Effects of burial of propagules on the emergence of submerged aquatic macrophytes

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Maaike Cazemier

Supervisors:
Bastiaan van Zuidam
Rudi Roijackers

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Preface

This report is the result of a thesis of the author: a requirement for the MSc study programme Hydrology and Water Quality, specialisation Aquatic Ecology and Water Quality Management at Wageningen University. The research was carried out at the Aquatic Ecology and Water Management Group. Supervision was also provided by this group. I would like to thank Bastiaan van Zuidam and Rudi Roijackers very much for their guidance and support. I would also like to thank another member of the Aquatic Ecology and Water Management Group, Edwin Peeters, for his help with interpreting the data and choosing the correct statistical tests. Finally, I would like to thank Eef Velthorst for his help by performing the particle size analysis.

Summary

Burial through sedimentation may negatively affect germination and establishment of aquatic macrophytes. The purpose of this study was to examine the effects of burial on the emergence of *Potamogeton pusillus*, *Chara* species and *Zannichellia palustris*. Two sediment types have been examined that are assumed to accumulate in lake Markermeer and IJsselmeer, i.e. clayish sediment and sandy sediment. Some differences were found between the two burial sediments. The clayish sediment consisted of smaller particles than the sandy sediment, redox values were about 50 mV higher and the sediment had a higher moisture content and higher concentrations of organic matter, nutrients and metals. Without burial, these sediments appeared to be a rather good growth medium for the species examined. The results show that emergence decreased with increasing burial depth for seedlings of *P. pusillus* and *Z. palustris*. For *P. pusillus* emergence was suppressed more under a burial layer of sandy sediment than a layer of clayish sediment. Linear regression showed that *Potamogeton pusillus* only emerges if the thickness of the burial layers does not exceed 4.5 cm in case of burial with sand and 5.8 cm in case of clayish sediment. Emergence of *Z. palustris* was lethally suppressed under a burial layer of 5.8 cm (linear regression). Emergence of *Chara spp.* decreased only slightly under a burial layer of clayish sediment. Burial with a sandy sediment had no significant effect on emergence of seedlings of these species.

Another experiment was performed to study the effects of organic matter content and related factors. The results revealed that emergence of *P. pusillus* was not influenced by partial or full replacement of the clayish sediment with an artificial Bentonite mixture with a very low organic matter content. The treatments showed a gradient in organic matter content and related factors such as redox potential and microbial activity. The results imply that emergence of *Potamogeton pusillus* is not influenced by these factors. However, the set up of this experiment was minimal and could be improved or expanded.

This research has confirmed earlier findings that state that different aquatic macrophyte species respond in a different way to burial with different sediments.

1 Introduction

Lake Ijsselmeer (Figure 1-1) was formed in 1932 after finalisation of the separation dam in the Zuiderzee (Noordhuis et al., 2010). In 1976, after construction of a second separation dam, lake Markermeer was separated from lake IJsselmeer. The lakes are both shallow. Lake Markermeer is mainly 2 to 5 m deep with an average depth of 3.8 m. Lake IJsselmeer is deeper, mainly 3 to 6 m deep and on average 4.6 m deep (Noordhuis et al., 2010). The soil in lake Markermeer mainly consists of clay and loam, while lake IJsselmeer mainly has areas with sandy soils. Both lakes have temperatures ranging from near-zero to >23 °C and are largely turbid due to continuous resuspension and sedimentation of bottom material (Kelderman et al., 2011). Suspended solids concentrations vary between 50 mg/L and 300 mg/L and lead to Secchi depths between 0.2 and 0.8 m. The resuspension is caused by wind induced waves creating orbital flow in the water column which results in shear stress at the sediment surface. Resuspended particles enter the circulating currents in the lake, created by the prevailing wind direction in the Netherlands (South-West). In the deep parts there is a current near the bottom that is opposite to the wind direction, and at the surface there is a current in the wind direction (Noordhuis *et al.*, 2010). In lake Markermeer, net sedimentation occurs in the south-eastern part of the lake, this is the deeper part of the lake where there is no wave-induced erosion. Net erosion occurs in the north-western, shallow part (Noordhuis *et al.*, 2010). Sedimentation in lake IJsselmeer mainly originates from riverine inputs (Klijn *et al.*, 2006). However, erosion and sedimentation mostly comprise of reallocation of material. For example, tidal channels from the period when the lakes were still open to the sea are currently filling up with sediments of more shallow parts (Noordhuis *et al.*, 2010). Sedimentation rates range from 0.9 to 3.6 cm per year, in the period between 1990 and 2001 (Klijn *et al.*, 2006).

Growth of rooted vegetation occurs between 40 cm water depth and 2.70 m water depth in the lakes, but in many areas there is no or little vegetation (Noordhuis et al., 2010). Submerged macrophytes are an important part of the aquatic ecosystem, because they promote clear water by taking up nutrients and decreasing resuspension by fixating the sediment and reducing water movement near the bed. Furthermore, plants (and clear water) promote biodiversity (Scheffer, 2004). Presence of propagules in or on the sediment is necessary for re-establishment of plant stands each spring and colonisation of unvegetated areas. Propagules are viable sexual and vegetative reproduction organs of aquatic vegetation (Barrat-Segretain, 1996). Resuspension of sediment could have a negative effect on submerged aquatic vegetation in several ways, for example by physical disturbance of the seedbank, burial of propagules and increased turbidity, causing lack of light at the lake bottom inhibiting plant growth. In areas where mainly sedimentation occurs, propagules on the bottom of a lake are buried to a certain depth every year. This may reduce the chance of successful (re)establishment of submerged vegetation.
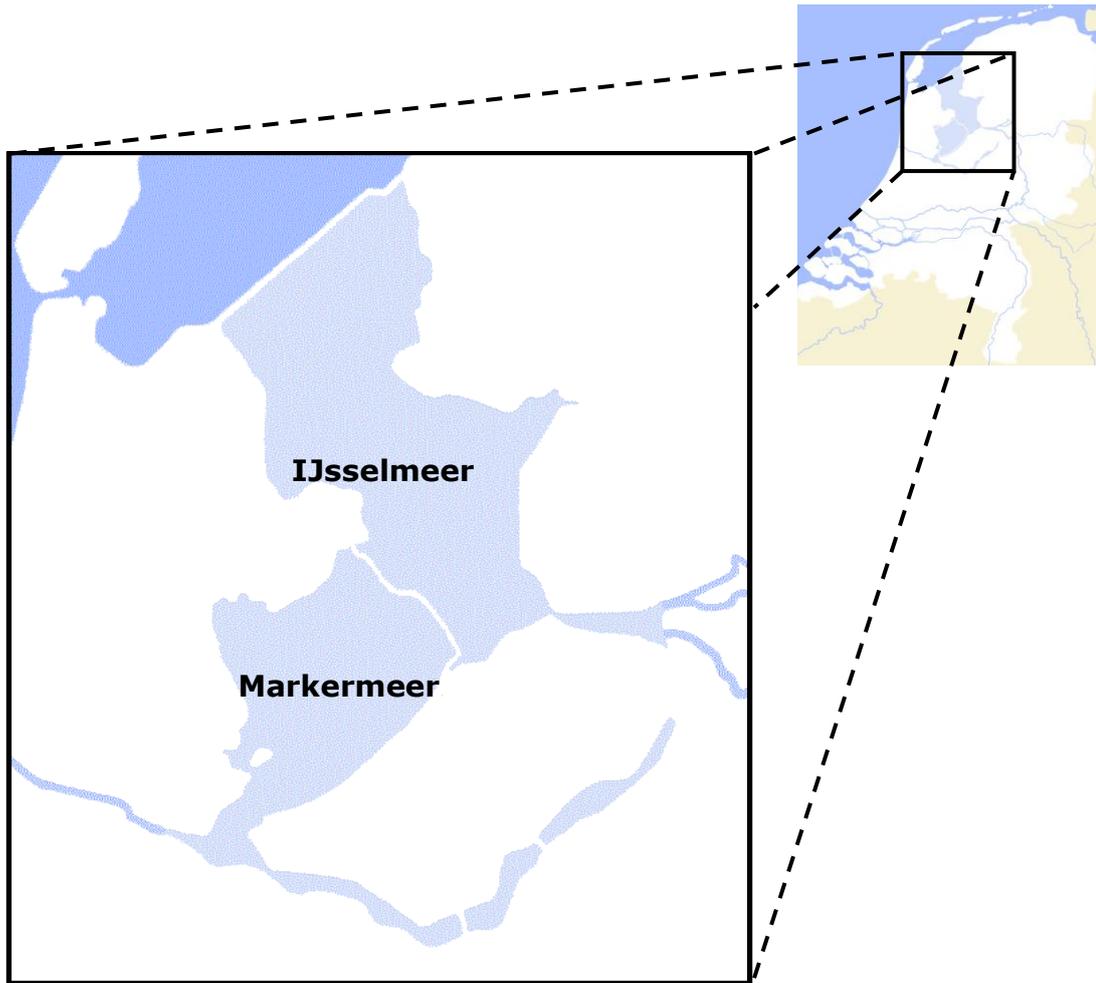
Figure 1-1 Location of the lakes IJsselmeer and Markermeer in the Netherlands

In the lakes, measures to increase biodiversity and coastal safety are considered. These may increase sedimentation, leading to increased propagule burial. In lake Markermeer, water managers are considering the development of sheltering measures in the lake (Noordhuis *et al.*, 2010). The objective is to increase habitat diversity in the lake by creating areas that are sheltered from wave forces. Through these measures, the lake water should become more clear, promoting submerged vegetation. On the other hand, such a measure will also cause an increase of the sedimentation rate in the sheltered area. To achieve the desired increase in diversity it is crucial that submerged vegetation establishes in these sheltered areas. Therefore, it is important that the vegetation is not inhibited by the increased sedimentation rates. In lake IJsselmeer a project has started called the 'Zachte zandmotor' [1]. This pilot aims to create an enforcement of the east coast of lake IJsselmeer by suppleting sand close to the coast. The currents in the lake are supposed to deposit the sand on the coastline. The increased net sedimentation of sand near the coast may affect the present

submerged vegetation and the propagule banks and decrease their ability to establish as a plant.

One of the objectives of this study is to quantify the effect of burial on top of the propagule bank for three different species from the study area: *Potamogeton pusillus, Chara spp.* and *Zannichellia palustris.* These species were selected because they occur in the shallow parts of the lakes, they are therefore easy to collect from the lake floor. Furthermore they are colonizing species and play a part in the development of vegetation fields: plant stands generally start to develop in shallow areas, and establishment of these species forms the first phase of the development of submerged vegetation stands.

Potamogeton pusillus or Lesser Pondweed is a pondweed that grows in nutrient-rich, fairly shallow, stagnant waters (Pot, 2007). The species is quite common in the Netherlands. It reproduces by producing fruits and turions (Barrat-Segretain and Bornette, 2000). A turion is a reproductive organ or a winter bud, consisting of a bud in two or three leaves (Figure 1-2). The turions are approximately 2 cm long. If the turion germinates, the head of the bud will form a shoot with leaves, while the tail develops roots. Turions of *Potamogeton* species are found to germinate under influence of light and in warm temperatures after a period of low temperatures (Soetikno, 1981).

Figure 1-2 Turion of *Potamogeton pusillus*

Charophytes are found to be early colonizers of shallow waters (Van den Berg, 1999). Charophytes reproduce through oospores, produced in large quantities (Sederias and Colman, 2009). Oospores are around 300 µm large and are round zygotes with striae. The oospore wall is black, gray or brown, but is often covered by a light colored lime encrustation (Haas, 1994). Some examples are shown in Figure 1-3. Viable oospores exude white starch grains when squeezed with a forceps. Some charophyte species also produce bulbils, which are vegetative propagules that are formed at the base of the plant on the main stem or in the sediment on the rhizoids (Raam, 1998). Bulbils are not considered in this study.

Figure 1-3 Oospores of *Chara sp.* (Photograph by Bastiaan van Zuidam)

A large variation in germination percentage of charophytes has been observed (Bonis and Grillas, 2002). Known germination cues are exposure to warm temperatures, the end of a period of low temperatures, light and changes in redox potential (Sederias and Colman, 2009, Bonis and Grillas, 2002). Furthermore, both the presence of oxygen and anoxia have been reported to be triggers for germination of different *Chara* species (Bonis and Lepart, 1994, Bonis and Grillas, 2002). Bonis and Grillas (2002) add that different *Chara* species respond to different germination triggers, however it is mostly not known which triggers a species has. Burial is generally found to limit Charophyte emergence (Van den Berg,1999). Finally it should be noted that *Chara* oospores can remain viable for many years (Bonis and Grillas, 2002).

Zannichellia palustris or Horned pondweed is a fast reproducing plant found in shallow, nutrient rich waters. The species occurs in slightly more shallow waters than the other two species studied and produces seeds of about 0.5 cm long [2]. The seeds have a distinct shape, as is shown in Figure 1-4. They are very firm and banana-shaped, with a horned back and two tails. If the seed germinates, the skin splits in the length of the seed and a germling emerges. *Zannichellia palustris* seeds need a period of cold temperatures to stimulate germination (Lombardi et al., 1996). Lombardi *et al.* (1996) also showed that germination of the seeds is optimal at 20 °C. *Zannichellia palustris* seeds have been found to experience negative effects from burial with sand (Spencer and Ksander, 2002).

Figure 1-4 Seed of *Zannichellia palustris* [2]

Research has already shown that propagule emergence and establishment is negatively affected by burial through sedimentation (Dugdale *et al.*, 2001, Bonis and Lepart, 1994, Dittmar and Neely, 1999). Emergence is defined here as the appearance of a young plant above the sediment surface. However, little is known about the processes involved in the germination and emergence of propagules when buried. Also, effects of sediment type, grain size and other physical conditions are unknown. The maximum burial depth that germlings can overcome differs per species and probably depends on energy reserves in the propagule (Dugdale *et al.*, 2001). The effect of burial on germination of propagules also differs between plant species (Dugdale *et al.*, 2001). The size of the propagule and the energy reserve stored in the propagule could be an important factor in determining the maximum burial depth that germlings can overcome (Dittmar and Neely, 1999). The differences in response to burial could also be caused by differences in germination cues, for example organic matter content, temperature and redox potential (Dugdale et al., 2001, Soetikno, 1981, Bonis and Lepart, 1994, Bonis and Grillas, 2002). Toxic concentrations of heavy metals, ammonium and sulphide may inhibit germination (van Wijck *et al.*, 1992). For many species of submerged aquatic macrophytes the response to burial of propagules is not quantified yet. Furthermore, it has been reported that burial with more coarse textured sediments resulted in lower emergence rates in a seed bank (Dittmar and Neely, 1999). Preliminary research by Van Zuidam (unpublished work) has shown that emergence of propagules of *Nitellopsis obtusa* and *Chara vulgaris* reduced with increasing burial depth. The inhibiting effect of burial with mud appeared to be stronger than burial with sand. It is not clear what caused this difference.

This research focuses on the effects of burial of propagules on plant establishment. Plant establishment is crucial for the development of macrophyte stands and water quality. The top layers of the sediment in lake IJsselmeer and Markermeer range from sand in the northeastern part of lake IJsselmeer and heavy clay in the south western part of lake Markermeer. All fractions participate in the resuspension and sedimentation processes in the lake (Noordhuis et al.,

2010). To cover the large range in burial materials the effects of a sandy sediment and a clayish sediment on seedling emergence was examined. The response of *Potamogeton pusillus, Chara spp. and Zannichellia palustris* to burial with these two different sediments was investigated, as well as some possible causes of the changes in emergence such as oxygen concentration, redox potential and organic matter content. This knowledge can aid in decision making to improve the water quality in the lakes and to anticipate consequences of measures considered for the lakes. Previous research on the effects of the burial layer is limited; therefore the intent of this study was to fill a gap and offer further research in this area.

1.1 Research questions and hypotheses

The main question in this research is: What is the effect of burial on the emergence of *Potamogeton pusillus, Chara* species and *Zannichellia palustris*? The aim is to answer the following research questions.

Q1: Is the sediment that accumulates in (parts of) lake Markermeer and Lake IJsselmeer suitable for germination of these species?

H1: The sediment that accumulates in lake Markermeer and lake IJsselmeer is suitable for germination of the studied plant species: a large part of the propagules develops to a plant.

Q2: What is the effect of burial depth on plant emergence of these three species?

H2: Germling emergence declines with increasing burial depth for all species.

Q3: What is the difference in germling emergence between burial with sediment accumulating in lake Markermeer (clayish) and the sediment accumulating in lake IJsselmeer (sandy)?

H3: Burial with clay has a stronger reducing effect on germling emergence than burial with sand, because the redox conditions in the clayish sediment are unfavourable for germination.

Q4: Does the presence of organic matter and corresponding factors, such as oxygen concentration and redox potential in the burial layer have an inhibiting or promoting effect on the emergence of these three species?

H4: The presence of organic matter in the sediment inhibits the emergence of germlings.

2 Material and methods

2.1 Collection and pretreatment of materials

The sediments and propagules used in the experiment have been collected from the field. All have been collected from lakes in the IJsselmeer area. This section discusses how and where the sediments and propagules have been collected, and how they have been treated before the start of the experiment.

2.1.1 Propagules

Oospores of *Chara* species were collected in April 2011 from the sediment of lake Markermeer, in a shallow area close to Muiderberg. The oospores were present in a sandy sediment which was later sieved in the laboratory. The size of the oospores was comparable to the grain size of the sediment, therefore sieving obtained the highest possible oospore density. Material smaller than 500 µm and that was retained on a 212 µm sieve was used. The density of oospores in the sediment has been estimated using a microscope.

Turions of *Potamogeton pusillus* and seeds of *Zannichellia palustris* have been collected from the bottom of lake Eemmeer in the beginning of October 2011. The top layer of the sediment has been collected and sieved in the field over a 1 mm sieve to remove the larger part of the sediment. In the laboratory, the collected material was sieved over a 2 mm sieve to remove large particles and again over a 1 mm sieve and rinsed in a bucket to remove the heavy particles (propagules are relatively light). Turions of *Potamogeton pusillus* and seeds of *Zannichellia palustris* were removed from the remaining material using a forceps. Propagules were identified based on their morphological features. Viability was estimated based on visible appearance: the firmness of the propagule and in case of *Potamogeton* turions the presence of green parts.

All propagules have been stored in tap water at 4 °C for at least one month. This procedure has been used before and has proven to be an effective way of storage and a possible germination trigger (Smart and Barko, 1985, Boedeltje *et al.*, 2002, Van Zuidam, unpublished work). The propagules of *Potamogeton pusillus* and *Zannichellia palustris* have been stored for 5 weeks, oospores of *Chara* sp. have been stored for 7 months.

2.1.2 Sediment

A standard soil was used for the experiments. It was decided to use this soil as a growth medium, because the propagules had already proven to germinate well on this soil. This eliminated the possible event that the sediment used for burial would not provide the factors needed for successful germination. This soil was

created by rewetting Dutch bricks that were not baked. This resulted in a red, clayish mixture with a moisture content of 28%. This clay was homogenised by mixing, to ensure a constant grain size distribution and moisture content. This mixture will hereafter be referred to as red brick clay.

The sediments used to cover the propagules were collected from the field. The clayish sediment was collected from lake Markermeer, close to the shore of Lelystad. The sediment was collected from a landing stage approximately 50 m from the shore, using an Ekman grabber. The sandy sediment was collected from the eastern shore of lake IJsselmeer, at the shore of Hindeloopen. The water was very shallow and it was possible to collect the sediment using a shovel. At both locations it was verified that the collected sediment did not contain vegetation or propagules. Closer examination in the laboratory confirmed this.

Upon return to the laboratory, the sediment was sieved over a 1 mm sieve to ensure that large particles, such as shells, were removed. The sediment was mixed to achieve a homogenised mixture. Until the start of the experiment, the sediment was stored at 4° C. The moisture content and organic matter content have been determined for all sediments. To determine the moisture content, four replicate samples of each sediment have been dried at 100°C for two days. All organic matter was removed from the already dry sediment by combusting the sediment for 5 hours at 550°C. From the weight loss, the organic matter content was calculated.

2.2 Experimental setup

2.2.1 Experiment 1

The experiment was set up in dark grey PVC tubes with a diameter of 125 mm. The propagules were placed on a standard soil of red brick clay. The thickness of this layer was chosen as such that the surface of the sediment, including the burial layers, was equally high for each treatment. In the case of *Potamogeton Pusillus* and *Zannichellia palustris*, 50 and 25 propagules respectively have been randomly taken from the batch with a forceps and placed on the sediment surface. In the case of *Chara spp.*, which were present in a sandy mixture, 4 teaspoons of the mixture have been spread over the sediment surface. The oospores of *Chara spp.* are hard to separate from sand particles. The four teaspoons contain an estimated 60 oospores, based on five replicate countings of the amount that was added to the treatments. It has been assumed that sand and other particles in the mixture were present in a relatively low amount and therefore did not influence germination and emergence.

There was one control treatments and 2 treatments to investigate the direct effects of the sediments, where the propagules were not buried. Propagules were placed on the standard soil (red clay), the sandy sediment or the clayish

sediment. The germination of propagules on standard soil, which is expected to be a good growth medium, can be compared with the germination on the burial sediments. In this way, direct effects of the presence of the sediments on germination will become visible. A schematic view of the different types of experimental units is shown in Figure 2-1 and Figure 2-2.

After placement of the propagules the burial layer was added, except for the treatments without burial. The propagules were buried with 2 types of sediment. The thickness and depth of the sediment layers has been measured from the top end of the units, to make sure that the sediment surface was at the same depth in every unit and therefore the water columns as well. These sediments were applied in different thicknesses, to test the effects of different burial depths. The burial depths are 1, 2.5 and 5 cm. 1 cm is the smallest burial depth practically feasible in this experiment, and 5 cm is a depth that is expected to have severe effect on the propagules (Van Zuidam, unpublished work).

To ensure that a statistical analysis (ANOVA) can show differences between the results of the different treatments, every treatment had 5 replications. An overview of the set-up of the experiment is shown in Table 2-1. These treatments are applied to the three species in 5 replicates. For capacity reasons, it was decided not to include *Zannichellia palustris* in the treatment with the sandy sediment.

Table 2-1 Experimental setup of the first experiment.

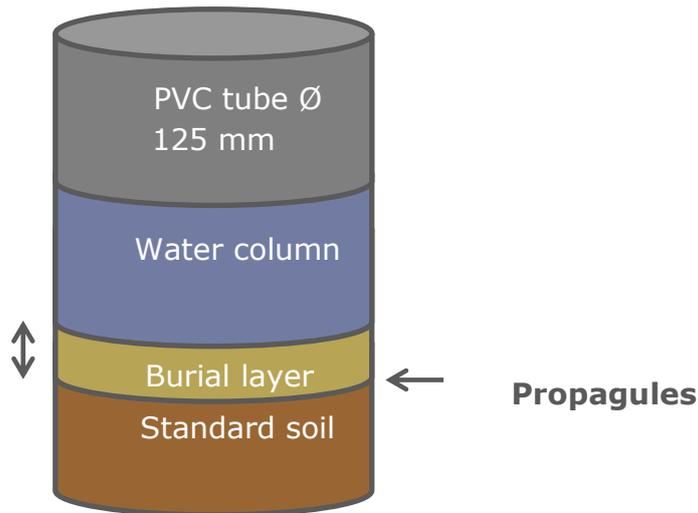| Treatment	Burial depth
Control	0 cm
Burial with clayish sediment	1 cm
	2.5 cm
	5 cm
Burial with sandy sediment	1 cm
	2.5 cm
	5 cm
Direct effect clayish sediment	0 cm
Direct effect sandy sediment	0 cm

Figure 2-1 Schematic view of the experimental setup for the burial treatments and the control treatment with the standard soil.
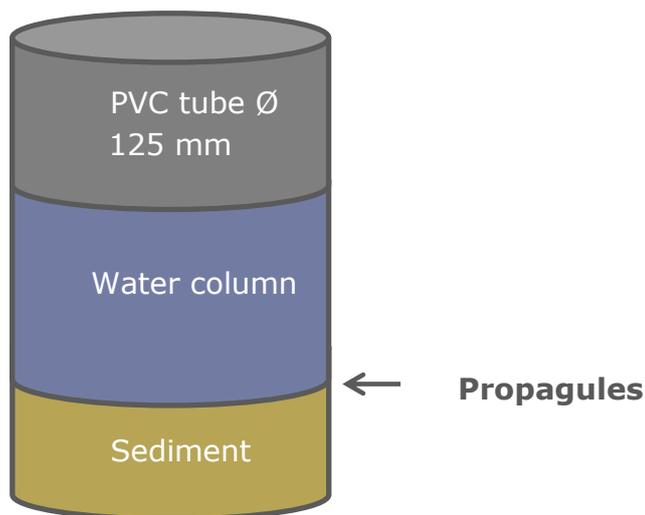

Figure 2-2 Schematic view of the experimental setup for the control treatment with sediment to test the effect of the clayish and sandy sediment without burial is shown.

The units were filled with tap water to create an environment favourable for germination. Tap water has been found to be a good environment for aquatic plants (Litav and Lehrer, 1978). The water was added very gently, to minimize resuspension of the sediment. The sediment surface remained visible through the water column of approximately 8 cm. The experimental units have been placed in a water bath that was kept at a constant temperature of 20°C. The set-up is shown in Figure 2-3. It was decided that this was the best temperature for propagule germination based on the results of earlier experiments and literature (Lombardi *et al.*, 1996).

Figure 2-3 The water bath with the experimental units

Six growth lights were suspended above the water bath, as shown in Figure 2-4. The lights produced an intensity of on average 400 µEinstein/m^2/s just below the water surface for 12 hours per day. The experimental units are placed randomly in the water bath to prevent any influence of the position of the lights on the results.

Figure 2-4 Growth lights suspended above the water bath

During a period of 2 months, emergence of germlings was monitored. Emerged germlings were removed carefully every week as soon as the shoots were recognizable and the plant species could be identified. Although this induced a slight disturbance of the sediment surface, competition between plants was

prevented and it was the only way to count the germlings. The germlings were counted and checked for presence of the propagule. Generally, the propagule is attached to the germling and is therefore removed with the plant. If this is not the case, the germling is not counted because the propagule that stays behind may form a new shoot inducing a double counting.

Apart from the removal of germlings, the experimental units required maintenance. During the two months the experiment was running, the water levels in the units and the water bath have been maintained using demineralized water. The water levels in the experimental units would drop almost 3 cm per week due to evaporation. Refilling the experimental units with demineralized prevented inputs of nutrients and salts. In the treatments with a top layer of clay, a high abundancy of algae and filamentous algae developed during the two months of the experiment. Transparency should be comparable between units to provide equal conditions for germination. To maintain visibility in these units, Daphnia magna were added to the water. The Daphnid population maintained clear water in most of the experimental units, although some additional removal of algae was needed to enable clear view of the sediment surface. This was done using a hand sieve and forceps, without disturbing the sediment.

At the end of the experiment, sediment of the treatments with *Potamogeton pusillus* and *Zannichellia palustris* was sieved over a 1 mm sieve to retrieve all propagules and non-emerged germlings. The propagules found were counted and visually examined to establish whether they had germinated but not emerged, and (in case of no germination) whether the propagule was still viable. This could not be done for *Chara spp.*, because the oospores are very small and have the size of sand grains.

2.2.2 Experiment 2

To explore the influence of organic matter in the sediment on emergence of germlings, a gradient in organic matter content has been made in the clayish sediment used in experiment 1. To do so, another smaller experiment was designed to dilute the organic matter content. This was done in 5 steps using a mixture of bentonite clay, pellets used to seal walls of auger holes, and white sand [3]. The bentonite mixture was artificially made using 1 part bentonite clay and 2 parts fine sand based on weight. The ratio between the clay and the sand was determined empirically to find the concentrations where the moisture content and texture of the mixture was as equal to that of the clayish sediment used as possible. It was assumed that mixing with this clay would dilute the organic matter content without influencing other factors such as density and grain size.

Five different mixtures of Markermeer clay and bentonite clay form a gradient of concentrations of organic matter and related substances. For this experiment,

propagules of *Potamogeton pusillus* was buried under 2.5 cm of mixed clay sediment and bentonite clay (in 5 different concentrations) as shown in Table 2-2. This species and burial depth were chosen because emergence of this species was expected to be reduced by about 50% when buried with the natural clayish sediment. The chance of good visibility of any effects of the sediment mixture on emergence rates would therefore be high. The setup of the experimental units was the same as experiment 1, as shown in Figure 2-1 and in terms of lighting and temperature.

Table 2-2 Experimental setup of the second experiment, exploring effects of organic matter.

Treatment	Potamogeton pusillus
100% clay sediment	2x
75% clay sediment/25% bentonite mixture	2x
50% clay sediment/50% bentonite mixture	2x
25% clay sediment/75% bentonite mixture	2x
100% bentonite mixture	2x
Total of units	**10 units x 50 propagules**

2.2.3 Measurements

In the final weeks of the experiment, oxygen and redox profiles in the first 3.5 cm of the sediment were measured using a two glass microsensor [4]. Due to time constraints, a limited amount of experimental units have been measured (two units of every treatment). A step size of 1 mm was used, with a stabilization time of 20 seconds.

For every sediment, the available PO_4, NO_3/NO_2 and NH_4 was measured in triplicate. Using the Psenner extraction method (Psenner & Pucsko 1988) the available P in the pore water and the organically and iron bound P were measured separately. The nutrients available in the pore water are directly available and are extracted using nanopure water. The organically and iron bound nutrients P becomes available in an anaerobic, reduced environment. These nutrients are extracted using $Na_2S_2O_4$ and $NaHCO_3$.

To explore the possibilities of sulphide toxicity in the sediments used, the total concentrations of Al, Fe, Mn and S were determined in duplo for each sediment type. These elements were measured with ICP-AES after destruction of air dry samples with HNO_3 – HCl. These measurements were used to calculate whether the sulphur present in the sediment could be bound by the metals present.

Because germlings may be obstructed by large grains present in the sediment, for every sediment used the grain size distribution was determined in duplo. The measurements were performed using the laser diffraction method with a Coulter LS230 instrument. This instrument measures 116 size fractions ranging from 0.04 to 2000 µm (Buurman *et al.*, 2001).

2.3 Data processing and analysis

After finalization of the experiments and measurements, the data was processed and analysed using Excel and SPSS. To do so, emergence was defined as the percentage of the propagules that had emerged as a plant and expressed as percentage of the total amount of propagules that emerged. Germination was defined as the percentage of the initial amount of propagules that had germinated and emerged or germinated but not emerged.

A statistical analysis was performed to assess the significance of the results. The results of experiment 1 were emergence rates for every treatment in 5 replicates. These were analysed using a 1-way and 2-way ANOVA. The 1-way ANOVA (analysis of variance) helps to determine whether there are any significant differences between the means of three or more groups (Dytham, 2011). The null hypothesis is that there is no significant difference between the groups. The test is based on the following assumptions. First of all, every case is independent and the dependent variable is continuous. Secondly, this variable is approximately normally distributed which was tested using the Shapiro-Wilk test for normality (Dytham, 2011). Finally, equal numbers of subjects in the various groups are needed. The 2-way ANOVA is comparable to the one-way ANOVA, but now the dependent variable can be tested for influence of two independent variables instead of one. Furthermore, interaction effects of the two variables can be tested, to find out whether there are two independent ways of grouping the data (Dytham, 2011). There are three null hypotheses: groups based on the first variable have the same mean, groups based on the second variable have the same mean and the two variables do not interact. The assumptions for this test are the same as for the one-way ANOVA.

For the emergence rates found in experiment 2, a linear relation was expected. Therefore the experiment was set up in a regression design (duplo). The results were analysed using linear regression. Linear regression is used to investigate the linear relationship between a dependent and an independent variable. The purpose is to describe the linear relationship between these two variables, to determine how much of the variation in the dependent variable can be explained by the linear relationship with the independent variable and to predict new values of the dependent variable from new values of the independent variable (Quinn and Keough, 2002). This analysis assumes that the values of the independent variable are arbitrarily chosen, that the relationship between the

variables is best described linearly and that the dependent variable is approximately normally distributed (Dytham, 2011).

Some other measurements performed during the experiments also required statistical analysis. Firstly, the measurements of N and P. A Kruskal Wallis test was performed to assess whether the medians of the groups of replicates differed significantly from each other. A Kruskal Wallis test is a distribution free rank test. It is the non-parametric version of the ANOVA and is used to compare the medians of 3 or more groups (Quinn and Keough, 2002). The null-hypothesis states that all samples are taken from populations with the same median (Dytham, 2011). The test does not make assumptions about normality or homogeneity of variances (Dytham, 2011). For post-hoc testing, to find out which two groups differ significantly, the Mann-Whitney U test is used. This test is also a rank-based, non-parametric test, but compares two groups. The test is more powerful for two samples than the Kruskal-Wallis test (Dytham, 2011). The null hypothesis of this test is that the two samples come from populations with identical distributions. To use this test for post-hoc testing the significance level α is adapted for the number of combinations made between the groups. α is therefore divided by the number of combinations of two groups made (Bonferroni correction, Quinn and Keough, 2002).

Finally, to assess the differences between the grain size distributions measured, the Kolmogorov-Smirnov test was used. This test is generally used to analyse frequencies or large samples of continuous data. The two sample version of this test used here tests whether 2 distributions are the same (Dytham, 2011). This question is answered calculating a test statistic, by dividing every value by the number of observations of each distribution and comparing them. The test statistic, D, is defined as the largest difference between the two cumulative frequency distributions (Sokal and Rohlf, 1981). This test statistic is then compared to the critical difference, D_α, based on the significance level (0.05). If D is larger than D_α, there is a significant difference between the 2 distributions (Sokal and Rohlf, 1981).

3 Results

3.1 Sediment characteristics

3.1.1 Moisture and organic matter content

The average moisture contents per sediment are shown in Table 3-3. The average percentages moisture and organic matter are shown in Table 3-1 and Table 3-2. The standard deviations are shown between brackets. These show that the variation between replicates was generally low.

Table 3-1 Moisture and organic matter content of the sediments used in experiment 1, expressed in weight percentage (std. deviation between brackets).

Sediment type	Moisture content	Organic matter content
Red brick clay	27.8% (0.1%)	2.8% (0.1%)
Clayish sediment	67.1% (0.1%)	6.5% (0.6%)
Sandy sediment	19.9% (0.2%)	0.1% (0.0%)

Table 3-2 Moisture and organic matter content of the sediment mixtures used in experiment 2, expressed in weight percentage (std. deviation between brackets).

Sediment type	Moisture content	Organic matter content
100% clay sediment	67.1%	6.5% (0.6%)
75% clay sediment/25% bentonite mixture	66.9%	5.0% (0.8%)
50% clay sediment/50% bentonite mixture	67.1%	3.4% (0.2%)
25% clay sediment/75% bentonite mixture	67.2%	1.9% (0.1%)
100% bentonite mixture	67.3%	0.5% (0.1%)

3.1.2 Particle size distribution

The grain size distribution was determined for every sediment type used. The results for the clayish sediment, the sandy sediment, the red brick clay sediment and the bentonite mixture respectively are shown in Appendix 1. The distribution is based on volume and not numbers. Large particles are therefore emphasized upon in this distribution. The particle size distribution over the classes of the Dutch NEN 5104 classification is shown in Table 3-3 (Meulen *et al.*, 2002).

Table 3-3 Particle size distribution of sediments used in this study, classified according to the Dutch classification (NEN 5104). The volume in each class is shown as a percentage of the total volume of particles in the sample.

NEN classification	Size range	Clayish sediment (%)	Sandy sediment (%)	Red brick clay (%)	Bentonite mixture (%)
clay	< 2 μm	8.0	0.0	18.7	3.7
silt	2 - 63 μm	26.2	0.0	80.9	7.6
extremely fine sand	63 - 105 μm	14.6	0.3	0.4	15.2
very fine sand	105 - 150 μm	9.3	2.2	0.0	33.5
moderately fine sand	150 - 210 μm	6.8	7.6	0.0	23.7
moderately coarse sand	210 - 300 μm	18.9	34.5	0.0	14.8
coarse sand	300 - 420 μm	10.3	39.2	0.0	1.5
very coarse sand	420 - 2000 μm	6.1	16.4	0.0	0.0

Table 3-3 shows that the clayish sediment contained clay, silt and sand particles. The presence of sand in this sediment was expected, because during processing of this sediment the larger particles could be felt. The sandy sediment contains more large particles than the clayish sediment. This sediment was also observed to be heavier than the clayish sediment. The table shows that in the sandy sediment there are no silt or clay particles present.

A large volume of the red brick clay used as standard soil consists of clay particles. The particle size distribution shows that this sediment only entails clay and silt. However, during processing of this sediment a number of large particles were found with a diameter of over 1 mm.

There is a statistically significant difference between the cumulative frequency distributions of the different sediment types (Kolmogorov-Smirnov, $D > D_{0.05}$ for every combination).

Table 3-3 also shows the particle size distribution of the bentonite mixture used in experiment 2. The Bentonite mixture has a particle size distribution comparable to the clayish sediment, however the distributions are statistically different ($D > D_{0.05}$).

3.1.3 Redox and oxygen

The oxygen profiles measured were similar for all treatments: saturated or supersaturated conditions just above the sediment (3 to up to 20 mg/L) and anoxic conditions from the first millimetres below the top of the sediment. The oxygen concentrations were therefore zero at the propagules for all burial treatments. The oxygen profiles are shown in Appendix 2. The redox profiles however differed between treatments. The range in redox potentials for every treatment is shown in Figure 3-1 trough Figure 3-8, along with the average redox potential profile. The redox potentials in the clayish sediment vary most, over the depth as well as between replica's. The redox profiles measured in treatments with sand are more consistent and vary less between measurements. In these profiles the effect of the underlying red brick clay is visible and therefore the location of the propagules. At this point, the redox potential increases again slightly. Figure 3-7 shows that the redox potential of the red brick clay is higher than the clayish and sandy sediments used. The redox potential of the treatments with the 100% bentonite mixture in experiment 2 remains relatively high throughout the depth profile with a minimum of 100 mV.
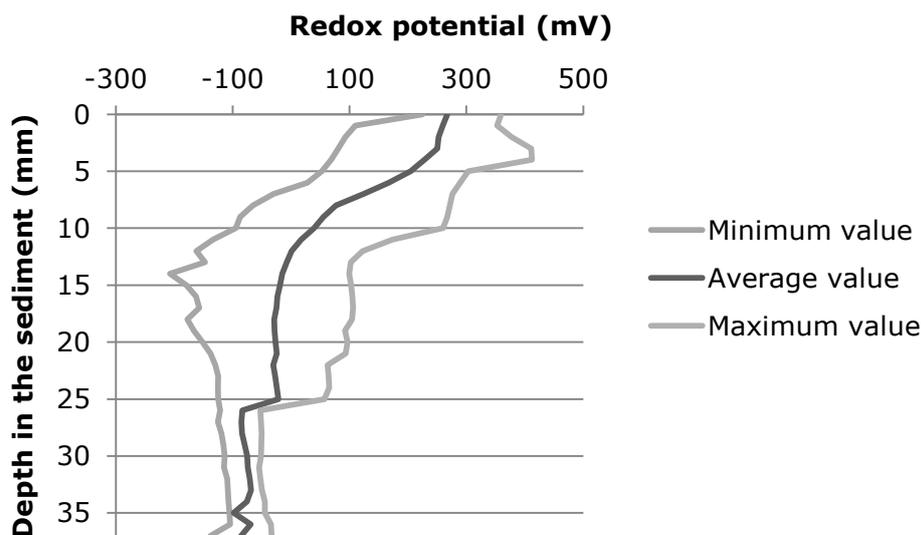
Figure 3-1 Depth profile of the redox potential for treatments with a burial layer of 1 cm clay (6 units, duplo measurements).
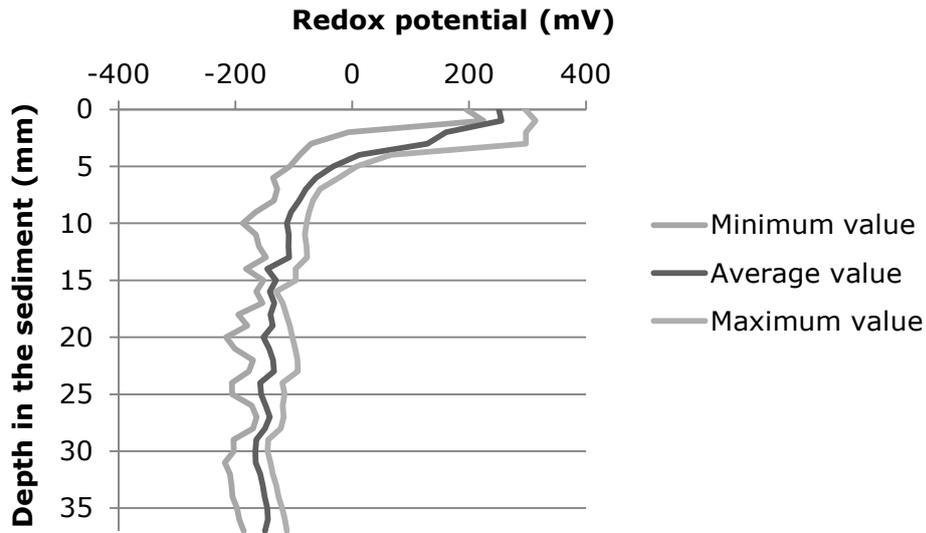
Figure 3-2 Depth profile of the redox potential for treatments with a burial layer of 2.5 cm clay (2 units, duplo measurements).
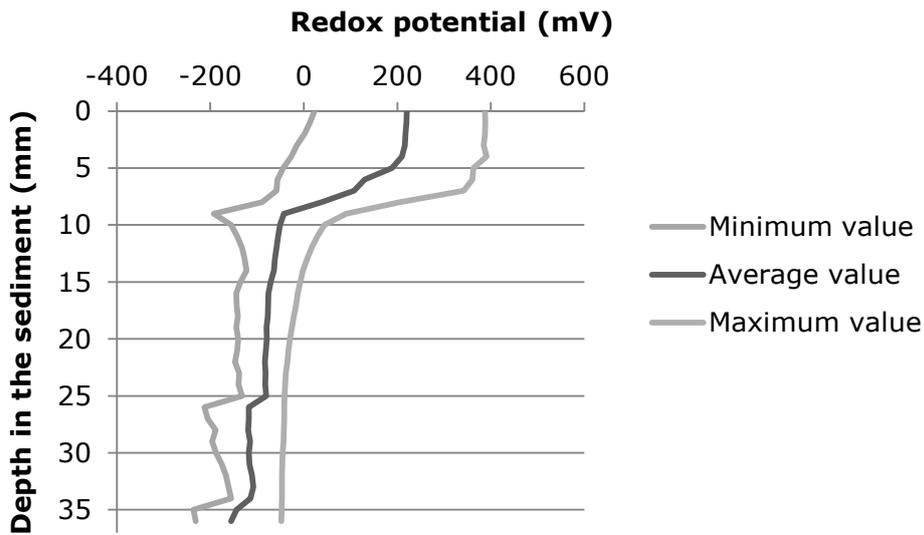
Figure 3-3 Depth profile of the redox potential for treatments with a burial layer of 5 cm clay (3 units, duplo measurements).
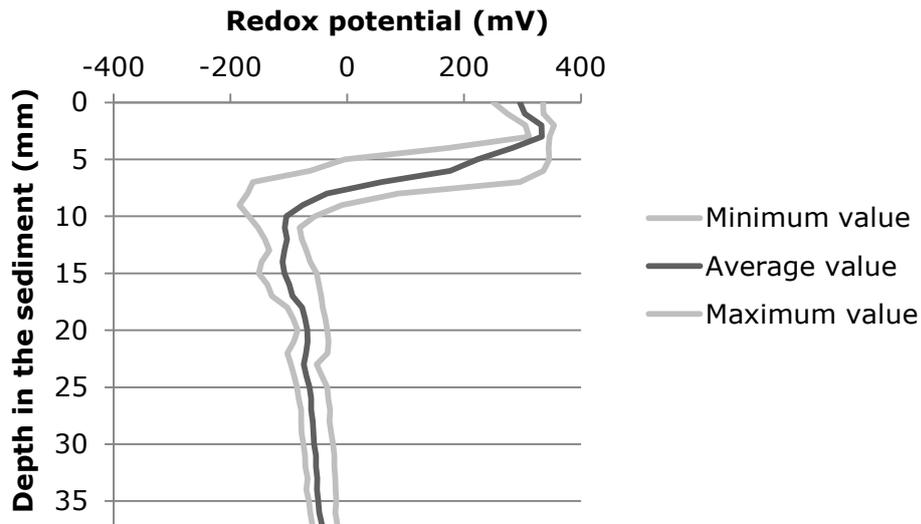
Figure 3-4 Depth profile of the redox potential for treatments with a burial layer of 1 cm sand (2 units, duplo measurements).
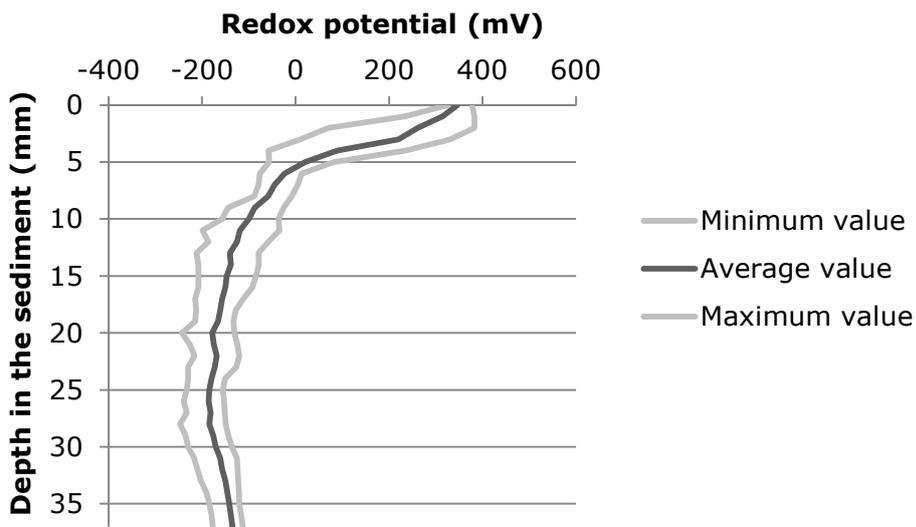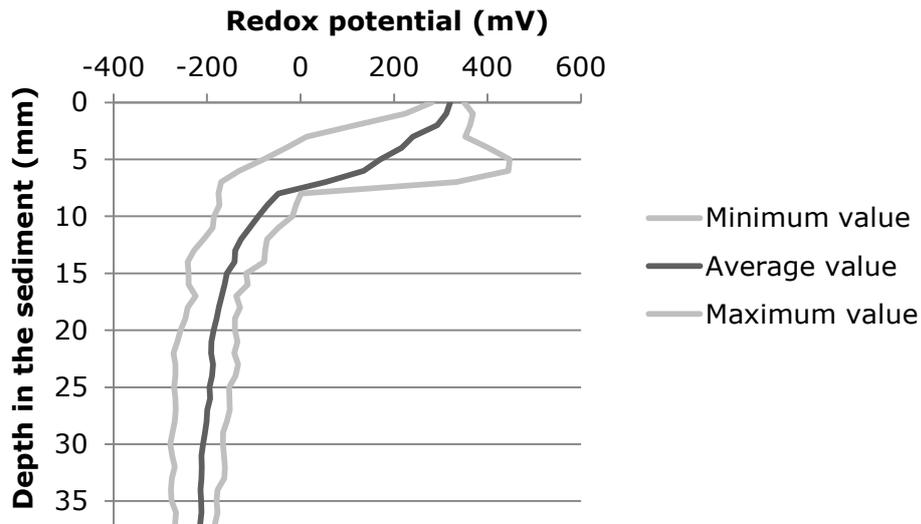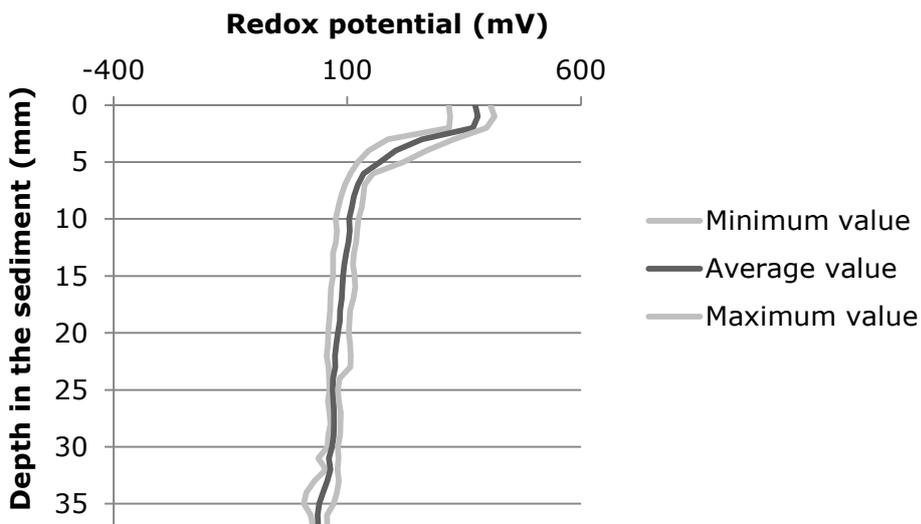

Figure 3-5 Depth profile of the redox potential for treatments with a burial layer of 2.5 cm sand (2 units, duplo measurements).

Figure 3-6 Depth profile of the redox potential for treatments with a burial depth of 5 cm sand (3 units, duplo measurements).

Figure 3-7 Depth profile of the redox potential for control treatments with a soil of red brick clay and no burial (2 units, duplo measurements).
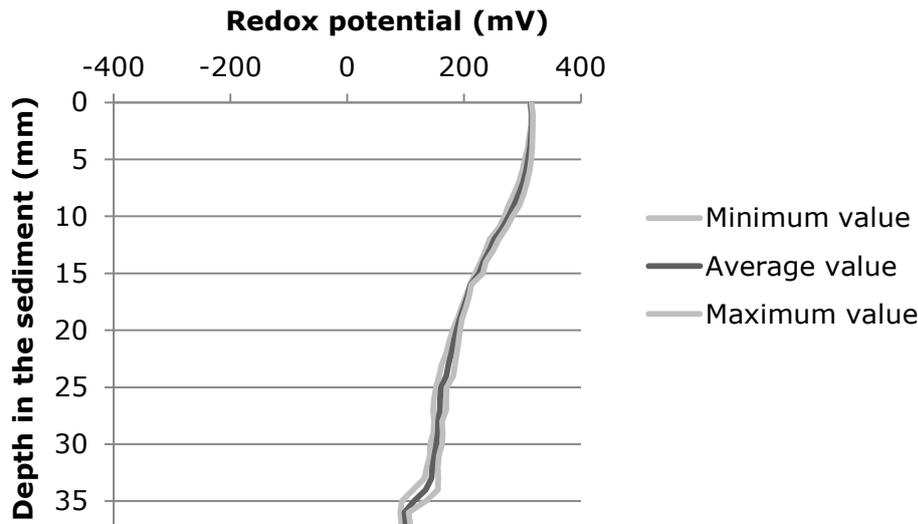
Figure 3-8 Depth profile of the redox potential for treatments with the pure Bentonite mixture, not mixed with clay (2 units, duplo measurements).

Table 3-4 shows the average estimated redox potential at the depth of the propagules of the different treatments. The redox potential at the propagules buried under sand is lower than burial with clay. This is probably caused by the organic matter in the clayish sediment. The redox potential is maintained at the level for oxidation or fermentation of organic material to CO_2 and methane respectively while there is enough material available (Georgio and Williams, 2005). There is very little organic matter present in the sandy material (chapter 3.1.1), allowing redox potential to become lower. The bentonite mixture had a high redox potential compared to the other sediments, while the mixture has an organic matter content of 0,5%. A reason might be that the bentonite mixture was the only sediment that had oxygen present for over 1 cm into the sediment. The mixture was laboratory-made and had no microbial life at the start of the experiment.

Table 3-4 Average estimated values for the redox potential (mV) at the depth of the propagules.

Burial depth	Clay	Sand	Red brick clay	Bentonite mixture
0 cm	350	300	350	
1 cm	-100	-150		
2.5 cm	-150	-180		150
5 cm	-200	-250		

3.1.4 Nutrients

The available nitrogen and phosphate measured is shown in Table 3-5. This is an average of three replicates, and the sum of both available and loosely sorbed N and P. For both N and P, a Kruskal Wallis test (chapter 2.3) showed a significant difference between the measurements for the different sediment types (H(4)=12.210, p=0.016 and H(4)=9.771, p=0.044 respectively). However, post-hoc testing using the Mann-Whitney U test (chapter 2.3) did not reveal which groups differed significantly. The standard deviations are shown between brackets in Table 3-5 to indicate that there is some overlap between the measurements.

Table 3-5 Measured values of available and loosely sorbed N and P in mg/g dry weight with standard deviation between brackets.

Sediment type	Average P in PO4	Average N in NO3, NO2 and NH3
Red brick clay	0.0529 (0.0140)	0.0053 (0.0018)
Clay	0.0371 (0.0033)	0.0188 (0.0017)
Sand	0.0165 (0.0013)	0.0138 (0.0140)
Bentonite mixture	0.0113 (0.0011)	0.0047 (0.0008)

3.1.5 Metals and sulphide

To assess the possibilities of sulphide or aluminium toxicity, the presence of a number of metals and sulphur in the sediment was measured. In Table 3-6 the total amounts of Al, Mn, Fe and S are shown that are present in the sediments used. The concentrations are given in moles, because S binds 1/1 with Fe and Mn (Wang and Chapman, 1999). The table shows that the total amount of S is much lower than the total amount of Fe and Mn. Under waterlogged conditions, sulfate present in organic material will be reduced to sulphide, which can be highly toxic to plants (Welle *et al.*, 2007). Sulfide reacts with iron and manganese to form iron and manganese sulfides in an anoxic environment (Wang and Chapman, 1999). It was already mentioned that, in the experimental units, oxygen was depleted within a few mm in the sediment. Therefore, Fe^{2+} and Mn^{2+} will probably be present to bind the sulphide formed. This means there will likely not be any influence of sulfide toxicity on emergence of the propagules.

Some differences in contents show from the table. The clayish sediment has by far the highest S concentration. The content of Fe is also high. The Fe concentration is the highest in the red brick clay, which was expected because this is a red clay which usually indicates high amounts of oxidised iron. The concentrations in the sandy sediment are lowest. The aluminium content of the sediments is quite high compared to the other metals. Toxic effects of aluminium

are possible, but not expected because the highest concentration is found in the red brick clay sediment which has already proven to be a good growth medium. The pH of the water in the treatments was around 7. The pH in the sediment is therefore expected to be 6 to 7, a value where solubility of aluminium is low (Gensemer and Playle, 1999).

Table 3-6 Metals and sulphur measured in the sediments used (mol/kg dry weight).

	Al	Mn	Fe	S
Red brick clay	0.834	0.016	0.507	0.004
Sandy sediment	0.011	0.000	0.006	0.002
Clayish sediment	0.438	0.006	0.272	0.185
Bentonite mixture	0.444	0.003	0.106	0.063

3.2 Experiment 1

Already one day after the start of the experiment the first results became visible: the propagules that were not buried started to germinate. During the two months of the experiment, changes in the experimental units became visible. The treatments with a top layer of red brick clay developed a film on top of the sediment but remained mostly unchanged. The treatments with a top layer of sand also remained mostly unchanged, but produced small gas bubbles. This may have been caused by air that was in the sediment when it was brought in the experimental units. The treatments with a top layer of clay were mostly unrecognizable at the end of the experiment. Microbial and biological activity in the sediment had led to an active, overgrown top layer. The sediment also appeared to be lifted up in some treatments, due to production of gas. After some time, the gas escaped in large bubbles. The gas had no specific smell. Furthermore, the treatments developed several populations of algae and filamentous algae.

When removing the germlings from the sediment, the propagule is usually still attached to the germling. Therefore the propagule is also removed. However, during the experiment it became clear that it was not always possible to remove the propagule with the germling. The propagule or a small part of the plant may remain in the sediment, possibly developing to another germling and inducing a double counting. Therefore the decision has been made to exclude the countings without the propagule attached to the germling. However, the number of times this happened was low: for *Potamogeton pusillus* and *Zannichellia palustris* 2 and 6% of the emerged germlings respectively. *Chara spp.* Had the largest chance of not retrieving the propagule: this happened for 27% of the germinated germlings. Not only because of the small size of the oospore, but also because

germlings of *Chara spp.* tended to split into several branches below the sediment surface. Therefore it was difficult to see whether there were two or more plants or only one. The decision not to include countings without retrieval of the propagule did not influence the significance or normality of the results.

3.2.1 Burial depth

Figure 3-9 shows the emergence percentages per sediment type against burial depth with a 95% confidence interval, for all species. There was a main effect of burial depth ($F_{(3,111)}=10.33$, $p<0.0005$), indicating that emergence generally decreases if burial depth increases.
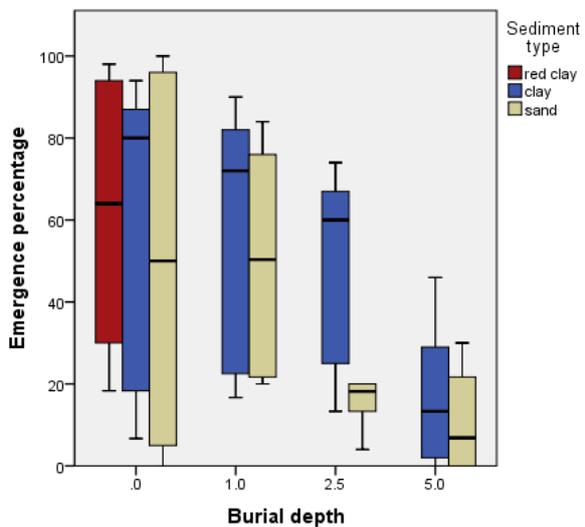
Figure 3-9 Boxplot of the emergence percentage per sediment type (N=115).

In Figure 3-10, Figure 3-12 and Figure 3-14 the response of the three plant species to burial is shown. Figure 3-10 shows the mean emergence percentage after 2 months for *Potamogeton pusillus*. The burial treatments are compared to the treatment with red brick clay without burial, because this layer of red brick clay is also present under the burial layers for reasons explained in the Material and methods section. The figure shows that the emergence decreases when the propagules are buried. For burial with sand germination is reduced more than for burial with clay. The difference between the sediments is largest for large burial depths. For burial with 5 cm sand, the emergence rate decreases to almost zero, while burial with 5 cm clay still results in an emergence of more than 30%. The decrease in emergence with burial depth is significant for both sediment types. One-way ANOVA showed that for sand $F_{(3,16)}=594.16$ and $p<0.0005$. For clay the analysis resulted in $F_{(3,16)}=79.95$ and $p<0.0005$.
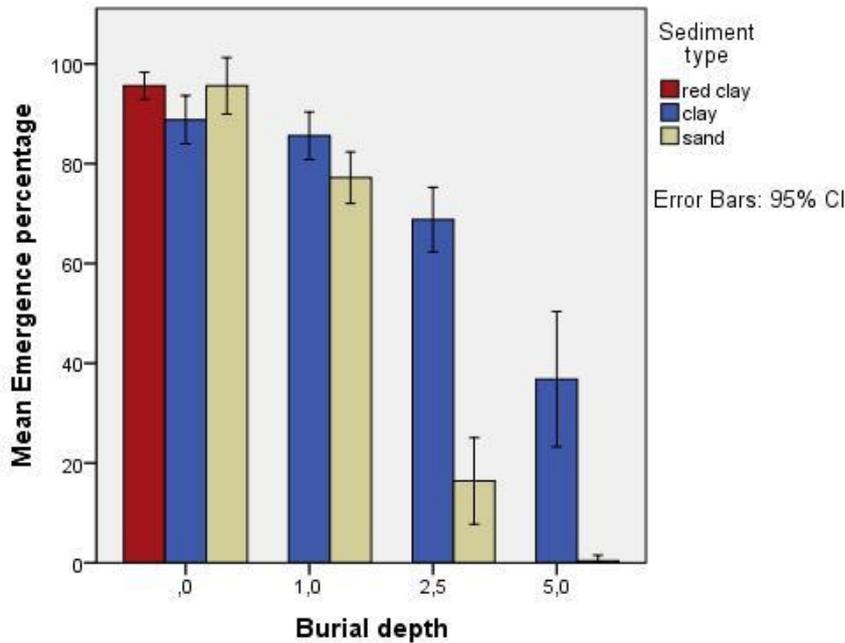
Figure 3-10 Mean emergence rate against burial depth (cm) for *Potamogeton pusillus*. The error bars show the 95% confidence interval (N=45).

A linear regression was performed for the two sediment types used for burial. The result is shown in Figure 3-11. Regression showed that for burial with sand the emergence decreased with 10.0 germlings per cm burial or with 19.9% of the propagules present. The coefficient of determination (R^2) was 0.875, indicating that 87.5% of the variation in emergence can be explained by burial depth. From the regression follows that, starting at an average germination of 89.7% germination at 0 cm burial, emergence will be reduced to zero at a burial depth of 4.5 cm. When buried with clay, emergence decreased with 5.9 propagules per cm burial depth or 11.8% of the propagules originally present (R^2 = 0.935). Starting at an average germination of 96.8% without burial, emergence will be reduced to zero at 8.2 cm burial depth.
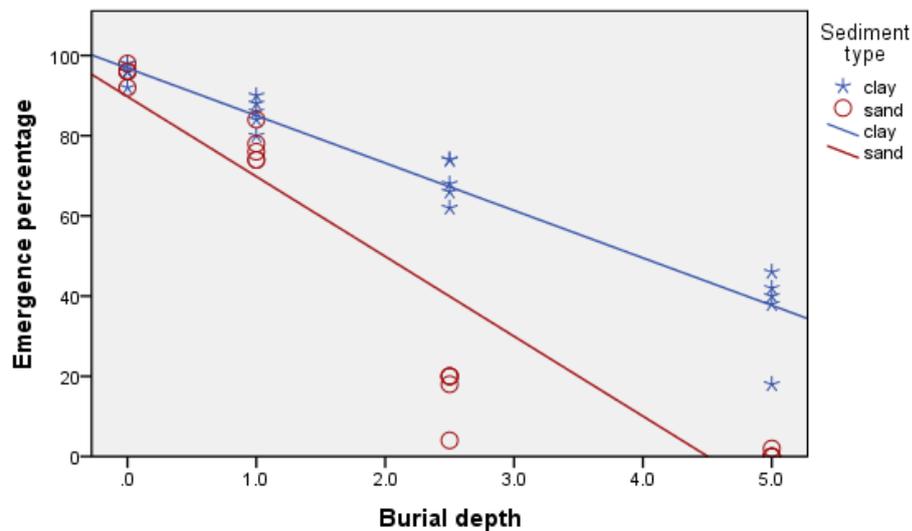
Figure 3-11 Measured emergence of *Potamogeton Pusillus* for burial with sand and clay (cm) and linear regression for the two sediment types (N=35).

Figure 3-12 shows the average emergence percentage of *Chara spp.* after 2 months. The emergence percentages are lower than for the other two species used in this experiment. The emergence percentages vary around 20%. Comparing the red brick clay treatment with the burial treatments, the results show a slight decrease in emergence rate with burial depth for burial with clay (F(3,16)=4.006, p=0.026). The emergence decreased with 1.9% per cm burial with a coefficient of determination of 0.392. Starting at an emergence rate of 23.3%, this means that emergence of *Chara spp.* is not depleted until the oospores are buried with 12.2 cm clay. The regression is shown in Figure 3-13.

For burial with sand, the emergence percentages do not show some decrease with burial depth, but this is not significant (F(3,16)=3.283, p=0.058). Linear regression showed a decrease of 0.7% in emergence rate per cm burial depth. The coefficient of determination for this regression was 0.051, meaning that only 5% of the change in emergence rate with burial can be explained with a linear model.
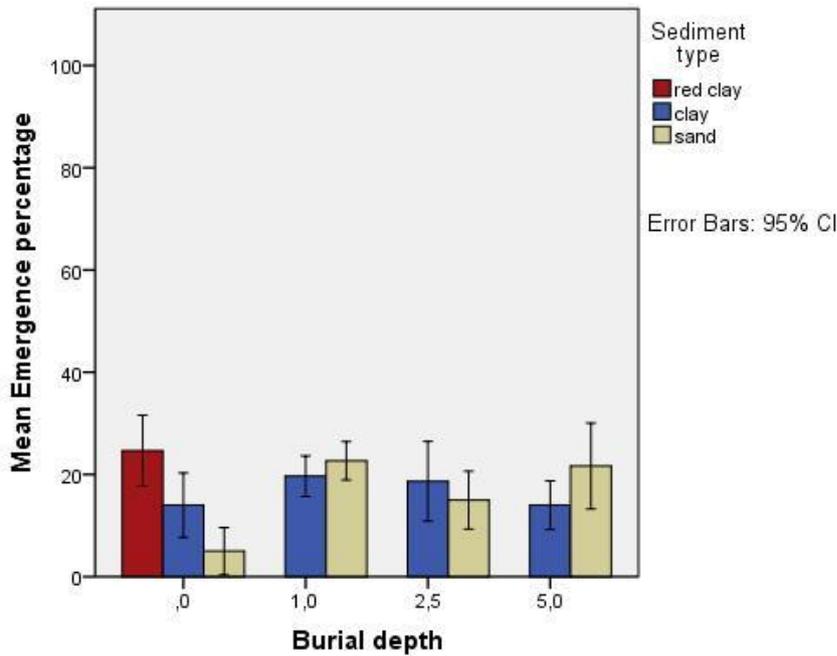
Figure 3-12 Mean emergence rate against burial depth (cm) for *Chara spp.* The error bars show the 95% confidence interval (N=45).
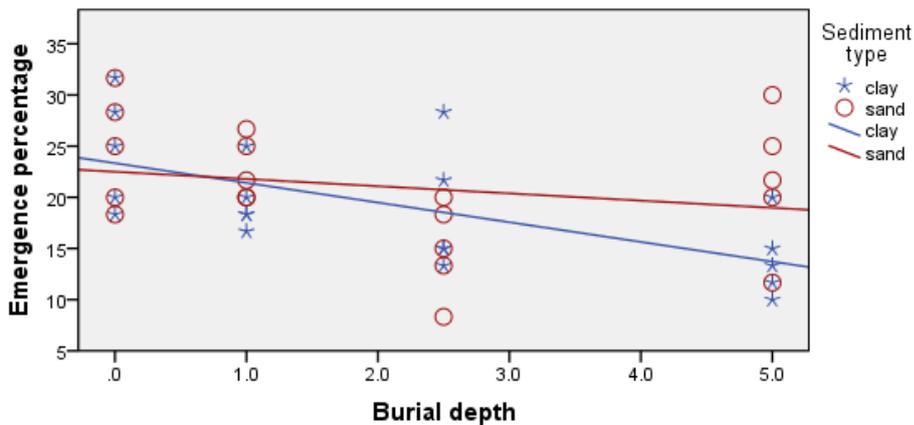

Figure 3-13 Measured emergence of *Chara spp.* for burial with sand and clay (cm) and linear regression for the two sediment types (N=35).

Figure 3-14 shows the mean emergence percentage after 2 months for *Zannichellia palustris* and the 95% confidence interval. This species only received burial treatments with clay. Comparing the burial treatments with the red brick clay treatment, the emergence rates of this species decrease significantly with burial depth (F(3,16)=81.802, p<0.0005). The results show that burial with 1 cm clay resulted in a small increase in emergence rate, when the emergence of the three treatments is compared with the control treatment without burial (in red). A burial layer of 5 cm appears to be lethal, the emergence percentage decreases from over 50% for burial with 2.5 cm to almost zero for burial with 5 cm.
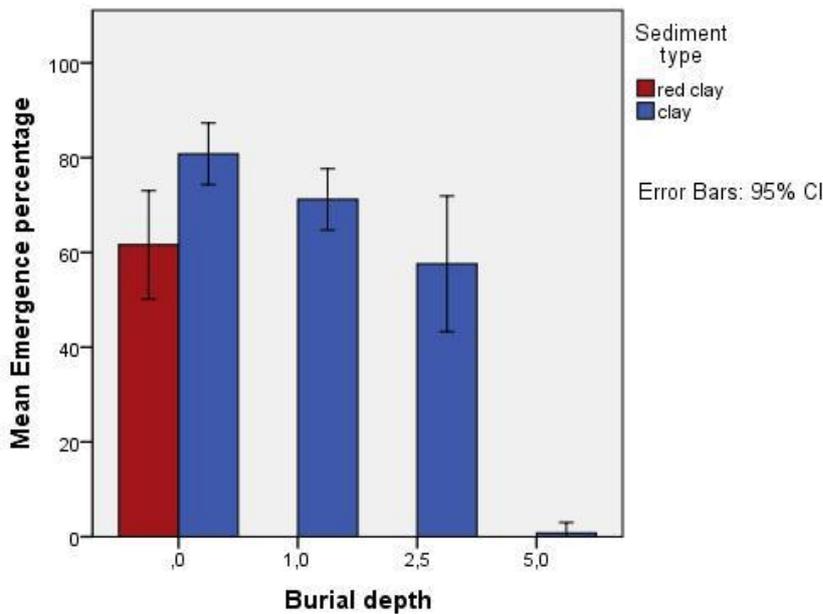
Figure 3-14 Mean emergence rates against burial depth (cm) for *Zannichellia palustris*. The error bars show the 95% confidence interval (N=25).

Linear regression shows a decrease of 3.3 germlings or 13.2% emergence per cm burial. Starting at 75.8%, emergence will therefore be reduced to zero at a burial depth of 5.8 cm. The coefficient of determination is 0.761 and the regression line is shown in Figure 3-15.
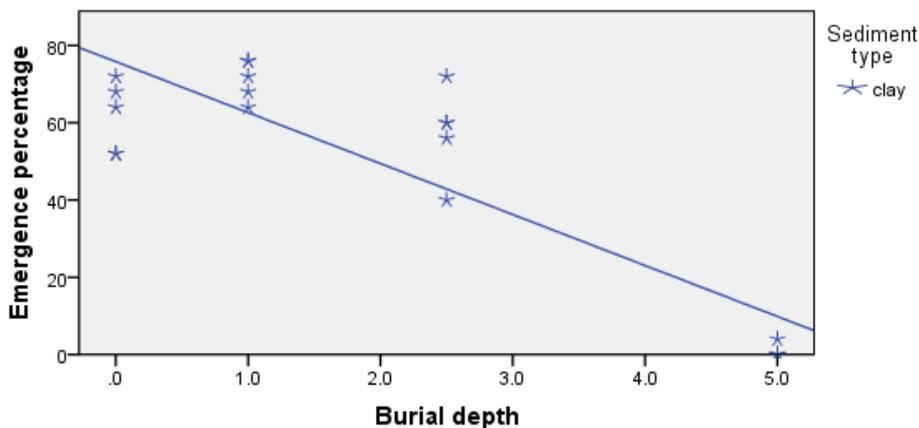

Figure 3-15 Measured emergence of *Zannichellia palustris* for burial with sand (cm) and linear regression (N=20).

3.2.2 Sediment type

The results presented in the previous section already showed a difference in emergence rates between burial with clay or sand. This difference will be further elaborated upon in this section. First of all, only *Potamogeton pusillus* and *Chara*

spp. are considered here, because *Zannichellia palustris* was only tested for clay. Emergence of all species is shown once more, but now per sediment type, in Figure 3-16. A one-way ANOVA shows that there was no significant interaction between sediment type if both species are considered ($F_{(1,78)}=2.622$, $p=0.109$). If the species are considered separately, there are some differences between burial with sand and burial with clay. For *Potamogeton Pusillus* there is a significant difference between burial with the two sediment types ($F_{(1,33)}=5.241$, $p=0.028$). For *Chara spp.* there is no significant difference between burial with sand or clay ($F_{(1,33)}=0.855$, $p=0.361$).

A two-way ANOVA reveals significant interaction effects of burial and sediment type for *Potamogeton pusillus* ($F_{(3,32)}=49.93$, $p<0.0005$). For *Chara spp.* there are no significant interaction effects of sediment and burial ($F_{(3,32)}=2.271$, $p=0.099$).
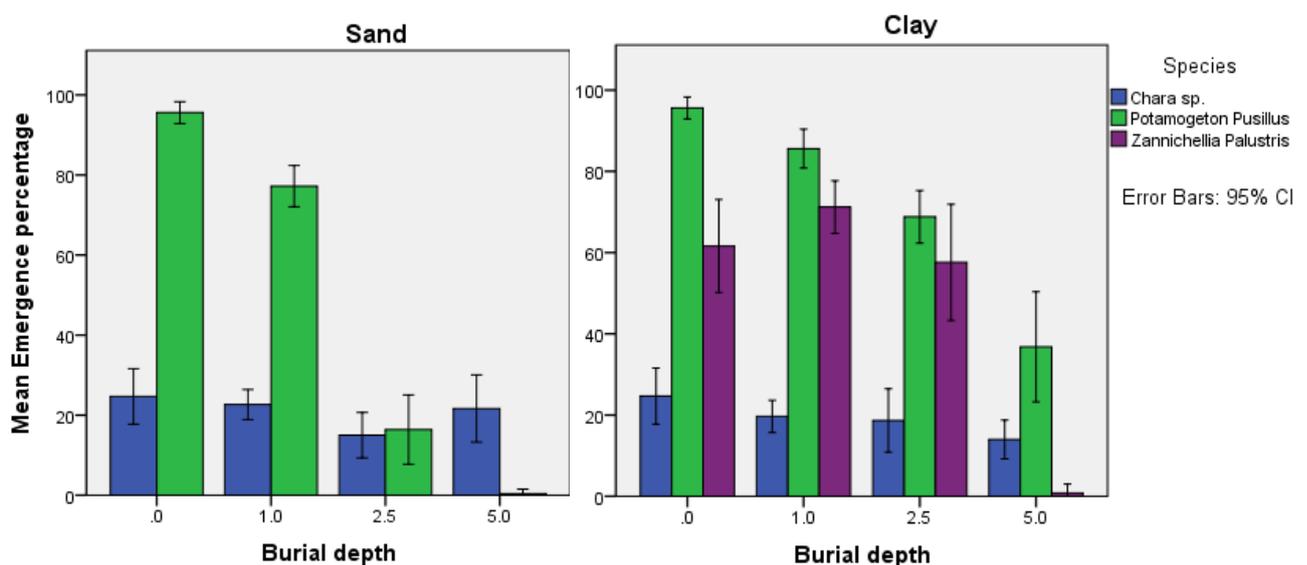

Figure 3-16 Emergence rates for the three species tested against burial depth (cm) for the two sediment types (sand and clay respectively, N=40 and N=60).

Figure 3-17 shows the emergence rates for the treatments without burial. These are three treatments, where the propagules have either been placed on red brick clay, clay or sand. The red brick clay was used as a standard soil below the propagules (chapter 2.1.2), because earlier experiments have shown that the plants would germinate well on this soil. In Figure 3-17 the emergence rates of propagules on the standard soil can be compared with the emergence rates of propagules placed on the two sediments used for burial. The germination rates in the figure show that *Chara spp.* germinates better on a subsoil of red brick clay than on a subsoil of clay or sand. ANOVA analysis shows that the differences are not significant ($F_{(1,13)}=3.82$, $p=0.073$). However, the range in germination of this species for these sediments indicates a decrease in germination of this

species for increasing grain size and decreasing nutrients and iron concentrations. Germination of *Potamogeton pusillus* is very high (> 90%), for all three sediment types. Although significant ($F_{(1,13)}=7.45$, $p=0.017$) the difference between sediment types is very small. For *Zannichellia palustris* germination rates of propagules placed on clay are higher than those placed on red brick clay ($F_{(1,8)}=16.46$, $p=0.004$).
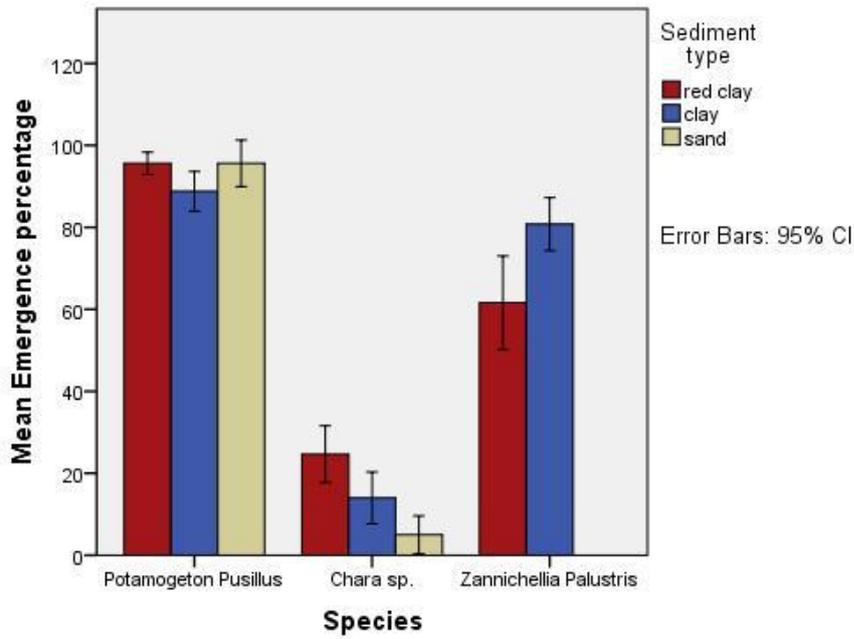

Figure 3-17 Mean emergence percentage for the three treatments without burial: propagules placed on brick clay, clay and sand (N=40).

3.2.3 Temporal aspects

The propagules of *Potamogeton pusillus* and *Zannichellia palustris* in the control treatments started germinating within days after placement. Also the propagules in the treatments with burial started emerging soon after the start of the experiment. Figure 3-18 shows the number of days after which the first germling appeared. The start of the experiment is day 0 and the experiment ended after 65 days. The first counting was after 6 days. For most burial treatments with *Zannichellia palustris* and *Potamogeton pusillus,* the first germling had already appeared at the first counting. For *Potamogeton pusillus* there is an increasing delay for 2.5 and 5 cm burial. For *Zannichellia palustris* the figure shows a strong delay for the 5 cm burial treatment. This is caused by the mere fact that only one germling appeared for this treatment during the experiment (65 days). The *Chara spp.* oospores needed almost two weeks to start germinating without burial and longer to emerge from under a burial layer. Figure 3-18 shows an increasing time needed for the first seedling to appear with burial depth, and a considerable difference between these species and *Potamogeton pusillus* and *Zannichellia palustris.* At the end of the experiment the emergence of Chara

germlings was still going on, but at a slower rate than at the peak of the seedling emergence after approximately 30 days.
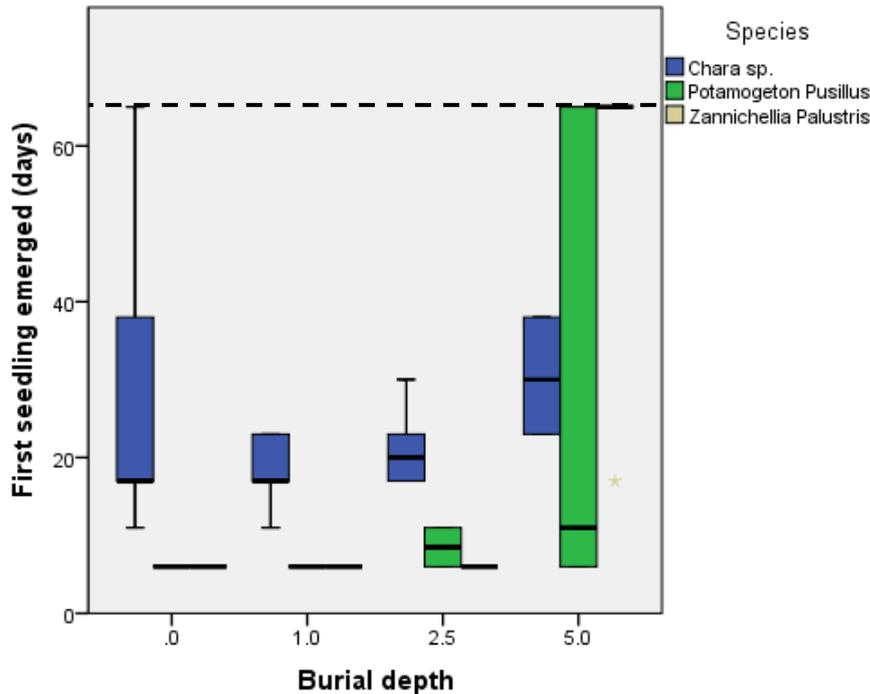

Figure 3-18 Number of days needed for the first seedling to emergence against burial depth (cm).

Figure 3-19 shows the number of days after which 90% of the germlings had emerged against burial depth. The 90% value was chosen to include the majority of germlings and to exclude outliers: one or two germlings that emerge later while the majority of germlings emerged weeks earlier. The figure shows that germlings of treatments with *Zannichellia palustris* emerged relatively soon, for some treatments already after the first two countings (on day 6 and 11 after the start of the experiment). This species shows no delay in emergence if the propagules are buried. For the 5 cm burial treatment only one germling emerged, after 17 days. For this germling, there was no considerable delay compared to the control treatments. *Potamogeton palustris* does show a delay in the time needed for the majority of the germlings to emerge with burial depth. Treatments with this species have a larger variation in the date at which the majority of germlings has emerged, especially for the 5 cm burial treatment. For *Chara spp.* the behaviour described before is visible: the emergence is delayed compared to the other two species and for a number of experimental units in every treatment the maximum emergence is not reached until the end of the experiment after 65 days.
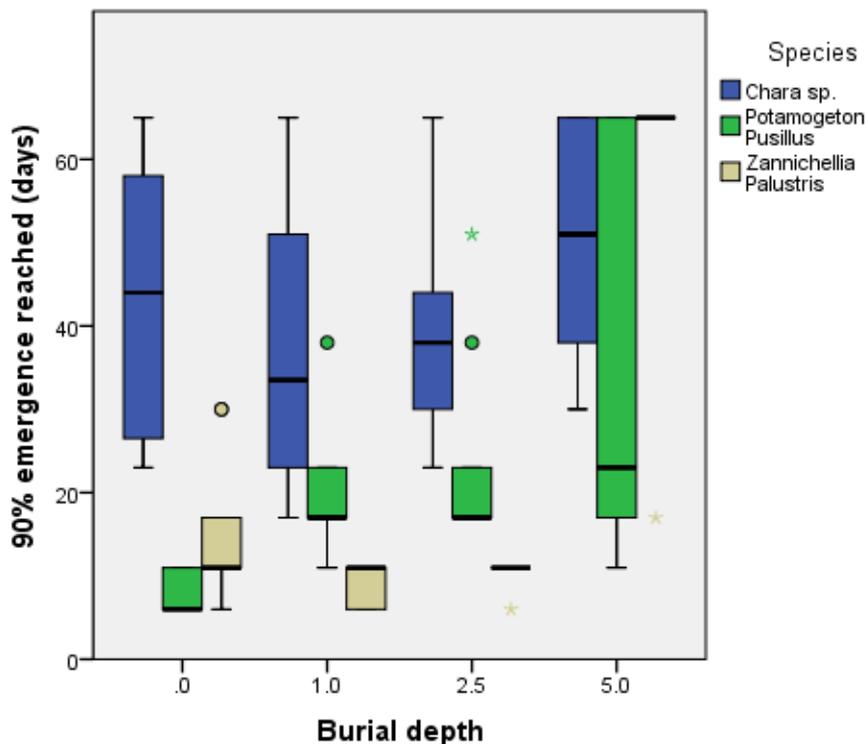
Figure 3-19 Number of days needed to reach 90% emergence against burial depth (cm), compared to the maximum counting (can also be 0).

3.3 Experiment 2

Experiment 2 was designed to investigate the effects of organic matter content on the emergence rate of one of the species used (*Potamogeton pusillus*). A series of sediments with decreasing organic matter content was attained by mixing the clayish sediment with an artificial bentonite mixture with the same moisture content (chapter 3.1.1). The organic matter content of the resulting sediment mixtures was measured (chapter 3.1.1).

The emergence of *Potamogeton pusillus* is shown against the organic matter content in Figure 3-20. Linear regression shows that there is no significant decrease or increase in emergence rate between the sediment mixtures ($F(1,8)=0.024$, $p=0.880$). The coefficient of determination (R^2) was 0.003, indicating that only 0.03% of the variation in emergence rate can be explained with a linear model.

The treatment with the pure bentonite mixture (lowest organic matter content) had a redox potential profile with much higher values than the treatment with only clay sediment (highest organic matter content) (chapter 3.1.3). This large difference of about 300 mV between the treatment with the highest and the lowest organic matter content did not show to have an influence on emergence for these cases.
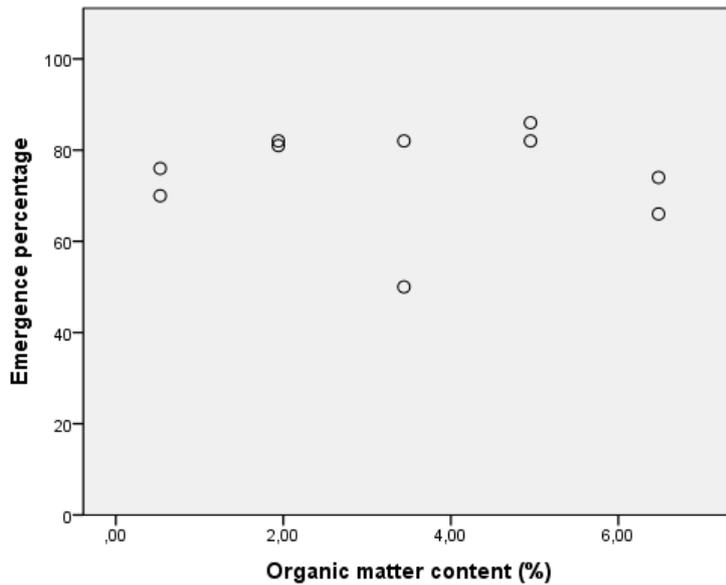
Figure 3-20 Emergence percentage of *P. pusillus* under burial of 2.5 cm sediment with varying organic matter content.

3.4 Retrieval of propagules

At the end of the experiment, after 65 days, the propagules were retrieved from the experimental units. This was achieved for *Potamogeton pusillus* and *Zannichellia palustris*, not for *Chara spp.* During retrieval it was monitored whether the propagules found were germinated and whether the non-germinated propagules were still viable. Viability has been determined similarly to the start of the experiment by the look and firmness of the propagules. The results are shown in Figure 3-21 for *Potamogeton pusillus* and Figure 3-22 for *Zannichellia palustris*. The figures show the part of the propagules that has germinated and emerged during the 2 months of the experiment and the part that has not emerged. The latter is divided in a part that has germinated but not emerged and a part that has not germinated and appears either still viable or not. Figure 3-21 shows that the larger part of the *Potamogeton pusillus* propagules had either already emerged or were not viable anymore. Since all propagules were viable at the start of the experiment, the propagules that are not viable anymore have lost their viability during the two months of the experiment. The number of non-viable propagules increases with burial depth for both clay and sand. The part of the propagules that is still viable also increases with burial depth. These propagules may have germinated and emerged if the experiment had lasted longer. The part of the propagules that has germinated but not emerged was very small for burial with clay. This means that the propagules that did germinate also emerged as a germling. For burial with sand the part with germinated but not emerged propagules is relatively large. These germlings did

not reach the sediment surface during the 2 months of the experiment and died during the way up or did not reach the surface in time. Part of the propagules in this group was actually found with a germling, which was often tortuous and winding in its attempt to reach the sediment surface. Therefore they appeared to be obstructed in some way. A small part of this group may be composed of propagules that did emerge, but stayed behind as the germling was removed. have emerged but the propagule has stayed behind when the germling was removed. Finally the figure shows that not 100% of the propagules was retrieved. Some propagules may have decayed, others may not have been retrieved despite our attempts.
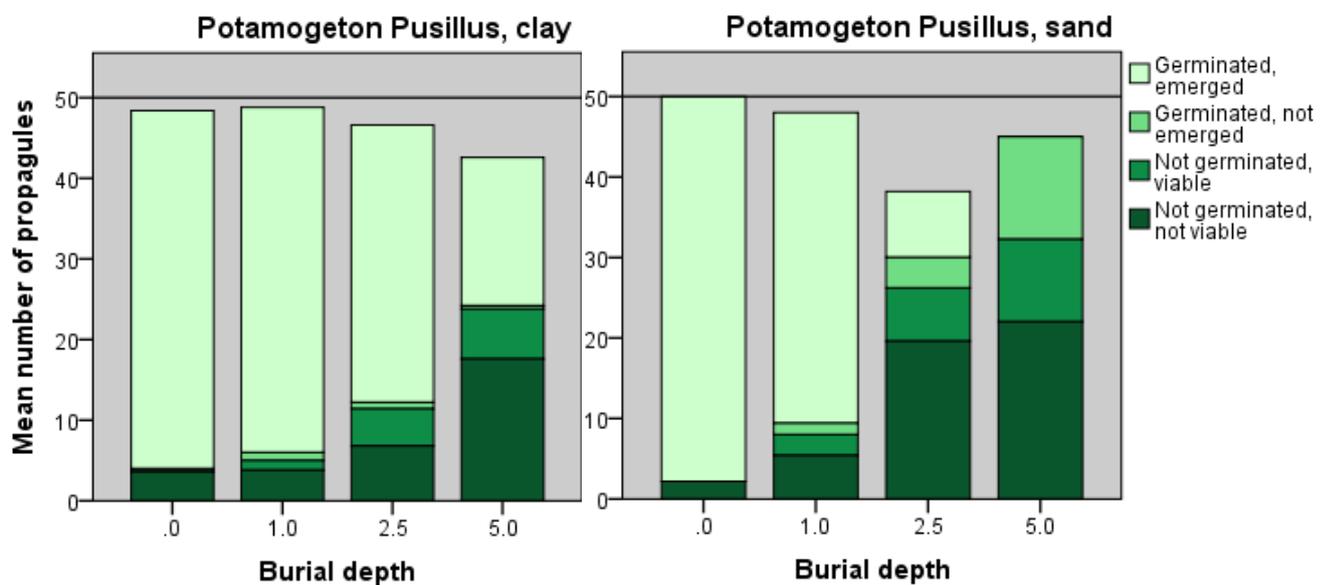

Figure 3-21 Mean number of *Potamogeton pusillus* propagules that emerged or were retrieved and found germinated, not germinated and viable or not germinated and not viable for burial with clay and burial with sand.

Figure 3-22 shows the retrieved propagules of *Zannichellia palustris*. The part of the seeds that had germinated but not emerged at 0 cm burial depth was left behind when the plants were removed. For this species, the amount of propagules that has germinated but not emerged increases with burial depth. At a burial depth of 5 cm, the larger part of the propagules has germinated but not emerged. A number of the germinated propagules retrieved were found with organic material attached, which may be a decaying shoot. The part of the retrieved propagules that has not germinated was small for 0, 1 and 2.5 cm burial but considerably larger for 5 cm burial. Around 30% of the propagules that was buried with 5 cm clay did not germinate.
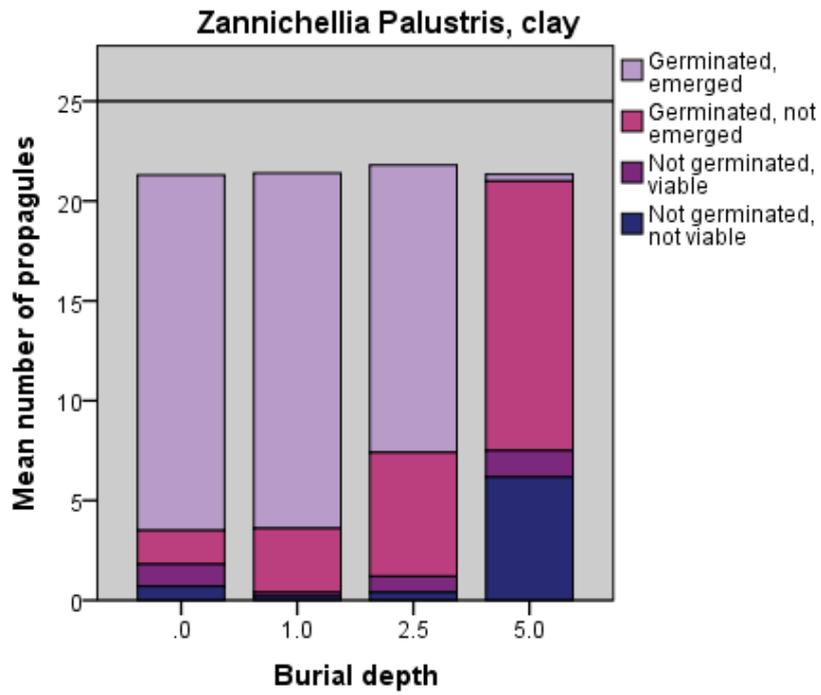
Figure 3-22 Mean number of *Zannichellia palustris* propagules that emerged or were retrieved and found germinated, not germinated and viable or not germinated and not viable.

4 Discussion and limitations

The purpose of this study was to examine the effects of burial on the emergence of *Potamogeton pusillus*, *Chara* species and *Zannichellia palustris*. This study also sought to expand knowledge about the aspects of the burial layer that inhibit germination. Therefore factors such as redox potential, nutrients, metals and organic matter content were studied.

Soon after the start of the experiments, the propagules in the treatments without burial started germinating. The propagules that were buried soon followed and germlings emerged for most treatments during the experiment. This indicated that the experiment was performed successfully. The emergence of germlings decreased significantly with increasing burial depth for almost all treatments. The plant species tested were buried with different sediments to assess differences between sediment types. In order to make a good comparison, a number of sediment characteristics were assessed. The red brick clay was found to be a viable soil for propagule germination and plant establishment. Compared to the sediments used for burial (sand and clay) this soil was high in iron and phosphate, low in nitrogen and this soil consisted of a range of mostly very small, but also very large particles. Although the experiments have led to valuable results, there were a number of limitations to this study. Hereafter, the results and limitations of the experiments will be discussed for every species.

Turions of *Potamogeton pusillus* reached 90% germination in the control treatments. Germination was however severely influenced by burial. The effects of burial with clay were less severe: emergence from under a burial layer of 5 cm was still almost 40%. Burial with sand was detrimental though: a burial layer of 5 cm reduced emergence to zero. This complies with earlier research that also found a larger reduction in emergence for larger grain sizes compared to burial with a fine textured sediment (Dittmar and Neely, 1999). Apparently, the sediment characteristics have led to changes in emergence. The sandy sediment consisted of larger grains, had a lower organic matter content, lower redox potential, lower moisture content and the concentrations of nutrients and metals were lower. A number of differences between the sediments can possibly be ruled out as a cause for differences in emergence of *Potamogeton pusillus*. The results of experiment 2 revealed that emergence was not influenced by partial or full replacement of the clayish sediment with an artificial Bentonite mixture with a very low organic matter content. The treatments showed a gradient in organic matter content or related factors such as redox potential and microbial activity. The results imply that emergence of *Potamogeton pusillus* is not influenced by these factors. Shortages of nutrients and metals are not expected, because these are supplied by the standard soil. Therefore, grain size distribution or moisture content may have caused the difference in emergence between burial with clay or sand. However, further research is needed to fully exclude the influence of

organic matter and related factors on emergence of Potamogeton, because the size and number of replicates for experiment 2 was limited. Also, the range in organic matter content was limited (up to 6.5%). Higher organic matter concentrations could have effects that did not show. Furthermore, it was decided only to examine *Potamogeton pusillus*. The use of one species limited the value of these results, because every species has different triggers and limitations. If this experiment would also be performed for the other two species the value of the results would have improved.

Of the propagules that were retrieved for *Potamogeton pusillus*, a relatively large amount germinated but the germlings had not emerged. A few germlings were retrieved from the burial layer with sand that showed deformation of the main stem. This indicates that the sand had obstructed the germlings in some way. Also a number of germlings was still viable and was not triggered to germinate. A part of these propagules loses viability and decays. The number of turions that was retrieved and had not germinated was slightly higher for sand. Also the number of turions that had lost viability was higher. This may be caused by the weight of the sand layer: the weight of the sand was higher than the weight of the clayish sediment.

Zannichellia palustris seeds were only treated with clay. Due to time constraints and restricted laboratory space, there was a limit to the size of the experiments and the number of measurements performed. Therefore this species did only receive burial treatment with clay and not with sand. This has limited the information gathered about the reaction of this species to burial. The oxygen concentration in the water columns was high, but reduced to zero within millimetres in the sediment. Therefore the propagules all burial treatments had no oxygen available during germination. However, this change in oxygen concentration between 0 cm and 1 cm burial could cause the increase in emergence between 0 and 1 cm for this species.

This species appears to withstand small burial layers rather well, but a burial depth of 5.8 cm is fatal. For this species, many germinated propagules were retrieved from the sediments at the end of the experiment, some with the remainings of the germling still attached. These results indicate that germination was triggered, but the germlings did not survive. This was possibly caused by a lack of energy to cross the distance to the sediment surface, or by the microbial activity and decaying processes in the clayish sediment.

Zannichellia palustris was the only species that did not respond as well as expected on the standard soil of red brick clay. Emergence was significantly lower than emergence of a treatment where the propagules were placed on the clayish sediment. The base layer of red clay may in this case have negatively influenced emergence of plants in the burial treatments. However, this does not

have an effect on the decrease in emergence with burial depth, because the relative differences remain.

Burial with sediment had remarkably little effect on emergence of *Chara spp.*. Generally the emergence rates of *Charophyte* oospores are low, around 20%. Previous research confirms this (Dugdale *et al.*, 2001, Casanova and Brock, 1990). This may be related to the survival strategy of the plant: quantity above quality or r-strategy (Barrat-Segretain, 1996). Many oospores are produced, but with a lower survival rate than seeds or turions. Finally, the results show equally high or higher emergence rates for 1 cm burial than for treatments without burial. This may indicate that *Chara spp.* oospores may be light inhibited to some extent. This has already been confirmed for some *Charophytes* in literature (Bonis and Grillas, 2002). For *Chara spp.* the emergence for burial with clay was slightly lower than for burial with sand. This states against the research of Dittmar and Neely (1999). A measured cause also mentioned in literature is redox potential, which was lower for sand (Kalin and Smith, 2007). Burial of *Chara spp.* oospores with sand did not show a significant decrease in emergence. Possibly, the shoot is affected by microbial activity and decomposing processes in the clayish sediment while travelling through the burial layer. This may decrease the success rate of the shoot in reaching the sediment surface. In the burial layer with sand, microbial activity and organic matter content were low. This would not have hampered the shoot and therefore the emergence levels are not influenced by burial depth. The redox potential decreased with over 500 mV as the oospores were buried deeper. There was hardly influence of these changes in redox potential to the emergence, since these barely changed.

The differences between the results for *Chara spp.* and the other two species were remarkable. *Chara spp.* did hardly respond to burial, but emergence rates were generally low compared to the other two species. This species also needed more time to germinate and emerge for all treatments. Possibly, there are overall differences in response to burial between charophytes and pondweeds. The difference in reproduction strategy mentioned earlier could also be a cause.

A limitation to the experiment with *Chara spp.* was the handling of the oospores. The method used for the propagules of *Chara spp.* differed from the handling of the other two species, which should not be the case. Firstly, a mixture of charophyte species was used. The number of species should be limited to find species-speciefic results. Secondly, the oospores were placed in the experimental units in a mixture with sand. Therefore the exact number of oospores was not known, which may have influenced the results.

A number of limitations was found in the sediments used. The fine, clayish sediment used for burial was actually rather coarse. Use of a very fine sediment might have resulted in different outcomes. Furthermore, both sediments started

to consolidate after the start of the experiment, leading to slightly different sediment surface depths. This consolidation was observed using the device that was used to measure the sediment depth at the start of the experiment and by visual examination of the stock. The consolidation was very small (no more than a few mm). The sediments in the treatments with large burial depths appeared to consolidate further (the red brick clay did not seem to consolidate) so that those sediment surfaces were further away from the light and heat source. However this is assumed not to have had significant effects. A problem caused by this effect was found during redox and oxygen profile measurements. These profiles are measured from the top of the sediment, but because the distance between the top of the sediment and the propagules was changed due to consolidation the exact depth of the propagules was not known. Another limitation was that, in some of the treatments with clay, the sediment was expanding and rising up because of gas production. Therefore, the redox potential at the propagules could only be estimated from the measurements. These processes could also have caused the differences in the redox potential profiles. If there would have been more time, a larger number of profile measurements would have given more insight in the variation between replicates of the measurements.

Finally, there were some concerns about the grain size analysis. The particles counted during the analysis were detached from each other using an ultrasonic treatment. For some types of clay particles, this treatment may not have fully separated the particles. This would then lead to a counting of a larger particle. This may have happened to the bentonite clay particles because the bentonite clay was found to be very sticky and plastic. Laser diffraction has been found to give deviating results for fine particles from the traditional method (Buurman *et al.*, 2001).

4.1 Conclusions

Based on the results of this study, the research questions formulated at the start of this study can largely be answered.

It was hypothesized that the sediment accumulating in lake Markermeer and IJsselmeer is suitable for germination of the studied plant species. The results show that this was indeed the case: propagules placed on the clayish and sandy sediments from the lakes germinated well compared to the standard soil used. Germination rates for *Chara spp.* were lower when placed on sediment from the lakes. Possible measured causes are that the red brick clay had smaller particle sizes and a higher iron and PO_4 content than the sediments from the lakes.

It was expected that the germling emergence would decrease with increasing burial depth. This was indeed the case for all but one treatments. Charophytes have shown to be most resistant against burial.

Furthermore it was expected that burial with clay would have a stronger reducing effect on emergence than burial with sand, because redox conditions would be unfavorable for germination. The results show that both the first and second part of this hypothesis can be rejected. For the two species that were treated with sand and clay, *Potamogeton pusillus* was influenced more by burial with sand and *Chara spp.* with clay. Emergence of *Potamogeton pusillus* was severely hampered by burial with sand, and less by burial with clay. Emergence of *Chara spp.* was influenced by burial with clay but not by burial with sand. The second part of this hypothesis stated that emergence would be negatively influenced by redox conditions in the clay sediments. For *Potamogeton pusillus* this hypothesis can be rejected. Burial with sediment that had decreasing organic matter concentrations did not significantly change the emergence of this species.

The final research question was based on the possible influence of organic matter content and corresponding factors. The hypothesis was that the presence of organic matter in the sediment inhibits germling emergence. The results have shown that changing the organic matter content and corresponding factors, such as redox potential, did not influence emergence.

This research has confirmed earlier findings that state that different aquatic macrophyte species respond in a different way to burial with different sediments. Therefore, findings in this research may not apply to other macrophyte species.

4.2 Recommendations for further research

Based on the results of the study, there are several recommendations for future research. First some of the limitations outlined in the previous section may be minimized or eliminated in a revised execution of the experiments. Furthermore, the experiment may be expanded to explore more sediment types and the responses of more species. This study has established the effects of burial depth on emergence of plants, but knowledge about the specific factors and processes underlying these results is still largely unknown. Further experiments in a controlled environment could rule out or confirm the influence of a number of factors or characteristics, such as organic matter content, redox potential or grain size. An example is to perform a burial experiment with an inert material (such as plastic grains) of different sizes or weights. Another experiment could be to do a burial experiment with sediment that has been ashed at 550°C to remove all organic materials and compare these to the results of this research.

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Appendix 1: Particle size distributions
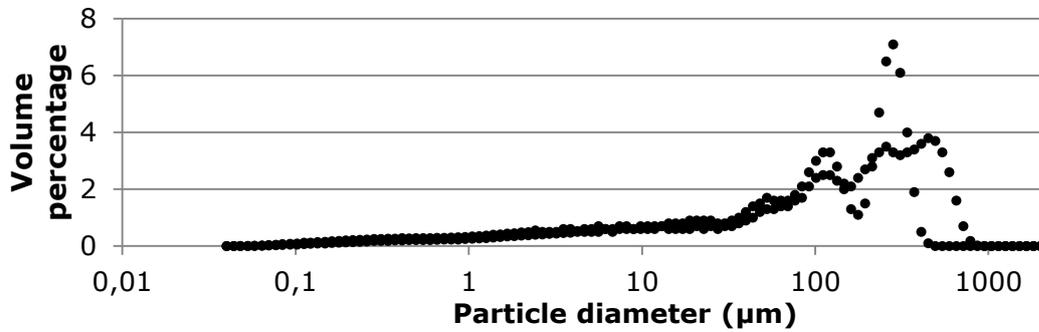
Figure 1 Particle size distribution in volume percentages for the clayish sediment
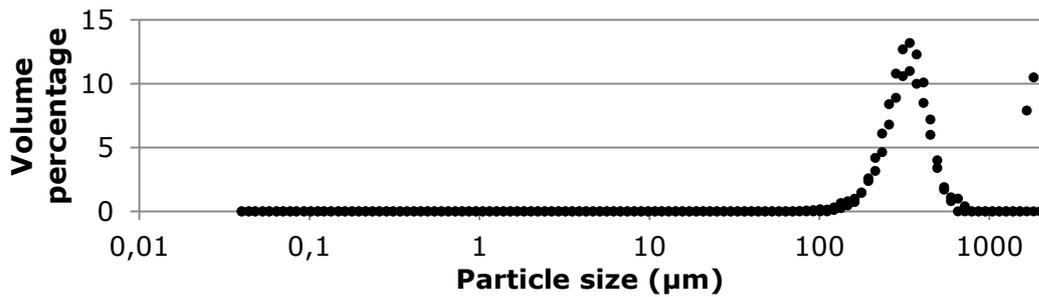
Figure 2 Particle size distribution in volume percentages for the sandy sediment
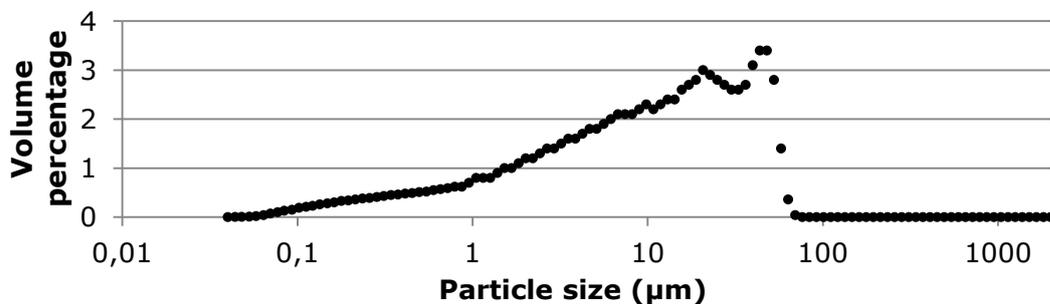
Figure 3 Particle size distribution in volume percentages for the red brick clay
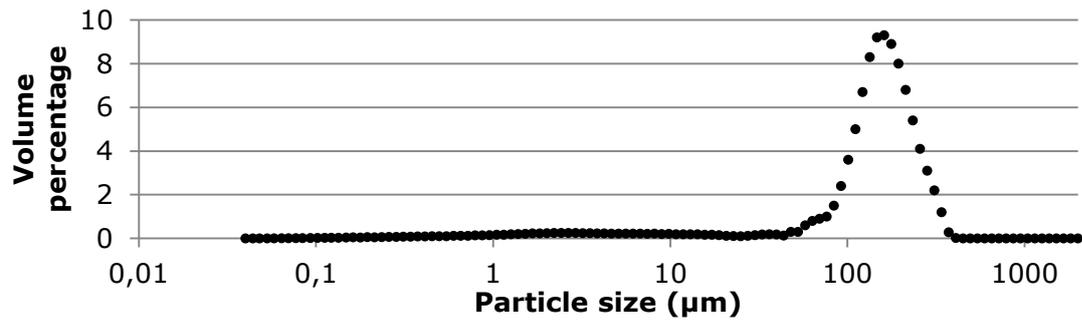
Figure 4 Particle size distribution in volume percentages for the bentonite mixture

Appendix 2: Oxygen profiles

Oxygen concentration (mg/L)
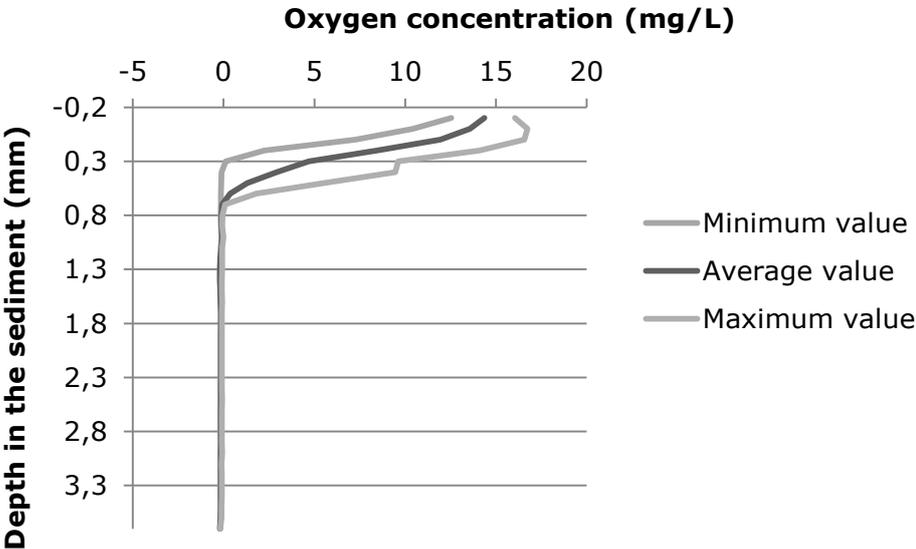
Figure 1 Depth profile of the oxygen concentration for treatments with a burial layer of 1 cm clay (2 units, duplo measurements).

Oxygen concentration (mg/L)
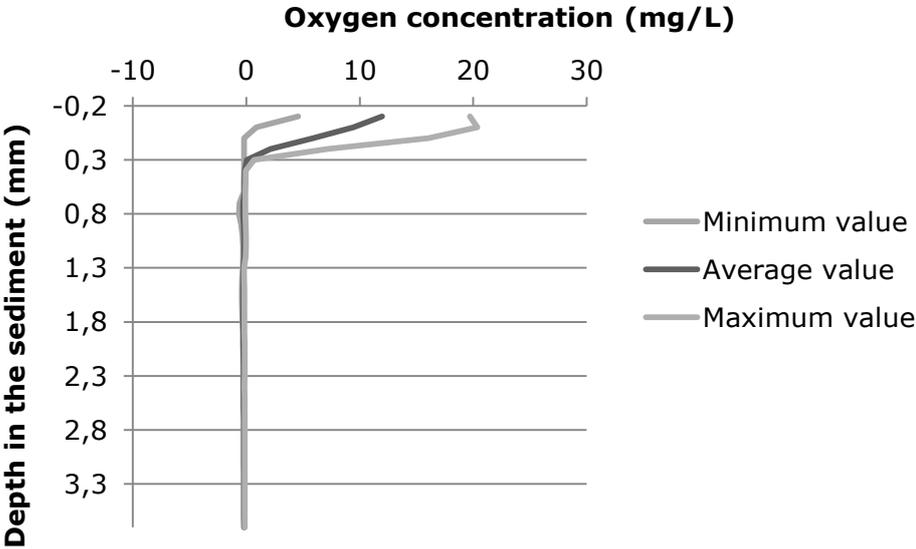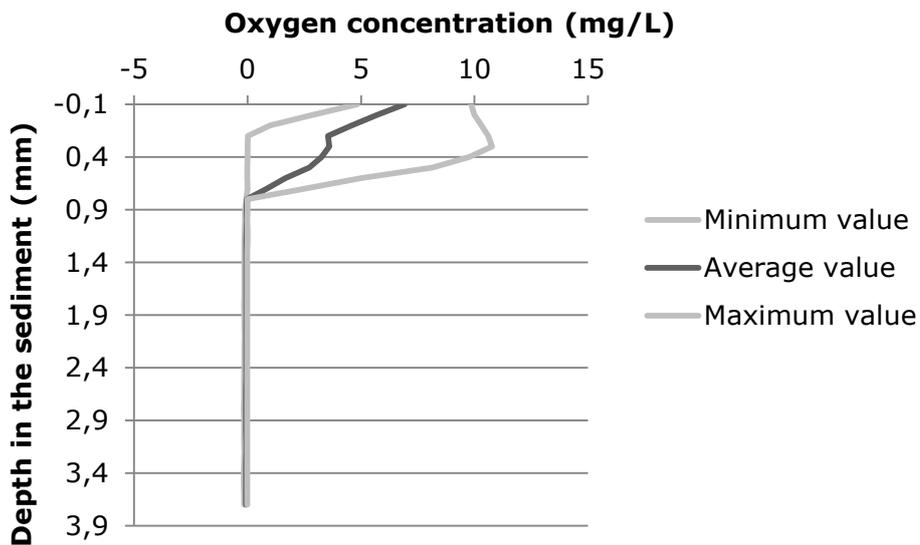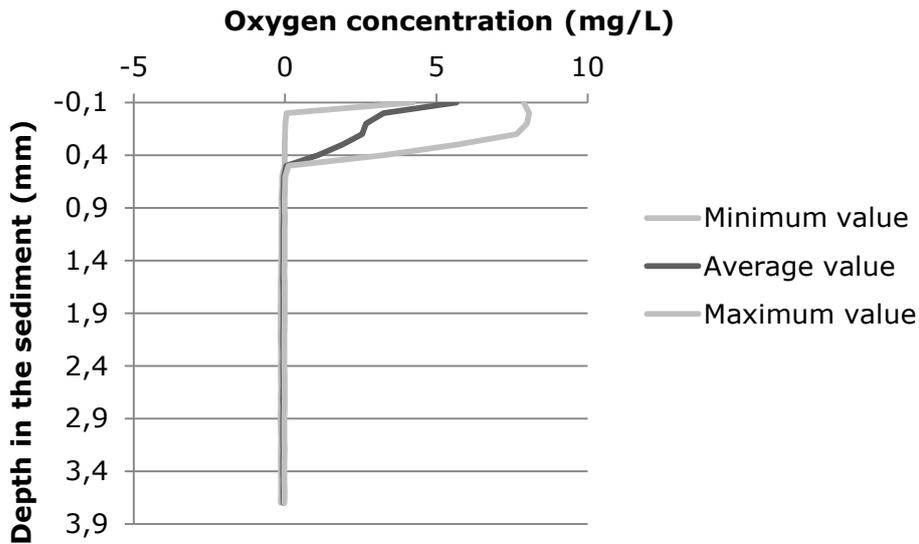

Figure 2 Depth profile of the oxygen concentration for treatments with a burial layer of 2.5 cm clay (2 units, duplo measurements).

Oxygen concentration (mg/L)

Depth in the sediment (mm)
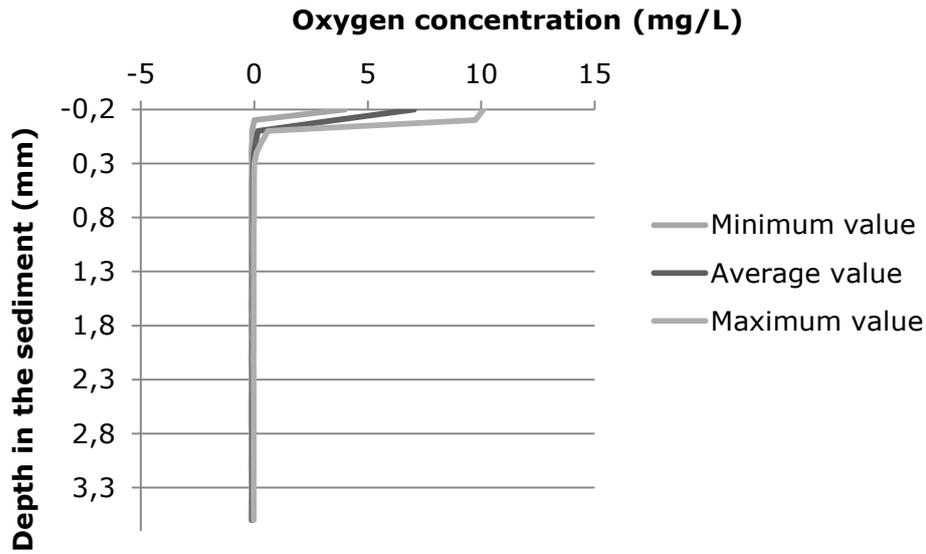
Figure 3 Depth profile of the oxygen concentration for treatments with a burial layer of 5 cm clay (3 units, duplo measurements).

Oxygen concentration (mg/L)

Depth in the sediment (mm)

Figure 4 Depth profile of the oxygen concentration for treatments with a burial layer of 1 cm sand (2 units, duplo measurements).

Oxygen concentration (mg/L)

Figure 5 Depth profile of the oxygen concentration for treatments with a burial layer of 2.5 cm sand (2 units, duplo measurements).
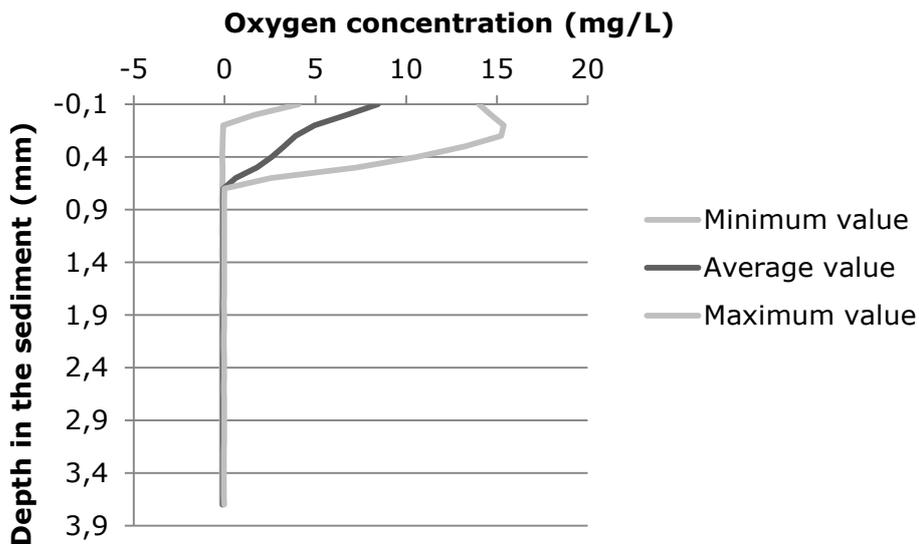
Oxygen concentration (mg/L)

Figure 6 Depth profile of the oxygen concentration for treatments with a burial depth of 5 cm sand (3 units, duplo measurements).
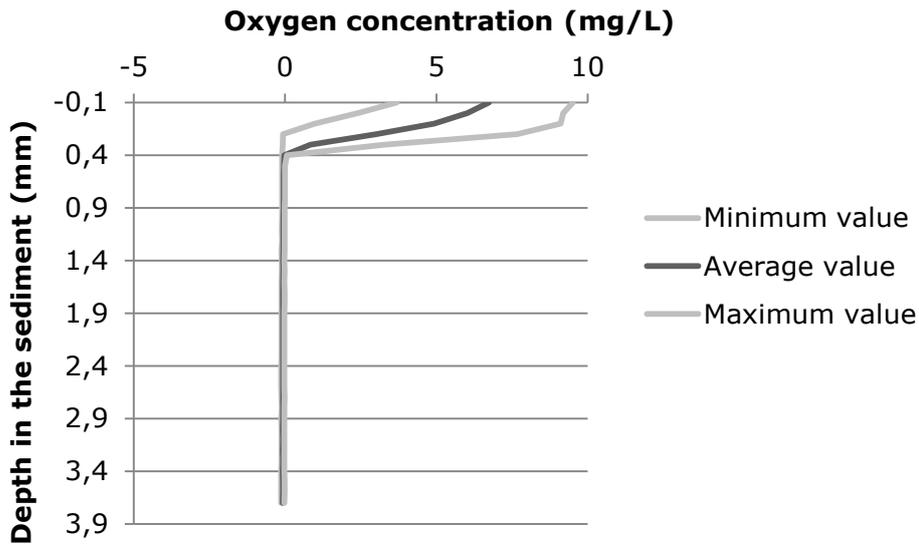
Figure 7 Depth profile of the oxygen concentration for control treatments with a soil of red brick clay and no burial (2 units, duplo measurements).
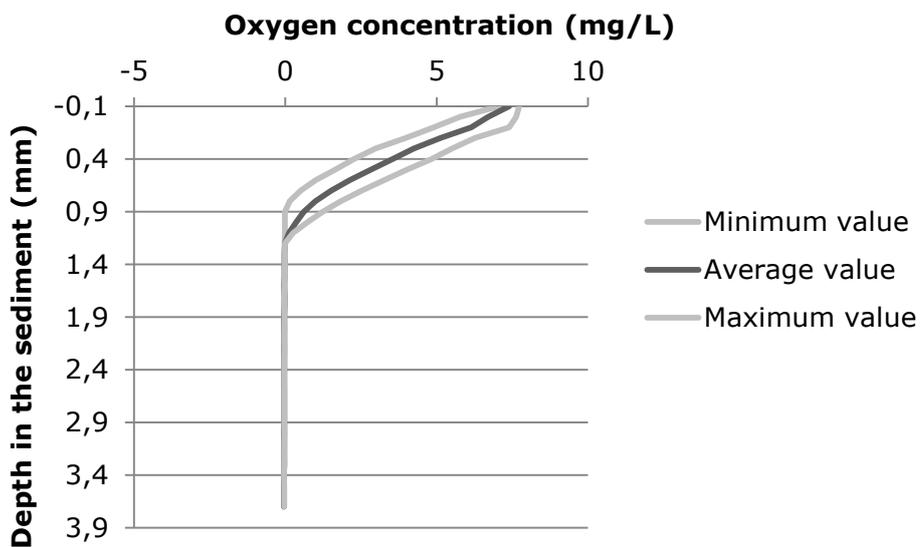

Figure 8 Depth profile of the oxygen concentration for treatments with the pure Bentonite mixture, not mixed with clay (2 units, duplo measurements).