNIEGOWSKI, K., MERTENS, J., DIELS, J., SMOLDERS, E., SPRINGAEL, D. (2009). Inverse modeling of pesticide degradation and pesticide-degrading population size dynamics in a bioremediation system: Parameterizing the Monod model. *Chemosphere*, 75 (6), 726-731.
- SNIEGOWSKI, K., BERS, K., VAN GOETEM, K., RYCKEBOER, J., JAEKEN, P., SPANOGHE, P., SPRINGAEL, D. (2011). Improvement of pesticide mineralization in on-farm biopurification systems by bioaugmentation with pesticide primed soil. *FEMS Microbiology Ecology* 76(1), 64-73.
- SØRENSEN SR, HOLTZE MS, SIMONSEN A, AAMAND J (2007) Degradation and mineralization of nanomolar concentrations of the herbicide dichlobenil and Its persistent metabolite 2,6-dichlorobenzamide by *Aminobacter* spp. isolated from dichlobenil-treated soils. *Appl Environ Microbiol* **73**: 399-406.
- SØRENSEN SR, RONEN Z, AAMAND J (2002) Growth in coculture stimulates metabolism of the phenylurea herbicide isoproturon by *Sphingomonas* sp. strain SRS2. *Appl Environ Microbiol* **68**: 3478-3485. doi: 10.1128/aem.68.7.3478-3485.2002
- SØRENSEN SR, SIMONSEN A, AAMAND J (2009) Constitutive mineralization of low concentrations of the herbicide linuron by a *Variovorax* sp. strain. *FEMS Microbiol Lett* **292**: 291-296.
- STAERMAN GK, GEORGE DB, CARLSON K, LANSFORD S (2003) Pesticide removal from container nursery runoff in constructed wetland cells. *J. Environ Quality*. 32:1548-1556.
- TAKAGI K, KATAOKA R, YAMAZAKI K (2011) Recent technology on bioremediation of POPs and persistent pesticides. *Jarq-Japan Agricultural Research Quarterly* 45: 129-136.
- TESORIERO, A. J., AND L. J. PUCKETT (2011), O<sub>2</sub> reduction and denitrification rates in shallow aquifers, *Water Resources Research*, 47(12), n/a–n/a, doi:10.1029/2011WR010471.
- VERHOEVEN, J.T.A., B. ARHEIMER, C. YIN, AND M.M. HEFTING. 2006. Regional and global concerns over wetlands and water quality. *Trends Ecol. Evol.* 21:96–103.
- WEAVER, M.A., ZABLOTOWICZ, R.M., LOCKE, M.A., 2004. Laboratory assessment of atrazine and fluometuron degradation in soils from a constructed wetland. *Chemosphere* 57, 853–62.
- YANG C, WANG M, CHEN H & LI J (2011) Responses of butachlor degradation and microbial properties in a riparian soil to the cultivation of three different plants. *Journal of Environmental Sciences-China* 23: 1437-1444.
- ZWIETERING, M.H., JONGENBURGER, I., ROMBOUTS, F.M., VAN 'T RIET, K., 1990. Modeling of the Bacterial Growth Curve. *Appl. Envir. Microbiol.* 56, 1875–1881.