SPSD II

CHANGES IN METAL BIOGEOCHEMISTRY RESULTING FROM WETLAND CREATION: BIOAVAILABILITY, TOXICITY AND RISK "WETMAT"

F. TACK, C. JANSSEN, P. MEIRE

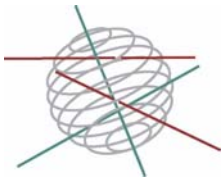
PART 2

GLOBAL CHANGE, ECOSYSTEMS AND BIODIVERSITY

- ATMOSPHERE AND CLIMATE
- MARINE ECOSYSTEMS AND BIODIVERSITY
- **TERRESTRIAL ECOSYSTEMS AND BIODIVERSITY**
- NORTH SEA
- ANTARCTICA
- **BIODIVERSITY**

.be

SCIENTIFIC SUPPORT PLAN FOR A SUSTAINABLE DEVELOPMENT POLICY
(SPSD II)



Part 2:
Global change, Ecosystems and Biodiversity

FINAL REPORT

CHANGES IN METAL BIOGEOCHEMISTRY RESULTING FROM WETLAND CREATION: BIOAVAILABILITY, TOXICITY AND RISK "WETMAT"

EV/32

Prof. dr. ir. F. Tack
GHENT UNIVERSITY
LABORATORY OF ANALYTICAL CHEMISTRY AND APPLIED ECOCHEMISTRY

Prof. dr. C. Janssen
GHENT UNIVERSITY
LABORATORY OF ENVIRONMENTAL TOXICOLOGY AND AQUATIC ECOLOGY

Prof. dr. P. Meire
UNIVERSITY OF ANTWERP
ECOSYSTEM MANAGEMENT RESEARCH GROUP

March 2006

BELGIAN SCIENCE POLICY

ONDERZOEKSGROEP MILIEUTOXICOLOGIE

Ecochem UGent

ECOSYSTEM MANAGEMENT RESEARCH GROUP

D/2006/1191/38
Published in 2006 by the Belgian Science Policy
Rue de la Science 8
Wetenschapsstraat 8
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be

Contact person:
Mrs Maaike Van Cauwenberghe
Secretariat: +32 (0)2 238 36 13

Neither the Belgian Science Policy nor any person acting on behalf of the Belgian Science Policy is responsible for the use which might be made of the following information. The authors are responsible for the content.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without indicating the reference.

Changes in metal biogeochemistry resulting from wetland creation: bioavailability, toxicity and risk

Authors:

Dieter Vanthuyne
Ghent University (UGent)
Laboratory for analytical chemistry and applied ecochemistry
Coupure Links 653
B-9000 Gent
Tel: +32 (0)9 264 60 94
Fax: +32 (0)9 264 62 32

Marjan Speelmans
Ghent University (UGent)
Laboratory of environmental toxicology and aquatic ecology
Jozef Plateaustraat 22
B-9000 Gent
Tel: +32 (0)9 264 89 19
Fax: +32 (0)9 264 37 66

Sander Jacobs and Sabine Van Regenmortel
University of Antwerp (UA)
Ecosystem management research group
Universiteitsplein 1C
B-2610 Wilrijk
Tel: +32 (0)3 820 22 74
Fax: +32 (0)3 820 22 71

Table of contents

1 Abstract 7
2 Introduction 9
3 Lab scale experimental setup 11
3.1 Materials and methods 11
3.1.1 Experimental setup 11
3.1.2 Sampling 13
3.1.3 Measurements and analysis 13
3.1.3.1 Soilcharacteristics 13
3.1.3.2 Temperature and ORP 14
3.1.3.3 Pore water characteristics 14
3.1.3.4 Reed plants 15
3.1.3.5 Invertebrates 16
3.1.4 Statistics 16
3.2 Results 17
3.2.1 Soil characteristics 17
3.2.2 Temperature and ORP 21
3.2.3 Pore water characteristics 23
3.2.4 Reed plants 33
3.2.5 Invertebrates 36
3.3 Discussion 43
3.4 Conclusions and recommendations 51
4 Field scale experimental setup 53
4.1 Materials and methods 53
4.1.1 Experimental setup 53
4.1.2 Sampling 54
4.1.3 Analysis 55
4.1.3.1 Soil characteristics 55
4.1.3.2 Pore water characteristics 55
4.1.3.3 Reed 55
4.1.3.4 Invertebrates 56
4.1.3.5 Suspended solids 56
4.1.3.6 Tidal characteristics 56
4.1.4 Statistics 56
4.2 Results 56
4.2.1 Soil characteristics 56
4.2.2 Pore water characteristics 60
4.2.3 Reed 66

4.2.4 Invertebrates 67
4.2.5 Sedimentation 70
4.2.6 Tidal characteristics 70
4.3 Discussion 71
4.4 Conclusions and recommendations 73
5 General conclusions and recommendations 75
6 Acknowledgements 77
7 References 79

1 ABSTRACT

In the vicinity of rivers, flooding of available lowlands can be considered as a possibility for temporal storage of river water during high water regimes to minimize the flooding danger of inhabited areas. The creation of wetlands for controlled flooding may also contribute to an increased ecological and biological value of the ecosystem area. Due to industrial activities, river water, sediments and soils are often contaminated with metals. The aim of this study was to determine the changes in the metal biogeochemistry under different hydrological conditions and in the presence of reed plants and how this affects the uptake of metals by plants and invertebrates. Two experimental setups were used. Lab scale experiments consisted of 42 barrels filled up with three different soils and flooded with water of different salinities. For the field scale experiment, 4 tanks filled with two soil types were placed on a platform in the river Scheldt and flooded daily by river water. Results showed that the metal mobility did not depend on the total metal content. Other factors such as soil pH, CEC, redox status and carbonate, OM and clay contents however play an important role in mediating metal behaviour. To prevent the mobilization of the metals, it is advisable to reduce redox variations in the soil. The two-year period of the experiment was too short to detect any measurable changes in most of the soil properties. The measured metal (im)mobilization kinetics can however already be incorporated in models in the near future. These models should allow to predict metal mobility changes at the oxic-anoxic interface upon changing hydrological conditions, where the already existing geochemical models fail due to the fact that they are based on steady state conditions.

In the two-year experimental period, large quantities of metal contaminated sediment were found to be deposited on the soils of the tanks which were placed in the river Scheldt. This sedimentation could pose a problem, as it minimizes the water storage capacity of a flood control area. Moreover, the contamination status of alluvial soils should be used as a criterion when selecting new flood control areas as uncontaminated flood control areas are expected to be contaminated when polluted sediments originating from the surrounding surface waters are being deposited or when the soil is contacted with metal-polluted surface water. The latter can be expected in the future, as upon increasing oxygen contents of the river waters due to decreasing organic matter loads, metals can be mobilised from metal-polluted river sediments. Reduction of metal mobility upon increasing oxygen contents of river waters therefore needs further investigation.

The metal accumulation in benthic organisms like *T. tubifex* was found to strongly depend on the inundation period. A higher Zn and Cu accumulation was recorded when substrates were allowed to oxidise. For the talitrid amphipod *O. gammarellus,* no clear influence was noted. Photosynthetic performance of the reed plants seemed

to be mainly determined by other factors than merely metal contamination levels. There was however a possible relation between the abundance of stress enzymes and the metal contamination, making it a more valuable parameter to assess the effect of metals on reed plants in wetlands. The reed itself did not have any measurable effect on the concentration of the metals in the soil or pore water. Also no differences in the concentrations of metals in the reed biomass or differences in the reed growth were observed between the contaminated and uncontaminated soil.

Key words: metal mobility, inundation period, flood control area, Phragmites australis, invertebrates

2 INTRODUCTION

The creation of wetlands in the densely populated Belgium is an ideal manner to minimize the flooding danger of inhabited areas during high discharge events, as reserves for water storage are created. Wetlands have also many other useful purposes. They are an ideal habitat for ecologically rare species (Tockner and Stanford, 2002) and they can be used for small scale waste water treatment (Werker et al., 2002). The construction of wetlands therefore has not only social and economical benefits but also an ecological relevance. In Belgium several former and current wetlands are polluted due to historical flooding and overbank sedimentation or disposal of dredged sediments (Vandecasteele et al., 2004a). These sediments contain a wide range of contaminants, including metals. Due to the high retention time of metals in most soils (Andreu and Gimeno-García, 1999), most wetlands currently still deal with a significant degree of metal contamination. This constitutes a large problem when managing, restoring or creating wetlands. Several parameters affect the mobility and bioavailability of metals. Thus a clear view on the possible consequences of the creation of a wetland in a contaminated environment is required to prevent the mobilization of metals, a higher bioavailability (Vandecasteele et al., 2005) and an increased transfer of metals to invertebrates and to the food web. When creating wetlands, large changes can occur in the hydrological regime and salinity of the designated area. Hydrological regime, average water storage period and salinity of the flooding water might be key issues in the development of a specific ecosystem and wetland management. The hydrological regime affects the pH, redox potential and indirectly also the organic matter and $CaCO_3$ content of the soil (van den Berg and Loch, 2000). It is not easy to control all the parameters that influence the metal mobility in wetlands, but the hydrological regime is a parameter that can be controlled fairly easily. It also influences the plant and animal species present in the wetland habitat. The aim of this study is to determine the changes in the metal biogeochemistry when creating wetlands and how this affects the uptake of metals by plants and invertebrates.

3 LAB SCALE EXPERIMENTAL SETUP

3.1 Materials and methods

3.1.1 Experimental setup

Barrels of 80 cm in height and a volume of 120 litres were equipped with drainage outlets and laid in with geotextile to prevent soil export. The barrels were filled with three different soil types. The first soil (soil L), was a metal contaminated dredged sediment originating from dredging activities in the harbour of Brussels (Belgium). This soil was selected for the presence of the metals combined with the absence of other contaminants. Before the experiment, the sediment was dewatered for a few months and a part of the sediment was then transported in a truck to the experiment site. A soil with a similar texture and metal contamination, but originating from a more oxic environment was selected as the second soil (Soil C). This soil originated from 'Het Lippensbroek', a site near the Scheldt in Hamme (Belgium). The third soil was a non contaminated soil (Soil N), delivered by a stone bakery in Boom (Belgium). This soil had a similar texture as the other two soils.

The barrels, filled with the soils, were put into larger barrels equipped with a faucet at the bottom to allow water drainage. The space between the two barrels was filled with sand to reduce the water volume and to minimize temperature fluctuations in the inner barrel. To flood the soils, water of three different salinities was used (0, 3 and 10 psu). The soils were subjected to two different flooding regimes. One half of the soils were permanently flooded (P) and the other half subjected to a summer dry/ winter wet regime (R) with periodic simulated showers in the summer (day 80 to 280 and day 480-680). After day 280 soil L was not flooded anymore, because the 200 day dry period was insufficient to allow any measurable changes in the metal biogeochemistry. The amount of water used to simulate the showers during the dry periods was equal to the average rainfall in summer in Belgium. At day 280 the amount of water that was used to simulate rainfall was drastically reduced to a minimum. This was done to oxidise the soil as much as possible but still allow pore water extraction. For the soils which were permanently flooded with water of 3 psu, there was also a treatment that was planted with reed (*Phragmites australis*). Every treatment was duplicated. One setup was used to take soil samples at regular intervals, the other setup was used to take pore water samples and perform redox measurements.

As external factors (e.g. temperature and light regime) could not be controlled in the outdoor experimental set-up, samples were taken from the reservoirs to perform toxicity tests in the laboratory under controlled, standardised conditions. For each combination of substrate and salinity, chronic sediment toxicity tests were performed.

A first test was performed prior to the start of the flooding and allowed to establish the base-line toxicity of the substrates; a second and a third test after 6 and 12 months of permanent inundation were carried out to assess toxicity changes as a function of time. The substrates subjected to the summer-dry/winter-wet regime were tested when all the substrates were oxidised due to the dry period. Two test organisms were used: the oligochaete *Tubifex tubifex*, a burrowing species, as a representative of benthic organisms and the talitrid amphipod *Orchestia gammarellus* which has a semi-terrestrial lifestyle and represents terrestrial organisms living in the upland part of a wetland. The proposed reference soil, soil N, was found to be unsuitable for toxicity testing, because of the very low pore water pH (± 2.6) and the presence of oxidised pyrite (and possibly associated metals) in the substrate. Both factors can influence the behaviour of the test organisms and the metal speciation thus possibly resulting in confounding effects. For the toxicity tests, this soil was replaced by formulated sediment. Each time a new laboratory test was performed, the control soil was freshly made as proposed by the OECD (2005) and was thus not subjected to the different treatments. The three tested substrates were thus the uncontaminated artificial sediment (OECD), soil C and soil L.

The chronic toxicity tests were performed with the oligochaete *T. tubifex* at salinities of 0 and 3 psu; the amphipod *O. gammarellus* was used when salinities of 3 and 10 psu were applied. Endpoints monitored in these tests include metal accumulation, growth and reproduction. All bioassays were conducted in a 20 ± 1 °C acclimatised room and a 16:8 light-dark cycle. After homogenisation of the substrate samples, the wet equivalent of 100 g dry weight was transferred to the test vessels.

A detailed description of the test performed with *T. tubifex* can be found in Speelmans et al. (submitted). Ten sexually mature specimens of *T. tubifex*, obtained from a healthy culture maintained in our laboratory, were exposed to the substrates differing in salinity of the overlying water (0 and 3 psu). Survival, reproduction, growth and metal concentrations were determined after 28 days of exposure. As additional food source, grounded Tetramin© fish flakes were provided three times a week at a rate of 200 µg/individual.

As the amphipod *O. gammarellus* has a semi-terrestrial life style, toxicity tests were performed in terrestrial conditions. The moisture content of the wet equivalent of 100 g dry weight was adjusted (by adding water or by allowing evaporation of excess water) to the maximal water retention capacity of the substrates. Before each test, amphipods were acclimated for one week to the salinity used in the test. Five amphipods were added to each test vessel, and fed ad libidum with decaying brown algae (*Fucus vesiculosus)* collected in the Yzermonding, Nieuwpoort. The moisture level of the substrates was adjusted once a week by adding deionised water.

3.1.2 Sampling

Undisturbed core samples were taken every six months with a liner sampler (04.15.SB, Eijkelkamp, Giesbeek, NL). In the duplicate barrel, the temperature and redox potential (ORP) measurements were performed and pore water was extracted at three different depths: 20, 40 and 60 cm below the soil surface. At each of the three depths, three Rhizon MOM soil moisture samplers (Eijkelkamp, Giesbeek, NL) were horizontally installed to extract the soil solution in vacuum tubes over a porous filter compartment with a pore size of 0.1 µm. The pore size of 0.1 µm is operationally chosen to extract metals which can be considered as available for plant uptake. Pore water samples were taken each two weeks during a two year period (2004-2005).
For the toxicity tests, the upper 10 cm of the substrates was sampled with a plastic scoop. If flooded, substrates were directly covered with a layer of the corresponding overlying water to avoid contact with the air. Covering the samples with water prevents oxidation of the substrates and associated sulphides (if present).

3.1.3 Measurements and analysis

3.1.3.1 Soil characteristics

The soil samples were dried and ground. The dry matter content was determined by measuring the weight loss after drying the samples in the oven (2 h at 105°C). The pH was measured in a 1:5 sediment/deionised water suspension after equilibration for 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1:5 sediment/deionised water suspension, shaken for 30 min (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H_2SO_4 added to 1 g of sediment. The cation exchange capacity was measured by percolation of 150 ml of 1 M NH_4OAc through a percolation tube filled with a mixture of 5 g of sediment and 35 g of quartz sand, followed by washing through the excess with 300 ml of denatured ethanol. The exchangeable ammonium ions were then eluted with 500 ml of 1 M KCl and analysed in the percolate by means of steam distillation (Tecator Kjeltec System 1002 distilling unit). The exchangeable cations were also analysed in this percolate using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA) (Van Ranst et al., 1999). Pseudo-total Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn contents were determined by aqua regia extraction (Ure, 1990). The metal content in the extracts was analysed using ICP-OES. Acid volatile sulphur (AVS) was determined by reduction of sulphur to H_2S and capitation in a Zn-acetate solution, followed by a back titration. This method is described by Tack et al. (1997). Chlorides were determined by potentiometric titration with $AgNO_3$ (Van Ranst et al., 1999). For the texture analysis the dried samples were pre-treated. This consisted out of the

removal of organic matter with H_2O_2 and the removal of $CaCO_3$ with an acetate buffer solution. The samples were then rinsed three times by decantation after sedimentation. After the addition of a dispersion agent, the samples were stirred for four hours. The texture of the soil samples was determined by laser diffraction (Coulter LS200, Miami, FL). The clay fraction is in this method defined by the 0-6 µm fraction, because this fraction showed the highest correlation with the 0-2 µm fraction, determined with the classic pipette method, except for soil samples with a clay content higher than 50 % (Vandecasteele & De Vos, 2001). The maximum clay content in the measured samples did not exceed values of 50 %. The sand fraction is determined by the > 50 µm fraction.

3.1.3.2 Temperature and ORP

The temperature was measured with a temperature probe (HI 7669/2W), permanently installed at the same three depths of the rhizon soil moisture sampling sets and connected to a HI 9025 microprocessor pH/ORP meter. The ORP was measured with a combined platina and gel reference electrode (HI 3090 B/5) that was permanently installed in each barrel at the same depths of the temperature measurements and the pore water extractions. The electrodes were connected to the previous mentioned pH/ORP meter. The measured value was corrected compared to the Standard Hydrogen Electrode by adding the difference of the measurement of the ORP in a ZoBells solution (0.033 M $K_3Fe(CN)_6$ and 0.033 M $K_4Fe(CN)_6$ in 0.1 M KCl) with the theoretical value of + 428 mV.

3.1.3.3 Pore water characteristics

Collected pore water samples were acidified and all samples of each depth were poured together in a polystyrene flask awaiting analysis. The pore water samples were analysed for Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn contents using ICP-OES. Concentrations of Cd, Cr, Cu, Ni and Pb in the pore water of the treatment flooded with the fresh flooding water that were below the determination limit, were determined using GF-AAS (SpectrAA-800/GTA-100, Varian, Palo Alto, CA). The pore water samples were diluted 50 times with $CsCl_2$ to determine Na, K, Ca and Mg concentrations using ICP-OES. After one year the pore water samples were alternately measured for metal content and for TOC, anions and pH. The TOC content was determined in fresh samples with a Total Organic Carbon Analyser (model TOC-5000, Shimadzu Corporation, Tokyo, Japan). The anions were determined using a Metrohm 761 Compact IC with a 788 IC filtration sample processor (Herisau, Switzerland) and the pH was measured using a pH-electrode (Orion model 520 A).

3.1.3.4 Reed plants

During the harvest of the reed plants the number of stems in each barrel was counted. Reed plants were weighed, stem thickness and height of the plants were measured and the number of leaves or nodes was counted. The dry weight of the reed plants and the biomass production were determined after drying (50 °C, 48h). Six plants were randomly selected for metal analysis. An overnight destruction with nitric acid and H_2O_2 (Du Laing, 2003) was used to destruct the plant material. Cd, Cr, Cu, Ni and Pb were determined with GF-AAS (Graphite Furnace Atomic Absorption Spectrometry) (SpectrAA-800/GRA-100). Fe, Mn and Zn were determined with ICP-OES (Inductive Coupled Plasma-Optical Emission Sprectrometry) (Varian Vista MPX, Varian, Palo Alto, CA).

Photosynthetic pigment content was determined spectrophotometrically as described by Wellburn (1994). Five plants per treatment were sampled in July and September 2004. From each plant two leaves were sampled, a young leaf (the second leaf) and an older leaf (the seventh leaf). Chlorophyll fluorescence was determined as a measure for physiological stress. It is based on the fact that when chlorophyll molecules absorb light, the electrons are temporarily boosted to a higher energy level. The absorbed energy can be re-emitted almost instantaneously as light energy of a longer wavelength, a phenomenon known as fluorescence. The amount of energy transmitted as fluorescence is inversely proportional to the rate of the photosynthetic activity. Based on this relation the measurement of chlorophyll a fluorescence can provide information on the physiological status of the plant. One of the most important parameters is the maximum quantum yield (variable fluorescence/maximum fluorescence: F_v/F_m). F_v/F_m is relatively constant in most plant species when measured under non-stressed conditions and equals 0.832 ± 0.004). Plants which are grown under stressing environmental conditions (e.g. metal stress, drought stress...) show remarkable lower F_v/F_m values (Rohacek, 2002). Sampling was conducted in the same way as for photosynthetic pigment content, measurements were performed by using a Plant Efficiency Analyser (PEA, Hansatech, Ltd, King's Lynn, Norfolk, UK).

Metal phytotoxicity is considered to result in oxidative stress (Cuypers et al. 1999). Elements such as Cu produce several reactive oxygen species; lipid peroxidation products and hydrogen peroxide can accumulate in plant tissues after exposure to metals. The increased activity of these enzymes caused by toxic amounts of metals is often a defence against metal induced oxidative stress (Koppenol, 1994; Weckx & Clijsters, 1997). Quantification of five enzymes (e.g. guiacol peroxidase (GPOD), malic enzyme (ME), ascorbate peroxidase (APOD), gluthation reductase (GR) and isocitraat dehydrogenase (ICDH)) was used to assess the stress status of the plant. Measurements of these enzymes were performed by the Centre for Environmental Science, Universiteit Hasselt, Diepenbeek as described in Van Assche et al. (1988)

and Van Assche & Clijsters (1990). Leaf samples for stress enzymes were taken in September 2004 and only the C and L substrate were sampled. Samples for stress-enzymes were taken from the first, third and the fourth leaf. When the first leaf was too small to take samples, the fifth leaf was used. Enzymatic activities are expressed as mU per sampled surface area. One unit (U) corresponds to the amount of substrate transformed by the enzyme in one minute at 30°C.

3.1.3.5 Invertebrates

- *Tubifex tubifex*

After 28 days, surviving organisms were retrieved by sieving over a 250 µm sieve. The cocoons and juveniles were transferred to a clean glass vessel to which 70 % alcohol and a few drops of Bengal red were added. Cocoons and juveniles were counted using a stereomicroscope**.** All surviving adults per replicate were placed in clean medium with a salinity corresponding to that of the treatment. After 24h of gut clearance, 5 individuals per replicate were pooled for metal analysis, rinsed in clean medium, dried to constant weight at 60°C and weighed with an analytical balance (± 0.01 mg). The remaining individuals were blotted dry with filter paper, weighed (± 0.01 mg), shock frozen in liquid nitrogen and stored at -80°C.

- *Orchestia gammarellus*

After 42 days of exposure, surviving amphipods were recollected and placed on filter paper moisturised with clean medium with a salinity corresponding to that of the treatment. After 24h of gut clearance 3 individuals per replicate were kept separate for metal analysis, rinsed with clean medium, dried to constant weight at 60°C and weighed with an analytical balance (± 0.01 mg). Remaining organisms were blotted dry, shock frozen in liquid nitrogen and stored at – 80°C. For the determination of total Zn and Cu concentrations, dried samples were digested in 1.5 ml of 14 M HNO3 with the aid of a microwave oven (4 min at 90 W, 6 min at 160 W, 4.5 min at 350 W). Every batch of samples included a blank (no organisms) and a certified reference (CRM 278R, mussel tissue, Community Bureau of Reference, Brussels, Belgium) for quality control. After cooling to room temperature, the digested samples were diluted with 8.5 ml of double distilled water. Zn was measured by ICP-OES (Varian, Vista MPX, Palo Alto, CA), Cu was analysed by graphite furnace atomic absorption spectrometry (GF-AAS, Varian, SpectrAA-800/GTA-100, Palo Alto, CA).

3.1.4 Statistics

All data of the toxicity tests were analysed by means of general linear models (GLM) as normality and homoscedasticity assumptions were met. Results of the laboratory bioassays are expressed as the ratio of the values obtained in the treatment to that of the control sediment (OECD). This enabled to correct for life cycle variation in the measured parameters. As no data were available for one treatment combination in

the *T. tubifex* tests (C, 6 months of inundation at 0 psu), Type IV sums of squares were used to test the hypotheses of interest. For *T. tubifex* growth, all parameters for *O. gammarellus* and reed (photosynthetic pigment content, F_v/F_m and stress enzymes) data for each treatment combination consisted on multiple measurements taken on replicate samples, which cause multiple error terms to enter into the statistical model. Therefore, general linear mixed models (GLMM) were fitted to the data in which replicates were treated as a random factor, nested within treatment combination. Standard errors were adjusted with the Tukey procedure if post-hoc pair wise comparisons were made among treatment means. Analyses were run with the procedures GLM and MIXED in SAS v. 9.1.3.

3.2 Results

3.2.1 Soil characteristics

The texture of the three soils differed only slightly. The texture of soil N was 48 ± 2 % clay, 34 ± 1 % loam and 18 ± 2 % sand (n=2). Soil C consisted of 46 ± 2 % clay, 53 ± 1 % loam and 1 ± 1 % sand (n=2). The variation in the soil texture of L was much larger: 36 ± 7 % clay, 48 ± 11 % loam and 16 ± 17 % sand. Most of the soil characteristics did not change during the course of the experiment and did not vary between the different depths. Therefore, for those characteristics, only summary statistics are given below (Table 1 and Table 2).

Table 1: Average values (av) and standard deviations (sd, n=6) of the soil characteristics of the different soils

	pH	OM (%)	EC ($mS.cm^{-1}$)	$CaCO_3$ (%)	CEC (meq $NH4^+.100g^{-1}$)
	av ± sd	av ± sd	av ± sd	av ± sd	av ± sd
N	5.5 ± 1.7	6.1 ± 1.0	3.1 ± 0.4	0.2 ± 0.3	16.6 ± 1.8
C	7.5 ± 0.1	10.0 ± 0.9	0.6 ± 0.5	4.5 ± 0.3	25.8 ± 1.2
L	7.7 ± 0.2	8.9 ± 0.5	1.0 ± 0.5	5.4 ± 0.5	16.1 ± 0.8

The metal content of the three soils is given in Table 2. Soil N has a much lower metal content than the other two soils. The values are below or just above the background value for Flemish soils (Vlarebo, 1996). The values of soil C and L are all much higher than the background value. For Cd, the sanitation level for nature areas (type I and II) is exceeded.

Table 2: Average values (av) and standard deviations (sd, n=6) of the metal content ($mg.kg^{-1}$ DM) of the three different soils

	Cd	Cr	Cu	Fe	Mn
	av ± sd	av ± sd	av ± sd	av ± sd	av ± sd
N	0.10 ± 0.09	61 ± 5	15 ± 2	27099 ± 2112	94 ± 33
C	8.31 ± 0.44	169 ± 4	91 ± 2	35824 ± 944	1178 ± 27

L	5.15 ± 0.41		85 ± 15		136 ± 95		25121 ± 1687		375 ± 28	
	Ni		Pb		Zn		Ca		Al	
	av	sd	av	sd	av	sd	av	sd	av	sd
N	31 ± 3		15 ± 2		60 ± 5		6349 ± 969		19120 ± 3476	
C	32 ± 1		192 ± 32		655 ± 20		23786 ± 1045		21724 ± 2065	
L	48 ± 8		150 ± 18		651 ± 49		30276 ± 4357		15128 ± 1495	

The difference in the chloride content between the different soils was smaller than the difference between the treatments since the soils were flooded with water of different salinities. Before flooding the soil, only measurable amounts of chlorides could be detected in soil L (26 ± 8 mg $Cl^-.kg^{-1}$ DM). After flooding the soil with water of 10, 3 and 0 psu, the chlorides were 960 ± 598 mg $Cl^-.kg^{-1}$ DM (n=24) in the most saline treatment, 314 ± 209 mg $Cl^-.kg^{-1}$ DM (n=33) in the brackish treatment and 48 ± 57 mg $Cl^-.kg^{-1}$ DM (n=24) in the treatment flooded with fresh water.

The only soil where detectable amounts of sulphides were present throughout the whole experiment was soil L. The amount of sulphides measured is shown in Figure 1. This soil was already reduced from the beginning of the test. After one year (nine months of oxidation) the sulphide content of the top layer already decreased in every treatment. During the second year (after 20 months of oxidation) the sulphide content also decreased in the deeper layers.

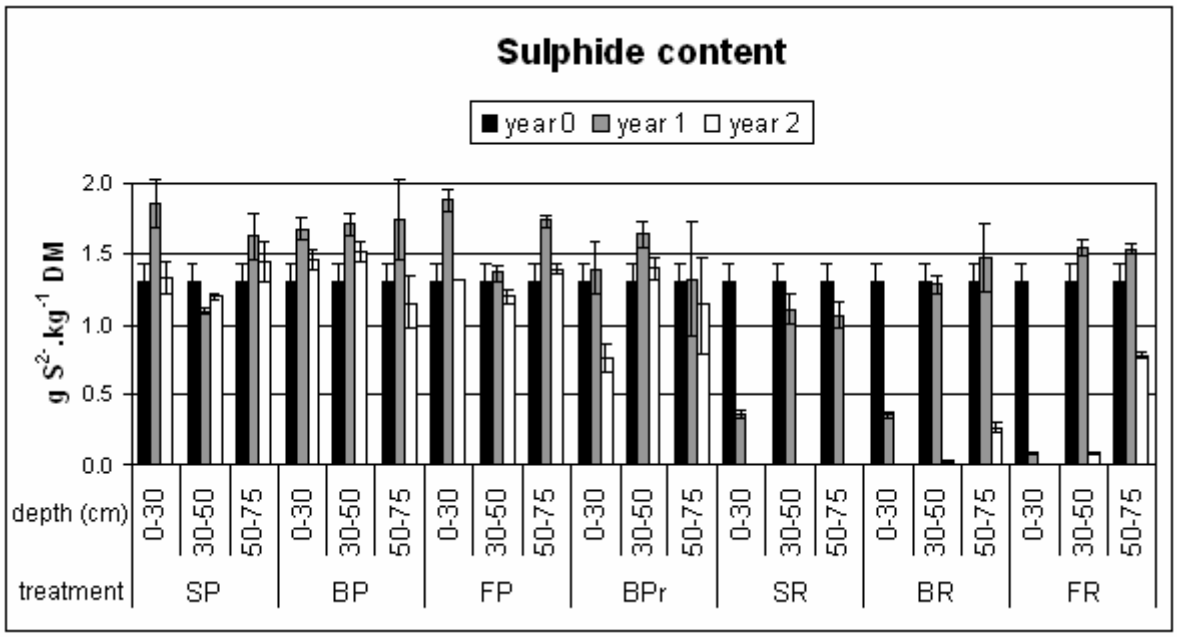


Figure 1: Sulphide content (g $S^{2-}.kg^{-1}$ DM) of soil L for the different treatments (salinity of flooding water: S = 10 psu, B = 3 psu, F = 0 psu; hydrological regime: P = permanently flooded, R = drying after a short flooded period) represented by mean ± sd (n=3)

The sequential extraction procedure was performed on the three soils, but only for soil C and L the recoveries were good (Table 3). The metal content of soil N was too

low to allow a reliable sequential extraction. Because of the bad recoveries for soil N, only the sequential extraction results for soil C and L are shown in Figure 2. The differences in the metal fractions between the three depths were minimal, except for Fe in soil L after a 200 day drying period. Also no clear differences were found between the treatments with different salinities of the flooding water. The results shown in Figure 2 are averages of the different depths and salinities. Pb and Cr show very little differences between the different treatments. For the other elements, the largest differences are observed between the two soil types (C and L) and between the two different hydrological regimes (P and R) of soil C.

Table 3: Average (n = 14) recoveries (%) of the metals in the sequential extraction procedure

	N	C	L
Cd	3051 ± 8990	99 ± 17	85 ± 18
Cr	67 ± 11	97 ± 14	74 ± 20
Cu	178 ± 130	104 ± 13	88 ± 26
Fe	94 ± 41	98 ± 11	97 ± 16
Mn	255 ± 528	106 ± 14	107 ± 29
Ni	217 ± 381	111 ± 11	85 ± 19
Pb	145 ± 109	104 ± 19	109 ± 26
Zn	256 ± 549	114 ± 16	114 ± 27

When looking at the differences between the soil types (C versus L), Ni, Fe and Mn show the same pattern. The acid extractable fraction is larger for those elements in soil L than in soil C at the expense of the reducible fraction. For Ni, the residual fraction in soil L is also smaller than in soil C and the oxidizable fraction is larger. Similar observations are made for Zn, while for Cd and Cu, the differences between the soils lie in the larger acid extractable and reducible fraction in soil C than in soil L, while the residual fraction is more or less the same.

When looking at the differences between the hydrological regimes (P versus R), it is obvious that the hydrological regime is not causing a significant change in the metal fractionation for soil L, except for Fe in the upper soil layer. This upper soil layer shows a smaller acid extractable Fe fraction in favour of the reducible fraction. For soil C, the differences between the regimes are more visible, except for Cu which shows no clear differences. For Cd on the other hand, the reducible fraction is smaller and the oxidizable fraction increases when the soil is flooded for extended periods. For Zn, it is the acid extractable fraction that increases at the expense of the reducible fraction. Fe, Mn, and Ni again show similar a pattern. When flooded, the acid extractable fraction increases and the reducible fraction decreases.

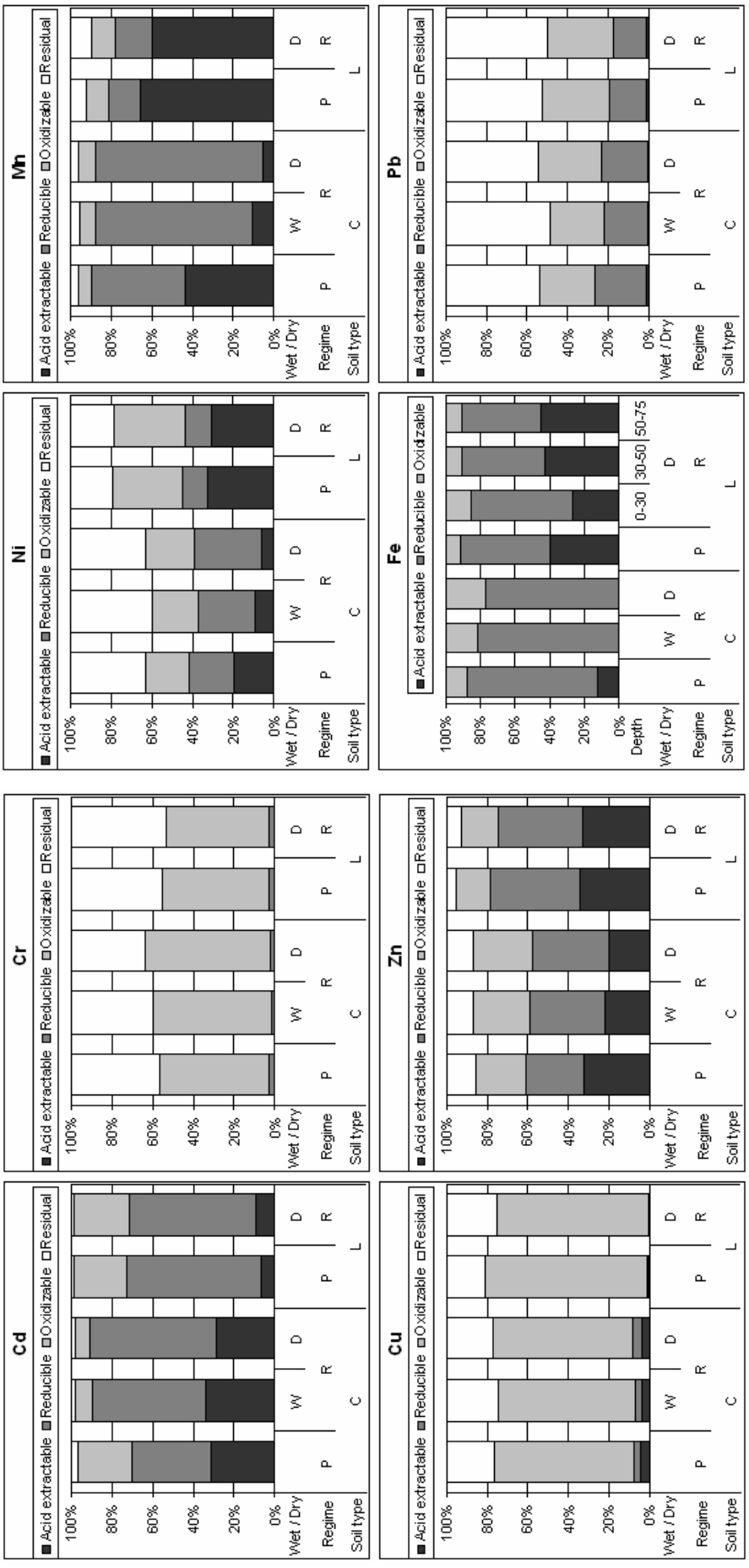


Figure 2: Sequential extraction of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn (W = flooded for 200 days, D = dry for 200 days)

3.2.2 Temperature and ORP

The temperature of the soil at the different depths was similar. Figure 3 shows the average temperature (separate dots) and the normal Kernel smoothing plot of the average soil temperature. This curve shows a minimum of 5 °C in winter and a maximum of 20 °C in summer.

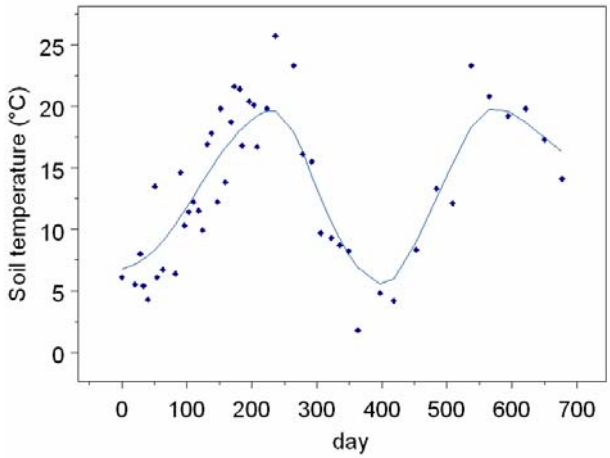


Figure 3: Average soil temperature (°C) during the course of the experiment with the normal Kernel smoothing plot (band width = 100 days)

The ORP of the different treatments at the different depths is shown in Figure 4. Soils N and C are oxidised in the beginning of the experiment, while soil L is strongly reduced. For the treatments that were not permanently flooded, the ORP increased during the dry periods (day 80-280 and day 480-680) in the top layers and at a later stage also in the deeper layers. The maximum value was higher for soil N than for soil C. The effect of the addition of water to simulate the rainfall during the first dry period is also visible in the ORP fluctuation. A strong decrease in the amount of water that was added in the second period does not lead to any visible fluctuations in the second dry period. The ORP of soil L starts to rise when the volume of the added water was reduced (day 280). Deeper layers of soil are being oxidised later. For the permanently flooded treatments, the ORP decreases or stays low for every treatment. The ORP of soil C decreases faster than that of soil N. The ORP at the end of the experiment of soil C reaches lower values than that of soil N. It is however comparable to the ORP of soil L, which stays fairly constant throughout the course of the experiment.

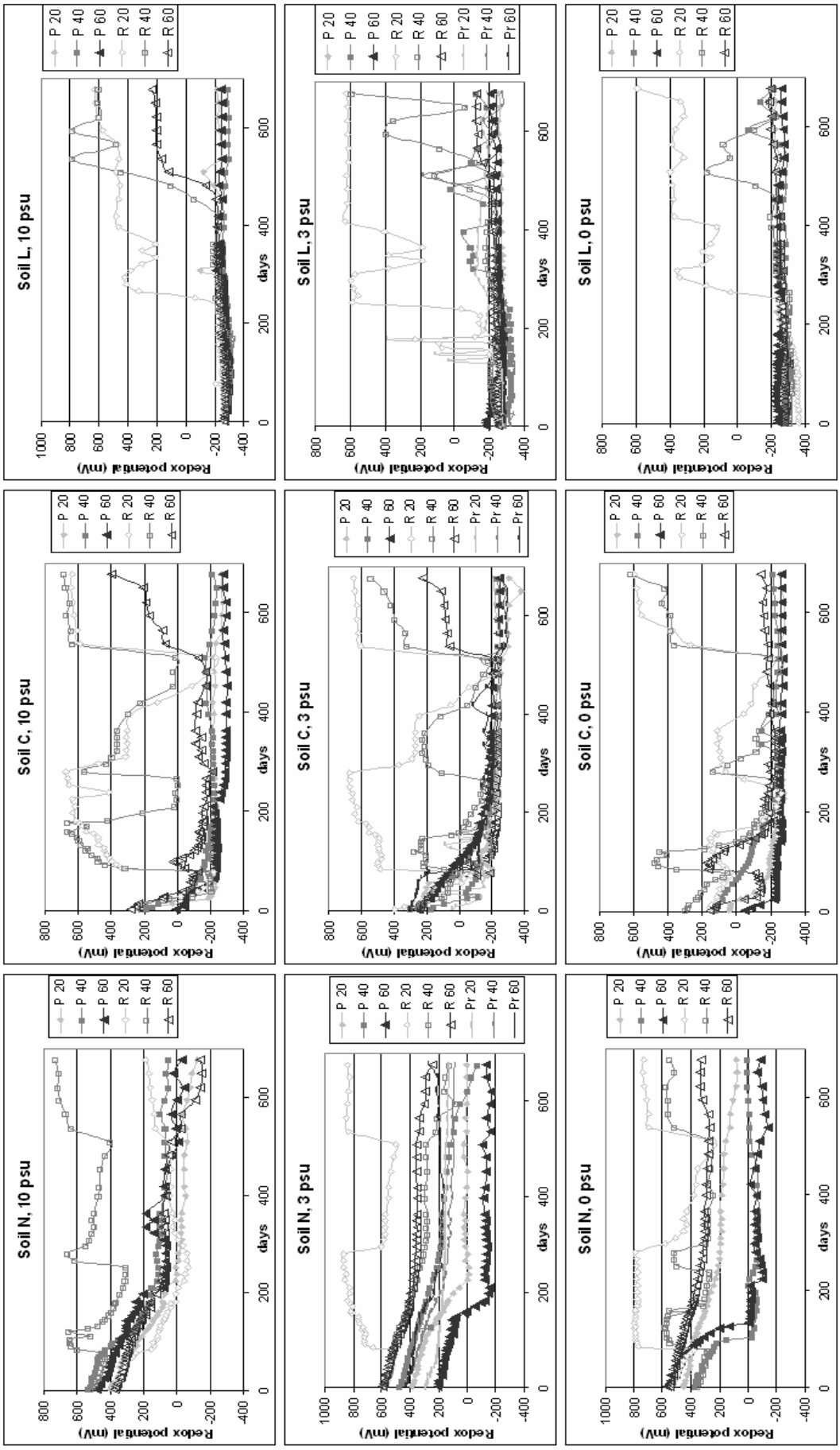


Figure 4: ORP (mV) in soils N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)

3.2.3 Pore water characteristics

In Figure 5 to Figure 10 the metal pore water concentrations which were not below the detection limits are presented. Those detection limits were higher for the treatments flooded with water of 10 and 3 psu, due to the high salt concentration. Pb is below the detection limit at every time, in the pore water of every treatment. The metal pore water concentration is the highest in soil N, although the total metal content is the lowest. Soil L shows the lowest pore metal concentrations. There is no clear relationship between the pore metal concentration and the depth for the treatments that were permanently flooded. Changes induced by the alterations in the hydrological regime were first visible in the upper layers of the soil. Metal pore water concentrations of soil L are all very low in the beginning of the experiment and do not change when the soil stays flooded or when the soil is set dry with a regular addition of small water amounts, equal to the average amount of rainfall in Belgium. The only changes occur in regime R at day 280, when the amount of added water is drastically reduced. The concentrations of all the measured elements, except Cr, start to rise in the upper layers and later on also in the deeper layers. In soil C, which was oxidised in the beginning, the metal pore water concentration of Fe, Mn, Ni and Cr increases in the treatments that are permanently flooded, while that of Cd, Cu and Zn decreases. In the alternating regime R, the opposite is observed during the dry periods. Although during the first dry period the concentration of the metals did not differ that much compared to the second dry period (when a lower amount of water was added to simulate rainfall). For soil N, the differences between the different depths and the different treatments are larger. The pore water metal concentrations of soil N did not show the same pattern as soil C, although both soils were oxidised in the beginning. There was no clear relationship between the changes in the hydrological regime and the pore water metal concentration. There was no influence of the reed on the pore metal concentration, except for Fe in soil L. In the growing season of the reed plants, the Fe concentration increased in the pore water of the upper soil layer. The amount of sulphides also decreased in that upper layer, in the second year of the experiment. This could indicate that Fe sulphides are being oxidised. In the second year of the experiment, not only the soil pH, but also the pH of the pore water was measured, along with anions and TOC in the pore water of the soils which were flooded with fresh water (0 psu). In Figure 11, the pH of the pore water is shown for all the treatments. Large variations were observed for soil N. The pH ranged from 2 to 6, depending on the depth. The pH stayed fairly constant in that soil, irrespective of the hydrological regime or treatment. The pH of the pore water of soil C showed less variability. When the soil was permanently flooded, the pH of the pore water fluctuated around 7. A slight increase was measured when the soil was set dry. The pH of soil L was the highest (between 7 and 8) and stayed relatively constant.

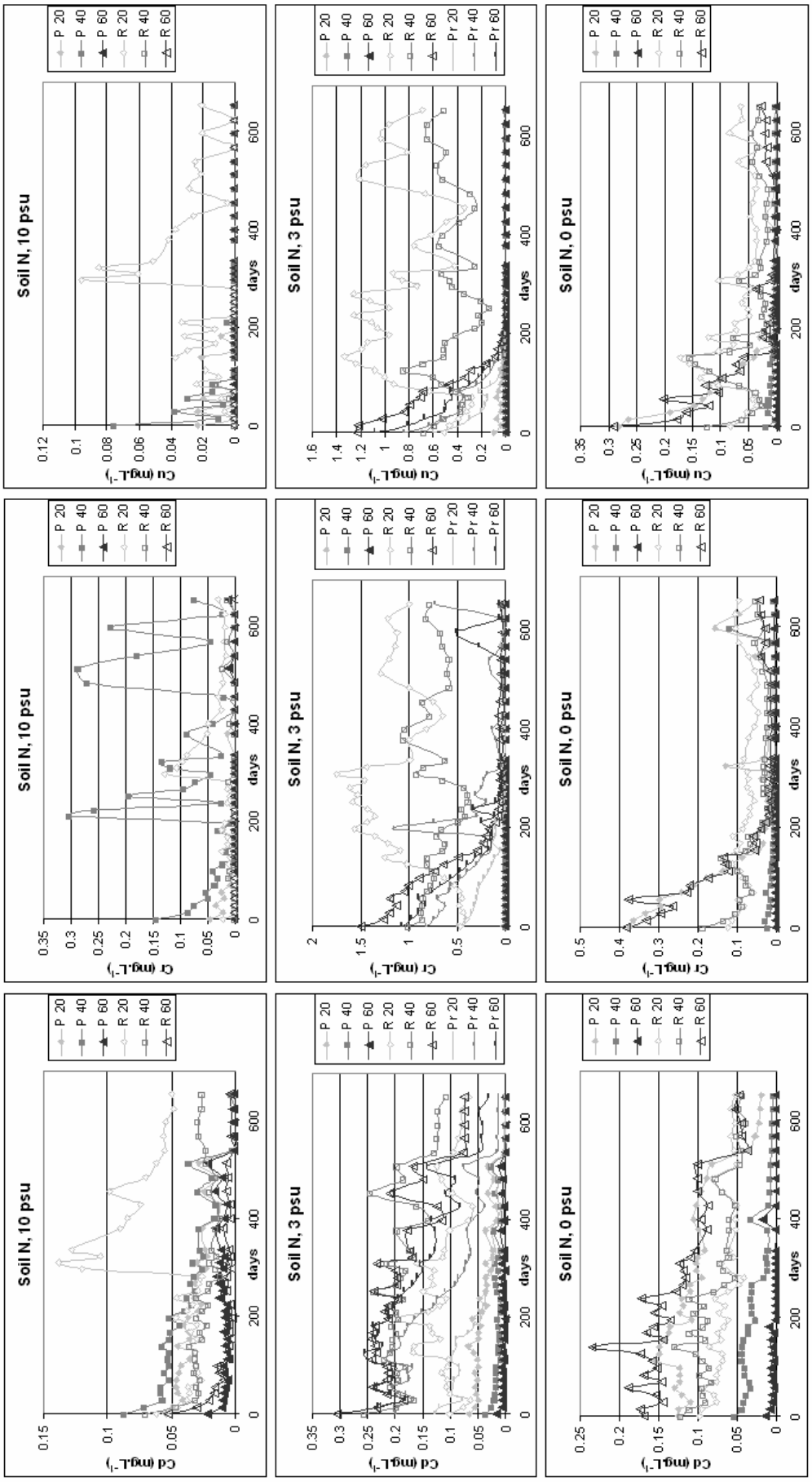


Figure 5: Cd, Cr and Cu pore water concentrations in soil N at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: flooded except from day 80-280 and from 480-680, small r denotes a treatment planted with reed)

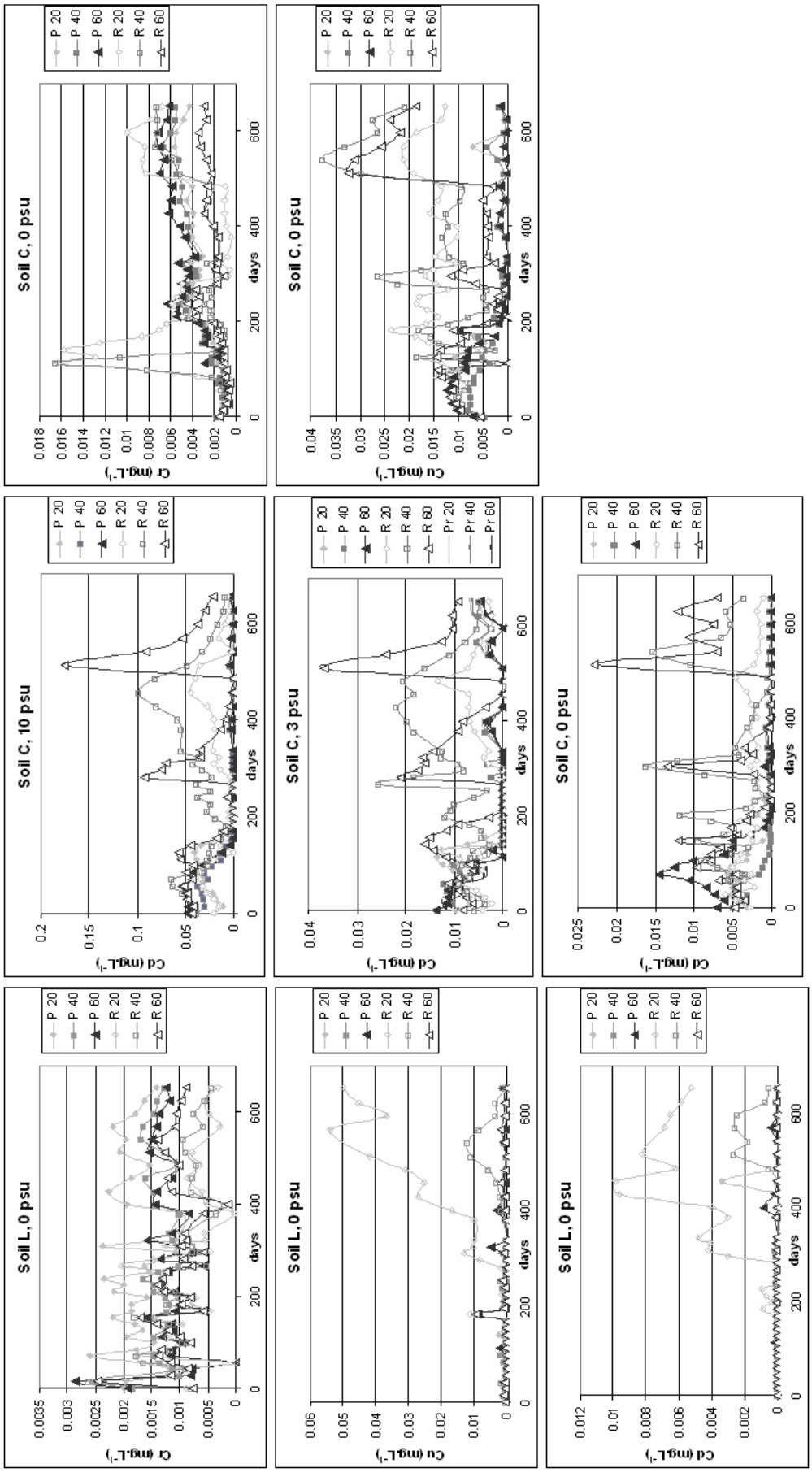


Figure 6: Cd, Cr and Cu pore water concentrations in soil C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) for Cd in soil C and with two hydrological regimes (P: permanently flooded, R: for C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)

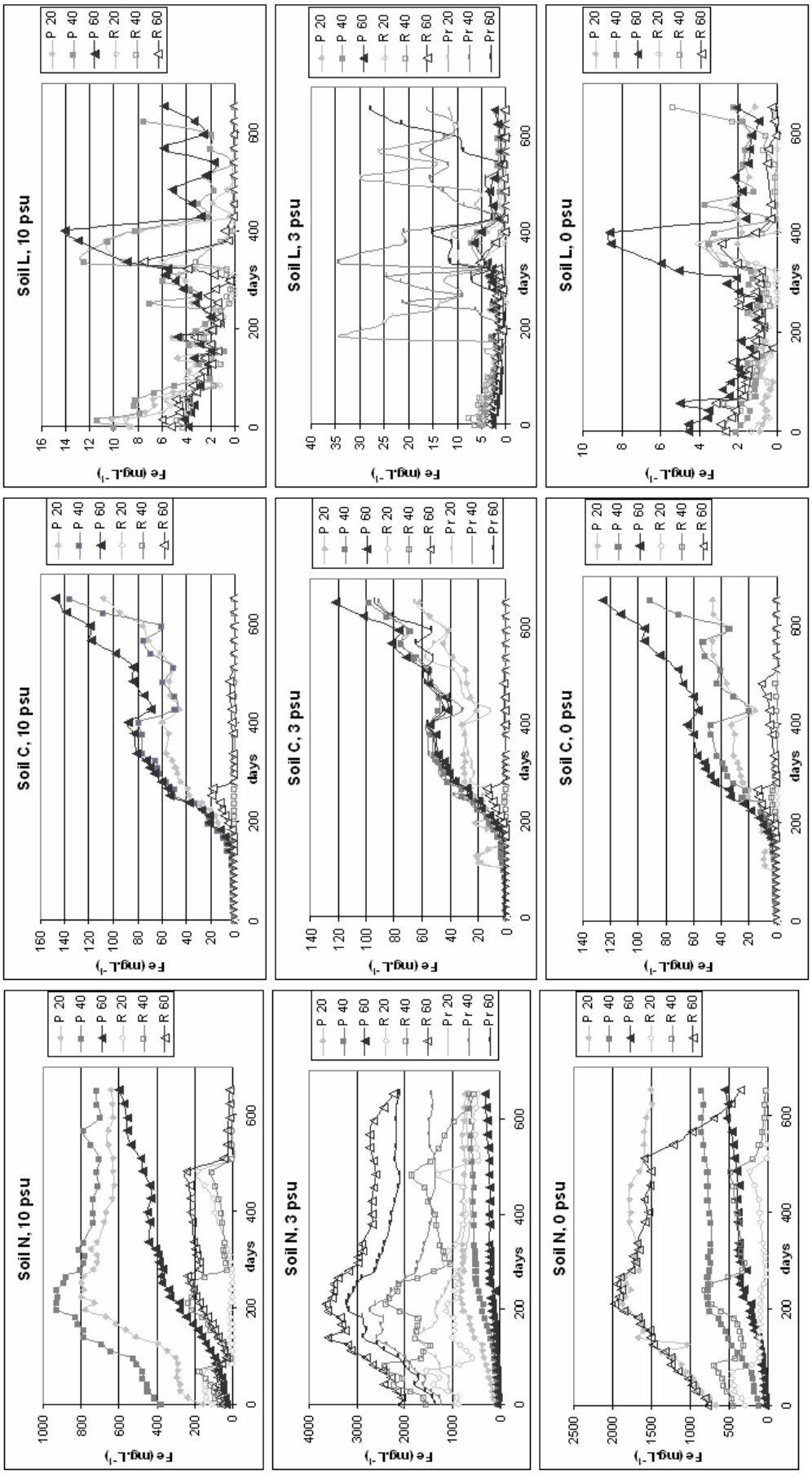


Figure 7: **Fe pore water concentrations in soil N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)**

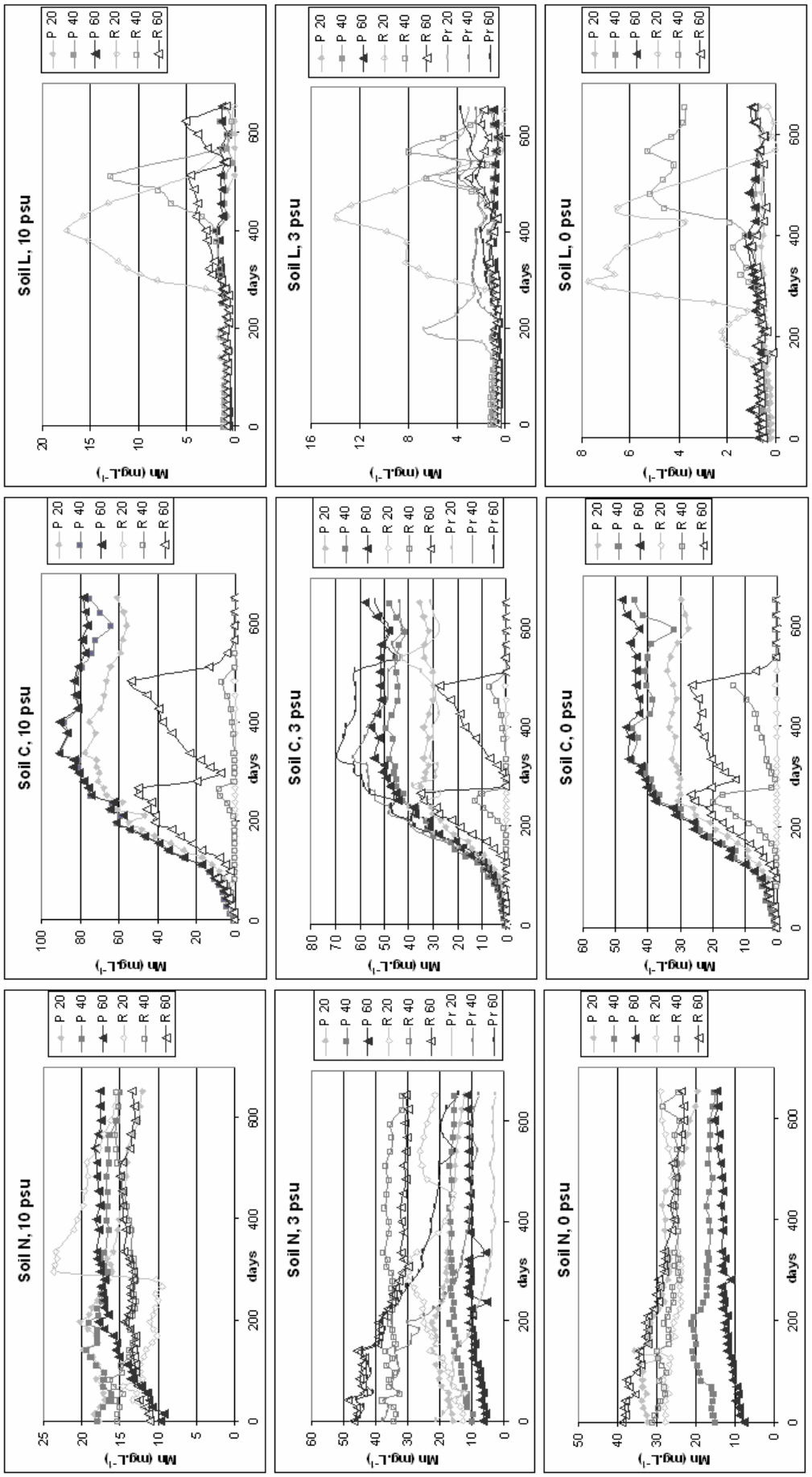


Figure 8: **Mn pore water concentrations in soil N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)**

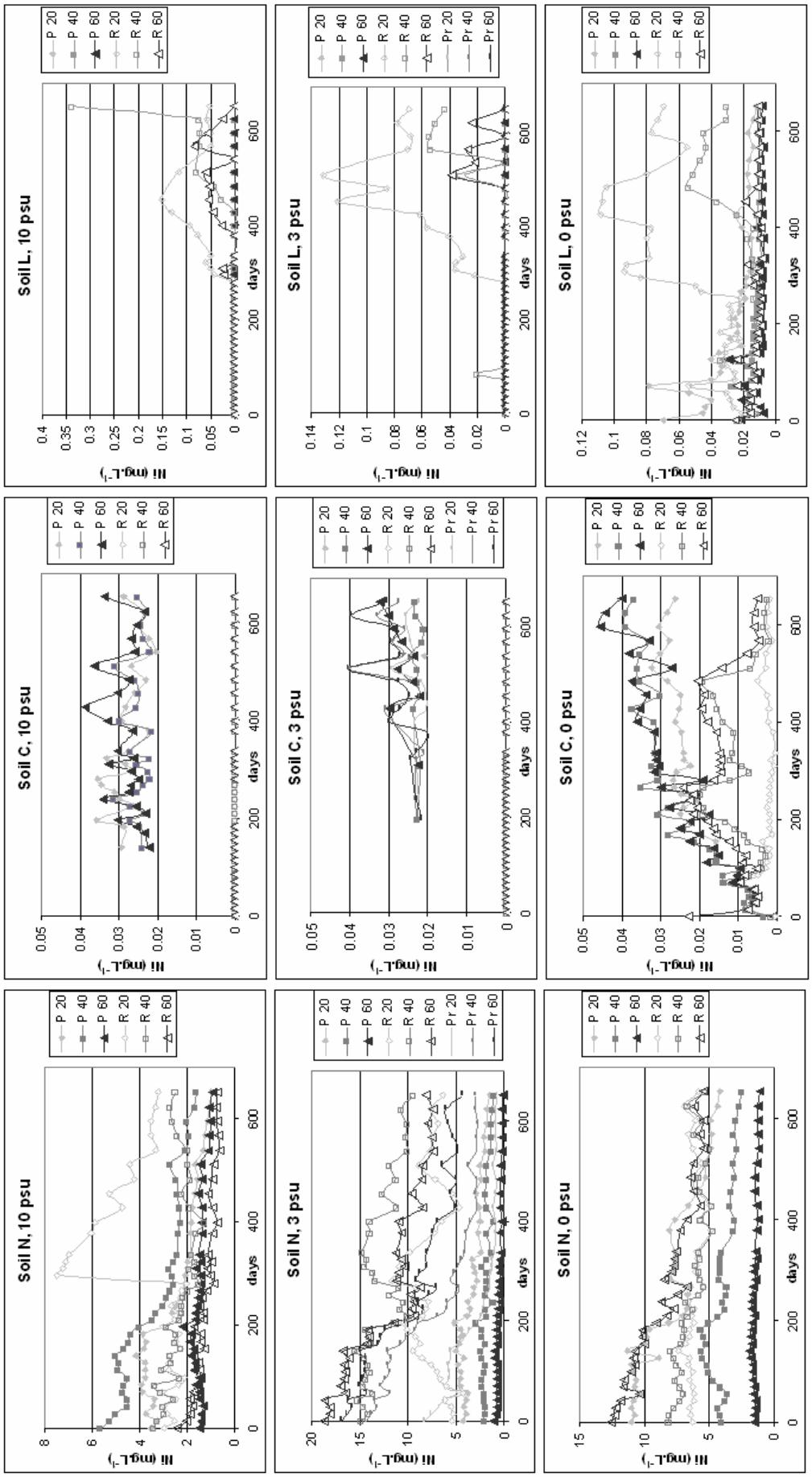


Figure 9: Ni pore water concentrations in soil N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)

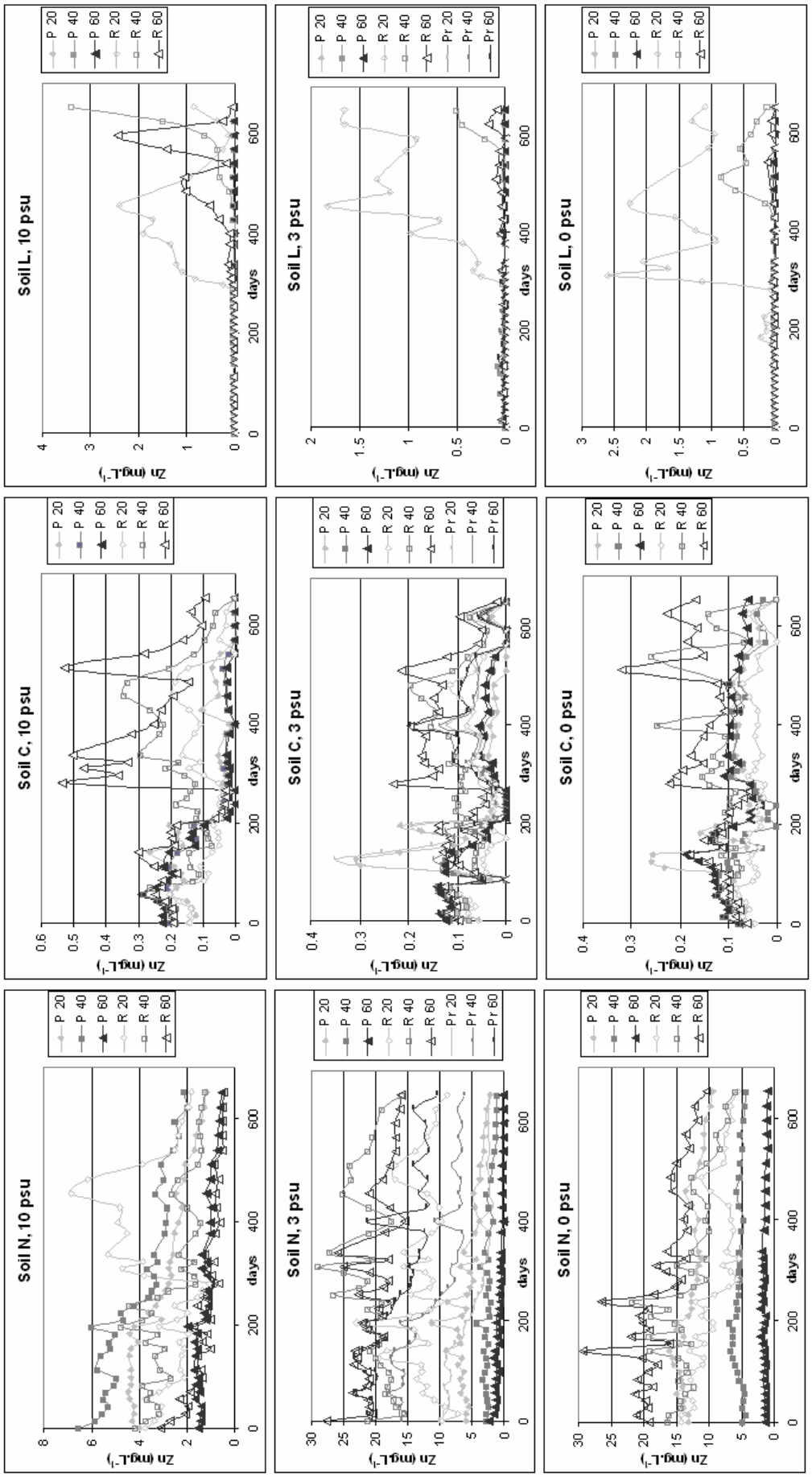


Figure 10: Zn pore water concentrations in soil N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)

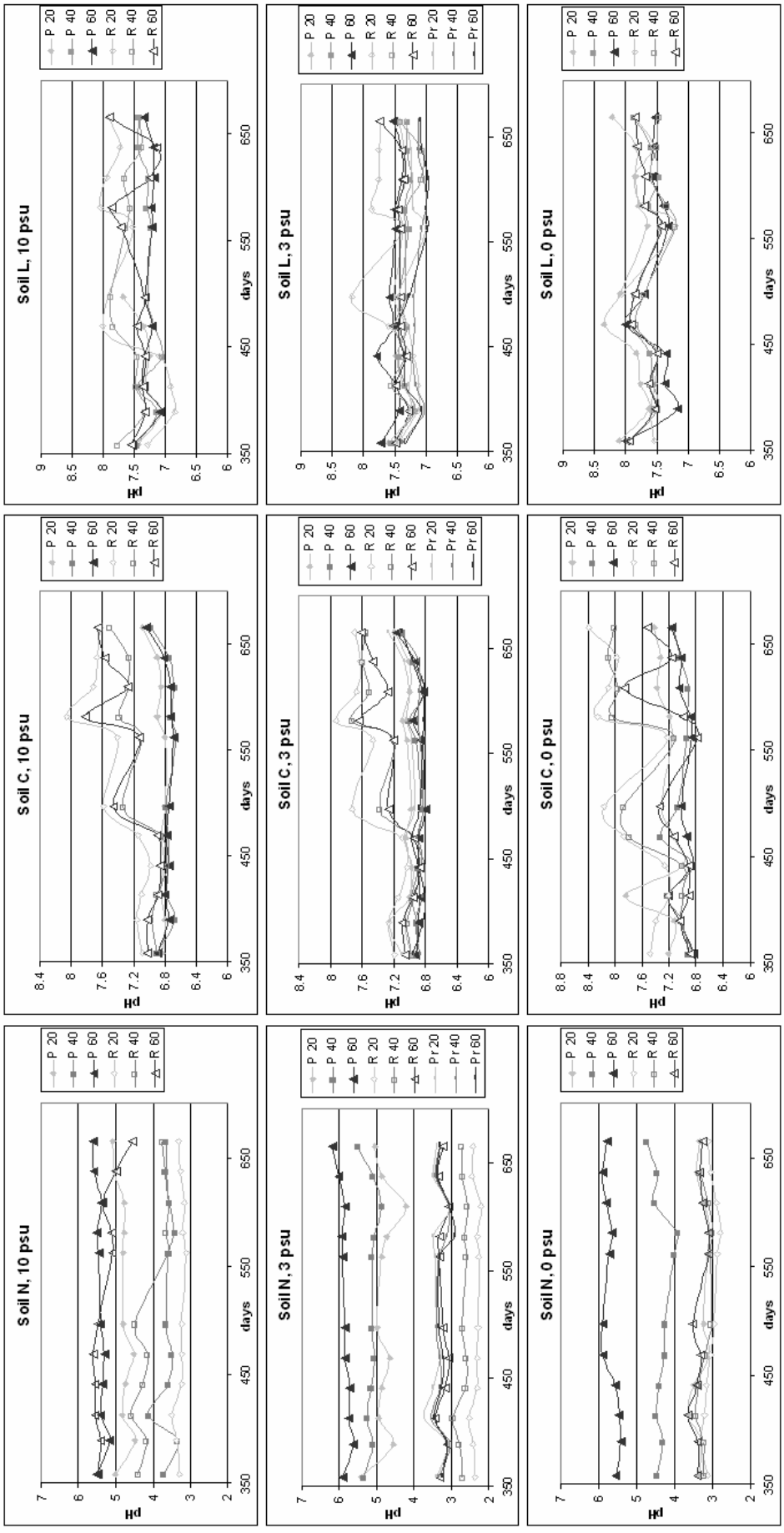


Figure 11: pH of the pore water in soil N, C and L at three depths (-20, -40 and -60 cm), for three salinities (0, 3 and 10 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days, small r denotes a treatment planted with reed)

In Figure 13, the chloride, nitrate and sulphate concentrations of the pore waters are shown. There was no relationship between the chloride concentration and the depth or the hydrological regime. The chloride concentration was the lowest in soil L, and the highest in soil N and C and was fairly constant throughout the year. The nitrate concentration did show differences between the hydrological regimes. In the upper layer of soil C and L, the nitrate concentration increased when the soil was not flooded. In soil N, the nitrates fluctuated around the detection limit (20 or 40 $mg.L^{-1}$, depending on the dilution factor). Sulphate concentration was the highest in the pore water of soil N and reached similar values in the pore water of the upper layer of soil L when it was being dried. In the pore water of soil C, the sulphate concentration also increased when the soil was being dried. Soils C and L show similar TOC concentrations (Figure 12), but the variability is high. In soil N, the TOC concentration is the lowest. No clear relationships are observed between TOC and depth or hydrological regime.

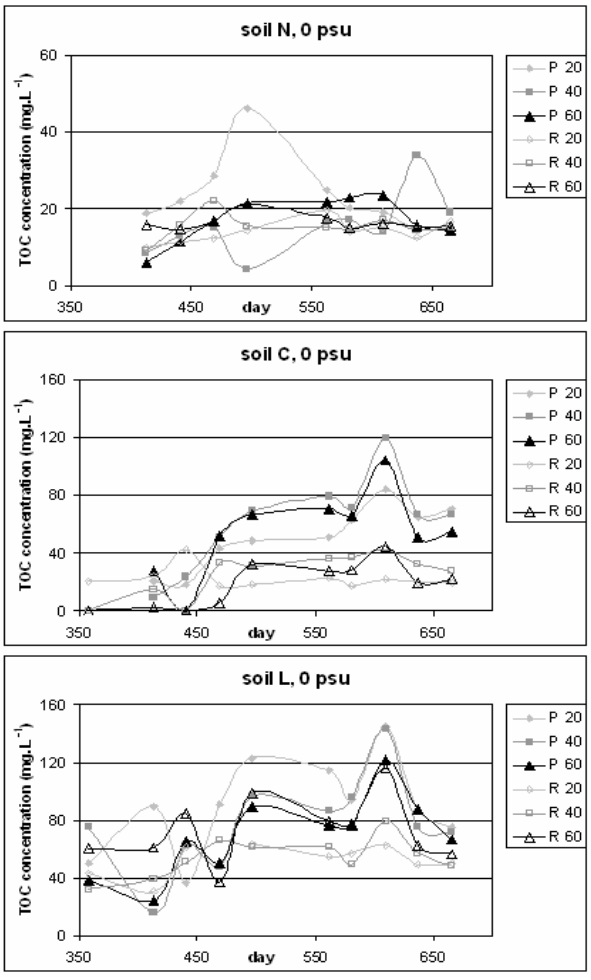


Figure 12: TOC concentration in the pore water in soil N, C and L at three depths (-20, -40 and -60 cm), for one salinity (0 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days)

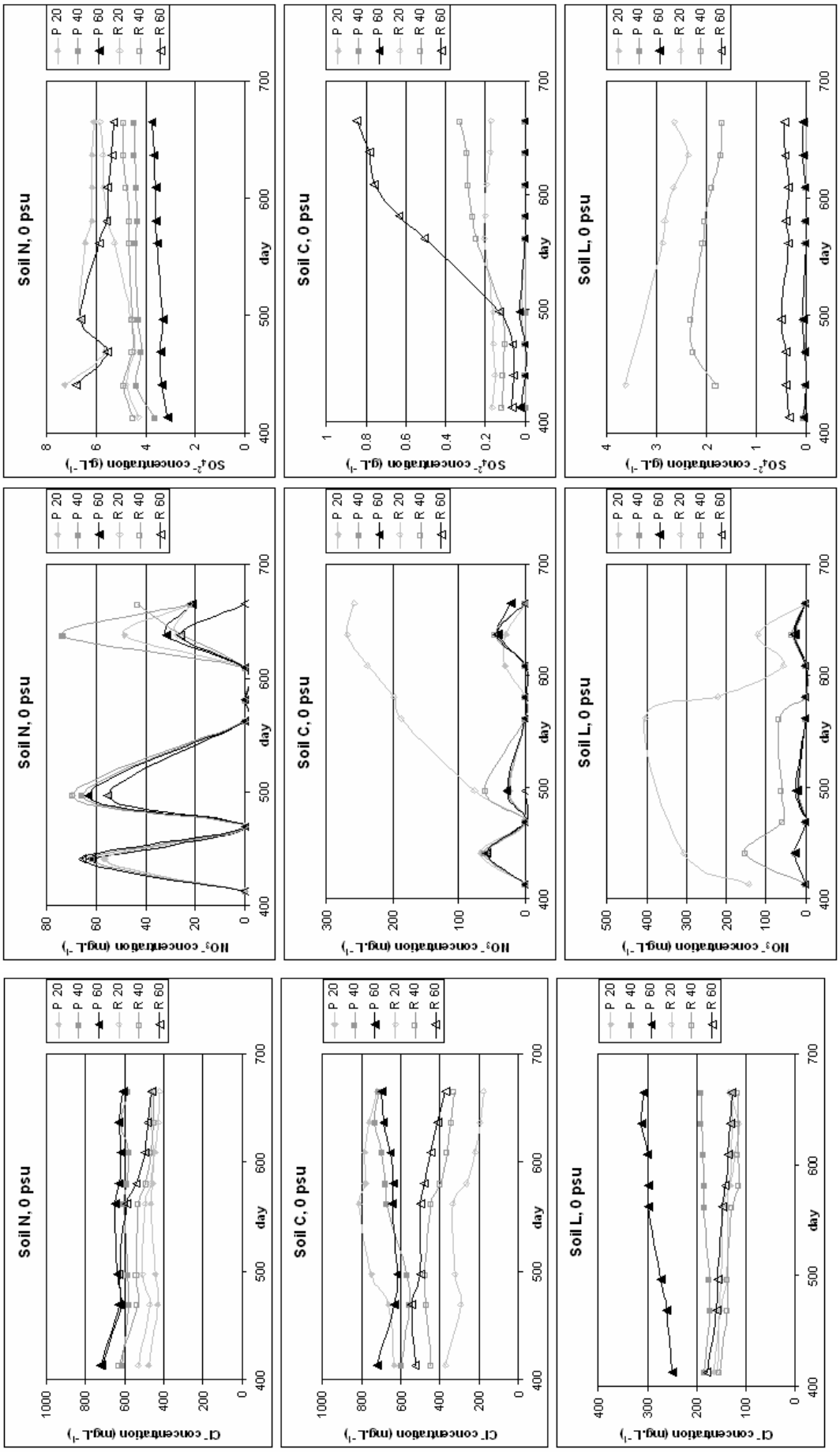


Figure 13: Anion (chlorides, nitrates and sulphates) concentration in the pore water in soil N, C and L at three depths (-20, -40 and -60 cm), for one salinity (0 psu) and with two hydrological regimes (P: permanently flooded, R: for N and C: flooded except from day 80-280 and from 480-680, for L: flooded during the first 80 days)

3.2.4 Reed plants

The reed plants that grew on different soils show in both growing seasons large differences (Table 4). The plants that grew on soil N were the smallest, had the smallest diameter and lowest productivity and density in both growing seasons ($p < 0.05$). The plants grown in soil L were the longest, thickest and were the most productive and had the highest shoot density ($p < 0.05$), although the length did not differ significantly in the first growing season from that of the plants that grew in soil C.

Table 4: Reed biomass characteristics of two growing seasons. Small letters denote homogeneous subsets of the reed characteristic within one year (Tuckey post-hoc test)

	N	C	L
	2004		
Length stems (m)	0.43 ± 0.19 [a]	1.16 ± 0.19 [b]	1.41 ± 0.27 [b]
Diameter stems (mm)	2.0 ± 0.6 [a]	4.1 ± 0.6 [b]	5.6 ± 1.3 [c]
Productivity (kg DM/m²)	0.20 - 0.23	2.14 - 3.05	8.32 - 8.85
Shoot density (# stems/m²)	676	2149	2531
	2005		
Length stems (m)	0.70 ± 0.15 [a]	1.50 ± 0.22 [b]	1.70 ± 0.20 [c]
Diameter stems (mm)	2.2 ± 0.3 [a]	3.6 ± 0.9 [b]	5.1 ± 1.0 [c]
Productivity (kg DM/m²)	0.25 - 0.30	3.04 - 3.07	11.81 - 14.02
Shoot density (# stems/m²)	525	1771	3302

The differences between the metal concentrations in the reed plants that grew on the different soils are shown in Table 5. The small letters denote homogeneous subsets within one year. Comparison of the metal concentrations between the two growing seasons showed that the metal concentrations in the leaves and stem of the reed are all lower in 2005 than in 2004, except for Zn. These differences are significant ($p<0.05$) for Al, Cr, Cu and Fe (for leaf and stem tissue), and also for the Cd concentration in the leaves. If the metal concentrations between the plant tissues are compared, metal concentrations in the leaves are higher ($p<0.05$) than in the stem tissue, except for Zn.

Table 5: Metal content (mg.kg^{-1} DM) of reed leaves and stem in two subsequent growing seasons. Small letters denote homogeneous subsets of the metal content within one year (Tuckey post-hoc test)

		2004					
		N		**C**		**L**	
LEAF	Cd	0.11 ± 0.02	a	0.05 ± 0.01	ab	0.07 ± 0.02	b
	Cr	4.3 ± 0.4	a	3.1 ± 0.4	b	2.1 ± 0.3	c
	Cu	2.5 ± 0.2	a	3.8 ± 0.3	b	4.2 ± 0.3	b
	Fe	201 ± 19	a	118 ± 17	b	176 ± 27	a
	Mn	268 ± 58	a	471 ± 118	a	277 ± 69	a
	Ni	1.6 ± 0.2	a	0.7 ± 0.2	b	0.5 ± 0.1	b
	Pb	1.5 ± 0.4	a	1.3 ± 0.3	a	1 ± 0.2	a
	Zn	16 ± 1	a	20 ± 6	a	26 ± 6	a
	Al	53 ± 7	a	36 ± 8	a	41 ± 8	a
STEM	Cd	0.04 ± 0.02	a	0.06 ± 0.03	a	0.01 ± 0	a
	Cr	2.4 ± 0.4	a	1.8 ± 0.1	b	1.5 ± 0.2	b
	Cu	3.8 ± 0.2	a	3.7 ± 0.6	a	1.3 ± 0.2	b
	Fe	74 ± 19	a	30 ± 3	b	39 ± 9	b
	Mn	101 ± 58	ab	139 ± 32	a	64 ± 38	b
	Ni	1.5 ± 0.2	a	0.3 ± 0	b	0.4 ± 0.1	b
	Pb	1 ± 0.4	a	0.5 ± 0.2	b	0.5 ± 0.2	b
	Zn	30 ± 1	a	17 ± 3	a	28 ± 11	a
	Al	18 ± 7	a	8 ± 2	b	7 ± 2	b
		2005					
		N		**C**		**L**	
LEAF	Cd	0.07 ± 0.01	a	0.05 ± 0.02	a	0.04 ± 0.01	b
	Cr	1.5 ± 0.2	a	1.3 ± 0.4	a	1.2 ± 0.4	a
	Cu	2.9 ± 0.7	a	3 ± 0.5	a	2.7 ± 0.5	a
	Fe	222 ± 66	a	75 ± 19	b	87 ± 20	b
	Mn	238 ± 73	a	461 ± 244	b	233 ± 74	a
	Ni	1.3 ± 0.2	a	0.7 ± 0.2	b	0.7 ± 0.3	b
	Pb	1.9 ± 0.6	a	1 ± 0.7	b	0.5 ± 0.2	c
	Zn	23 ± 5	a	20 ± 7	a	26 ± 10	a
	Al	53 ± 8	a	25 ± 10	b	24 ± 7	b
STEM	Cd	0.02 ± 0.01	a	0.03 ± 0.02	b	0.02 ± 0.01	a
	Cr	1.2 ± 0.2	a	0.9 ± 0.3	a	0.8 ± 0.4	a
	Cu	1.6 ± 0.9	a	1.8 ± 1.2	a	0.8 ± 1.2	a
	Fe	70 ± 20	a	17 ± 4	b	22 ± 10	b
	Mn	58 ± 11	a	55 ± 29	a	20 ± 9	b
	Ni	1.4 ± 0.6	a	0.4 ± 0.2	b	0.6 ± 0.3	b
	Pb	0.3 ± 0.1	a	0.2 ± 0.1	b	0.2 ± 0.1	b
	Zn	33 ± 9	a	34 ± 17	a	38 ± 22	a
	Al	8 ± 2	a	5 ± 1	b	5 ± 3	b

Substrate type is the main factor that explains observed differences in chl a content (substrate effect, $p<0.001$). Plants grown on the uncontaminated clay soil (N) have a significantly lower content of this pigment (1164.2 ± 116.8 µg/g fresh weight) compared to the contaminated upland soil (C) (1985.3 ± 106.1 µg/g fresh weight) and the contaminated dredged sediment (L) (1940.3 ± 104.1 µg/g fresh weight) (Figure 14). Younger leaves have on average a higher chl a content than older leaves (leaf effect, $p<0.05$). Date of sampling and all possible interactions between substrate type, leaf and date of sampling were not significant ($p_{all}>0.17$). Substrate type exerted a similar effect on the chl b content of the leaves (substrate effect, $p<0.001$). The amount of chl b in reed on the clay soil was almost 50 % of the amount measured in the other two substrates (N: 346.1 ± 42.8 compared to C: 611.4 ± 38.3 and L: 656.7 ± 37.6 µg/g fresh weight). Leave age, date of sampling and all the interactions between the factors were not significant ($p_{all}>0.11$).

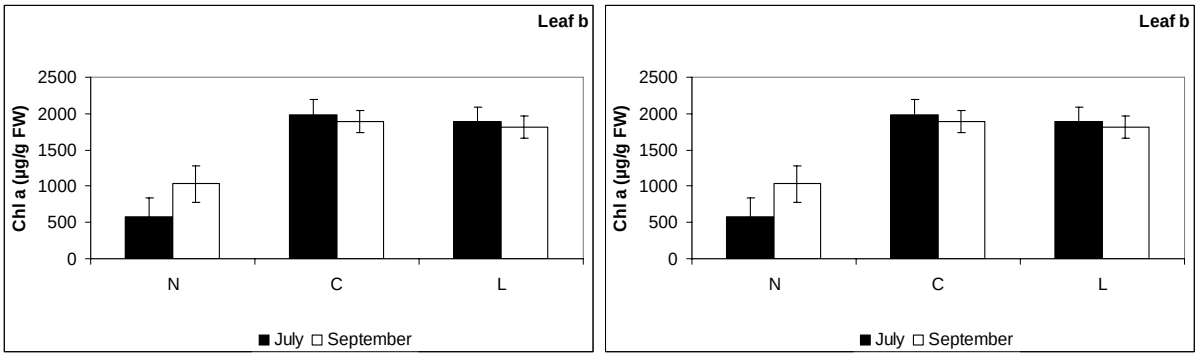


Figure 14: Chlorophyll a content of a young leaf (leaf a) and an older leaf (leaf b) of *Phragmites australis* grown on different substrates (N, C and L). Data are presented as mean ± SE

Carotenoid content was only related to sampling time (date effect, $p<0.001$), with the highest values noted in September (296.4 ± 11.6 µg/g fresh weight compared to July values of 213.6 ± 15.8 µg/g fresh weight). Substrate type, leave age and all interactions were not significant ($p_{all}>0.09$).

Fluorescence values were influenced by substrate type. On average the lowest values were noted in the uncontaminated clay soil (N) (substrate effect, $p<0.05$). Average values of F_v/F_m were lower in leaf b (leaf effect, $p<0.01$) (Figure 15). Substrate type and leaf interact indicating that the quantum yield in each leaf is differently affected by substrate type (substrate*leaf interaction, $p<0.01$). F_v/F_m shows similar values in the younger (leaf a) and the older leaf (leaf b) for the C and the L substrates, but fluorescence values measured for leaf b in the N substrate are significantly lower than those in leaf a. All the other effects and interactions were not significant ($p_{all}>0.06$). No significant effects on leaf enzyme activity between plants grown on the different substrates were noted.

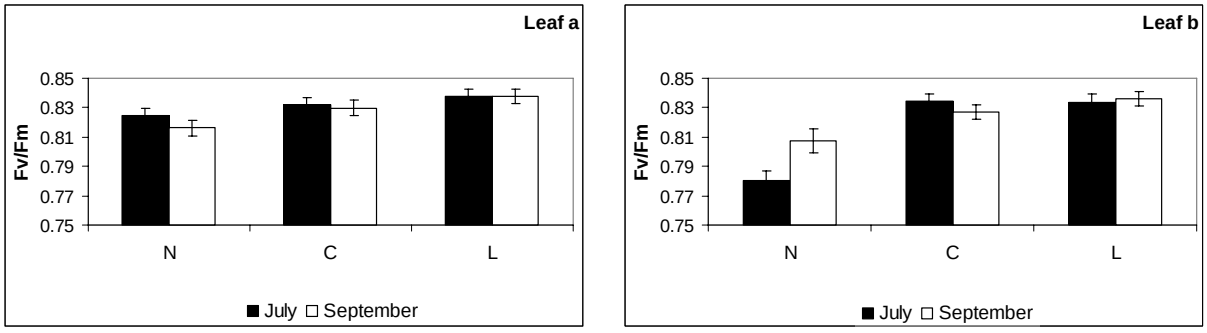


Figure 15: Chlorophyll a fluorescence measured in a young leaf (leaf a) and an older leaf (leaf b) of *Phragmites australis* grown on different substrates (N, C and L). Data are presented as mean ± SE

3.2.5 Invertebrates

- *Tubifex tubifex*

In the permanently flooded treatments (P) the Zn body burdens were on average significantly lower after a prolonged period of inundation (inundation effect, $p<0.001$), and a lower salinity (salinity effect, $p<0.05$), and were lower in L compared to C (substrate effect, $p<0.001$) (Figure 16). However, these effects interacted significantly with each other, indicating that the effects differed among substrate types and salinity treatments.

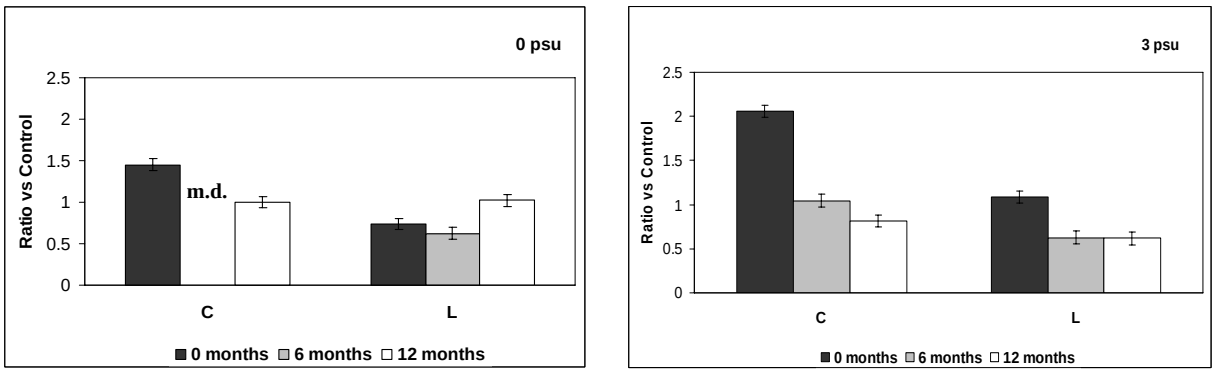


Figure 16: Zn accumulation in *Tubifex tubifex* in two contaminated substrates (C and L) after 0, 6 and 12 months of inundation with water of different salinity (0 and 3 psu). Data are presented as mean ± SE. m.d. = missing data

Accumulation in each substrate is differently affected by the applied salinity treatment (substrate*salinity interaction, $p<0.05$) and inundation period (substrate*inundation interaction, $p<0.001$). Both factors did not affect Zn accumulation in the L, but prolonged inundation period led to a distinct decrease in Zn accumulation in C and accumulation was on average higher when brackish water was applied. Salinity and inundation period interacted (salinity*inundation interaction, $p<0.001$), the decline in Zn accumulation with prolonged flooding was more pronounced when a higher salinity was applied. The three-way interaction between substrate type, salinity and

inundation period was not significant (substrate*salinity*inundation interaction, p=0.85).

Results for Cu accumulation were similar to those obtained for Zn. Prolonged flooding generally resulted in a decrease of Cu body burdens (inundation effect, p<0.001), as did a lower salinity (salinity effect, p<0.01). On average, accumulation in the C substrate was about three times higher than in the L substrate (5.03 ± 0.21 compared to 1.46 ± 0.14) (substrate effect, p<0.001). Moreover, complex interactions between substrate type, salinity and inundation were noted. The effect of inundation period depended on the substrate type (substrate*inundation interaction, p<0.001). For C, a significant decrease in body burden as a function of time was noted: highest accumulation was observed in the test conducted before the start of the flooding (10.27 ± 0.27) and this was significantly higher than the results obtained after 6 and 12 months of permanent inundation (1.90 ± 0.52 and 2.93 ± 0.24, respectively). In the upland soil, Cu accumulation was slightly, but significantly, higher in the 3 psu treatments. The dredged sediment, on the other hand, was not affected by salinity (substrate*salinity interaction, p<0.001). The interactions between inundation and salinity; substrate, salinity and inundation period were not significant (inundation*substrate*soil interaction, p_{all}>0.9).

The weight of the retrieved animals depended on substrate type (substrate effect, p<0.001) and inundation period (inundation effect, p<0.05) (Figure 17). On average, animals collected from the substrates before the start of the flooding treatment were larger (1.76 ± 0.11) than animals retrieved from the substrates that had been inundated for 6 and 12 months (respectively 1.21 ± 0.14 and 1.51 ± 0.12). In addition, individuals retrieved from the sediment (1.93 ± 0.10) weighed significantly more than animals maintained in the upland soil (1.07 ± 0.10). Inundation time and salinity interacted (salinity*inundation interaction, p<0.01): a decrease in growth was observed in the 0 psu treatments, while growth in the 3 psu treatments showed a slight increase. Each substrate type seemed to react differently to the prolonged inundation period (substrate*inundation interaction, p<0.05). Salinity, the interaction between substrate and salinity and the three-way interaction between substrate, salinity and inundation period were not significant (substrate*inundation*salinity interaction, p_{all}>0.13). In conclusion, growth in the 0 psu treatments seems to be negatively affected by inundation time, which was the most obvious in the dredged sediment. An opposite but less clear trend was observed in the 3 psu treatments.

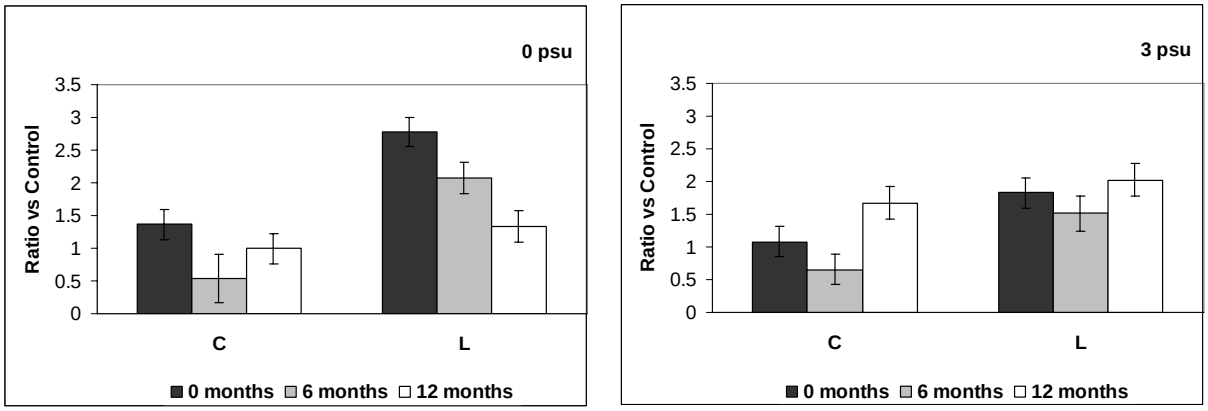


Figure 17: Growth of the oligochaete *Tubifex tubifex* in two contaminated substrates (C and L) after 0, 6 and 12 months of inundation with water of different salinity (0 and 3 psu). Data are presented as mean ± SE

Regarding cocoon production, no general trend could be detected in the treatments. The influence of inundation time on reproduction depended on the applied salinity and substrate type (inundation*substrate*salinity interaction, $p<0.001$). Cocoon production was significantly lower in C (1.02 ± 0.03) compared to L (1.24 ± 0.03) (soil effect, $p<0.001$). In general, a higher salinity had a negative effect on cocoon production (salinity effect, $p<0.01$). A longer inundation period resulted on average in a lower cocoon production (inundation effect, $p<0.01$). Moreover, cocoon production showed a significant decrease in L with prolonged inundation period (soil* inundation interaction, $p<0.05$). With prolonged inundation a significant decrease in cocoon production was noted at 0 psu, while no significant differences were observed at 3 psu (psu*inundation interaction, $p<0.01$). The interaction between substrate and salinity was not significant (substrate*salinity interaction, $p>0.07$).

Results from the bioassay with the substrates subjected to the summer dry/winter wet regime were compared to the results of the test conducted with the substrates which had been permanently flooded during 12 months. Zn body burdens were significantly lower in the L substrate (substrate effect, $p<0.001$) and in the permanently flooded treatment (water regime effect, $p<0.001$). However, complex interactions were found between salinity, substrate type and water regime. Accumulation in each substrate is differently affected by salinity (substrate*salinity interaction, $p<0.05$) and the applied water regime (substrate*water regime interaction, $p<0.001$). In the upland soil accumulation was highest in the freshwater treatments while in the dredged sediment accumulation was higher in the brackish treatments; however none of these differences were significant. The effect of the applied water regime was higher in C; the permanently flooded treatments showed on average a 3.4 times lower Zn accumulation in C, while in L this was only 1.7. The three way interaction between salinity, substrate and applied water regime was significant (substrate*salinity*water regime interaction, $p<0.01$). All but one substrate-salinity combinations react strongly

to the different water regimes; the summer dry/winter wet treatment causes significantly higher body burdens of Zn. Only L in the 0 psu treatment shows no reaction to this treatment. Salinity, and the interaction between salinity and water regime were not significant (p_{all}>0.054).

Average Cu body burdens were significantly lower in the freshwater treatment (salinity effect, p<0.001), lower in the permanently flooded treatment (water regime effect, p<0.001) and lower in the sediment compared to the soil (substrate effect, p<0.001) (Figure 18). However, these factors interacted significantly with each other, indicating that the effects differed among substrate and salinity treatments. Accumulation in each substrate type was differently affected by salinity (salinity*substrate interaction, p<0.01). Salinity had significant effects on Cu accumulation in both substrates, but this was more pronounced in L. Cu accumulation was always higher in the brackish treatments; for L an increase of a factor 3.6 when comparing the freshwater and the brackish water treatments was noted while in C this was only 1.4. Salinity and the applied water regime interacted (salinity*water regime interaction, p<0.001).

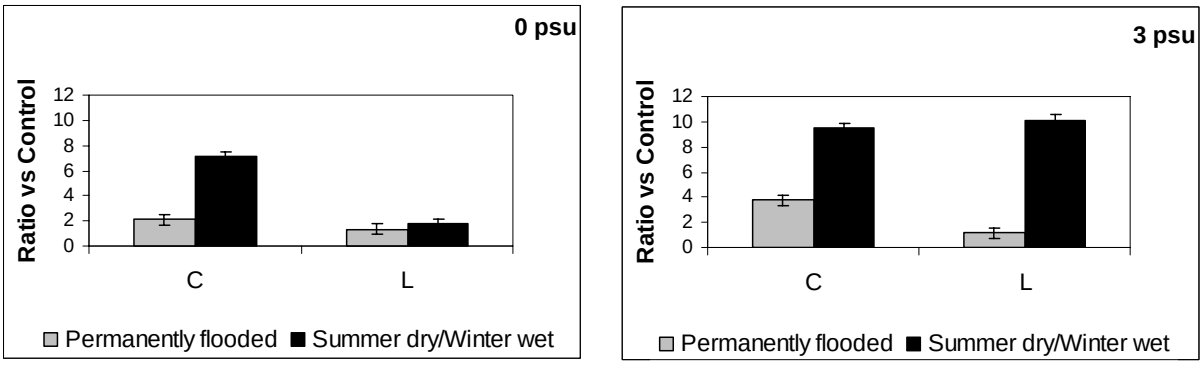


Figure 18: Cu accumulation in *Tubifex tubifex* in two contaminated substrates (C and L) after 12 months of inundation with water of different salinity (0 and 3 psu) and subjected to different water regimes (permanently flooded and summer dry/winter wet). Data are presented as mean ± SE

The increase in Cu accumulation was on average more pronounced in the summer dry/winter wet regime when a higher salinity was applied. The three way interaction between salinity, substrate type and water regime was significant (salinity*substrate*water regime interaction, p<0.001). Almost all substrate-salinity combinations show strong significant increases in Cu accumulation when comparing the permanently flooded and summer dry/winter wet regimes, except the dredged sediment in the 0 psu treatment which showed almost no increase in Cu body burdens. The interaction between substrate and applied water regime was not significant (substrate*water regime interaction, p=0.31).

On average individuals were larger in the dredged sediment (substrate effect, p<0.05) and in the brackish water treatments (salinity effect, p<0.05). Average weight did not differ in the summer dry/winter wet regime when a different salinity was

applied. On the other hand when the substrates were permanently flooded, average weight of the organisms was significantly higher in the brackish treatments (water regime*salinity interaction, $p<0.05$). Applied water regime, the interactions between substrate and salinity, substrate and applied water regime and the three way interaction between substrate, salinity and water regime were not significant ($p_{all}>0.76$).

Cocoon production was significantly affected by the applied water regime, with on average the highest production in the permanently flooded substrates (water regime effect, $p<0.001$) (Figure 19). Substrate type and applied water regime interacted (substrate*water regime interaction, $p<0.01$). Average cocoon production in the upland soil does not seem to be affected by regime type, while production was significantly lower in the sediment in the summer dry/winter wet regime when compared to the permanently flooded treatment. The three way interaction between substrate, salinity and water regime was significant; cocoon production showed no significant decreases in C for each salinity-water regime combination.

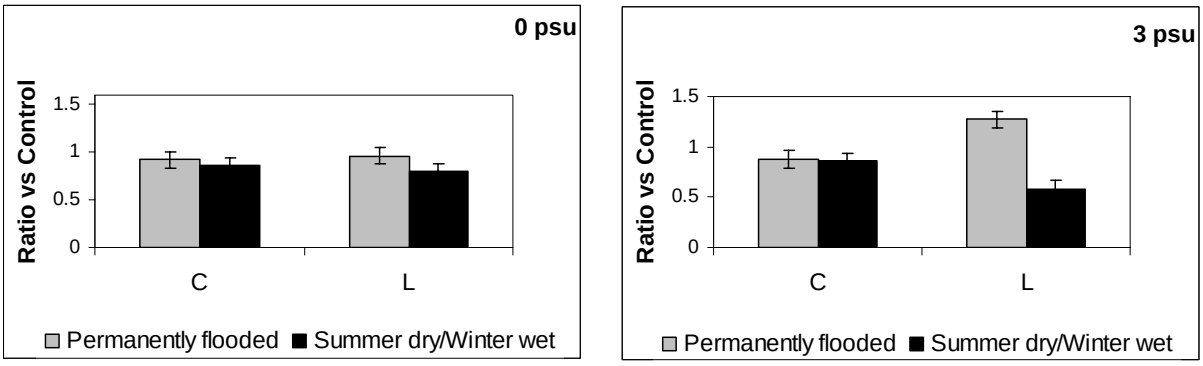


Figure 19: Cocoon production per adult of *Tubifex tubifex* in two contaminated substrates (C and L) after 12 months of inundation with water of different salinity (0 and 3 psu) and subjected to different water regimes (permanently flooded and summer dry/winter wet). Data are presented as mean ± SE

However, while no decrease in cocoon production was noted for L in the freshwater treatments, a significant decrease was noted in the 3 psu treatments when comparing the permanently flooded and the summer dry/winter wet regime. Substrate type, salinity, the interaction between substrate and salinity, and applied water regime and salinity were not significant ($p_{all}>0.052$).

- *Orchestia gammarellus*

For the permanently flooded treatments (P) the Zn body burdens were, on average, significantly lower at the start of the flooding treatment (inundation effect, $p<0.001$)

(Figure 20). Inundation and salinity interacted (salinity*inundation interaction, p<0.001): in the 3 psu treatments, highest Zn accumulation was observed after 12 months of permanent flooding; in the saltwater treatments accumulation was higher after 6 months of inundation. Salinity, substrate type, the interaction between substrate type and salinity, substrate type and inundation period, and the three way interaction between salinity, substrate type and inundation period were not significant (p_{all}>0.086).

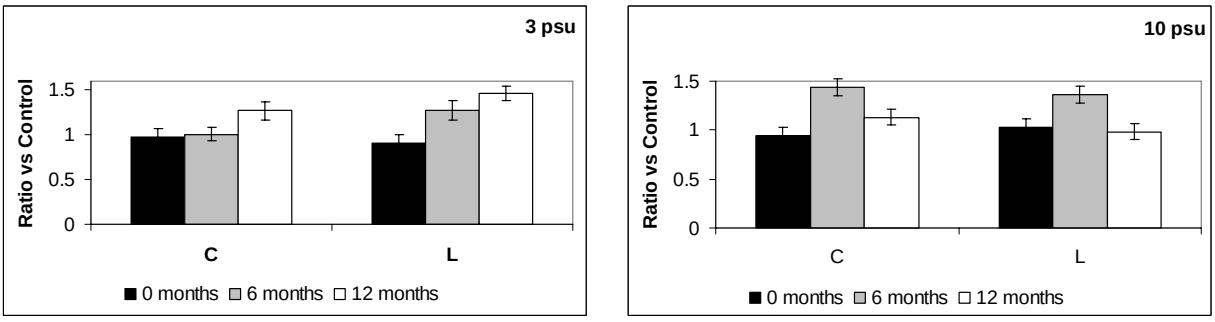


Figure 20: Zn accumulation in *Orchestia gammarellus* in two contaminated substrates (C and L) after 0, 6 and 12 months of inundation with water of different salinity (3 and 10 psu). Data are presented as mean ± SE

On average, Cu accumulation was lower in the 10 psu treatments and after 6 months of flooding (salinity effect, p<0.01; inundation effect, p<0.001). Substrate type, the interactions between substrate type and salinity, substrate type and inundation period, salinity and inundation period, and the three way interaction between salinity, substrate type and inundation were not significant (p_{all}>0.09). Amphipods were on average larger in the saltwater treatments (salinity effect, p<0.001) and were larger when substrates had been flooded during 6 months (inundation effect, p<0.001). No significant effects were found for substrate type, the interaction between substrate type and salinity, substrate type and inundation, salinity and inundation, and the three way interaction between salinity, inundation period and substrate type (p_{all}>0.07).

For regime R, the Zn accumulation was on average significantly higher in the dredged sediment (L), in the brackish treatments and in the permanently flooded substrates (substrate effect, p<0.05; salinity effect, p<0.01 and water regime effect, p<0.05). Furthermore, salinity and substrate type interacted: Zn body burdens were significantly lower when saltwater was used in the sediment, while accumulation in the upland soil did not seem to be affected by the salinity treatments (substrate*salinity interaction: p<0.05). All the other interactions were not significant (p_{all}>0.08).

Cu body burdens were significantly higher in the summer dry/winter wet treatments (water regime effect, p<0.05) (Figure 21). However, substrate type and applied water regime interacted: Cu body burdens in C were significantly higher when subjected to the summer dry/winter wet regime; accumulation in L was not affected by applied

water regime (water regime*substrate type interaction, $p<0.05$). All other effects and possible interaction types were not significant ($p_{all}>0.05$).

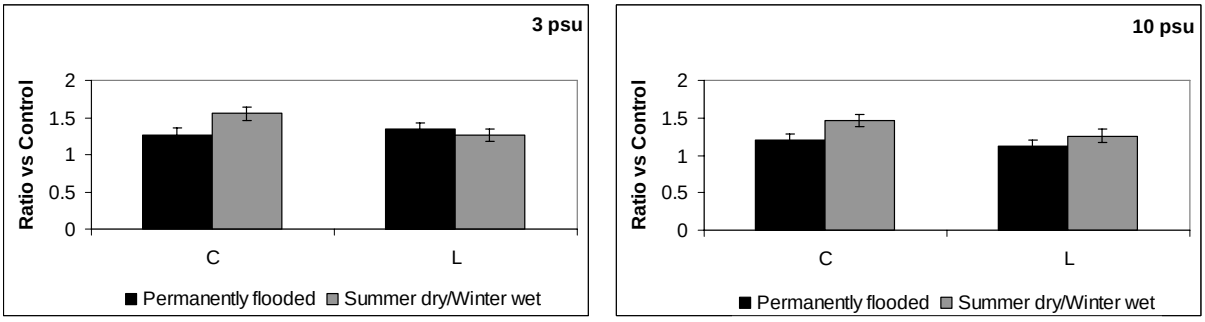


Figure 21: Cu accumulation in *Orchestia gammarellus* in two contaminated substrates (C and L) after 12 months of inundation with water of different salinity (0 and 3 psu) and subjected to different water regimes (permanently flooded and summer dry/winter wet). Data are presented as mean ± SE

Amphipods were on average larger in the summer dry/winter wet regime (water regime effect, $p<0.05$) (Figure 22). Significant interactions exist between water regime, substrate type and salinity. Smallest individuals were retrieved from the permanently flooded dredged sediment, growth in the upland substrate was not influenced by the applied water regime (water regime*substrate interaction, $p<0.05$). Salinity and water regime interacted: individuals from the permanently flooded substrates treated with brackish water were significantly smaller when compared to the 3 psu summer dry/winter wet treatment; no differences in growth were observed between both water regimes when salt water was used (salinity*water regime interaction, $p<0.05$). The three way interaction was significant (substrate*water regime*salinity interaction, $p<0.05$). No differences in growth were noted for C in none of the salinity treatments; on the other hand while L showed no difference in the saltwater treatments, an increase in growth was noted in the brackish water treatments when comparing the permanently flooded and the summer dry/winter wet regimes. All other effects and possible interaction were not significant ($p_{all}>0.06$).

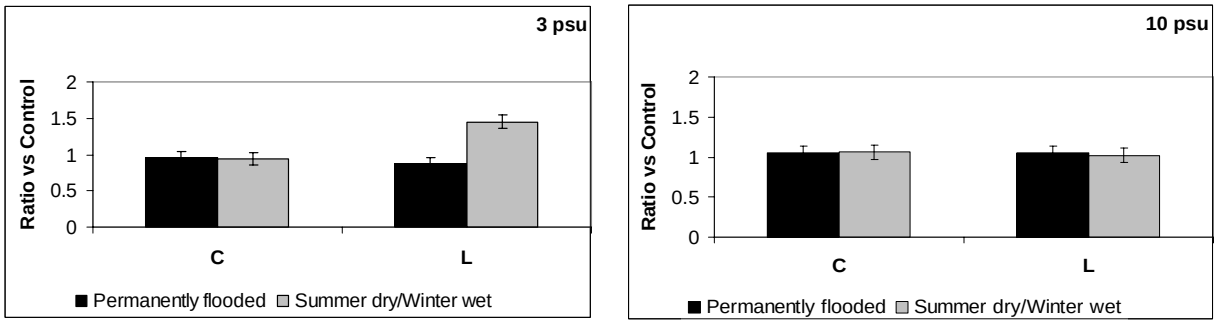


Figure 22: Growth of *Orchestia gammarellus* in two contaminated substrates (C and L) after 12 months of inundation with water of different salinity (0 and 3 psu) and subjected to different water regimes (permanently flooded and summer dry/winter wet). Data are presented as mean ± SE

3.3 Discussion

Soil characteristics

The three soils that were used in the experiment have a similar texture, but differ in a few other characteristics. Soil C and L are both contaminated with metals. The total metal content of both soils exceeded the background concentrations in Flanders (Vlarebo, 1996). The Cd level in soil C exceeded the soil sanitation values for agricultural and nature areas. The metal content in soil N was much lower than the background concentration for all metals, except for Ni and Cr which were just above that value. Metal concentrations and general soil characteristics of soil C, an alluvial soil along the Zeeschelde, were within the range observed for operational flood control areas in the freshwater part of the Zeeschelde, with average Cd and Zn concentrations between 3.7-22.6 and 475-1900 $mg.kg^{-1}$ dry soil for Cd and Zn respectively (Vandecasteele et al., 2004b). Soil N had a rather low $CaCO_3$ content in comparison with unpolluted alluvial soils sampled in the same area (Vandecasteele et al., 2004b).

The two contaminated soils differed in ORP status. Soil C is an upland soil that was oxidised, while soil L is a reduced dredged sediment. Soil N was selected as an uncontaminated control. Due to the previous oxidation of pyrite (FeS_2) and the absence of a pH buffering agent such as $CaCO_3$, the pH of soil N was very low, which made it interesting for studying the effect of the low soil pH on the metal mobility. The pH of that soil was not homogenous, which is reflected in the high standard deviation of the soil pH (Table 1) and the high variability of the pore water pH (Figure 11). The low pH also resulted in a higher conductivity. The other two soils showed a high content of $CaCO_3$ and a neutral or slight alkaline pH. The CEC of soil C was the highest, due to the high OM content and the low sand content. The high variability in the chloride content is due to the differences between the depths, which are probably caused by the low permeability. The total Cd, Cu, Pb and Zn concentrations were lower in soil N than in the other two soils, but the pore water concentrations of all the metals (except Mn) were much higher.

The low pH of soil N can explain this phenomenon. The metal pore water concentrations (Figure 5 to Figure 10) of soil N show much variation between the treatments and between the depths. These variations can be coupled to the pore water pH (Figure 11). The highest metal concentrations correspond with the lowest pH values. The ORP also seems to affect the metal pore water concentration. When comparing the two oxidised soils (soil N and C), the effect is clearer in soil C. The ORP of soil N reaches in general higher values than in soil C, but that is probably due to the pH effect. A decrease of 1 pH unit accounts for an increase of 59 mV in the ORP. Taking this into account, the ORP in both soils is pretty much comparable.

However, faster changes of the ORP are observed in soil C, which could due to a higher microbial activity. An environment with a more neutral pH is generally more favourable for micro-organisms. Moreover, the higher OM-content in soil C also provides electron donors, which are necessary for microbial growth.

Soil chemistry

The influence of the ORP on the metal pore water concentration is different for each metal and also depends on the initial redox status of the soil. Under oxidized conditions the behaviour of Zn and Cu is mainly regulated by organic carbon, Fe and Mn oxides, which efficiently adsorb or occlude trace metals (Guo et al., 1997; Emmerson et al., 2000; Huggett et al., 2001). A decrease of the redox potential may cause changes in metal oxidation state, leading to the formation of new low-soluble minerals, and reduction of Fe and Mn oxides, resulting in the release of associated metals (Tack et al., 1998; Ma & Dong, 2004). This can be seen in the metal pore water concentration of soil C. Fe, Mn and Ni concentrations increase when the soil is being flooded and decrease when the soil is being dried. In the first dry period the Fe and Mn concentrations also increase, which indicate that the water, periodically added to simulate showers, induced reducing conditions. In soil N those mechanisms are harder to verify by looking at the metal pore water concentrations. Only Fe in that soil shows the same concentration profile as in the pore water of soil C. The predominant influence of the low pH maybe is an explanation for this.

Different processes occur when the soil was subjected to reducing conditions during a longer period of time. Although the proportion Zn and Cu associated with Fe and Mn oxides in sediments decreases under reducing conditions; the proportion associated with carbonates, insoluble humic substances and sulphides increases (Guo et al., 1997; Chapman et al., 1998; Chapman & Wang, 2001).

During a drying period however, the sulphides are oxidised again and the metals are released. Of the three soils, soil L is the only soil which contained measurable amounts of sulphides in the beginning (Figure 1). After a dry period (with periodic simulated showers) of 200 days (at day 280) the soil is not oxidised at a depth of -20 cm. The ORP of soil L (Figure 4) at that time is still low and the metal pore water concentrations are also very low and constant. From that moment, the ORP value rises in the top layers (and later also in the deeper layers) and the concentration of Cd, Cu, Mn, Ni and Zn in the pore water increases. This, together with the lower measured values of sulphides after one year, indicates that the metal sulphides are being oxidised and the metals are released. The pH of the pore water did not decrease due to the formation of sulphate, probably because of the high $CaCO_3$ content, present in that soil. Fe concentrations in the pore water did not increase

much, indicating that no large amounts of Fe sulphides were present in the soil or that the released Fe is quickly oxidised and immobilised as Fe oxides.

Pore water

When the pore water concentrations are compared with soil sanitation values for groundwater (ssv_g), different conclusions should be drawn than when looking only at the total metal content. In general, the metal concentrations in the pore water of the uncontaminated soil N always exceed the sanitation value for Cd (5 $\mu g.L^{-1}$), Ni (40 $\mu g.L^{-1}$) and Zn (500 $\mu g.L^{-1}$) (Vlarebo, 1996), while this is most of the time not the case in the contaminated soils C and L. However, when soil L is being oxidised, the Cd, Ni and Zn concentrations also exceed the ssv_g. The ssv_g of Cu (100 $\mu g.L^{-1}$) and Cr (50 $\mu g.L^{-1}$) is never exceeded in the pore water of the contaminated soils, while in the pore water of soil N with the lowest pH, the ssv_g is frequently exceeded. It has to be mentioned that the deionised, oxygen-rich water used in the experiment mimics the worst case scenario. River water is likely to contain OM which can be used as a food source for sulphate reducing bacteria and thus hindering a rapid oxidation which is seen in these tests.

It is clear that when a reduced sulphide-rich soil is allowed to oxidise, this can pose much problems concerning metal mobility. When the buffering capacity for the pH of the soil is depleted (e.g. through dissolution of carbonates), soils with a very low metal content can pose huge problems for a prolonged period of time. One should not allow this to happen, as after initial oxidation, it takes over two years of inundation to reduce pore water concentrations again below the sanitation thresholds. In soils which are well buffered and reduced on the other hand, the metal mobility is minimal. The pore water concentrations remain very low, even if the total metal content is higher than the widely accepted and used soil sanitation values. As long as sulphides are being oxidised, but the buffering capacity has not been depleted, sanitation thresholds can also be exceeded in soils with a high total metal content, but the metal mobility increases are much smaller than when no buffer is present. A reduced sulphide rich soil or sediment should thus not be oxidised to preserve its buffering capacity and to prevent that metals would be mobilised.

Reed growth and metal uptake

The variations in reed characteristics are also significant. The reed that grew on soil N was smaller, thinner, less dense and had a lower productivity than the reed that grew on the other two soils. The concentrations of most metals (except Mn) did not differ significantly between reed plants that grew on the contaminated soils (C and L). The differences in the reed growth between these two soils could thus not be attributed to the metal contamination, but is most likely due to the better nutrient status of the dredged sediment. Reed, an important plant species in the study area,

is generally not known as an accumulator of Cd and Zn on polluted soils. However, metal concentrations in decaying reed litter may significantly increase due to contact with polluted sediments (Du Laing et al., 2006). In contrast to reed, willows may be used as good indicators for Cd and Zn bioavailability on sediments, freshwater tidal marshes and alluvial soils (Vandecasteele et al., 2005). In the Scheldt estuary, freshwater tidal marshes with willow woods occur as a climax stage between the Rupel and the Dender embouchures.

The lower amount of chl a and chl b in reed leaves grown on the uncontaminated clay soil is probably due to the low pore water pH and related to the high sulphate concentrations in this substrate. Low pore water pH does not only have an influence on the growing performance of reed, but also promotes a higher metal availability (see also "total metal contents in reed"). It has been recognised that metals directly or indirectly inhibit photosynthesis through different mechanisms: one of them being through interference with chlorophyll synthesis (Lagriffoul et al., 1998). During sampling, one could already visually observe differences between the three substrates: reed growing on C and L was larger and much greener than reed grown on N, the latter being smaller, having a lower density and a yellow colour. Higher pigment content of 'leaf a' could be related to the age and associated physiological status of the leaf. Carotenoid content was only related to sampling time with the highest values noted in September 2004, which may be attributed to the die back of the reed at the end of the growing season.

Although one would expect the lowest fluorescence values in reed grown on metal contaminated substrates (C and L), the opposite was observed: lowest values of F_v/F_m were observed in the uncontaminated clay soil (N). In the conducted experiment other substrate properties, like the high sulphate content and especially the lower pH, which influences metal availability, caused the higher stress in the uncontaminated clay soil. Metals can influence the photosynthesis directly by inhibiting the photosynthetic electron transport as well as activities in the Calvin cycle or the assimilation of CO_2. Indirectly they interact with photosynthesis through a decrease in photosynthetic pigments (see above), or damaging the structure of the chloroplasts, lipid and protein composition of the thylakoid membranes (Burzynski & Klobus, 2004). So a higher amount of metals in the leaves probably interferes with photosynthesis, and as fluorescence is inversely proportional to the rate of the photosynthetic activity, this is reflected in the F_v/F_m measurements.

Tubifex tubifex

For soil and sediment associated metals; bioavailability is expected to vary with environmental variables such as pH, organic matter, redox potential, acid volatile sulphide and cation exchange capacity (Tessier & Campbell, 1987; Van Gestel et al., 1995; Ankley et al., 1996; Bervoets et al., 1997; Hund-Rinke & Kördel, 2003) and

biological factors such as vegetation, species dependent regulation mechanisms, habitat and feeding behaviour (Hund-Rinke & Kördel, 2003; Peijnenburg & Jager, 2003; Hendrickx et al., 2004). Although Zn and Cu are metals which are known to be essential for normal metabolic functioning, and are assumed to be physiologically regulated by some freshwater invertebrates, there is clearly an influence of inundation period on the accumulation of both metals (Rainbow & Dallinger, 1993; Timmermans, 1993; Parametrix, 1995). The impact of inundation period is closely linked to the redox conditions in the substrates at the beginning of experiment, which may also explain the different accumulation patterns between the substrate types. At the start of the experiment, the dredged sediment was already strongly reduced (-275 mV in 0 psu; -245 mV in 3 psu) while conditions in the upland soil point to a rather oxidized state (45 mV in 0 psu; 251.7 mV in 3 psu). The lower accumulation of Zn and Cu with prolonged flooding in the upland soil (C) is probably associated with reduced conditions under which Zn and Cu become less mobile and less available to *T. tubifex*. The sediment, which was not influenced by inundation period, showed a more stable redox profile during the course of the experiment, so that no major changes in bioavailability would be expected.

In addition to the effect of flooding, a higher accumulation of Zn and Cu was observed for the upland soil in the brackish treatment (compared to the freshwater treatment), which is probably due to a salt effect. The reaction of bound metals to flooding with saline water depends on two counteracting processes: (1) desorption regulated by complexation with salt water anions (Cl^- and SO_4^{2-}) and/or competition for sorption sites with cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and (2) coagulation, flocculation and precipitation (Chapman & Wang, 2001). Initial accumulation was always higher at the start of the experiment (0 months of incubation) in the 3 psu treatments. Inundation with saline water (of substrates that were initially not saline) may hence cause the dissolution of minerals such as Fe and Mn oxides and thus result in the release of associated trace metals (Emmerson et al., 2000). However, while desorption of metals with increasing salinity can result in increasing metal concentrations in the overlying water, concurrent increases in Ca^{2+} and Cl^- can decrease bioavailability (Chapman & Wang, 2001; Riba et al., 2004). It seems likely, as only the upland soil was affected by the salinity treatment, that during the first weeks of inundation – i.e. while the substrate is still oxidised – enhanced bioavailability of Zn occurs because of dissolution of Fe and Mn oxides. Later on, when due to the inundation the substrate becomes more reduced, the amount of dissolved Zn decreases due to binding to e.g. carbonates, sulphides or organic carbon.

Equivocal effects of salinity and flooding on the growth and reproduction of *T. tubifex* remain unclear. Growth seems to decrease with prolonged inundation in the freshwater treatments, while an opposite but less clear trend was observed in the

brackish treatments. Cocoon production was clearly adversely affected at 0 psu in the sediment, while the other treatments were unaffected. However, life history traits can be expected to be strongly dependent on substrate characteristics. Lacey et al. (1999) stated that in sediments with similar loss-on-ignition values, the quality and type of the available organic matter can strongly influence growth and can give rise to confounding effects in ecotoxicological tests. It is also possible that other contaminants, e.g. organic substances, are present that may have influenced reproduction and growth.

The impact of the different water regimes is linked to redox potential; oxidation of the substrates due to the dry period will alter metal bioavailability. As Cu and Zn become less mobile and less available with prolonged flooding due to more reduced substrate conditions (see above), oxidation of the substrates will initiate processes which promote a higher metal availability. Oxidation of reduced soils and sediments can cause the release of the associated metals through different mechanisms e.g. due to oxidation of sulphides and/or a decrease in pH due to oxidation (Cappuyns & Swennen, 2005). In our experiments no significant decrease in pH was noted upon oxidation of the substrates (probably due to buffering by carbonates present in the substrates), so the higher availability of Cu and Zn can not be explained by pH changes. Riedel et al. (1997) stated that higher oxygen levels caused the oxidation of organic substances and sulphides to which Cu was bound, resulting in the release of sediment associated Cu. The amount of organic substances in dredged sediment tends to decrease when exposed to oxidising conditions. This loss of organic matter is associated with chemical changes, e.g. humic material becoming structurally less complex (Gambrell, 1994; Stephens et al., 2001). Metals, which were under reduced conditions strongly bound to sediment organic matter, tend to become less tightly bound e.g. less stable-bound carbonate and exchangeable fractions (Eggleton & Thomas, 2004). Not all metals show a strong response to this loss of organic matter because organic matter in upland soils is also effective in immobilizing them. However, significant releases have been shown for Zn and Cd in particular (Gambrell, 1994). Different, less strong binding mechanisms which are more sensitive to the oxidising conditions are probably the cause of the higher Cu and Zn accumulation in the upland soil. The absence of a response to the freshwater summer dry/winter wet treatment in the dredged sediment remains unclear and can possibly be attributed to substrate variability.

As stated above, the effect of saline water depends on two opposing processes: (1) desorption and/or competition for sorption sites and (2) coagulation, flocculation and precipitation (Chapman & Wang, 2001). When assuming that Cu and Zn interact with different binding sites or that Zn, as stated by Eggleton & Thomas (2004), rapidly readsorbs to particles upon oxidation, the competition between salt water cations as Na^+, K^+, Ca^{2+}, Mg^{2+} and Cu for sorption sites can explain the higher bioavailability of

Cu in the brackish treatments. Another possibility comprises the transformation of Cu from a filterable state into a particulate state (organic and inorganic), as often happens in estuaries, which results in a decrease in dissolved Cu (Hall & Anderson, 1999). As Tubificidae are bottom dwelling organisms which feed on the sediment, such an increase in particulate Cu can cause elevated internal concentrations.
Although average weight of the retrieved organisms was higher in the permanently flooded treatments when brackish water was applied, no effect of salinity was noted for the summer dry/winter wet treatments. Interpretation of these observations is difficult, especially as the variation in growth in the permanently flooded treatments tested after 0, 6 and 12 months was high. The same goes for the cocoon production: only the dredged sediment in the brackish treatments showed a noticeable difference in reproduction when comparing both water regimes. No link was found between Zn or Cu accumulation and the measured population parameters. As stated before, the influence of substrate characteristics and the possible presence of other contaminants can cause these unclear effects. Due to methodological constraints, Cd concentrations could not be measured in *T. tubifex*. Since Cd is an important pollutant in the study area, assessment of the effect of flooding, inundation period and salinity on Cd availability for this organism is an important future research need.

Orchestia gammarellus
Contrary to the *T. tubifex* experiments, none of the observed differences in Zn or Cu accumulation in the *O. gammarellus* experiments could be attributed to the different substrate types. Talitrid amphipods, like *O. gammarellus,* get most of their Cu and Zn from their food rather than from solution; which can explain why no differences in accumulation between the substrates were noted (Weeks & Rainbow, 1993). Zn accumulation was not affected by salinity which is in line with the observations of Weeks & Rainbow (1992). However, although the same authors found no differences in Cu body burdens under different salinity regimes, we observed significant effects of salinity on Cu accumulation. It must be stressed that they exposed amphipods only for a short period (72h) to different salinity regimes while in our bioassay exposure took place during 42 days. Additionally, they used spiked waters while we exposed the organisms to contaminated substrates which makes the comparison even more complicated. It is possible that the inevitable presence of green algae on the substrates causes confounding effects. When such an additional food source is available this can interfere with metal accumulation because next to the substrates and the presented food there is now an additional factor which can mediate with metal uptake.
Amphipods were always larger in the saltwater treatments. The salinity of the brackish water (3 psu) probably coincides with their lower limit of salinity tolerance

and could induce an energy consuming physiological response to maintain their osmoregulatotory balance.

For *O. gammarellus* it has been known that accumulated metals, like Cu and Zn, are detoxified and stored in the form of metal rich granules in the ventral caeca of the midgut (Nassiri et al., 2000). These granules will eventually be removed when these cells complete the cell cycle and leave the body through discharge into and from the gut (Rainbow, 2002). This is not a process of regulation of body metal concentrations, because internal body burdens will still vary with the external availability of metals and in proportion to the amount of metal rich granules in the ventral caeca (Marsden & Rainbow, 2004). This probably explains why no link between metal accumulation and growth was found. Subjecting both substrates to the different water regimes gave rise to different accumulation patterns for Cu and Zn. Zn body burdens were higher in the permanently flooded treatments and Cu accumulation was higher in the summer dry/winter wet treatment. These results can not be satisfactory interpreted in terms of the changing redox conditions in both of the substrates as the summer dry/winter wet regime would promote more oxidised substrate conditions which in turn would cause a higher bioavailability of both metals. Only Cu accumulation in the upland soil seems to react according to this pattern. Furthermore, only Zn body burdens were influenced by salinity; lowest Zn ratios were observed in the sediment treated with salt water. Several studies reported that changes in the salinity of the food or the habitat should have little or no effect on the internal concentrations of Cu or Zn in semi-terrestrial amphipods (Marsden & Rainbow, 2004). As high littoral species depend more on metals from the diet than species that are submerged each tidal cycle, it seems likely that the presence of green algae on the substrate mediated directly or indirectly metal accumulation. King et al. (2005) observed that amphipods in the presence of algae showed an increase in Cu accumulation, which was probably due to a more rapid, but not necessary selective feeding behaviour. So metal accumulation could increase due to an increased partitioning to the pore waters or if the ingestion of metal contaminated algae (growing on the surface of the substrates) would increase (in preference to the offered food source). Nevertheless, the precise origin of the observed differences in metal accumulation remains unclear. Growth was, in general, not affected by the different flooding regimes. Only in the dredged sediment treated with brackish water, growth was significantly higher in the summer dry/winter wet treatment. More importantly, there does not seem to be a link between metal accumulation and growth: i.e. none of the observed accumulation patterns for Cu and Zn are reflected in the growth ratio. This is possibly due to storage of the detoxified metals in granules in the midgut as described higher.

Due to methodological constraints, Cd concentrations could not be measured in *O. gammarellus*. Since Cd is an important pollutant in the study area, assessment of the

effect of flooding, inundation period and salinity on Cd availability for this organism is an important future research need.

3.4 Conclusions and recommendations

Metal (im)mobilization kinetics were assessed with a very high resolution for different soils upon changing hydrological conditions. These are primarily important to be incorporated in models in the near future. These models should allow to predict metal mobility changes at the oxic-anoxic interface, where the already existing geochemical models fail due to the fact that they are based on steady state conditions. Moreover, the results confirmed that the metal mobility in the soil does not depend on the total metal content. When assessing potential risks involving metal mobility and toxicity, different factors such as soil pH, CEC, salinity, redox status and carbonate, OM and clay content must be considered. The hydrological regime is found to be a very important factor controlling metal mobility in contaminated flood control areas. It is clear that variations in the redox status of the soil significantly affect the amount of metals in the pore water. When contaminated reduced sediment is being oxidised, metal availability increases quite rapidly. Flooded conditions minimize the availability of metals in the long run. It is clear that when a reduced non-calcareous, sulphide-rich soil is allowed to oxidise, this can pose much problems concerning metal mobility. When the buffering capacity for the soil pH is depleted (e.g. through dissolution of carbonates), even soils with a very low total metal content can pose huge problems for a prolonged period of time. One should not allow this to happen, as after initial oxidation, it takes over two years of inundation to reduce pore water concentrations again to acceptable levels (e.g. below sanitation tresholds for groundwater). In soils which are well buffered and reduced on the other hand, the metal mobility is minimal. The pore water concentrations remain very low, even if the total metal content exceeds the soil sanitation values, which are currenly being used. As long as sulphides are being oxidised, but the buffering capacity has not been depleted, metal contents in pore water can also be very high (e.g. exceed sanitation tresholds for groundwater) in soils with a high total metal content, but the metal mobility increases are much smaller than when no buffer is present. A reduced sulphide rich soil or sediment should thus not be oxidised to preserve its buffering capacity and to prevent that metals would be mobilised. However, some other elements, such as arsenic (As), may become more mobile in these circumstances and breakdown of organic pollutants may be inhibited, which needs further investigation.

Also the salinity of the flooding water primarily affects the mobilization of Cd in contaminated soils. The Cd concentration in the pore water increases with increasing

salinity of the flooding water. This is an important aspect to take into consideration when creating wetlands in the brackish parts of the Scheldt estuary.

Similar observations were made for the accumulation of metals such as Zn and Cu by benthic organisms like *T. tubifex*. The metal accumulation was found to strongly depend on the inundation period. Although initial accumulation of Cu and Zn was higher in the contaminated upland soil (C) at the start of the permanent flooding treatment, these differences seem to disappear after 6 months of permanent inundation, and are probably associated with the change in redox potential as they were not observed in the reduced sediment (L). When the same substrates were allowed to oxidise in the summer dry/winter wet treatment, Zn and Cu became on average more mobile, resulting in a higher accumulation. Due to methodological constraints, Cd concentrations could not be measured in *O. gammarellus* and *T. tubifex*. Assessment of the effect of flooding, inundation period and salinity on Cd availability for these organisms is an important future research need. When a wetland is constructed in a historically metal-polluted area, permanently leaving a minimal amount of water in the area can reduce the metal transfer from the sediment to the benthic fauna, and possibly to higher trophic levels.

The reed growth was negatively affected by the low pH which was initiated by the oxidation of the pyrite in the uncontaminated soil. To prevent such negative effects on reed growth, the oxidation of sulphide rich soils should be prevented. Reed growth does not seem to be affected by environmental metal pollution levels and the plants do not seem to accumulate metals as Cd, Zn and Cu. The latter however also implies that other plants, e.g. willows or *Scirpus maritimus*, may be better suited for measuring metal availability for plants.

in aboveground plant parts.

4 FIELD SCALE EXPERIMENTAL SETUP

4.1 Materials and methods

4.1.1 Experimental setup

The mesocosm site consists of a metal construction of 4 tanks (2.56 m²) (Figure 24), placed on a platform in the Schelde estuary approximately 30 m off the left river bank at Kruibeke, which lies about 100 km from the mouth. Tanks are flooded every high tide of 4.80 m TAW, which means twice a day. The tanks are filled with contaminated and non-contaminated soils from two nearby polders (Lippenbroek polder and Niel polder), and in 2 tanks reed (*Phragmites australis* (Cav.) Steud) was planted to result in a 2*2 experimental design. Reed rhizomes were harvested in Kijkverdriet (Steendorp). Reed was planted in one contaminated and one less contaminated soil. In both treatments, the same amount of reed was planted, taking into account the number of living nodes at the rhizomes and the weight of the rhizomes. The tanks were numbered A, B, C and D (Figure 23).

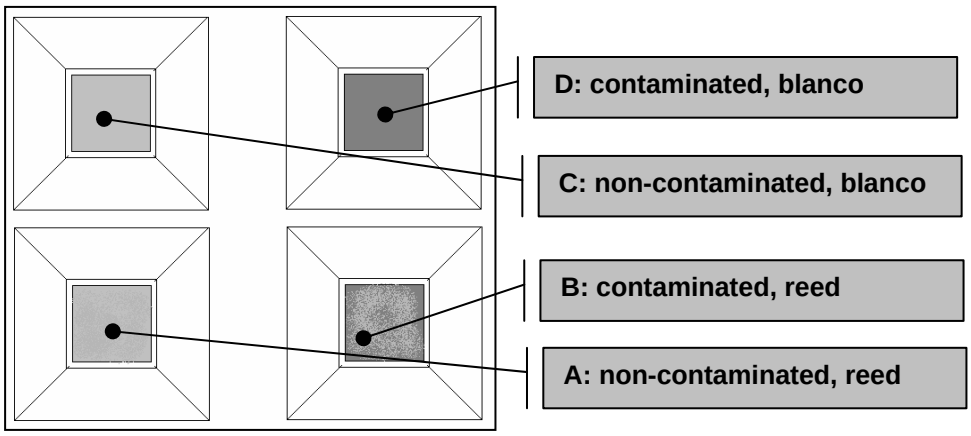


Figure 23: Numbering of tanks and treatments. A and B are closest to the river bank, B is the upstream direction

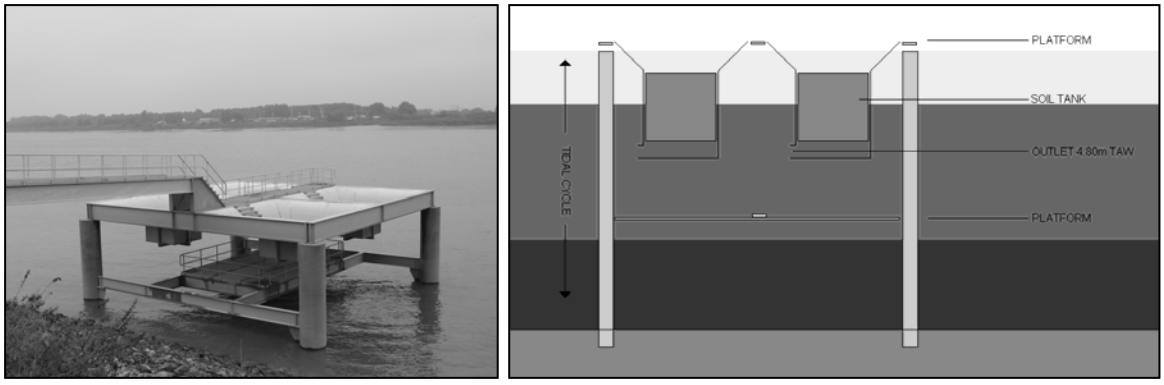


Figure 24: Photograph and schematic view of the mesocosm. Water inlet of the tanks is at 4.80mTAW, ground level at approx. 5.80mTAW

Additional containers with the different types of test soil (the same types as used for the experiment in Ghent) were installed in the mesocosmos at Kruibeke. Each container consists of a flower box filled with substrate, in which small sieves are placed. After one month of equilibration, animals (*T. tubifex*) were transferred into these reservoirs.

4.1.2 Sampling

To estimate the sedimentation (vertical accretion), a marker horizon (50*50 cm) of fine white kaolin clay and silver sand (1:3) was placed on the marsh surface. This layer was permeable for water and vegetation. The sedimentation was estimated as the accumulation of sediment above these kaolin clay fields. A minimum of 4 measurements - by taking cores (∅ 8 mm) and retracing the white layer - were taken at each plot.

Suspended solids in the river water were sampled monthly at the 'pontoon' of Kruibeke by the Flemish Environment Agency and the University of Antwerp. Samples were collected approximately 0.5 m below the water surface at 4 stages in the tidal cycle (4.80 m - between 4.80 m and high water - high water - after high water). Soil pore water was sampled with "peepers", in situ samplers which were placed in April 2003, to measure pore depth profiles (Figure 25).

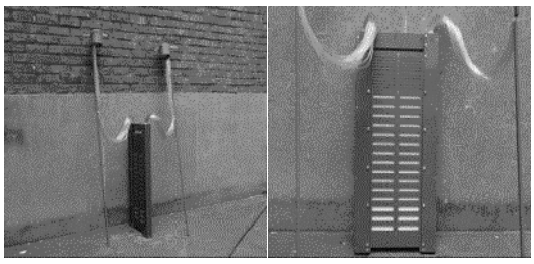


Figure 25: "peeper" polycarbonate membrane pore water sampler

The samplers consist of PVC-plates, with 25 cavities, which can be seen as cells. A polycarbonate membrane separates these cells from the soil. The first cell is situated 0.5 cm below the soil surface, the last one about 62 cm. Each cell has an in- and outflow. Every two months, a homeostasis between the pore water and the cell (filled with demineralized water) is established, and pore water can be sampled. The first measurements started in June 2003.

In the added containers, three sieves per container were seeded with 10 mature oligochaetes every two months, since September 2004. Two months after seeding, the old sieves containing the test organisms were retrieved and three new replicates

were seeded. In May, July and September 2004, 10 reed plants were sampled according to the procedure as described in Chapter 3. The older leaf was not sampled in May as insufficient leaves were available. Sampling of undisturbed core soil samples in the tanks was done with a liner sampler (04.15.SB, Eijkelkamp, Giesbeek, NL) every two months the first year and every four months the second year. The core samples were divided into five sections: the deposited sediment was separated from the rest of the soil, which was divided into four segments of 20 cm: 0-20, 20-40, 40-60 and 60-80 cm below the soil surface.

4.1.3 Analysis

4.1.3.1 Soil characteristics

The soil characteristics such as pH, OM, EC, CEC and $CaCO_3$ were determined as described in paragraph 3.1.3.1. The same methodology was used as described in the previous mentioned paragraph to determine total heavy metal content of the soil and the sulphide content of the soil samples.

4.1.3.2 Pore water characteristics

In the pore water, the following metals were measured every two months: Al, As, Cd, Cr, Cu, Ni, Zn, Pb, Fe and Mn. They were analysed using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES). Ammonium, nitrate, nitrite, sulphate and phosphate contents were also measured colorimetrically every two months.

4.1.3.3 Reed

At the beginning of November 2003 and 2004, all the reed shoots were harvested for biomass determination. The shoots were dried at 70°C for 24 h and dry weight was measured. During the growing season 2003 and 2004, the density of reed was measured every month until the end of September, and panicles were counted. For 2003, 50 shoots per treatment were measured in detail (shoot length, shoot diameter, number of leaves, presence of panicle). The different parts were washed, oven dried at 70°C for 24 h, and dry weight was determined. Heavy metal content was measured yearly in different parts (shoot-leaf-panicle). The different parts were washed and oven dried at 70°C for 24 h. Heavy metals were analyzed using ICP-OES. The photosynthetic pigment content was measured and the measurement of chl a fluorescence was performed as described in Chapter 3. The same procedure as described in Chapter 3 was used to determine the activity of the stress enzymes.

4.1.3.4 Invertebrates

The oligochaetes were sorted out in the laboratory and gut clearance was allowed for 24 h. The retrieved animals were digested in hot acid as described in Chapter 3 and internal concentrations of Zn and Cu were measured.

4.1.3.5 Suspended solids

Suspended solids were analyzed for grain size distribution (laser diffraction method, Malvern Mastersizer). They were also separated from the water by sedimentation, dried at 70°C and analysed for metals using ICP-OES after destruction with *aqua regia* (1/3 HNO_3 / HCl).

4.1.3.6 Tidal characteristics

Tidal water levels were recorded continuously (every 15 min) in a tube of 80 cm depth with a data logger (data logger, type Diver, Eijkelkamp Agrisearch Equipment).

4.1.4 Statistics

The same statistical procedures as described in Chapter 3 were used. GLMM's were fitted for the photosynthetic pigment content, the fluorescence measurements and the stress enzymes determined in reed.

4.2 Results

4.2.1 Soil characteristics

The soil characteristics at different depths are depicted in Figure 26 to Figure 28. They differed significantly ($p < 0.05$) between the contaminated soil (beds B and D) and the uncontaminated soil (beds A and C), except for the chloride content. No differences were observed between the treatments planted with reed and the ones without reed, except for the pH and EC of the uncontaminated soil (A and C). The pH values were slightly, but significantly higher in the soil without reed, whereas the EC values were lower. This could be due to e.g. the excretion of siderophores by the reed. The differences were however not significant in the contaminated soil. The total Cd concentration in the contaminated soil exceeds the soil sanitation tresholds for nature and agricultural areas. Cr and Cu concentrations do not exceed any sanitation threshold, but they significantly exceed background levels.

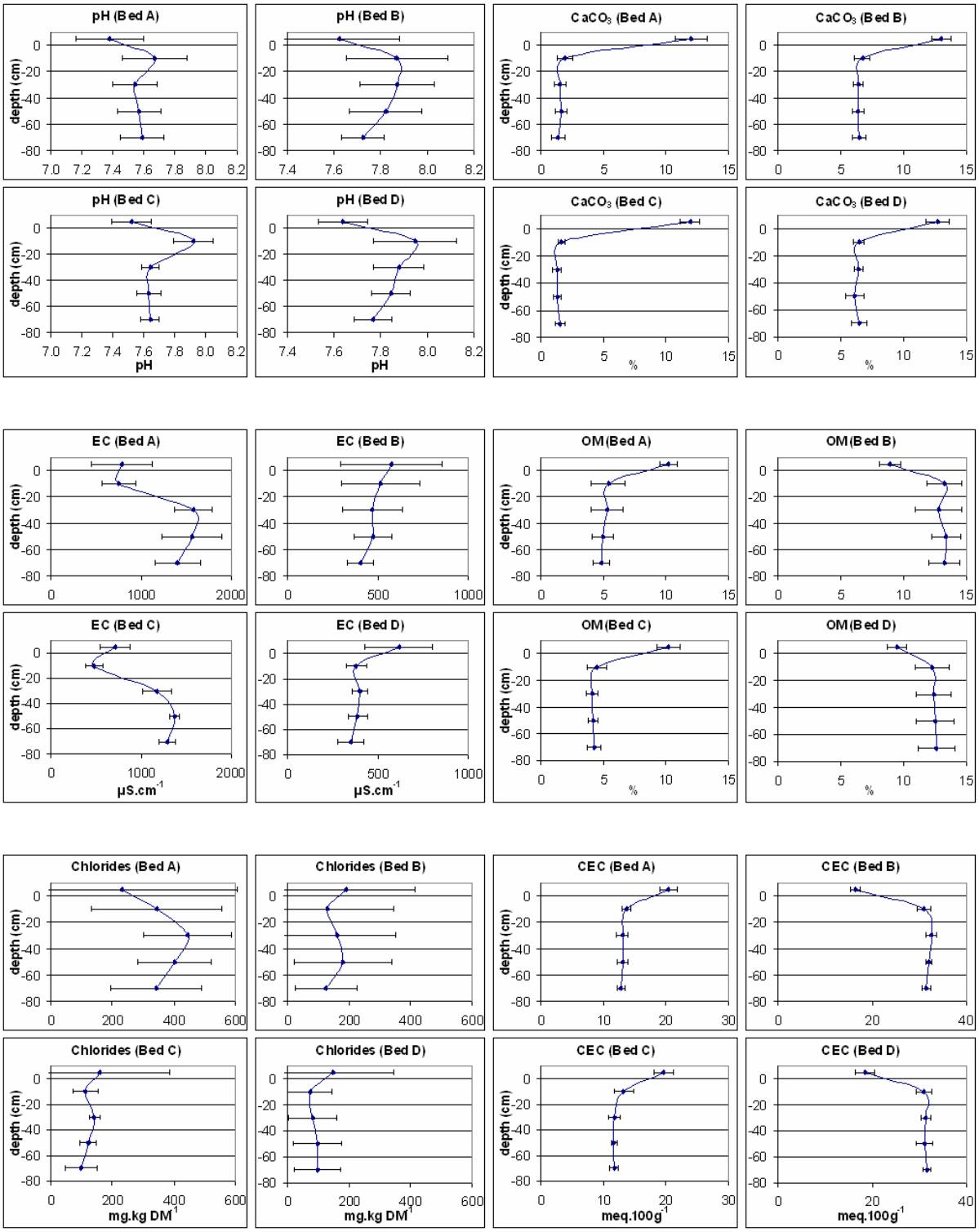


Figure 26: Average pH, EC, CEC, OM-content and $CaCO_3$ and chloride concentrations throughout the depth of the four beds. Horizontal bars indicate standard deviations (n =7)

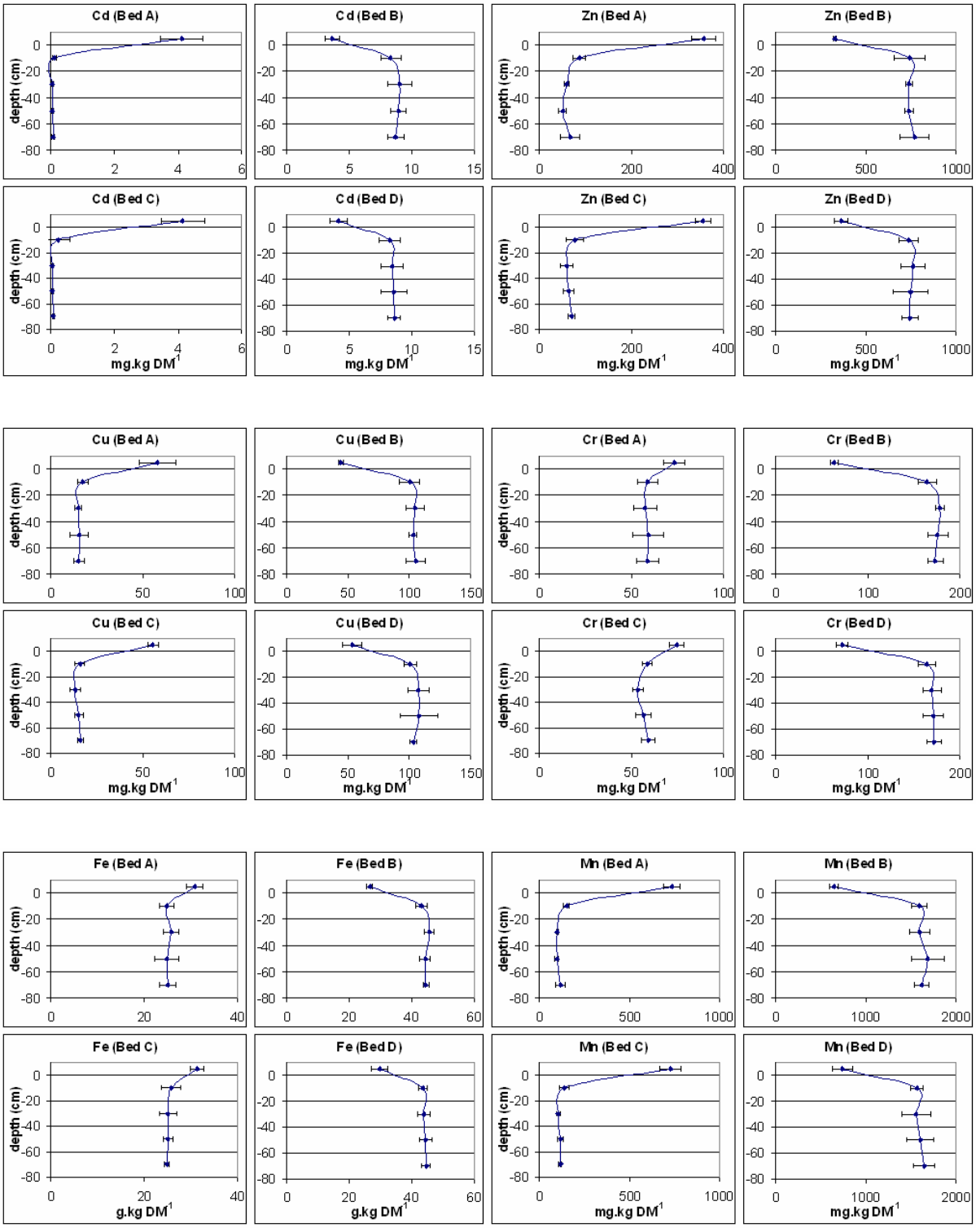


Figure 27: Average metal (Cd, Cr, Cu, Fe, Mn and Zn) concentrations throughout the depth of the four beds. Horizontal bars indicate standard deviations (n =7)

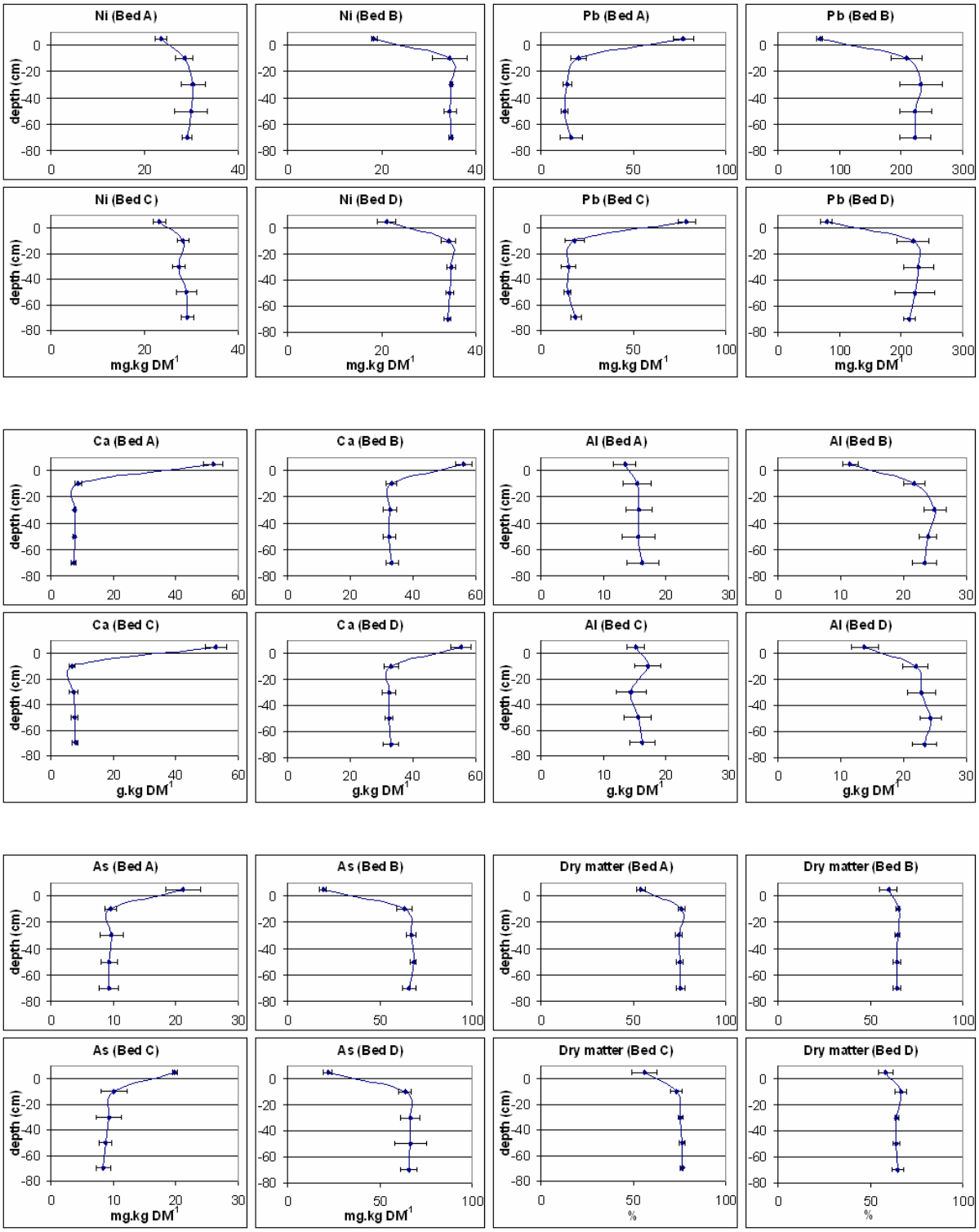


Figure 28: Average metal (Ni, Pb, Al, Ca and As) concentrations and dry matter content throughout the depth of the four beds. Horizontal bars indicate standard deviations (n =7)

The characteristics of the top layer (above 0 cm) differed from those of the underlying soils. This is due to the fact that this layer consists of deposited sediment (see also 4.2.5). The metal contents of the deposited sediments are much higher (except for Ni and Al) compared to the metal contents of the uncontaminated soil and lower (except for Ca) compared to the metal contents of the contaminated soil. Similar conclusions can be drawn for the OM content and the CEC. The $CaCO_3$ content of the deposited sediment is much higher than the ones of the soils. The soil pH was nonetheless lower in the sediment.

The total Cu, Cr, Cd and Zn concentrations in the deposited sediments exceeded the background values and are according to the Flemish legislation (Vlarebo, 1996) too high to allow this sediment to be deposited in nature areas. Cd levels in the sediment are even too high to deposit this sediment anywhere without any treatment.

In October 2003 and September 2004, no sulphides were detected in the soil and deposited sediments of the four beds. In May 2005, variable amounts were detected in the deposited sediments (see Table 6). Small amounts were detected in the upper half of the soil layer of beds A, B and C. The sulphide contents of the sediments in the beds planted with reed (A and B) were lower than in the ones without reed (C and D). This could indicate that reed slows down the sulphide formation or increase sulphide oxidation, which could be due to oxygen supply to its roots.

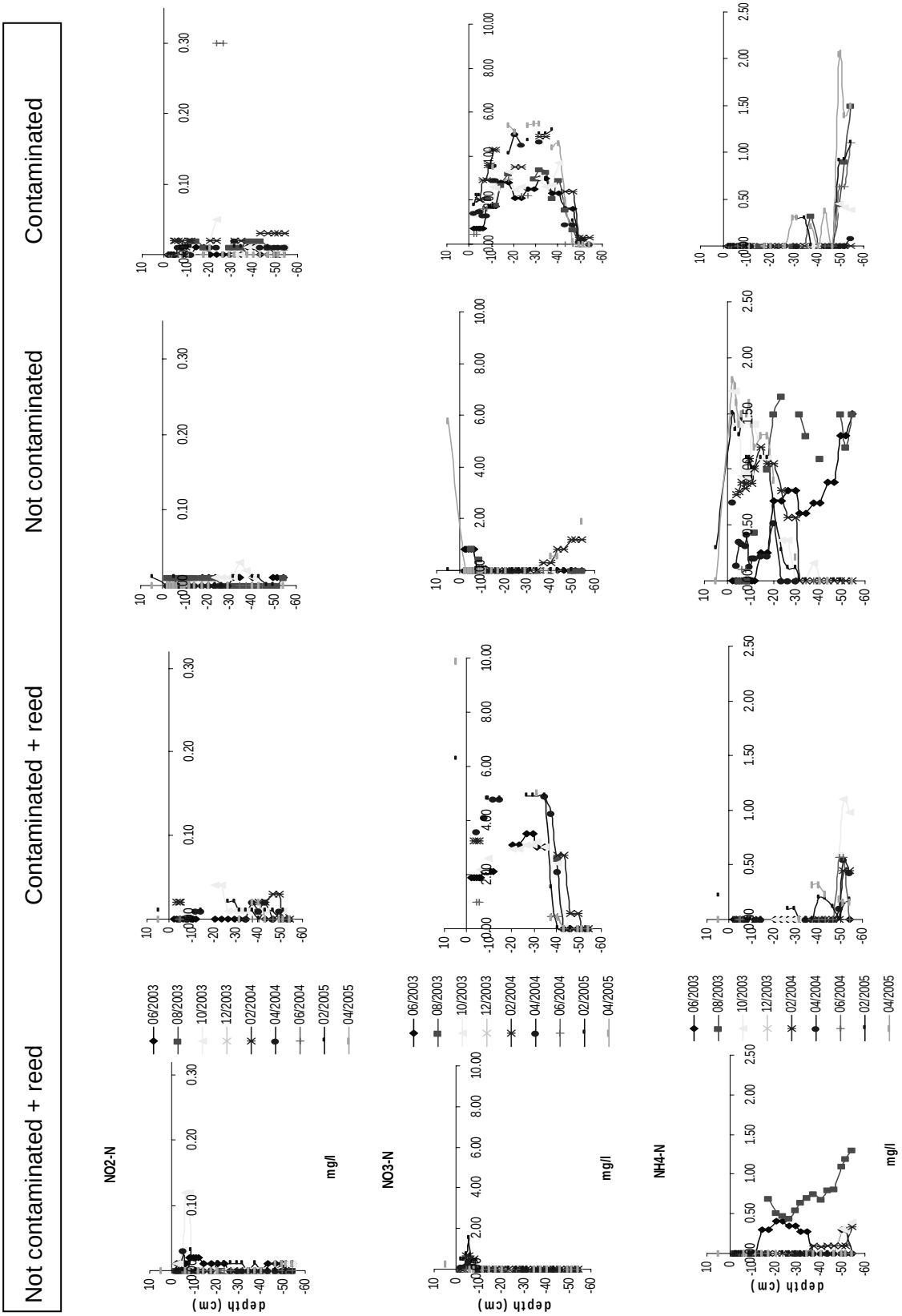
Table 6: Sulphide content of the four beds in May 2005

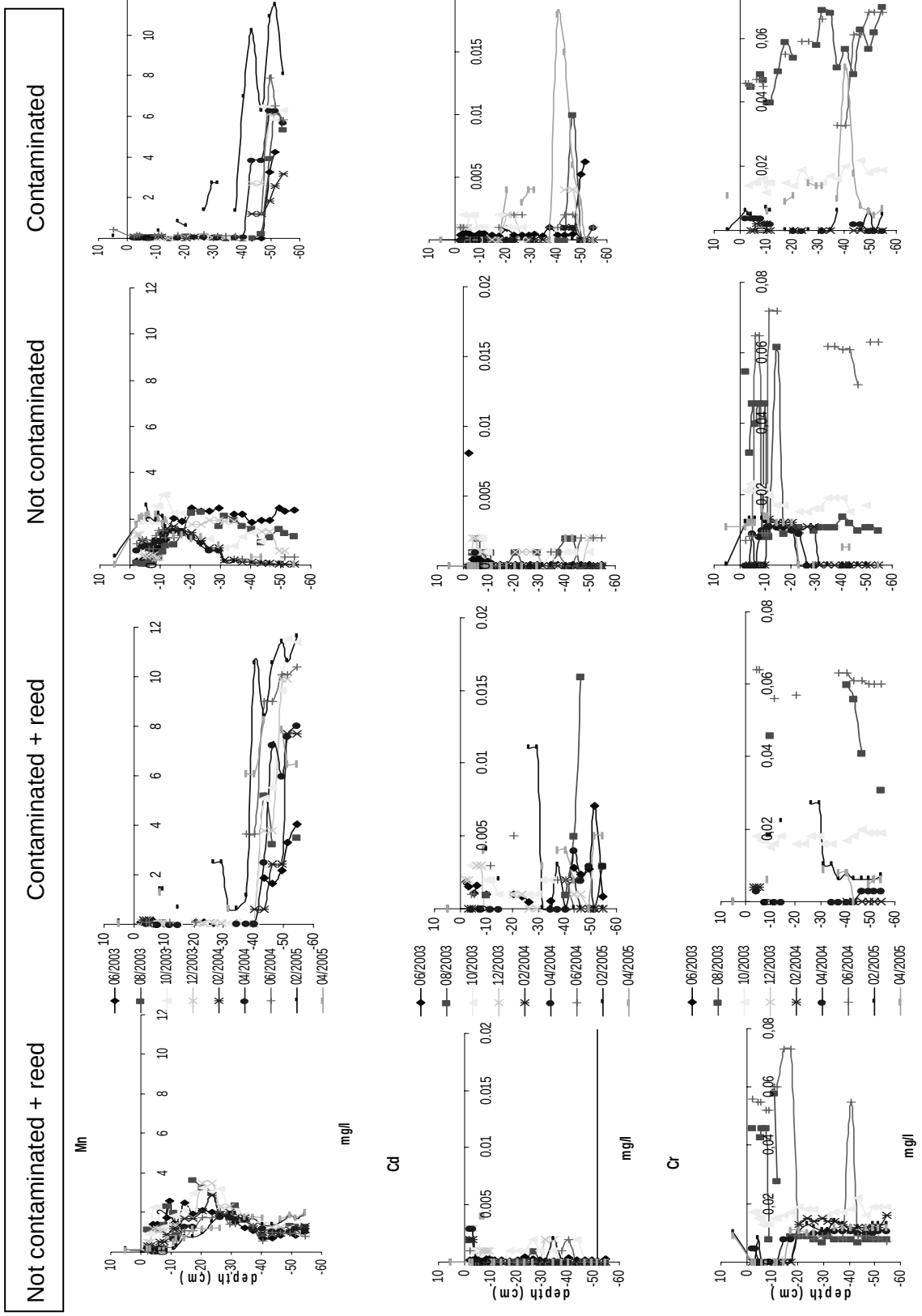
	Sulphides ($mg.kg^{-1}$ DM)			
	A	B	C	D
Sediment	< 10	71	399	1542
0-20 cm	73	79	83	< 10
20-40 cm	24	13	129	< 10
40-60 cm	< 10	< 10	< 10	< 10
60-80 cm	< 10	< 10	< 10	< 10

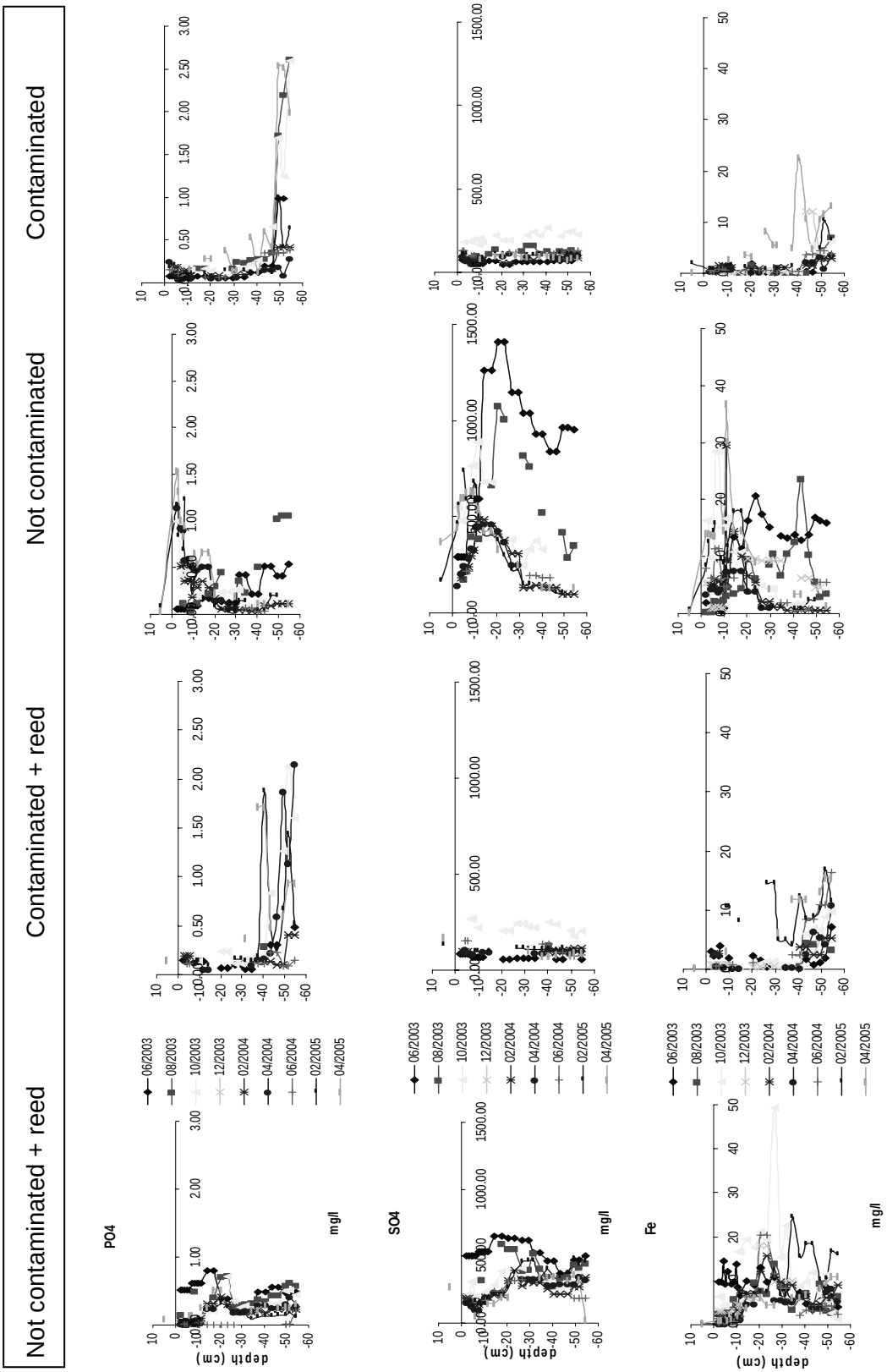
4.2.2 Pore water characteristics

The concentrations of (NO_3^-)-N nitrate were lower than the detection limit in almost all samples of the uncontaminated treatment. In the contaminated treatment, concentrations were low in the upper soil level but increased with depth and reached highest concentrations (3-5 mg $N.L^{-1}$) around -30 cm. Below -30 cm, concentrations started to decrease towards the detection limit, which was reached at -50 cm. Ammonium concentrations ranged between less than the detection limit and 1.50 mg $N.L^{-1}$. No trend was found as a function of depth. (NO_2^-)-N nitrite-concentrations were mostly lower than the detection limit. No seasonal trends were found for all three N-parameters (Figure 29). For orthophosphate, no trends were found. The

concentration ranged between 0 and 2.5 mg $P.L^{-1}$ (Figure 29). SO_4^{2-} concentrations were much higher in the uncontaminated than in the contaminated treatments. No seasonal trend was found (Figure 29). Also for Fe and Mn, no seasonal trend was found. In the contaminated treatment however, the concentrations of both Fe and Mn increased (Figure 29). No differences in Cd, Cr, Cu, Ni, Pb and Zn concentrations were found between the contaminated and the non-contaminated soils and no seasonal trends were detected (Figure 29).


Not contaminated + reed
Contaminated + reed
Not contaminated
Contaminated
NO2-N
NO3-N
NH4-N
mg/l
depth (cm)
06/2003
08/2003
10/2003
12/2003
02/2004
04/2004
06/2004
02/2005
04/2005


Not contaminated + reed
Contaminated + reed
Not contaminated
Contaminated
Mn
Cd
Cr
mg/l
depth (cm)
06/2003
08/2003
10/2003
12/2003
02/2004
04/2004
06/2004
02/2005
04/2005


Not contaminated + reed
Contaminated + reed
Not contaminated
Contaminated
PO4
SO4
Fe
mg/l
depth (cm)
06/2003
08/2003
10/2003
12/2003
02/2004
04/2004
06/2004
02/2005
04/2005

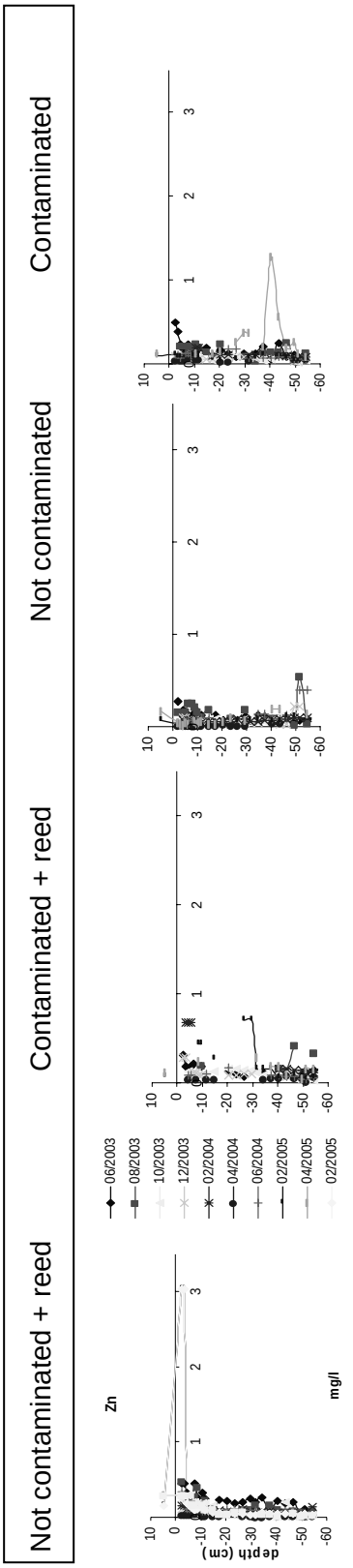


Figure 29: Concentration profiles at different sampling times

4.2.3 Reed

No significant differences in chl a, chl b or total carotenoid content were detected between the contaminated (B) and the uncontaminated substrate (A). On average, lowest concentrations of chl a, chl b and total carotenoids were found in July (sampling date effect, $p<0.001$). Analysis of the the chlorophyll a fluorescence data revealed an interaction between substrate type and leaf (substrate type*leaf interaction, $p<0.05$) (Figure 30). Values for leaf a were always lower in the non-contaminated soil (A), which indicates higher stress in leaf a. The interaction between substrate type and date was significant (substrate type*sampling date interaction, $p<0.05$). The B substrate showed the highest values of F_v/F_m in September, while it was the other way around for the A substrate. All other effects and interactions were not significant ($p_{all}>0.09$).

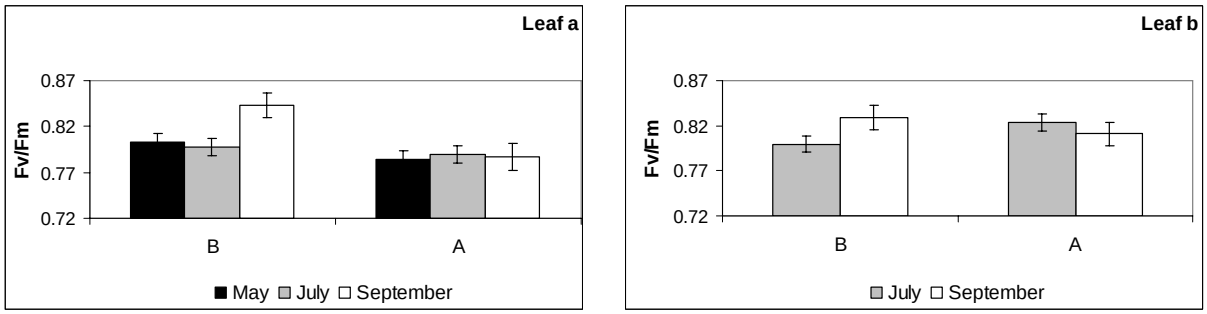


Figure 30: F_v/F_m of a young leaf (leaf a) and an older leaf (leaf b) of *Phragmites australis* grown on different substrates (A and B) under field conditions. Data are presented as mean ± SE

The activity of the stress enzymes ME, GR and ICDH was not influenced by substrate type (p all>0.15) (Figure 31). The activity of GPOD and APOD was significantly different between both substrates (substrate effect, $p<0.05$), activities of both enzymes were doubled when comparing the A with the B substrate.

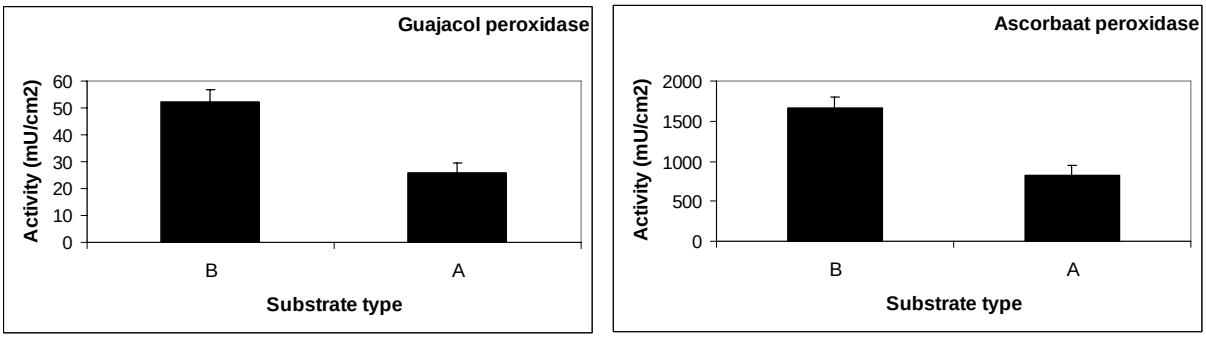


Figure 31: Activity of GPOD and APOD in leaves of *Phragmites australis* grown on different substrates (A and B) under field conditions. Data are presented as mean ± SE

The biomass did not differ significantly between the two treatments. The productivity of the reed was 268 $g.m^{-2}$ for bed A and 303 $g.m^{-2}$ for bed B. The reed density in September 2003 was around 120 shoots/m^2. This density resembles the density of

reed in the marshes near the river Scheldt. In September 2004, densities were much higher (400 shoots/m^2). There was no difference in density of shoots between the treatments (Figure 32).

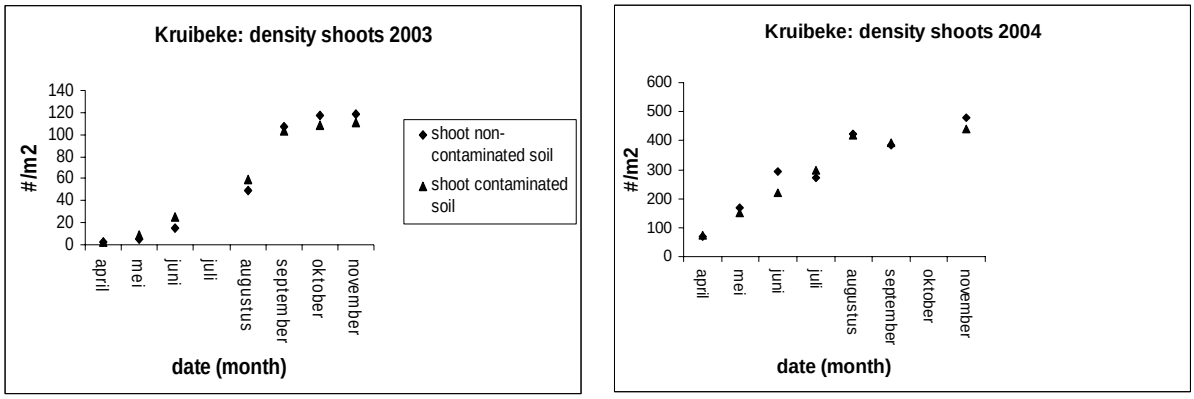
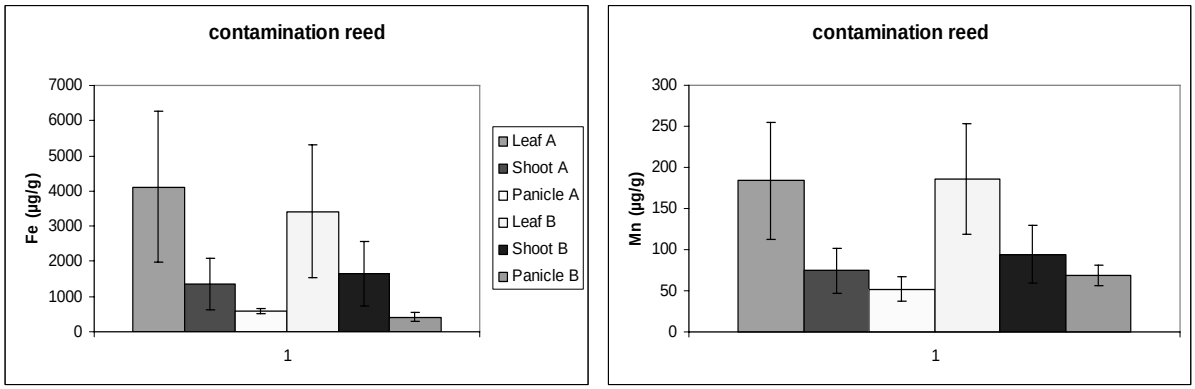


Figure 32: Measurements of density during the growing season 2003 and 2004

Allometric parameters did not differ between the treatments. The average length of the shoot was 152 cm in bed A and 159 in bed B, the average diameter was 5,4 mm in bed A and 5,5 mm in bed B. No significant differences in reed metal content were found between the treatments (Figure 33).

4.2.4 Invertebrates

Average Zn body burdens differed significantly between sampling dates (date effect, $p<0.001$) but not between substrate type ($p=1.19$) (Figure 34). Highest Zn accumulation was noted in the animals retrieved from the samples from September and December 2005 (787.93 ± 54.24 and 744.78 ± 44.29 respectively). However, substrate type and date of sampling interacted, indicating that the effect of sampling date is not independent of the substrate type (substrate type *date interaction, $p<0.05$).



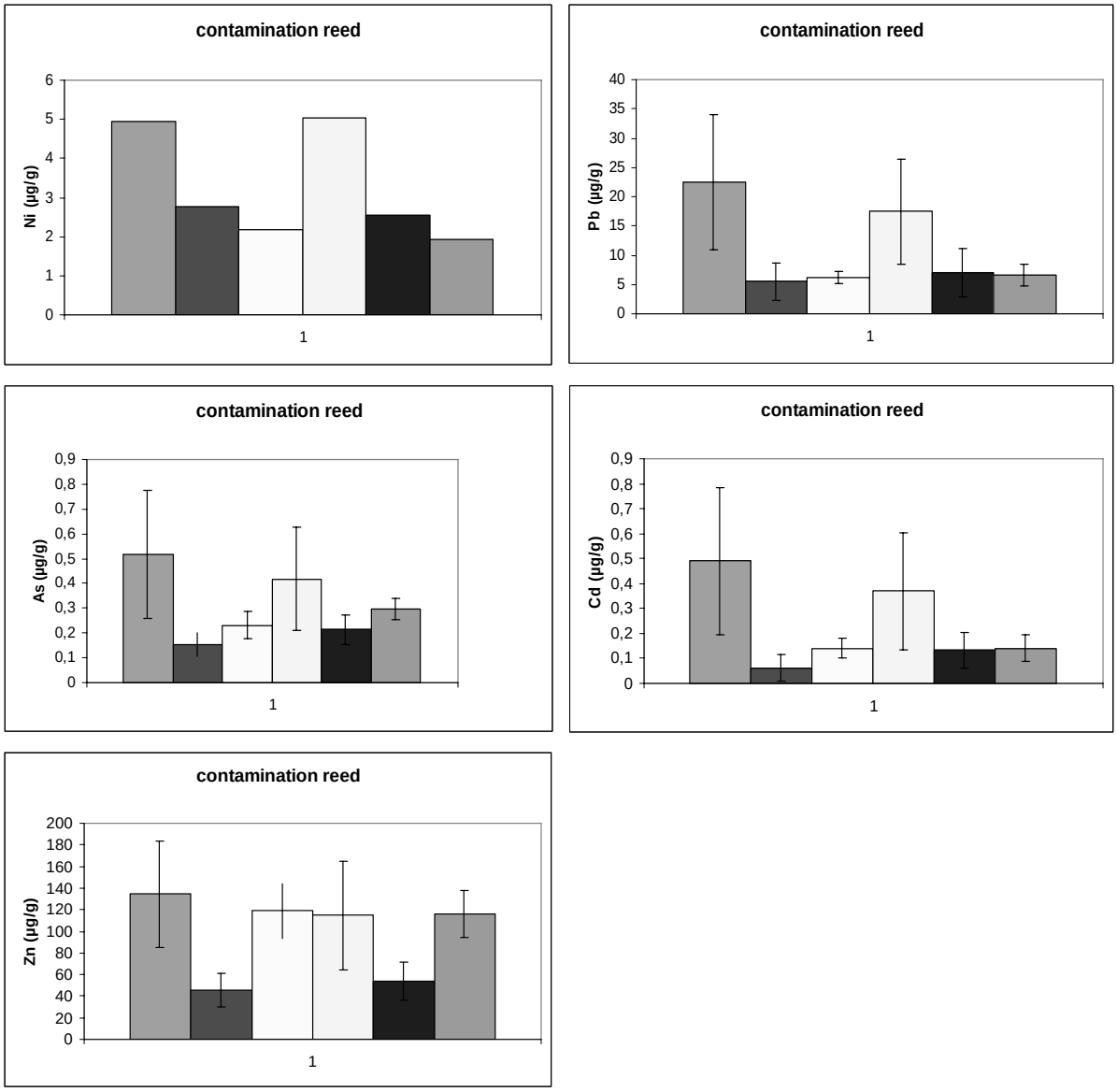


Figure 33: Heavy metal content in *Phragmites australis*. Treatment A is reed growing in the not contaminated soil, treatment B is reed growing in the contaminated soil

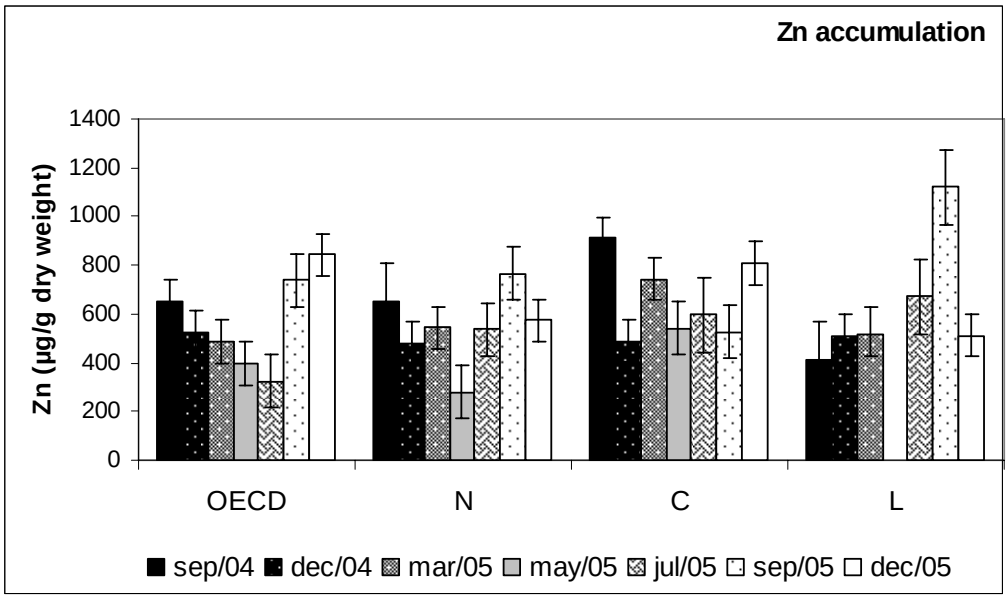


Figure 34: Zn accumulation in *Tubifex tubifex* exposed to four different substrates (OECD, N, C and L) under field conditions at the mesocosmos site at Kruibeke. Data are presented as mean ± SE

Cu accumulation was influenced by substrate type (substrate effect $p<0.001$) and by sampling date ($p<0.001$) (Figure 35).

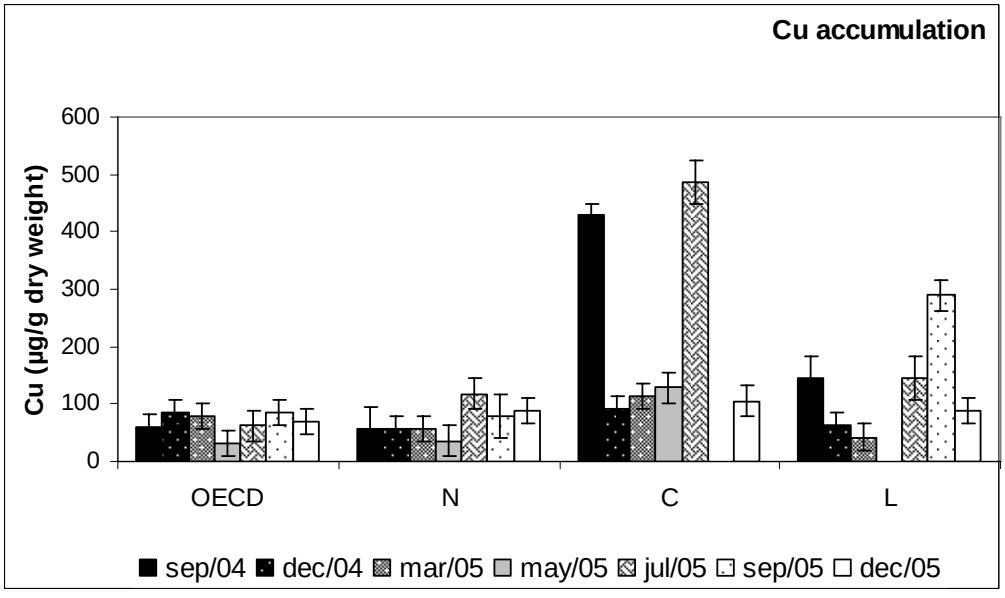


Figure 35: Cu accumulation in *Tubifex tubifex* exposed to four different substrates (OECD, N, C and L) under field conditions at the mesocosmos site at Kruibeke. Data are presented as mean ± SE

Average Cu body burdens were lowest in the OECD and the uncontaminated clay substrate. Lowest Cu accumulation was noted in March 2005 and highest in July 2005. The interaction between substrate type and date of sampling was significant ($p<0.001$).

4.2.5 Sedimentation

Sedimentation took place at a constant rate of approx 6mm/month until month 20, when sedimentation suddenly drops to zero or even below (erosion). Ground level is approx. at 5.80m TAW. Average sedimentation did not differ between the 4 treatments (Figure 36).

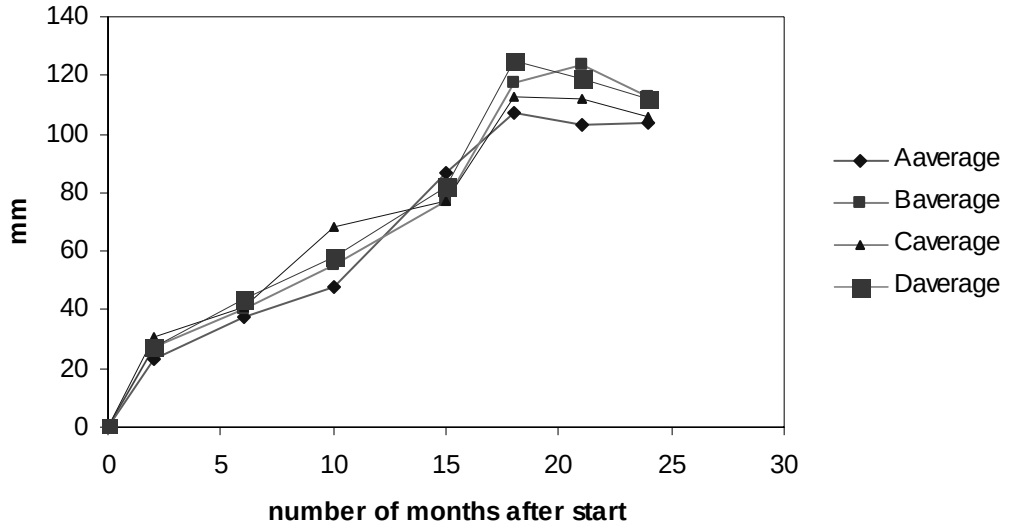


Figure 36: Sedimentation in the experiment in Kruibeke. Treatments A with reed and C without reed are the less contaminated treatments. Treatments B with reed and D without reed are the contaminated treatments

4.2.6 Tidal characteristics

The tidal regime follows the regime of the river. At high water above 4.80 m TAW, water flows in the experimental set-up. The groundwater starts rising 30 minutes later than in the outside of the experiment. The average duration of flooding above soil level was 1.6 h, the average height of flooding above soil was 10 cm (Figure 37).

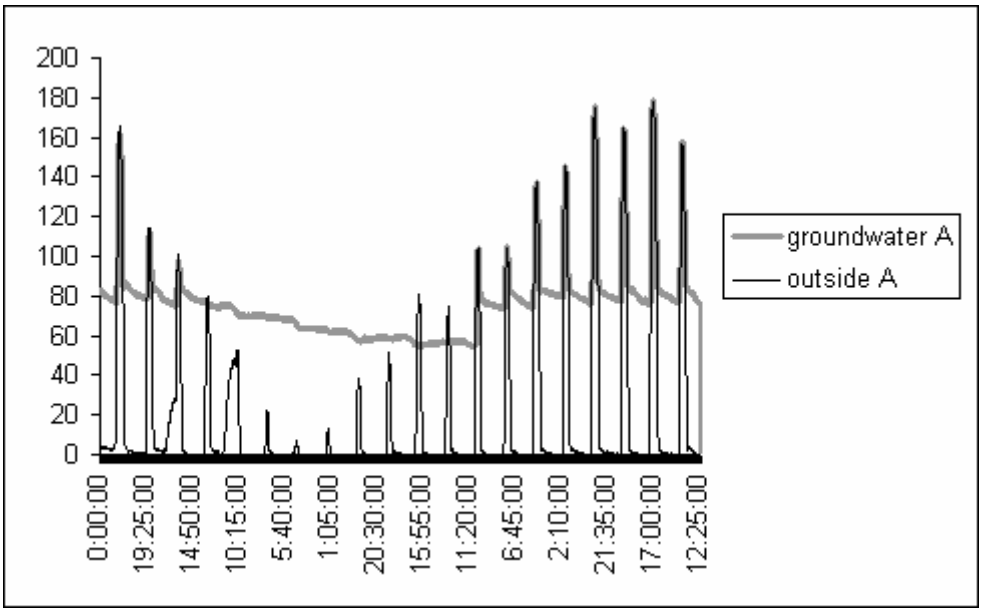


Figure 37: Tidal and ground water level in the mesocosm during a spring-neap tidal cycle. Levels in cm, zero is at 4.80 m TAW, soil surface of mesocosm at 100 cm

4.3 Discussion

The sedimentation of suspended material proved to be an important process for soils which are daily flooded by river water *in situ*. Several centimetres of contaminated sediment are being deposited each year. The quality of the sediments that are nowadays being deposited in tidal marshes and flooding areas along the river Scheldt is too low to allow for a legal sediment deposition in nature areas, and in the case of Cd also too low to allow for a legal deposition in other types of areas. In areas where the soil is historically very contaminated, the deposition of still, but less contaminated sediment could however be beneficial for nature development, since the organisms live in or on the top layer of the soil and no adverse effects were observed for reed growth and the invertebrate population in the contaminated, reduced soils. Moreover, the deposited sediments could limit the oxygen transfer to the underlying contaminated soils, which would reduce the metal mobility. As also mentioned in chapter 3, these conclusions should still be drawn with great caution as the mobility of some other elements, such as arsenic (As), is expected to increase under these reducing conditions, which first needs further investigation.

Vandecasteele et al. (2004b) also proposed to include the current soil contamination status of alluvial soils along the Zeeschelde and the Durme as a criterion in the selection procedure for new flood control areas. Otherwise, sediment of which the quality does not comply with the Flemish legislation regarding re-use of soils, will be deposited in areas that are not yet contaminated. The top soil layer will become contaminated with metals, which will be dispersed. As a result, the contaminated land surface will be enlarged. The two-year period in which the measurements were performed, was too short to detect significant changes in the metal biogeochemistry

and soil properties of the underlying soils. Therefore, the duration of the experiment should be extended.

Moreover, interpretation of metal concentrations in pore water and assessment of metal fluxes is very difficult in the experimental setup *in situ* due to the fact that the Scheldt water flushes away all mobile metals in an uncontrollable manner during high tides.

One can however suspect the beginning of Mn en Fe-oxide reduction in the deeper layers of the soil. Concentrations of most elements in the pore water also did not seem to differ significantly between the contaminated and the uncontaminated treatments.

The observed differences in the measured photosynthetic pigments, chl a fluorescence and stress enzymes can rarely be explained by substrate type alone. The photosynthetic pigment content is not influenced by substrate type, but displays a seasonal pattern. Average values of F_v/F_m for 'leaf a' were lower (thus higher stress) in the non-contaminated soil, while one would expect that the reed would perform better in this substrate. In this case, other factors than metal contamination had an influence on the photosynthetic performance of the reed. For the stress enzymes, however, differences were observed. The highest values (highest stress) are found in the contaminated substrates, which could indicate a possible relation with metal contamination. APOD en GPOD are both involved in the detoxification of peroxides, GPOD mainly in the vacuole, cytosol and the cell wall while APOD is principally active in the cytosol and the chloroplast.

As toxicological testing most of the time takes place in standardised and optimised conditions in the laboratory, it does not describe the situation in real environments. From ecological point of view, field studies are the most relevant way to assess the impact of metal contaminated soils and sediments. However, in case of moderate contamination it is extremely difficult to distinguish between effects originating from natural factors and those from the impact of metals (Chapman et al., 1998). The interaction between climatic conditions, other environmental factors (e.g. food stress, anoxia and salinity) and other pollutants has recently been acknowledged as an area of research that has been neglected in ecotoxicology (Spurgeon et al., 2005). Nevertheless, metal accumulation patterns displayed no general trend indicating that, as all samples were exposed to the same environmental conditions, other factors are responsible for the observed differences. Initially, animals were only exposed to the substrates that were added to the sieves, but after two months of exposure under field conditions the sieves became silted up with autochthonous sediment (with a different contamination degree). The moving behaviour of the oligochaetes is probably affected by season and temperature and hence the time they spend in the different compartments of the sieves. This probably explains partly why the observed metal concentrations do not follow the contamination degree measured in the original

substrates. Although the number of produced cocoons per sieve was counted, it seemed not to be as reliable as in the laboratory tests. Tidal action can cause the wash out of cocoons and the crawling around of the oligochaetes (which is affected by temperature and thus by date of sampling) obscures the real amount of cocoons produced.

For the performance of the reed, it is difficult to make unambiguous conclusions as observed differences are rarely explained by substrate type alone. The different parameters also gave different results: while the fluorescence measurements point to more stress in the non-contaminated soil, stress enzymes indicate stressed growing conditions in the contaminated substrate. The absence of a link between metal accumulation in the oligochaete *T. tubifex* and the contamination degree of the substrates is probably related to the silting up of the sieves. If so, metal accumulation of oligochaetes in wetlands will eventually be related to the contamination degree of deposited materials. However, more detailed research is needed to gain more insight into the exact behaviour of oligochaetes during such a sedimentation process and the time lag effects of such an event. Due to methodological constraints, Cd concentrations could not be measured in *O. gammarellus* and *T. tubifex*. Assessment of the effect of flooding, inundation period and salinity on Cd availability for these organisms is an important future research need.

Helophyte plants, including *Phragmites australis,* which are growing in wetlands in anoxic soils, release oxygen in the rhizosphere (Armstrong, J. 1978 & Armstrong, J & Armstrong, W. 1990). Because of the oxygen release, we expected a difference in the metal bioavailability between the treatments with and without reed. Because the pore water concentrations were not measured regularly in the deposited sediment, no influence of the reed on the pore water concentrations were detected. The sulphide content seemed to be affected by the reed growth.

4.4 Conclusions and recommendations

The creation of additional flood control areas will cause the spreading of metal contaminated river sediment. If uncontaminated areas will be used to create flood control areas, it will give rise to additional areas of contamination. On the other hand, in severely contaminated alluvial areas, the deposition of still, but less contaminated sediment could have beneficial effects by limiting the oxygen supply to the polluted underlying soil and reducing the metal content in the top soil layer. In this study, no adverse effects on metal mobility, invertebrate toxicity and reed growth were detected when the sediment stayed reduced. When soils are being flooded daily, they will most likely not oxidise, but the amount of contaminated sediment that is then being deposited could reduce the water storage capacity and thus the effectiveness of the flood control area. If the area is just being flooded once or twice a year, when

peek flows occur, deposition of polluted sediments is expected to be lower, but the deposited contaminated sediments are expected to oxidise and metals present as sulphides could become mobile. Since the top layer of the soil is an important habitat for much invertebrates, they could become exposed to elevated metal concentrations in the pore water. As long as the soil buffering capacity (related to $CaCO_3$) is sufficient to prevent the pH to decrease during oxidation, metal mobilisation will be restricted. The two year period was too short to detect any measurable changes in the soil properties and to see any effect of the deposited sediment on the underlying soil layers. Future measurements within five, ten or even twenty years would be interesting to investigate longer term alterations in the soil biogeochemistry.

The photosynthetic pigment content of the reed was not influenced by substrate type, but displayed a seasonal pattern. Other factors than metal contamination had an influence on the photosynthetic performance of the reed. Other plants, e.g. willows, may be better suited for measuring metal bioavailability in aboveground plant parts. For the stress enzymes there was a possible relation with the metal contamination, making it a more valuable parameter to determine metal toxicity for reed in this case. The reed did not have any measurable effect on the concentration of the metals in the soil or pore water. Also no differences in the concentrations of metals in the reed biomass were observed between the two treatments. Also in both treatments, no significant differences in reed growth were observed.

The toxicological testing with invertebrates in situ seemed not as reliable as in the laboratory tests. The observed metal concentrations did not follow the contamination degree of the substrates. The moving of the invertebrates to the deposited autochthonous sediment probably nullified the possible and expected differences between the two substrates. More detailed research is needed to gain more insight into the exact behaviour of oligochaetes during such a sedimentation process and the time lag effects of such an event. Due to methodological constraints, Cd concentrations could not be measured in *O. gammarellus* and *T. tubifex*. Assessment of the effect of flooding, inundation period and salinity on Cd availability for these organisms is an important future research need.

5 GENERAL CONCLUSIONS AND RECOMMENDATIONS

Risk assessment only based on total metal content for flood control areas is not advisable, as the metal mobility seems to be independent of the total metal content. Other factors such as soil pH, CEC, salinity, redox status and carbonate, OM and clay content also play an important role. When constructing flood control areas in a contaminated area, it is advisable to reduce the variability in the redox status of the soil to prevent the mobilization of the metals and the uptake by benthic organisms. This could be done by permanently flooding the soil or by flooding it daily to prevent oxidation. If not, the deposited contaminated sediment will oxidise and the metals present as sulphides will become mobile, even if the soil pH is buffered by carbonates. Since the top layer of the soil is an important habitat for much invertebrates, they can be exposed to elevated metal concentrations in the pore water.

Two very important factors have to be taken into account and investigated when considering the creation of new flood control areas. The first one is the amount of the deposited contaminated sediment when the flood control area is being flooded daily. Large amounts of contaminated sediment could be deposited and reduce the water storage volume of a flood control area, thus minimizing its effect. The second and equally important factor is the quality of the deposited sediment and flooding water. The water quality of the rivers in Belgium is slowly improving, but the sediment quality is poor due to historical contamination. Results of this study confirm the idea that soil contamination status of alluvial soils should be used as a criterion when selecting new flood control areas. Uncontaminated flood control areas will be contaminated when sediment is being deposited. Moreover, upon increasing oxygen contents of the river waters, due to decreasing organic matter loads, the redox status of the sediments can change and metals could be released from the sediments, even when permanently flooded. As such, newly created wetlands can be confronted with increasing metal contents in its flooding water. The effect of the improving water quality on the metal release from sediments should certainly be further investigated in the future.

The two year period was too short to detect any measurable changes in the soil properties and to see any effect of deposited sediments on the underlying soil layers. Future measurements within five, ten or even twenty years would be interesting to investigate longer term alterations in the soil biogeochemistry. The measured metal (im)mobilization kinetics can however already be incorporated in models in the near future. These models should allow to predict metal mobility changes at the oxic-anoxic interface upon changing hydrological conditions, where the already existing

geochemical models fail due to the fact that they are based on steady state conditions.

There are no clear indications that reed growth and metal uptake by reed plants are affected by the pollution status of the soils and sediments. The latter also implies that other plants may be better suited for measuring metal bioavailability in aboveground plant parts.

6 ACKNOWLEDGEMENTS

The authors wish to acknowledge several persons for their help and participation in the realisation of this project. First of all we wish to thank our promoters F. Tack, C. Janssen and P. Meire for giving us the opportunity to perform this research. Without G. Du Laing, who wrote the project proposal, this project would have never existed. Thank you for your efforts and support! The authors would also like to thank S. Van den bossche for the many analyses he did in the lab and the numerous sampling campaigns. He made our results so accurate and extensive. We are grateful to K. Lock for his help with the setup of the toxicological tests. E. Pequeur, J. Van Reybrouck, L. Clement, E. De Bruyn and L. Bervoets are very much acknowledged for their technical assistance, and DEME Environmental Contractors (DEC) for collecting and delivering the dredged sediment. Also, thanks to Dr. W. Geebelen for his advice and assistance during the sampling for the stress enzymes. We are grateful to Prof. Dr. R. Valcke and Dr. B. Gielen for lending us the Plant Efficiency Analyser for the fluorescence measurements. Thanks to the users of the project for their useful scientific input when attending the user meetings. Last, but not least we would like to thank the Belgian Science Policy Office for their funding and for the good cooperation with the project supervisors M. Van Cauwenberghe, A. Van Der Werf and D. Cox.

The authors,

Dieter, Marjan, Sabine and Sander.

7 REFERENCES

Andreu V, Gimeno-García E. Evolution of heavy metals in marsh areas under rice farming. Environ Pollut 1999; 104: 271-282.

Ankley GT, Di Torro DM, Hansen DJ, Berry WJ. Technical basis and proposal for deriving sediment quality criteria for metals. Environ Toxicol Chem 1996; 15: 2056-2066.

Armstrong J, Armstrong W. Light enhanced convective throughflow increases oxygenation in rhizomes and rhizosphere of Phragmites australis (Cav.) Trin.ex Steud. New Phytol 1990; 114: 121-130.

Armstrong J. Root aeration in the wetland condition. In: Hook, D.E. & Crawford, R.M. (eds.) Environment and plant ecology. John Wiley & Sons Canada Ltd., Toronto, Canada, 1978, pp. 181-218.

Bervoets L, Blust R, de Wit M, Verheyen R. Relationships between river sediment characteristics and trace metal concentrations in tubificid worms and chironomid larvae. Environ Pollut 1997; 95: 345-356.

Burzynski M, Klobus G. Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. Photosynthetica 2004; 42: 505-510.

Cappuyns V, Swennen R. Kinetics of element release during combined oxidation and pH_{stat} leaching of anoxic river sediments. Appl Geochem 2005; 20: 1169-1179.

Chapman PM, Wang F, Janssen C, Persoone G, Allen HE. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment and remediation. Can J Fish Aquat Sci 1998; 55: 2221-2243.

Chapman PM, Wang F. Assessing sediment contamination in estuaries. Environ Toxicol Chem 2001; 20:3-22.

Cuypers A, Vangronsveld J, Clijsters H. The chemical behaviour of heavy metals plays a prominent role in the induction of oxidative stress. Free radical res 1999; 31: 839-843.

Deleebeeck N. Zware metalen in rietplanten (Phragmites australis (Cav.) Steudel) langsheen de oevers van de Schelde. Scriptie voorgedragen tot het behalen van de academische graad van Gediplomeerde in de Aanvullende Studies van Milieuwetenschappen en –technologieën. Universiteit Gent, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, 2001. 79 pp.

Du Laing G, Bogaert N, Tack FMG, Verloo MG, Hendrickx F. Heavy metal contents (Cd, Cu, Zn) in spiders (Pirata piraticus) living in intertidal sediments of the river Scheldt estuary (Belgium) as affected by substrate characteristics. Sci Total Environ 2002; 289: 71-81.

Du Laing G, Tack FMG, Verloo MG. Performance of selected destruction methods for the determination of heavy metals in reed plants (Phragmites australis). Anal Chim Acta 2003; 497: 191-198.

Du Laing G, Van Ryckegem G, Tack FMG, Verloo, MG. Metal accumulation in intertidal litter through decomposing leaf blades, sheats and stems of Phragmites australis. Chemosphere, 2006, in press. doi: 10.1016/j.chemosphere.2005.10.034.

Eggleton J, Thomas KV. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ Int 2004; 30: 973-980.

Emmerson RHC, Birkett JW, Scrimshaw M, Lester JN. Solid phase partitioning of metals in managed retreat soils: field changes over the first year of tidal inundation. Sci Total Environ 2000; 254: 75-92.

Gambrell RP, Collard V, Patrick WH Jr. Cadmium uptake by marsh plants as affected by sediment physicochemical conditions. In: Baker, R.A. ed. Contaminants and sediments, Vol. 2. Michigan, USA: Ann Arbor Science, 1980, pp. 425-444.

Gambrell RP Jr, Patrick WH. Cu, Zn and Cd availability in a sludge-amended soil under controlled pH and redox potential conditions. In: Bar-Yosef B. ed. Inorganic contaminants in the vadose zone. Berlin: Springer-Verlag, 1989 pp. 89-106.

Gambrell RP. Trace and toxic metals in wetlands – A review. J Environ Qual 1994; 23: 883-891.

Guo T, DeLaune RD, Patrick WH. The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium, and zinc in estuarine sediment. Environ Int 1997; 23: 305-316.

Hall LW, Anderson RD. A deterministic ecological risk assessment for copper in European saltwater environments. Mar Pollut Bull 1999; 38: 207-218.

Hendrickx F, Maelfait JP, Bogaert N, Tojal C, Du Laing G, Tack FMG, Verloo MG. The importance of biological factors affecting trace metal concentration as revealed from accumulation patterns in co-occuring terrestrial invertebrates. Environ Pollut 2004; 127: 335-341.

Huggett DB, Schlenk D, Griffin BR. Toxicity of copper in an oxic stream sediment receiving aquaculture effluent. Chemosphere 2001; 44: 361-367.

Hund-Rinke K, Kördel W. Underlying issues in bioaccessibility and bioavailability: experimental methods. Ecotox Environ Safe 2003; 56: 52-62.

King CK, Simpson SL, Smith SV, Stauber JL, Batley GE. Short term accumulation of Cd and Cu from water, sediment and algae by the amphipod *Melita plumulosa* and the bivalve *Tellina deltoidalis*. Mar Ecol-Prog Ser 2005; 287: 177-188.

Koppenol WH. Chemistry of iron and copper in radical reactions. In: Rice-Evans CA, Burdon RH editors. Free radical damage and its control. New comprehensive biochemistry 28. Elsevier, Amsterdam, 1994, pp. 3-24.

Lacey R, Watzin MC, McIntosh AW. Sediment organic matter content as a confounding factor in toxicity tests with *Chironomus tentans*. Environ Toxicol Chem 1999; 18: 231-236.

Lagriffoul A, Mocquot B, Mench M, Vangronsveld J. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.). Plant Soil 1998; 200: 241-250.

Ma LQ, Dong Y. Effects of incubation on solubility and mobility of trace metals in two contaminated soils. Environ Pollut 2004; 134: 301-307.

Marsden ID, Rainbow PS. Does the accumulation of trace metals in crustaceans affect their ecology – The amphipod example? J Exp Mar Biol Ecol 2004; 300: 373-408.

Organisation for Economic Co-operation and Development (OECD). Proposal for an OECD guideline for testing of chemicals: Sediment-Water *Lumbriculus* toxicity test using spiked sediment (Final draft). March 2005, 35 pp.

Parametrix. Persistence, bioaccumulation and toxicity of metals and metal compounds. International Council on Metals and the Environment (ICME), Ottawa, Ontario, Canada, 1995, 93 pp.

Peijnenburg WJGM, Jager T. Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. Ecotox Environ Safe 2003; 56: 63-77.

Rainbow PS, Dallinger R. Metal uptake, regulation, and excretion in freshwater invertebrates. In: Dallinger R, Rainbow PS editors. Ecotoxicology of Metals in Invertebrates. SETAC special publications series, Lewis publishers, Boca Raton, Florida, USA, 1993, pp. 120-131.

Rainbow PS. Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 2002; 120: 497-507.

Raven PH, Evert RF, Eichhorn SE. Biology of plants. Fifth edition. Worth Publishers, New York. 1992, 791pp.

Riba I, Del Valls TA, Forja JM, Gomez-Parra A. The influence of pH and salinity on the toxicity of heavy metals in sediment to the estuarine clam *Ruditapes philippinarum*. Environ Toxicol Chem 2004; 23: 1100-1107.

Riedel GF, Sanders, JG, Osman, RW. Biogeochemical control on the flux of trace elements from estuarine sediments: water column oxygen concentrations and benthic infauna. Estuar Coast Shelf Sci 1997; 44: 23-38.

Rohacek K. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica 2002; 40:13-29.

Speelmans M, Vanthuyne DRJ, Lock K, Hendrickx F, Du Laing G, Tack FMG, Janssen CR. Influence of flooding, salinity and inundation time on the bioavailability of metals in wetlands. Sci Total Environ, submitted.

Spurgeon DJ, Svendsen C, Lister LJ, Hankard PK, Kille P. Earthworm responses to Cd and Cu under fluctuating environmental conditions: A comparison with results from laboratory exposures. Environ Poll 2005; 136: 443-452.

Stephens SR, Alloway BJ, Parker A, Carter JE, Hodson, ME. Changes in the leachability of metals from dredged canal sediments during drying and oxidation. Environ Pollut 2001; 114: 407-413.

Tack FM, Lapauw F, Verloo MG. Determination and fractionation of sulphur in a contaminated dredged sediment. Talanta 1997; 44: 2185-2192.

Tack FMG, Singh SP, Verloo MG. Heavy metal concentrations in consecutive saturation extracts of dredged sediment derived surface soils. Environ Pollut 1998; 103: 109-115.

Tack FMG, Verloo MG. Chemical speciation and fractionation in soil and sediment heavy metal analysis: a review. Int. J. Environ. Anal. Chem 1995; 59: 225-238.

Tessier A, Campbell PGC. Partitioning of trace metals in sediments: Relationships with bioavailability. Hydrobiologia 1987; 149: 43-52.

Timmermans KR. Accumulation and effects of trace metals in freshwater invertebrates. In: Dallinger R, Rainbow PS editors. Ecotoxicology of Metals in Invertebrates. SETAC special publications series, Lewis publishers, Boca Raton, Florida, USA, 1993, pp. 133-148.

Tockner K, Stanford JA. Riverine flood plains: present state and future trends. Environ Conserv 2002; 29: 308-330.

Ure AM. Trace-elements in soil – their determination and speciation. Fresenius J Anal Chem 1990; 337: 577-581.

Van Assche F, Cardinaels C, Clijsters H. Induction of enzyme capacity in plants as a result of metal toxicity: Dose-response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. Environ Poll 1988; 52: 103-115.

Van Assche F, Clijsters H. A biological test system for the evaluation of the phytotoxicity of metal-contaminated soils. Environ Poll 1990; 66: 157-172.

Van Brempt A. Invloed van ijzer-, zink- en koperverontreiniging op een aantal fotosynthese- en groeideterminerende karakteristieken van Riet (Phragmites australis). Afstudeerwerk voorgedragen tot het behalen van de graad Bioingenieur in de Milieutechnologie. Universitet Gent, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen. 1994, 172pp.

Vandecasteele B, De Vos B. Relationship between soil textural fractions determined by the sieve-pipette method and laser diffractometry. Vergelijkende studie tussen laserdiffractie en de zeef- en pipetmethode om bodemtextuur te meten. Januari 2001. Instituut voor Bosbouw & Wildbeheer, Ministerie van de Vlaamse Gemeenschap 2001; IBW Bb R 2001.003.

Vandecasteele B, Quataert P, De Vos B, Tack FMG. Assessment of the pollution status of alluvial plains: a case study for the dredged sediment-derived soils along the Leie river. Arch Envrion Con Tox 2004a; 47: 14-22.

Vandecasteele B, De Vos B, Buysse C, Van Ham R, Dauwe, W. Baggergronden in Vlaanderen. Bodemkwaliteit van de alluviale gebieden en gecontroleerde overstromingsgebieden langs de Zeeschelde en bijrivieren. Juli 2004. IBW Bb R 2004.003. In opdracht van AWZ. Instituut voor Bosbouw en Wildbeheer, Geraardsbergen. 2005b, 130 p.

Vandecasteele B, Quataert P, Tack FMG. The effect of hydrological regime on the bioavailability of Cd, Mn and Zn for the wetland plant species Salix cinerea. Environ Pollut 2005; 135: 303-312.

van den Berg GA, Loch JPG. Decalcification of soils subject to periodic waterlogging. Eur J Soil Sci 2000; 51: 27-33.

Van Gestel CAM, Rademaker, MCJ, van Straalen, NM. Capacity controlling parameters and their impact on metal toxicity. In: Salomons W, Stigliani WM editors. Biogeodynamics of pollutants in soils and sediments. Springer-Verlag, Berlin, 1995 pp. 171-192.

Van Ranst E, Verloo M, Demeyer A, Pauwels JM. Manual for the soil chemistry and fertility laboratory. International Training Centre for Post-Graduate Soil Scientists University of Ghent, Gent, 1999, 100 pp.

VLAREBO, 1996. Besluit van de Vlaamse regering houdende vaststelling van het Vlaams reglement betreffende de bodemsanering. Belgisch Staatsblad, 27 maart 1996.

Weckx JEJ, Clijsters H. Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Phys Biochem 1997; 35: 405-410.

Weeks JM, Rainbow PS. The effect of salinity on copper and zinc concentrations in three species of talitrid amphipods (Crustacea). Comp Biochem Physiol 1992; 101(C)2: 399-405.

Weeks JM, Rainbow PS. The relative importance of food and seawater as sources of copper and zinc to talitrid amphipods (Crustacea; Amphipoda; Talitridae). J Appl Ecol 1993; 30: 722-735.

Wellburn AR. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 1994; 144: 307-313.

Werker AG, Dougherty JM, McHenry JL, Van Loon WA. Treatment variability for wetland wastewater treatment design in cold climates. Ecol Eng 2002; 19: 1-11.