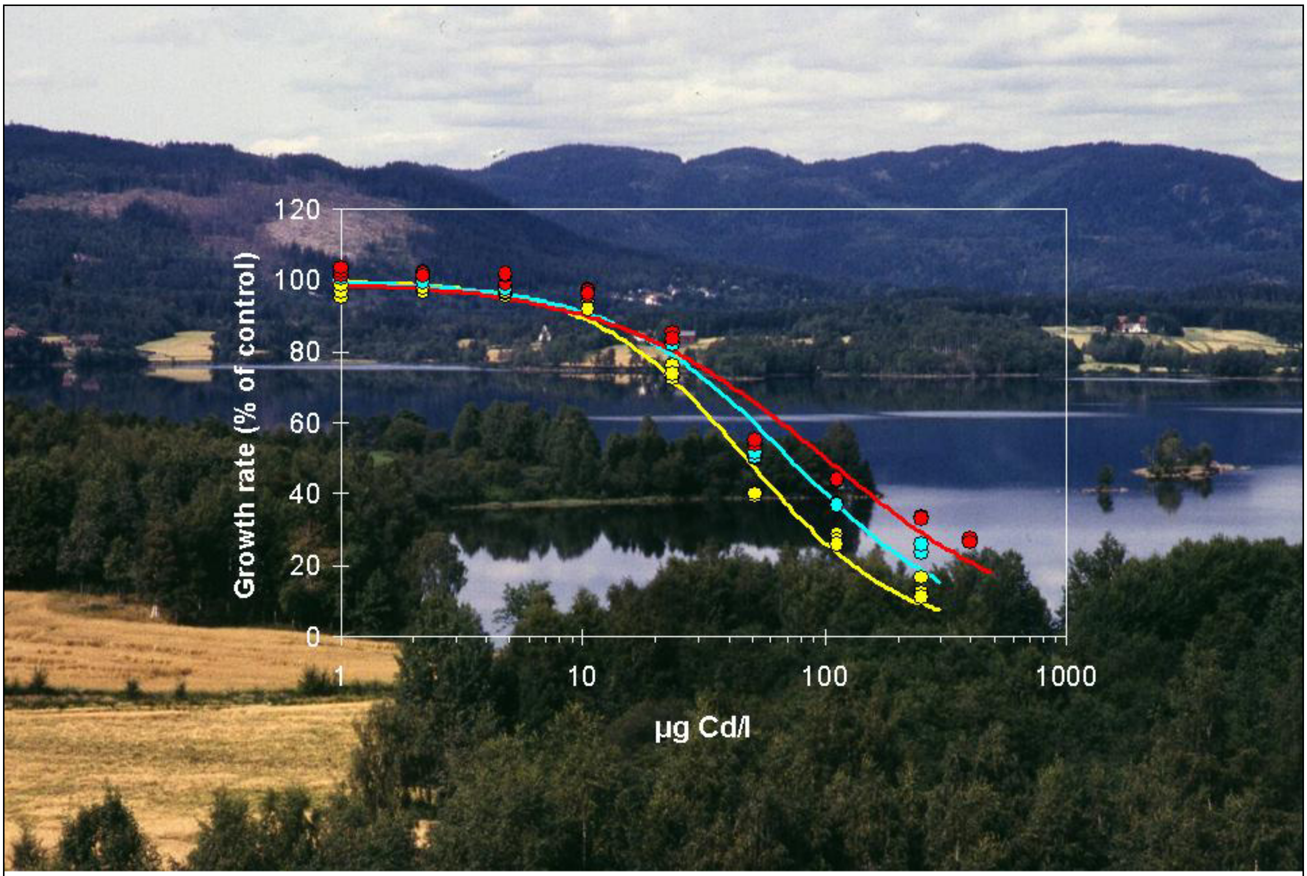
REPORT SNO 5422-2007

Effect of water hardness on the toxicity of cadmium to the alga *Pseudokirchneriella subcapitata*



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REPORT

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Abstract

The growth inhibiting effect of cadmium on the green alga *Pseudokirchneriella subcapitata* has been investigated in an artificial medium and three natural soft lake waters. The hardness of the media was manipulated by addition of calcium. Increasing hardness resulted in a lower slope of the concentration/response curve in all media. The EC_{50} increased with hardness (reduced toxicity), but the results for EC_{10} were less consistent. Slopes in the hardness/toxicity relationship were calculated. The maximum slope of EC_{50} versus hardness in the hardness range 3.4-15 mg $CaCO_3$/l was 5.44 µg Cd/mg $CaCO_3$/l. The corresponding slope for EC_{10} was 1.59 µg Cd/mg $CaCO_3$/l. At the higher hardness range (15-45 mg $CaCO_3$) the maximum slopes were lower.

The highest growth inhibiting effect of Cd was found in Lake Byglandsfjorden with hardness 3.5 mg $CaCO_3$ and total organic carbon 1.8 mg/l. When tested without addition of artificial chelators the EC_{50} for inhibition of growth rate in this lake was 9.4 µg Cd/l and EC_{10} was 2.8 µg Cd/l.

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Effect of water hardness on the toxicity of cadmium to the alga *Pseudokirchneriella subcapitata*

Preface

In connection with the risk assessment of cadmium under the EU Program for Risk Assessment of Existing Chemicals, there is a need for additional information on the toxicity of cadmium in waters with low hardness. This is of particular importance for the Nordic region where a large portion of the lakes are classified as “very soft waters” i.e. hardness less than 10 mg $CaCO_3$/l.

The Nordic Chemicals Group has contracted the Norwegian Institute for Water Research (NIVA) to compile data on toxicity of Cd on algae at different hardness levels from experiments with natural and modified Norwegian lake waters.

Results from algal growth inhibition tests with the green alga *Pseudokirchneriella subcapitata* in artificial media and nutrient spiked natural soft lake waters carried out at NIVA have been compiled and analysed for effect of water hardness. The results are presented in the present report.

Oslo, 02.05 2007

Torsten Källqvist

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Summary

The effect of cadmium on the growth rate of the green alga *Pseudokirchneriella subcapitata* has been investigated at three different level of water hardness in an artificial growth medium and three nutrient spiked, Norwegian natural soft lake waters. Different hardness levels were achieved by addition of calcium. The lake water with the lowest levels of hardness and total organic carbon (Lake Byglandsfjorden) showed the highest toxicity of Cd. In water from this lake, without the addition of calcium and artificial chelators, the EC_{50} for inhibition of growth rate was 9.4 µg Cd/l and EC_{10} was 2.8 µg Cd/l. When the hardness of the waters was increased by the addition of calcium the toxic effect of Cd was in general reduced (i.e. the EC_{10} and EC_{50} increased). In all tested media, the slope of the concentration/effect slope was reduced with increasing hardness. This led to a higher effect of hardness on the EC_{50} than on EC_{10}. In one of the lake waters (Lake Store Sandungen) which had the highest concentration of TOC (4.1 mg/l), a slight decrease of EC_{10} with increasing hardness was observed. The maximum slope of EC_{50} versus hardness in the hardness range 3.4-15 mg $CaCO_3$/l was 5.44 µg Cd/mg $CaCO_3$/l. The corresponding slope for EC_{10} was 1.59 µg Cd/mg $CaCO_3$/l. At the higher hardness range (15-45 mg $CaCO_3$) the maximum slopes were 3.06 for EC_{50} and 0.14 for EC_{10}.

1. Background

A risk assessment of cadmium is performed under the European Union program for risk assessment of existing chemicals. Data of acute and chronic toxicity of Cd to freshwater organisms have been compiled and analysed and a Predicted No Effect Concentration (PNEC) has been derived from the species sensitivity distribution and presented in the draft Risk Assessment Report (RAR) .

It is realized that that water characteristics affect Cd toxicity. Toxicity generally increases with reducing hardness, reducing concentrations of dissolved organic matter an increasing pH. The existing data on toxicity are, however too scarce to allow a quantitative analysis of the effects of pH and organic matter. For hardness, a correction of chronic values has been proposed, based on the slopes in plots of natural logarithm of chronic values against water hardness for *Daphnia magna* and two species of fish (US-EPA 2001). This hardness correction is recommended for the calculation of $PNEC_{water,regional}$ for regions with hardness levels in the range 40-200 mg $CaCO_3$/l. In the preliminary Risk Assessment Report the PNEC for water with hardness 40 mg/l has been calculated to 0.08 µg Cd/l, and it has been concluded that down to water hardness of 7-10 mg/l there is no indication of Cd toxicity below 0.08 µg/l. However, data are lacking for effects of Cd in very soft waters (hardness below about 10 mg $CaCO_3$/l) and it is not known if these waters are protected by the proposed PNEC for soft water (0.08 µg/l). It has therefore been suggested to perform further testing to assess the risks of Cd in very soft waters.

A large portion of the lakes in Nordic countries (Finland, Norway and Sweden) have a hardness level below 10. To allow the risk assessment of Cd in this region it is therefore necessary to extend the hardness correction below hardness 10 mg $CaCO_3$/l. The present study was initiated to provide information of the effect of hardness on the toxicity of Cd to the green alga *Pseudokirchneriella subcapitata.*

2. Methods

Algal growth inhibition tests of Cd were performed with the green alga *Pseudokirchneriella subcapitat*a (formerly known as *Selenastrum capricornutum*) in one artificial medium and three nutrient spiked natural lake waters. The tests were performed at three hardness levels (+0, +10 and +40 mg $CaCO_3$ above the background level of the medium).

The test alga, *P. subcapitata* was obtained from the culture collection of Norwegian Institute for Water Research. The strain NIVA CHL1 was originally isolated from a Norwegian soft water river in 1959 and is maintained in a soft water medium (10 % Z8, hardness 3.5 mg $CaCO_3$/l). The same medium, with some modifications were used in a screening test of Cd. The modifications were exchange of the trace metal solution with that of the ISO 8692 medium (ISO 2004) but with the concentration iron reduced to 32 µg Fe/l, (0.12 µM/l) and EDTA to 0.13 µM/l. The composition of the artificial test medium is shown in table 1.

Table 1. Chemical composition of the artificial growth medium.

Chemical	**mg/l**	**µM**
$NaNO_3$	46.7	549
$Ca(NO_3)_2 . 4H_2O$	5.9	25
$MgSO_4 . 7H_2O$	2.5	10.2
Na_2CO_3	2.1	19.8
K_2HPO_4	3.1	17.8
$FeCl_3 \cdot 6(H_2O)$	0.032	0.12
$Na_2EDTA \cdot 2(H_2O)$	0.05	0.13
H_3BO3	0.185	2.99
$MnCl_2 \cdot 4(H_2O)$	0.415	2.09
$ZnCl_2$	0.003	0.022
$CoCl_2 \cdot 6(H_2O)$	0.0015	0.0063

Samples of natural lake waters were collected from three Norwegian soft water lakes in December 2006. These were Lake Store Sandungen and Lake Maridalsvann in the Oslo area, and Lake Byglandsfjorden in the Southern part of Norway. The water was stored in polyethylene canisters until start of the tests.

The lake waters were filtered through acid-washed membrane filters (0.45 µm). The filtered lake waters were analysed for pH, electrolytic conductivity, total organic carbon, alkalinity, calcium, magnesium and metals. pH was measured with a Radiometer PHM 210 equipped with a combined glass electrode (Radiometer GK 2401 C). Conductivity was measured with a WTW inoLab Level 3 conductivity meter. Total organic carbon was analysed by wet chemical oxidation (peroxisulphate + UV) and IR-detection on a Phoenix 8000-TOC-TC analyzer. Alkalinity was measured with a Mettler DL 40 RC Memo titrator. Calcium and Magnesium was analysed by ionic chromatography Dionex IC 25 Ion chromaptograph. Metals (Cu, Cd, Cr, Ni, Fe, Zn) were analysed on a Perkin Elmer Sciex ELAN 6000 ICP-MS.

Filtered lake water was spiked with nutrients as shown in table 2. Initial trials in spiked lake water without EDTA showed slow and irregular growth in the waters from Lakes Maridalsvann and Byglandsfjorden. It was therefore decided to include EDTA in the lake water media. To investigate the effect of EDTA on the toxicity of Cd, the tests in Lake Store Sandungen were repeated without EDTA. Also a test in Lake Byglandsfjorden at the lowest hardness level was performed without EDTA in the medium. The levels of P, N, Fe and EDTA were the same as in the ISO 8692 medium, but nitrogen was supplied as NO^{3-} instead of NH^{4+}. This was done to avoid acidification of the medium due to assimilation of ammonium by the algae in media with low buffering capacity. The tests were carried out with the basal media described above and with addition of Ca^{2+} to obtain two additional hardness levels. Ca^{2+} was added from a stock solution of $CaCl_2 \cdot 2H_2O$. The additions were 4 and 16 mg/l of Ca, which corresponds to hardness levels 10 and 40 mg $CaCO_3$/l.

Table 2. Nutrient spiking used to prepared media based on natural lake waters.

Chemical	**mg/l**	**µM**
$NaNO_3$	23.8	280
KH_2PO_4	1.6	11.8
$FeCl_3 \cdot 6(H_2O)$	0.064	0.236
$Na_2EDTA \cdot 2(H_2O)$	0.1	0.269

Altogether 17 tests were performed as shown in table 3. The toxicity tests were performed as described in OECD Test Guideline 201 (OECD 2006) and ISO 8692 (ISO 2004). Briefly, test solutions were prepared by adding Cd from a stock solution of $CdCl_2$ to the growth medium and inoculating with 5×10^6 cells/l from an exponentially growing culture of *P. subcapitata* in the same basal medium (lowest hardness) as used in the test. Eight concentrations of Cd ranging from 1 µg/l to 249 µg/l were tested. The spacing of the concentration was a factor 2.2. An additional Cd concentration, 400 µg/l was included at the highest hardness level (H 40+). In the additional test with Lake Byglandsfjorden without EDTA, eight concentrations between 1 and 40 µg/l were tested with spacing factor 1.6.

Table 3. Overview of toxicity tests of Cd performed with *P. subcapitata*.

Test nr.	**Medium**	**EDTA**	**Hardness mg $CaCO_3$/l**	**Initial pH**	**Comment**
1	Artificial	0.13 µM	3.5	7.03	
2	Artificial	0.13 µM	3.5+10	7.04	
3	Artificial	0.13 µM	3.5+40	7.03	
4	Lake Store Sandungen	0.27 µM	6.2	6.79	
5	Lake Store Sandungen	0.27 µM	6.2+10	6.71	
6	Lake Store Sandungen	0.27 µM	6.2+40	6.65	
7	Lake Store Sandungen	0.27 µM		6.83	Inoculated one day after preparation
8	Lake Store Sandungen	-		6.85	
9	Lake Store Sandungen	-	6.2+10	6.74	
10	Lake Store Sandungen		6.2+40	6.65	
11	Lake Maridalsvann	0.27 µM	8.1	6.98	
12	Lake Maridalsvann	0.27 µM	8.1+10	6.91	
13	Lake Maridalsvann	0.27 µM	8.1+40	6.85	
14	Lake Byglandsfjorden	0.27 µM	3.4	6.72	
15	Lake Byglandsfjorden	0.27 µM	3.4+10	6.62	
16	Lake Byglandsfjorden	0.27 µM	3.4+40	6.54	
17	Lake Byglandsfjorden	-	3.4	6.71	

The test design included three replicate cultures at each concentration of Cd and six control replicates except for th etetsts in the artificial medium where only three control replicates were used.. The additional test with Lake Byglandsfjorden without EDTA, was performed with four replicates at each Cd level and eight control replicates in order to improve the statistical power.

For each basal medium, the tests at the three hardness levels (0+, 10+ and 40+) were performed simultaneously and inoculum from the same inoculum culture.

The cultures were incubated in glass vials with 12 ml culture volume. The vials were incubated on a shaking table under continuous illumination from fluorescent tubes (Philips TLD/950) providing approximately 70 µM m^{-2} s^{-1} photosynthetic active radiation (PAR). The temperature was kept at 21 ± 1 °C.

The cell density in the cultures was measured at 24±2 hours intervals for 72 hours. In the tests in artificial growth medium the cell density was only measured at the end of the test (72 hours). The measurements were made with an electronic particle counter (Coulter Multisizer M3) with a 100 µm orifice tube.

The average growth rate during 72 hours was calculated from the logarithmic increase in cell density for each replicate culture. The section-by-section growth rate was calculated for each 24 h interval (0-24, 24-48 and 48-72) in the control cultures in order to check the fulfilment of exponential growth according to the OECD Guideline 201.

For each test, the growth rates in the replicate cultures were calculated as percentage of the average growth rate in the control cultures. The concentration/response relationship was analyzed by non linear regression using a log-logistic model (Hill). The calculations were performed using the software REGTOX EV6.3, 2002 (Eric Vindimian, Paris, France, http://eric.vindimian.9online.fr/). The median effect concentrations EC_{10}, EC_{20} and EC_{50} with 95 % confidence intervals were calculated.

The pH was measured in the medium at the start of the test and in each treatment after 72 hours.

The initial concentrations of Cd were measured by chemical analysis (ICP) in four test solutions.

Adsorption of Cd on algal biomass was investigated in distilled water buffered with $NaHCO_3$ to pH 6.8. 100 µg Cd/l was added to water containing 330×10^6 and 3300×10^6 cells/l of *P. subcapitata*. The samples were agitated for 24 hours in the dark at 20 °C. After incubation, the algal suspensions were centrifuged for 10 min. at 6000 RPM. The supernatants were analyzed for Cd using ICP.

3. Results

3.1 Chemical composition of the lake waters

The chemical composition of the lake waters is shown in table 4. The hardness level was highest in Lake Maridalsvann (8.05 mg $CaCO_3$/l), intermediate in Lake Store Sandungen (6.21 mg/l) and lowest in Lake Byglandsfjorden (3.42 mg/l). The background concentration of cadmium was from 0.024 µg/l in Lake Store Sandungen to 0.010 µg/L in Lake Byglandsfjorden.

Table 4. Chemical characterization of lake waters used in growth inhibition tests.

	Unit	Lake Maridalsvann	Lake Store Sandungen	Lake Byglandsfjorden
pH		6.8	6.7	6.5
Conductivity	mS/m	2.7	2.0	1.3
TOC	mg/l	3.8	4.1	1.8
Ca	mg/l	2.52	1.95	1.04
Mg	mg/l	0.43	0.33	0.2
Hardness	mg $CaCO_3$/l	8.05	6.21	3.42
Cd	µg/l	0.022	0.024	0.010
Cr	µg/l	0.1	<0.1	n.a.
Cu	µg/l	6.88	1.90	n.a.
Fe	µg/l	<10	10	n.a.
Ni	µg/l	0.28	0.20	n.a.
Zn	µg/l	13.1	10.4	n.a.

3.2 Analytic verification of Cd concentrations in test solutions

The measured concentrations of Cd in test solutions are shown in table 5. The measured concentrations were 83-87% of the nominal concentration which indicates that the concentration in the stock solution was 15 % lower than the nominal concentration.

Table 5. Measured concentrations of Cd in freshly prepared test solutions.

Nominal (µg Cd/l)	Measured (µg Cd/l)	Measured (% of nominal)
4.84	4.0	83
10.6	9.0	85
51	44.5	87
113	96.5	85
Average	-	85

3.3 Adsorption of Cd on algal biomass

After 24 hours incubation in the dark, the cell density of the algal suspensions was 406×10^6 and 3470×10^6 cells/l respectively. The mean cell weight of *P. subcapitata* has been measured to be 3.0×10^{-8}mg/cell (dry weight). Thus, the biomass density in the suspensions were 12 and 104 mg/l respectively. The nominal concentration of Cd in the solutions was 100 µg/l, added from the same stock solutions of $CdCl_2$ as was used for the toxicity tests. The concentration of dissolved Cd was analyzed in the solutions after the removal of algae by centrifugation. The analyses showed 78 µg/l in the control solution (without algae), 63 µg/l in the water with 12 mg/l of algal biomass and 65 µg/l in water with 104 mg/l algal biomass. The concentration of Cd in the algal biomass can be calculated from the decrease in dissolved Cd concentrations in the algal suspension as compared to the control solution. Surprisingly, the decrease in dissolved Cd was slightly higher in the 12 mg/l algae suspension than in the 104 mg/l suspension. Thus the K_d-values that can be calculated from this experiment are a factor 10 different at the two algal biomass levels. Based on the experiment with 12 mg/l, the K_d is 12500 l/kg. For the higher biomass level (104 mg/l), the K_d was calculated to be 1250 l/kg.

3.4 Growth inhibition tests

The cell densities, growth rates and pH-values recorded in each test is shown in Appendix 1.
The performance of the control cultures in the growth inhibition tests is summarised in table 6. In all the tests in media with EDTA, the average control growth rates (0-72 h) were between 1.58 and 1.76 d^{-1}. The lowest control growth rate was 0.96 d^{-1} in the Lake Byglandsfjorden without EDTA (test 17). In Lake Sandungen without EDTA, the control growth rates decreased with hardness from 1.63 d^{-1} at 6.2 mg $CaCO_3$/l to 1.31 d^{-1} at 46.2 mg $CaCO_3$/l. The coefficient of variation of growth rates in replicate control cultures was between 0.57 and 9.74 %. The highest variation was found in test nr. 10 (Store Sandungen without EDTA at hardness level 40+). All the other tests fulfilled the validity criteria in OECD 201 of ≤7 % variation coefficient in replicate control cultures. The variation in section-by-section growth rate indicates that the growth in the control cultures was satisfactory exponential and fulfils the validity criterion in OECD 201 (coefficient of variation ≤35 %) in 16 of the 17 test performed. Again it was the test with Lake Store Sandungen without EDTA and hardness level 40+ which did not fulfil the criterion.

Table 6. Performance of control cultures in growth inhibition tests with *P. subcapitata*, and validity criteria according to OECD (2005).

Test nr	**Medium**	**Control growth rate (d^{-1})**	**St. dev.**	**Coef. Var. (%)**	**Section-by-section variation (%)**
1	Artificial,	1.69	0.11	6.46	n.a.
2	Artificial, H 10+	1.76	0.04	3.29	n.a.
3	Artificial, H 40+	1.70	0.06	3.29	n.a.
4	Lake Store Sandungen +EDTA,	1.69	0.019	1.14	10.5
5	Lake Store Sandungen+EDTA, H 10+	1.74	0.028	1.61	10.2
6	Lake Store Sandungen+EDTA, H 40+	1.73	0.010	0.57	22
7	Lake Store Sandungen+EDTA inoculated after 24 h	1.70	0.04	2.3	4.1
8	Lake Store Sandungen-EDTA	1.63	0.034	2.08	11.1
9	Lake Store Sandungen-EDTA, H 10+	1.50	0.092	6.14	25.4
10	Lake Store Sandungen-EDTA, H 40+	1.31	0.128	9.74	39.6
11	Lake Maridalsvann+EDTA	1.71	0.03	1.51	9.4
12	Lake Maridalsvann+EDTA, H 10+	1.68	0.02	1.21	4.6
13	Lake Maridalsvann+EDTA, H 40+	1.66	0.010	0.61	3.7
14	Lake Byglandsfjorden+EDTA	1.58	0.013	0.82	11
15	Lake Byglandsfjorden+EDTA, H 10+	1.65	0.019	1.15	5.3
16	Lake Byglandsfjorden+EDTA, H 40+	1.62	0.010	0.60	20.5
17	Lake Byglandsfjorden-EDTA	0.96	0.057	5.94	20.4
Val. Crit.		**0.92**		**≤7**	**≤35**

The results of the toxicity tests are shown below. All concentrations are nominal concentrations unless specifically mentioned.

3.5 Artificial medium

Test 1. Artificial medium, hardness 0+.

The growth curves of test nr. 1 are shown in figure 1. More than 10 % reduction of the growth rate was observed at Cd concentrations above 4.84 µg/l, and the growth inhibition was almost total at 113 µg/l.

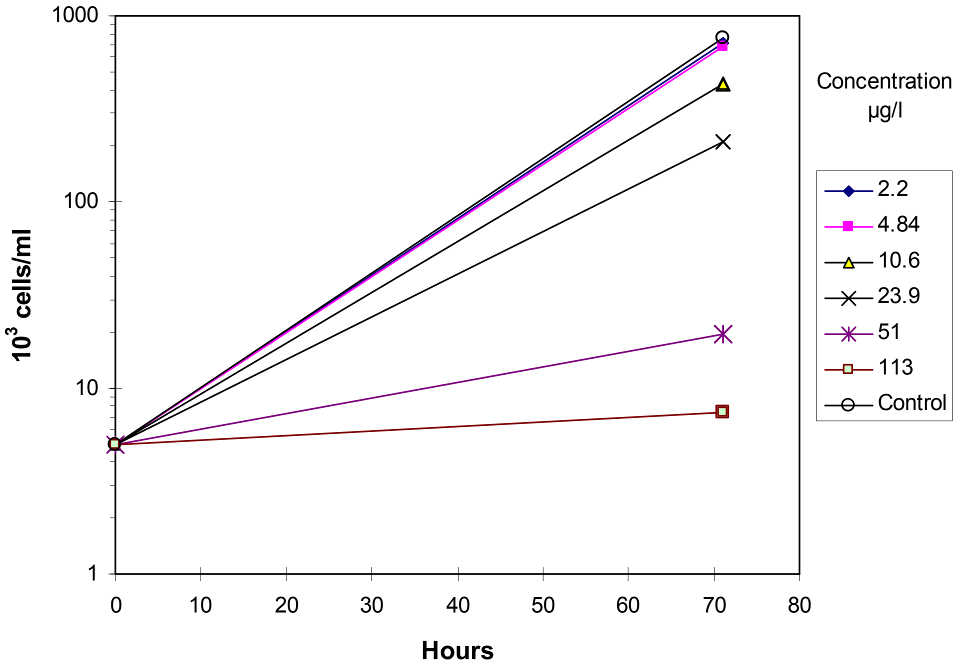

Figure 1. Growth curves for test of Cd in artificial medium. Hardness 0+. (Note that cell density was measured only after 72 hours).

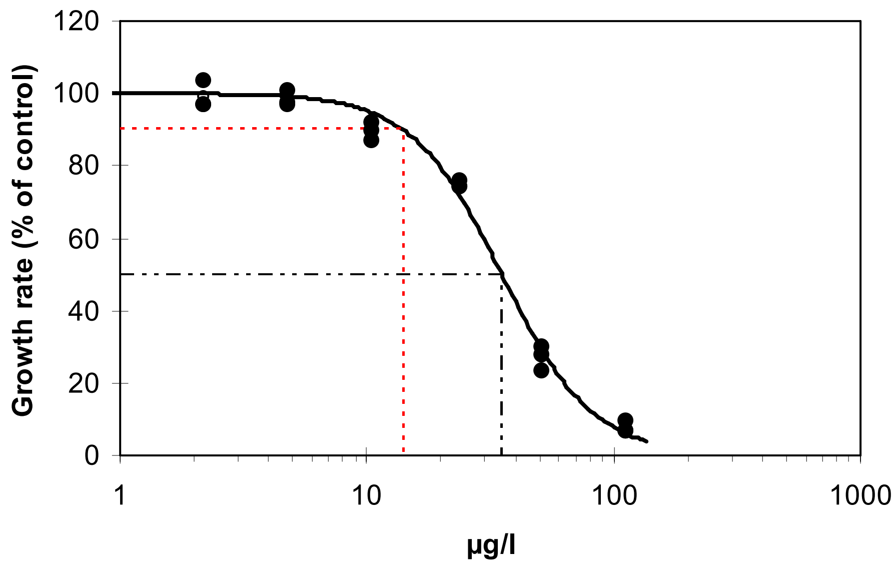

Fig. 2. Effect of Cd on growth rate of *P. subcapitata* in artificial medium at hardness 0+.

Test 2. Artificial medium, hardness 10+.

More than 10 % inhibition of the growth rate was observed at Cd concentrations above 10.6 µg/l. The growth was almost totally inhibited at 249 µg/l.

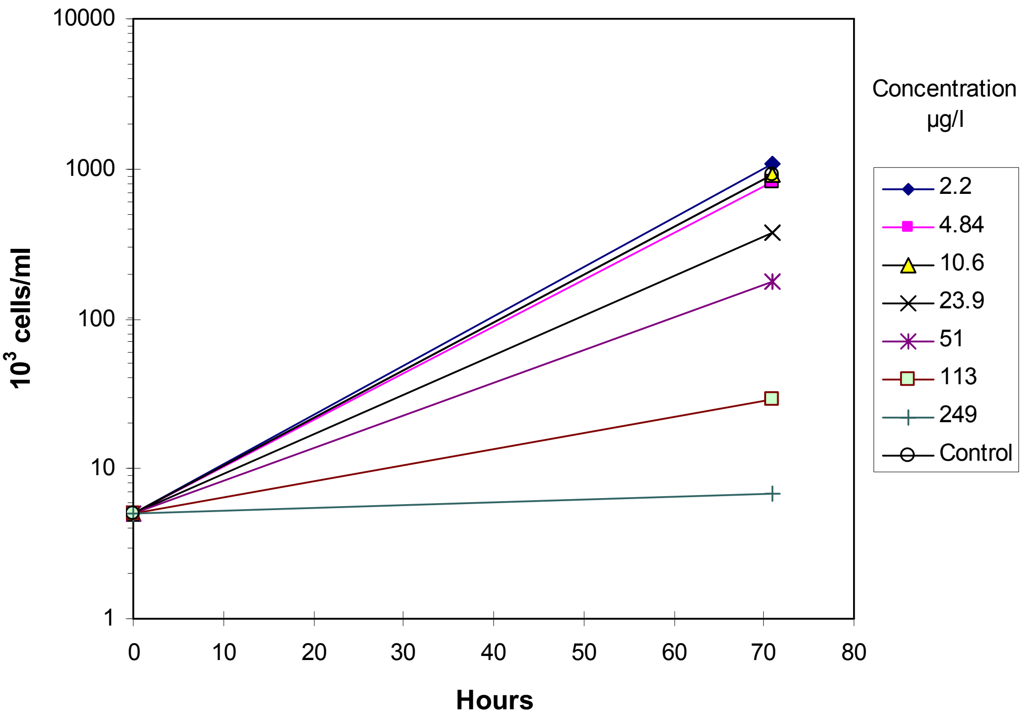

Figure 3. Growth curves for test of Cd in artificial medium. Hardness 10+. (Note that cell density was measured only after 72 hours).

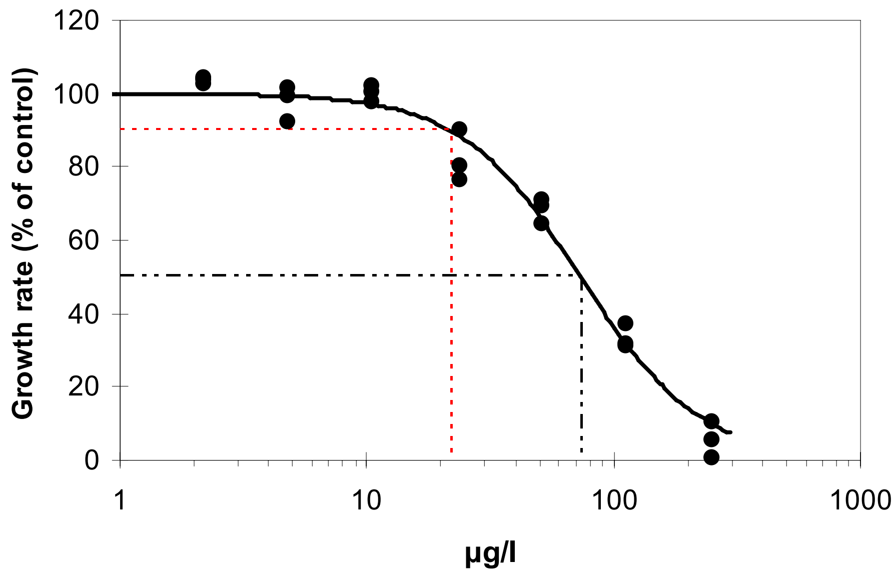

Figure 4. Effect of Cd on growth rate of *P. subcapitata* in artificial medium at hardness 10+.

Test 3. Artificial medium, hardness 40+.

More than 10 % growth inhibition was observed at concentrations above 10.6 µg/l. The inhibition at 249 µg/l was approximately 60 %.

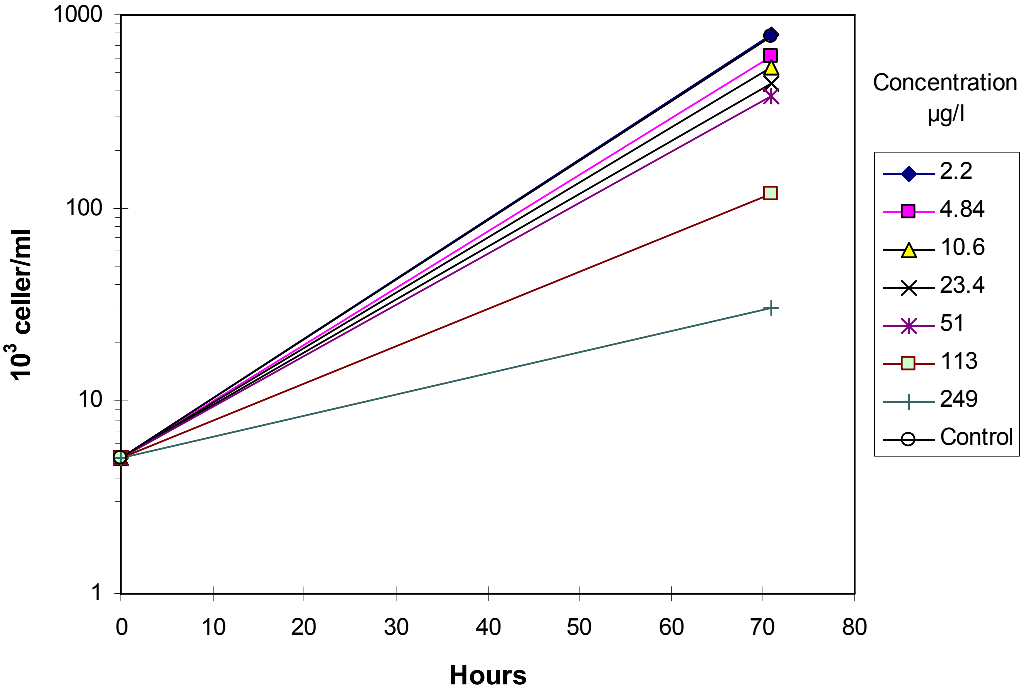

Figure 5. Growth curves for test of Cd in artificial medium. Hardness 40+. (Note that cell density was measured only after 72 hours).

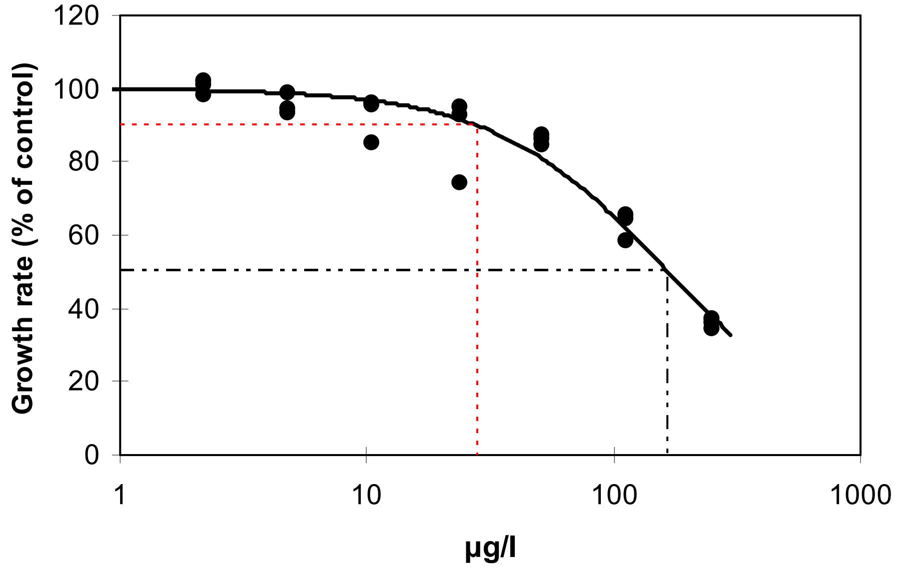

Figure 6. Effect of Cd on growth rate of *P. subcapitata* in artificial medium at hardness 40+.

Table 7. Effect concentrations calculated for tests in artificial medium. (Nominal concentrations, µg Cd/l).

Hardness	**EC_{10}**	**95 % c.i,**	**EC_{20}**	**95 % c.i.**	**EC_{50}**	**95 % c.i.**
3.5 mg $CaCO_3$/l	14	11 - 16	19	17 - 22	36	33-38
13.5 mg $CaCO_3$/l	21	18-26	34	29-38	73	66-79
43.5 mg $CaCO_3$/l	26	18-34	51	40-40	154	140-180

3.5.1 Lake Store Sandungen with EDTA

Test 4. Lake Store Sandungen with EDTA. Hardness 0+

More than 10 % inhibition of the growth rate was observed at concentrations above 23.4 µg/l. Complete inhibition was found at 249 µg/l. A constant exponential growth was observed in the control and at concentrations of Cd up to 23.4 µg/l. At 51 and 113 µg Cd/l, the growth rate after 48 hours was slightly lower than in the initial phase of the test.

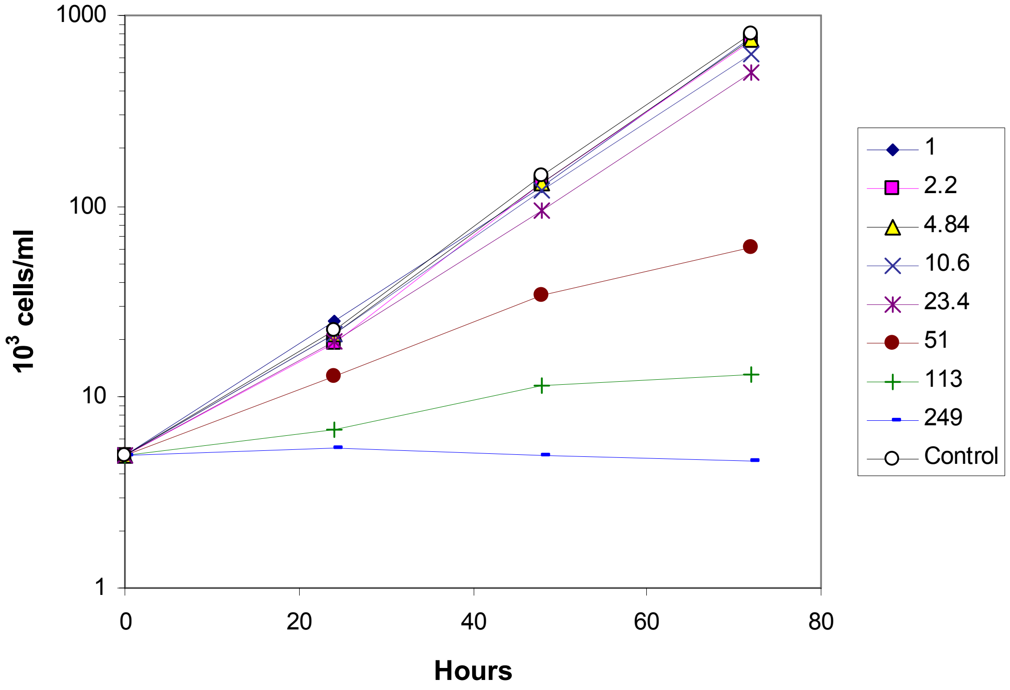

Figure 7. Growth curves for test of Cd in Lake Store Sandungen with EDTA. Hardness 0+.

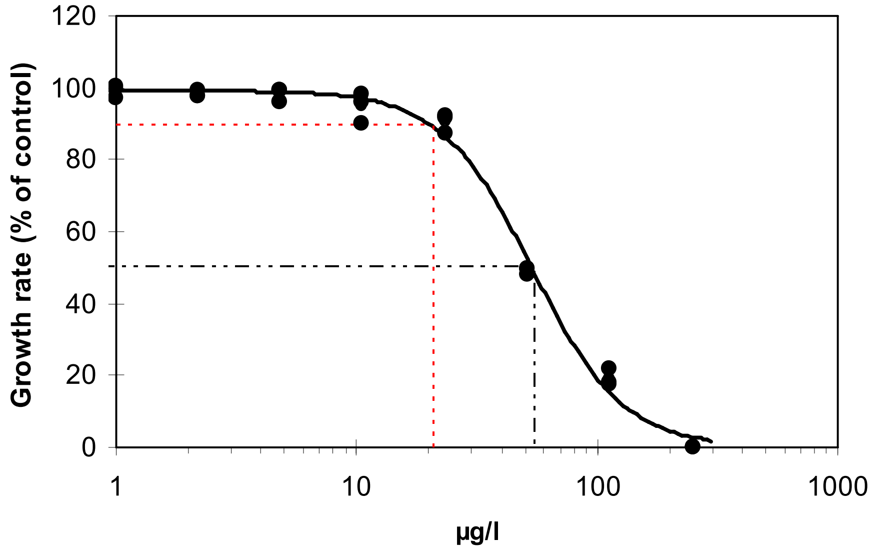

Figure 8. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen with EDTA. Hardness 0+.

Test 5. Lake Store Sandungen with EDTA. Hardness 10+

More than 10 % inhibition of the growth rate was observed at concentrations above 23.4 µg/l. The inhibition at 249 µg/l was approximately 80 %. A constant exponential growth was observed in the control and at all concentrations of Cd except for 51 and 113 µg Cd/l where the growth rate after 48 hours was slightly lower than in the initial phase of the test.

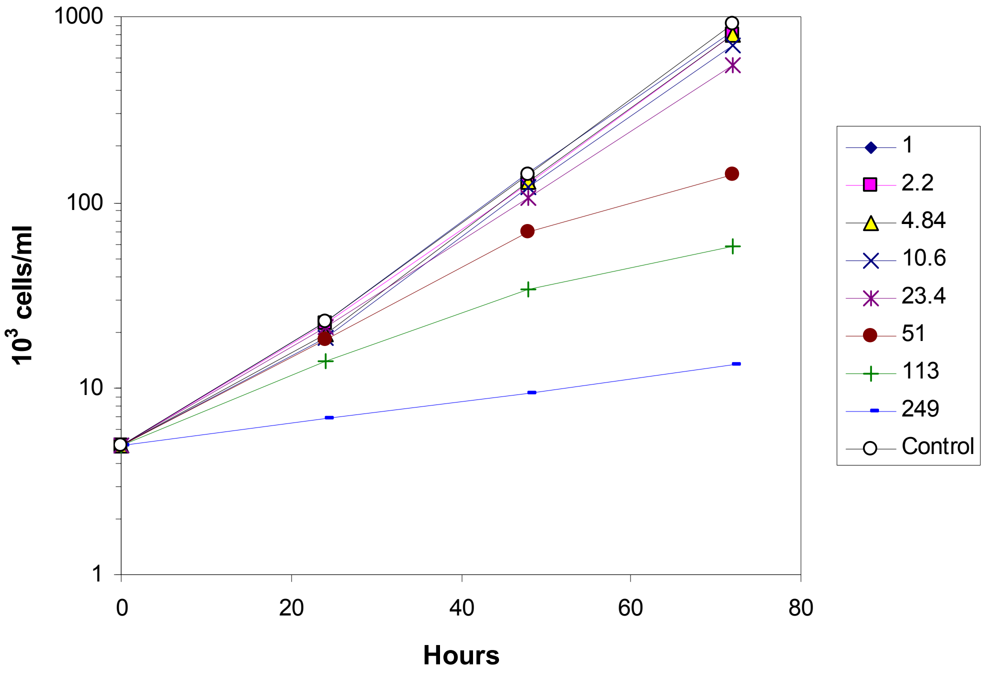

Figure 9. Growth curves for test of Cd in Lake Store Sandungen with EDTA. Hardness 10+.

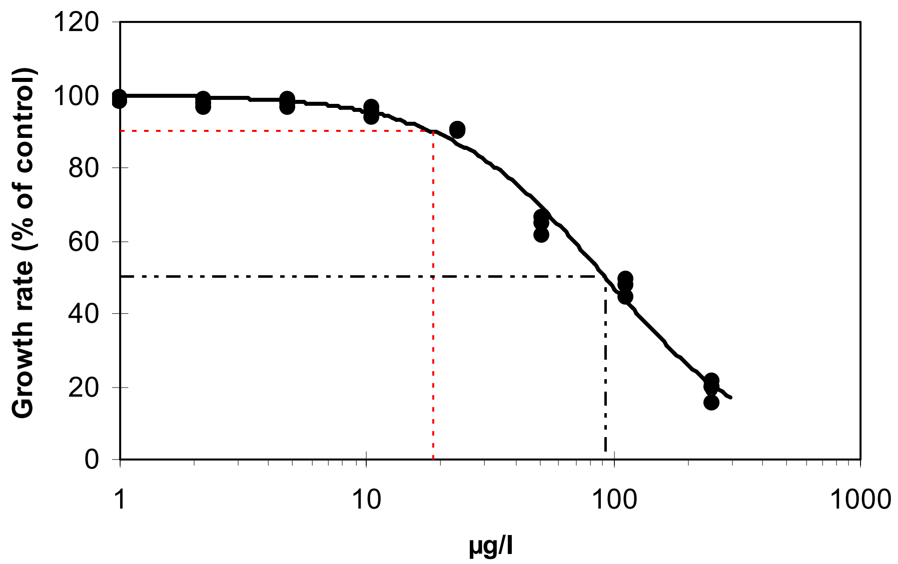

Figure 10. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen with EDTA. Hardness 10+.

Test 6. Lake Store Sandungen with EDTA. Hardness 40+

More than 10 % inhibition of the growth rate was observed at concentrations above 23.4 µg/l. The inhibition at 400 µg/l was approximately 70 %. At intermediate concentrations of Cd (51 and 113 µg/l) the growth was slightly lower after 48 hours than during the first 48 hours of exposure.

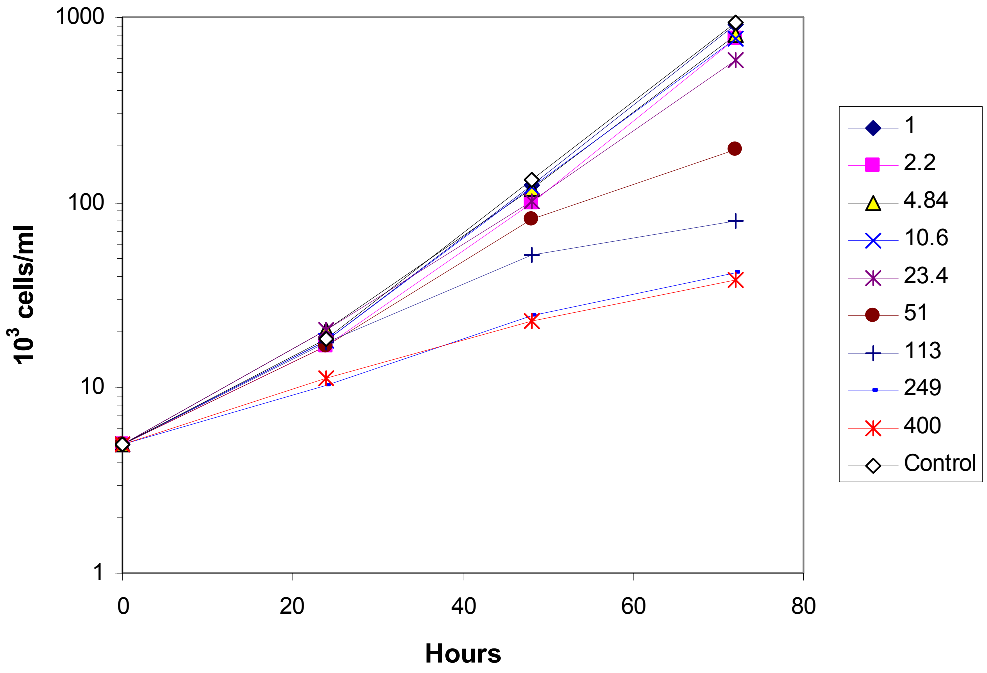

Figure 11. Growth curves for test of Cd in Lake Store Sandungen with EDTA. Hardness 40+.

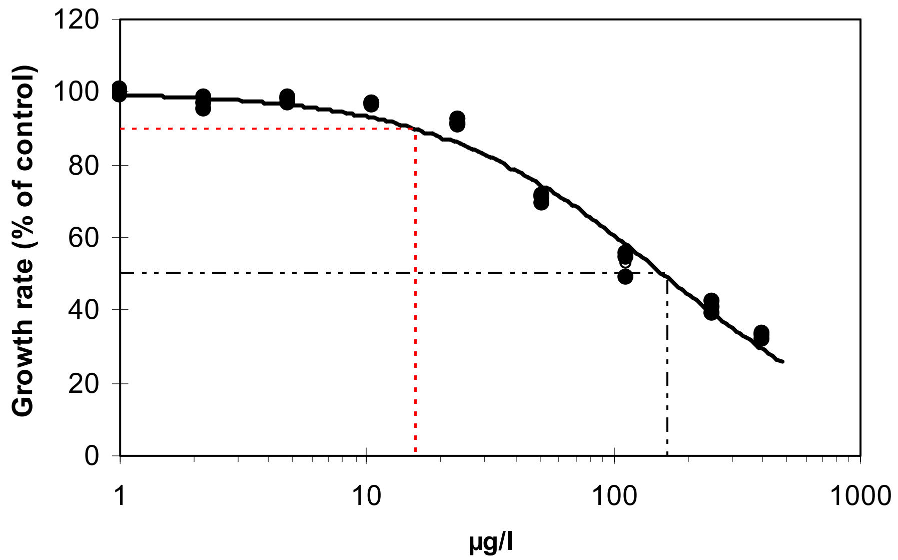

Figure 12. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen with EDTA. Hardness 40+.

Test 7. Lake Store Sandungen with EDTA. Hardness 0+. Cultures inoculated one day after preparation of test solutions.

More than 10 % inhibition of the growth rate was observed at concentrations above 10.6 µg/l. Complete inhibition was found at 249 µg/l. At 23 µg Cd/l, the growth rate was lower during the first day than during the last two days of exposure. At 113 µg/l the algae grew slowly for two days but were completely inhibited after 48 hours.

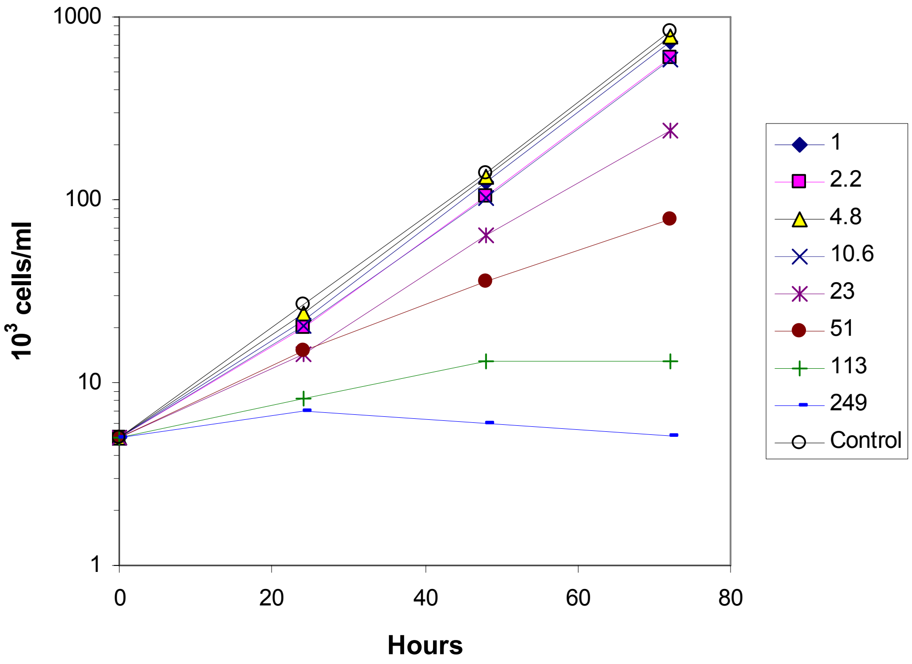

Figure 13. Growth curves for test of Cd in Lake Store Sandungen with EDTA. Hardness 0+. Cultures inoculated one day after preparation of test solutions.

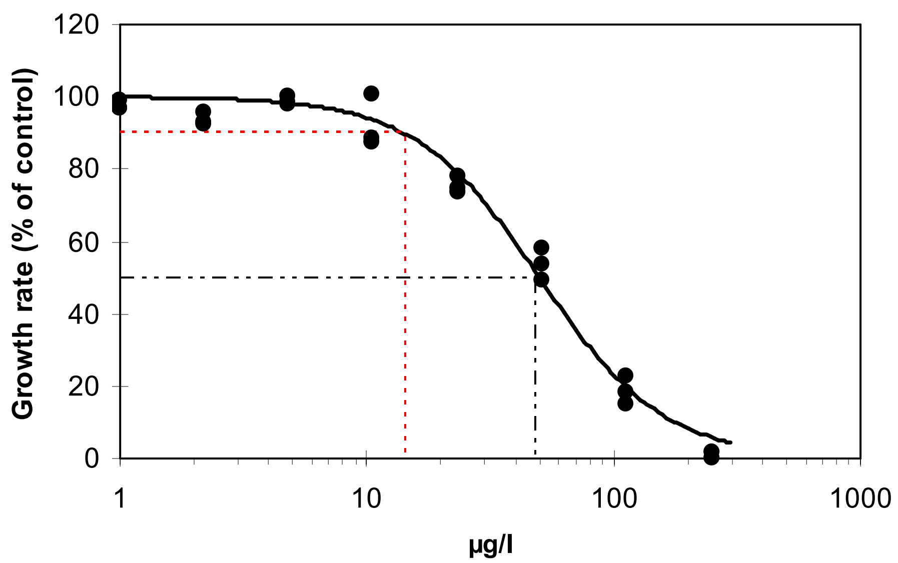

Figure 14. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen with EDTA. Hardness 0+. Test inoculated one day after preparation of test solutions.

Table 8. Effect concentrations calculated for tests in Lake Store Sandungen with EDTA . (Nominal concentrations, µg Cd/l).

Hardness	**EC_{10}**	**95 % c.i.**	**EC_{20}**	**95 % c.i.**	**EC_{50}**	**95 % c.i.**
6.2 mg $CaCO_3$/l	21	19 - 24	30	27 - 33	54	51 - 56
16.2 mg $CaCO_3$/l	18	15 - 21	33	30 - 36	91	87 – 96
46.2 mg $CaCO_3$/l	16	13 - 20	38	32 - 43	162	148 – 175
6.2 mg $CaCO_3$/l*	13	11 - 16	21	18 - 24	47	44 -51

* Cultures inoculated one day after preparation of test solutions.

3.5.2 Lake Store Sandungen without EDTA

Test 8. Lake Store Sandungen without EDTA. Hardness 0+.

More than 10 % growth inhibition was observed at 10.6 µg/l and higher concentrations of Cd. At concentrations of Cd between 23.4 and 249 µg/l, a gradual decrease of growth rate (increasing inhibition) with time was observed.

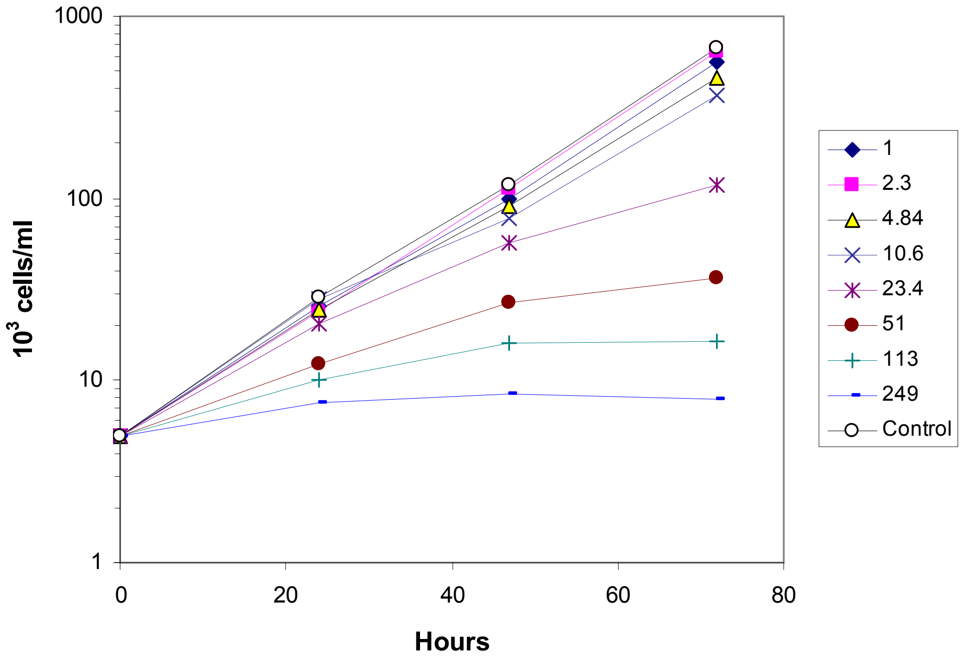

Figure 15. Growth curves for test of Cd in Lake Store Sandungen without EDTA. Hardness 0+.

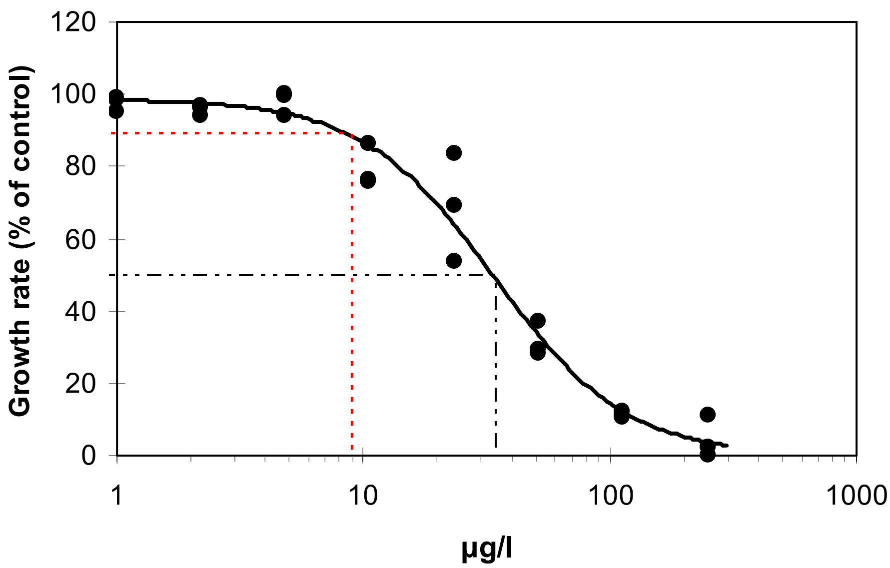

Figure 16. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen without EDTA. Hardness 0+.

Test nr. 9. Lake Store Sandungen without EDTA. Hardness 10+.

The average growth rate over the exposure period (0-72 hours) was inhibited more than 10 % as compared to the control at 23.4 µg/l and higher Cd concentrations. Deviation from constant, exponential growth was observed in all treatments including the control. Increasing growth inhibition with time was seen especially in the concentration range 51-249 µg Cd/l.

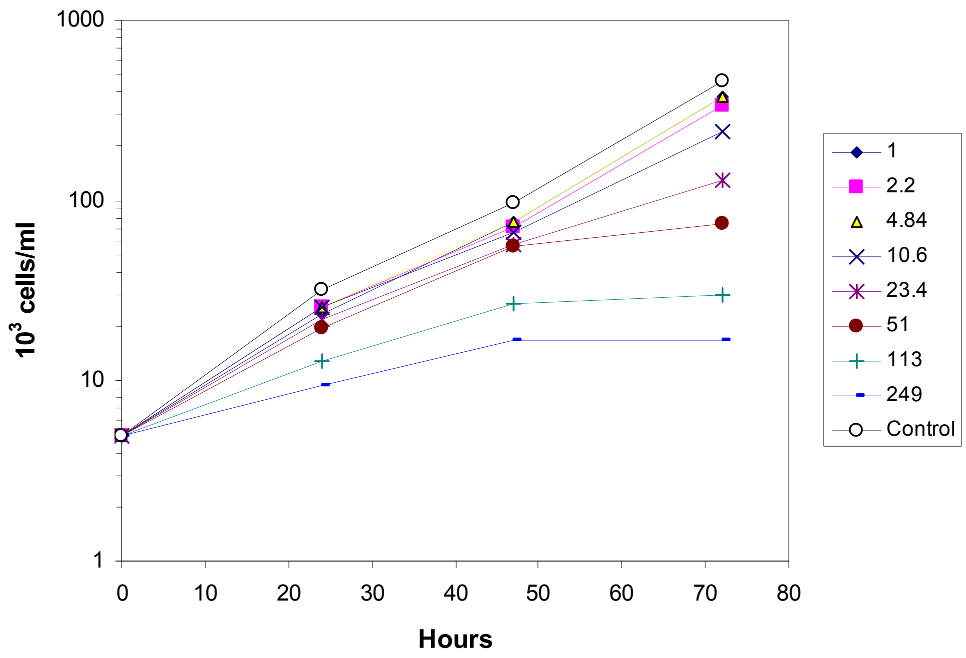

Figure 17. Growth curves for test of Cd in Lake Store Sandungen without EDTA. Hardness 10+.

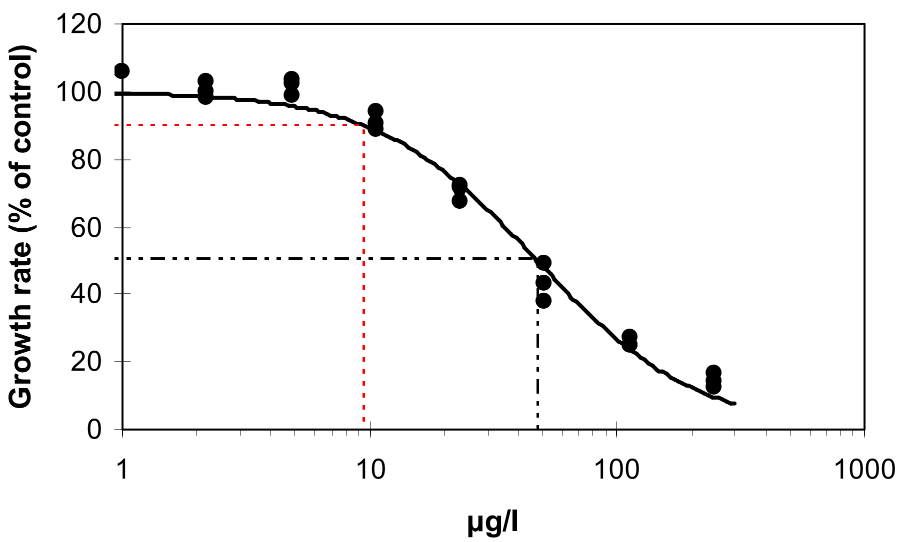

Figure 18. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen without EDTA. Hardness 10+.

Test nr. 10 Lake Store Sandungen without EDTA. Hardness 40+.

Irregular growth was observed at all treatments and variation between replicates was higher than normal. More than 10 % reduction in average growth rate (0-72 h) was observed at 23.4 µg/l and higher Cd concentrations.

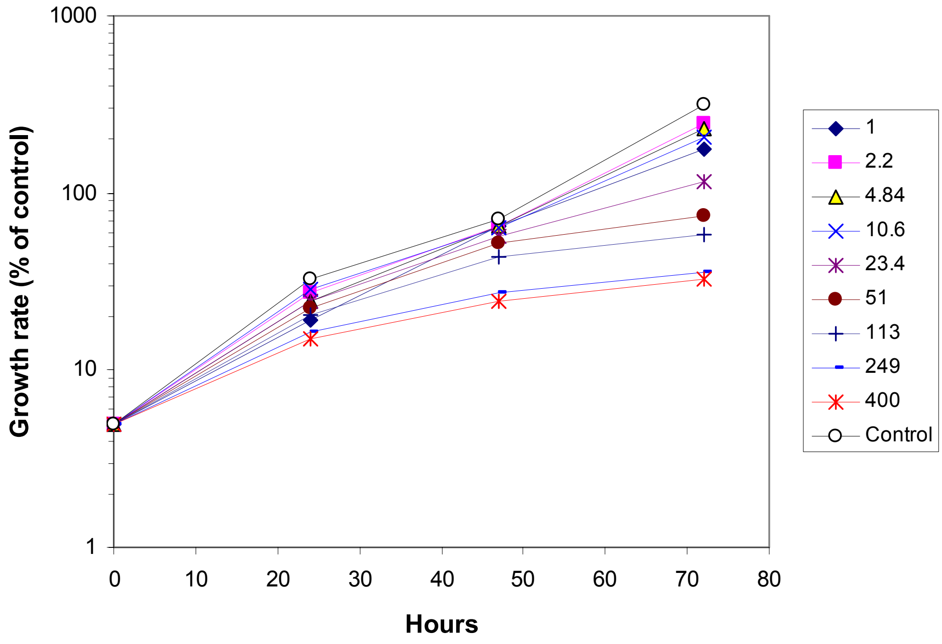

Figure 19. Growth curves for test of Cd in Lake Store Sandungen without EDTA. Hardness 40+.

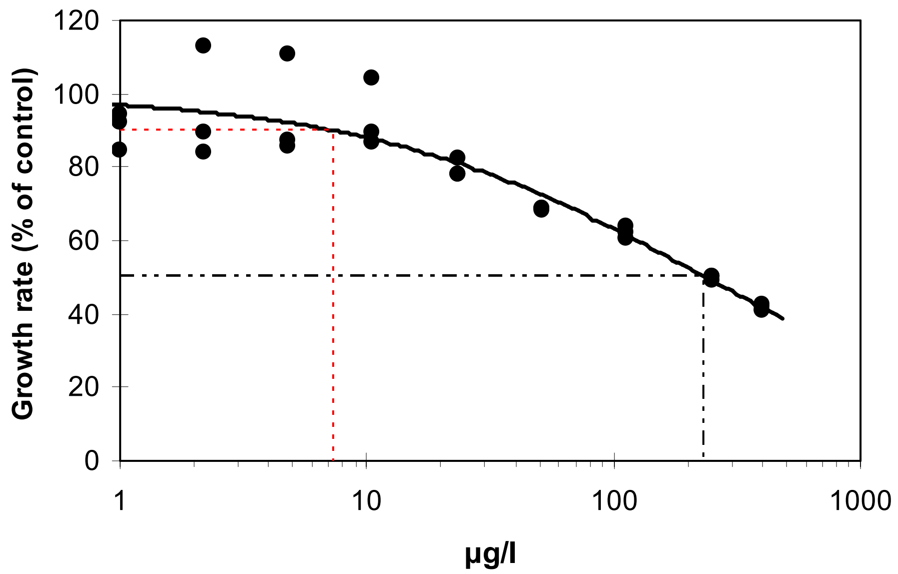

Figure 20. Effect of Cd on growth rate of *P. subcapitata* in Lake Store Sandungen without EDTA. Hardness 40+.

Table 9. Effect concentrations calculated for tests in Lake Store Sandungen without EDTA. (Nominal concentrations, µg Cd/l).

Hardness	**EC_{10}**	**95 % c.i.**	**EC_{20}**	**95 % c.i.**	**EC_{50}**	**95 % c.i.**
6.2 mg $CaCO_3$/l	8.8	6.5 - 12	15	12 - 18	34	30 - 39
16.2 mg $CaCO_3$/l	10	8.4 - 12	19	16 - 21	51	43 - 55
46.2 mg $CaCO_3$/l	7	4.1 – 14	26	18 - 39	234	186 - 312

3.5.3 Lake Maridalsvann with EDTA

Test 11. Lake Maridalsvann with EDTA. Hardness 0+.

More than 10 % reduction of average growth rate (0-72 h) was observed at 23.4 and higher Cd concentrations. Slightly declining growth rate with time was seen at 51 µg Cd/l and higher concentrations.

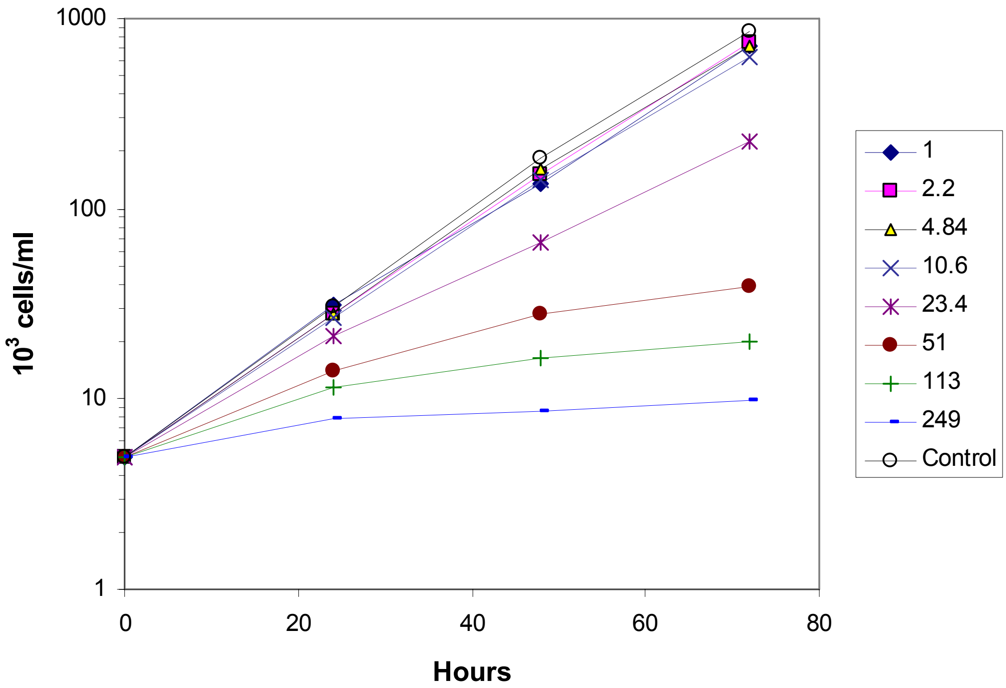

Figure 21. Growth curves for test of Cd in Lake Maridalsvann with EDTA. Hardness 0+.

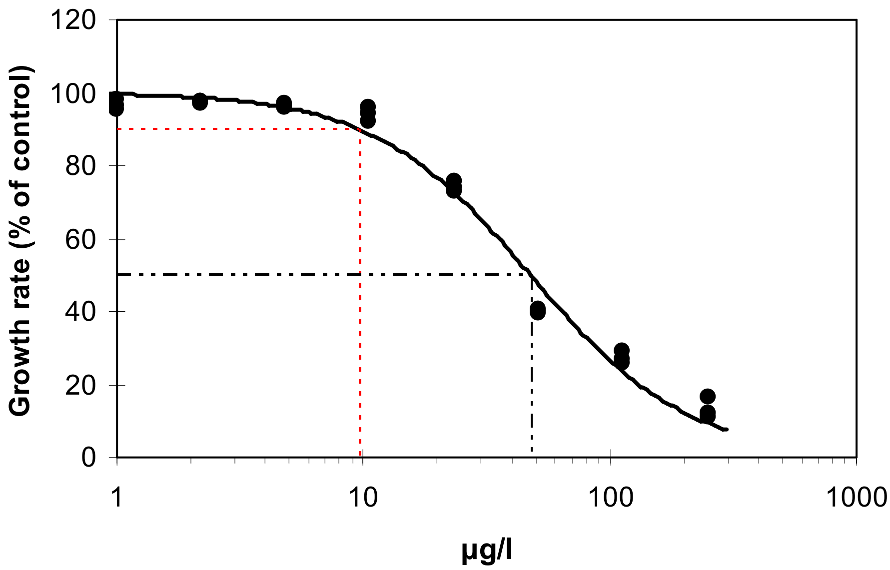

Figure 22. Effect of Cd on growth rate of *P. subcapitata* in Lake Maridalsvann. Hardness 0+.

Test 12. Lake Maridalsvann with EDTA. Hardness 10+.

More than 10 % reduction of average growth rate (0-472 h) as compared to the control was observed at 23.4 and higher Cd concentrations. The growth rate was declining slightly with time at 23.4 µg/l and higher Cd concentrations, indicating that growth inhibition increased with duration of exposure.

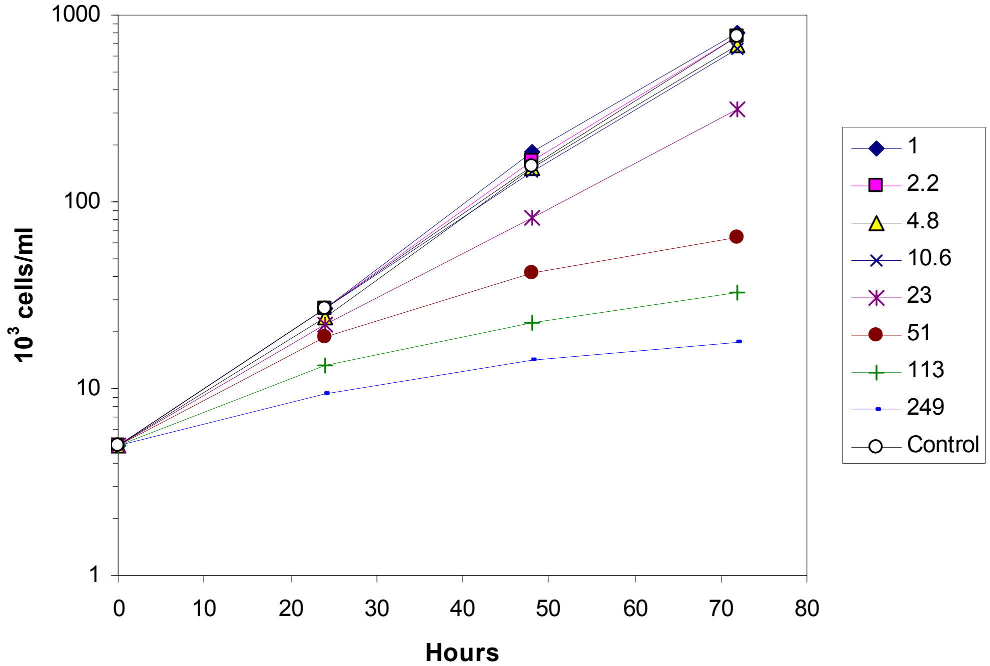

Figure 23. Growth curves for test of Cd in Lake Maridalsvann with EDTA. Hardness 10+.

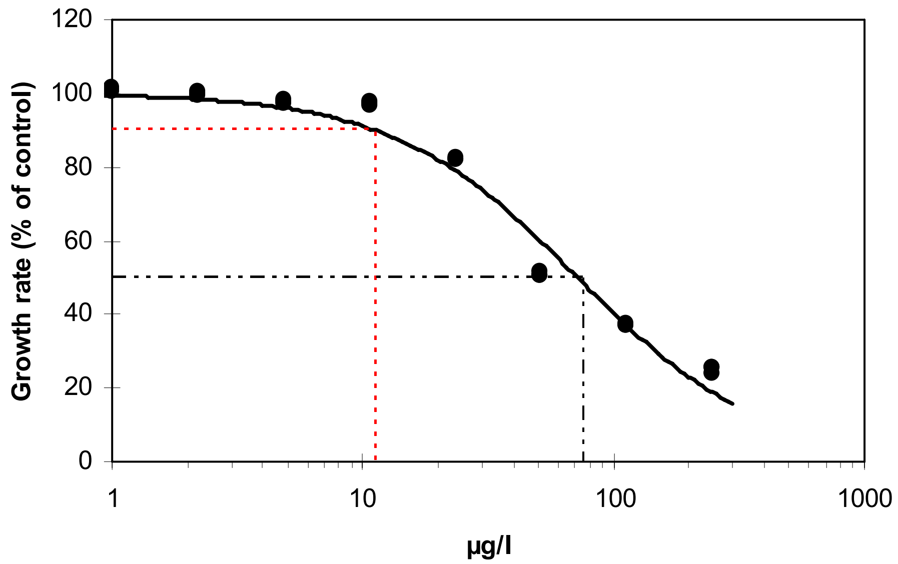

Figure 24. Effect of Cd on growth rate of *P. subcapitata* in Lake Maridalsvann. Hardness 10+.

Test 13. Lake Maridalsvann with EDTA. Hardness 40+.

The average growth rate (0-72 h) was reduced more than 10 % as compared to the control at 23 and higher concentrations of Cd. The growth rate declined with time at 23.4 µg/l and higher Cd concentrations, especially after 24 hours, indicating that growth inhibition increased with duration of exposure.

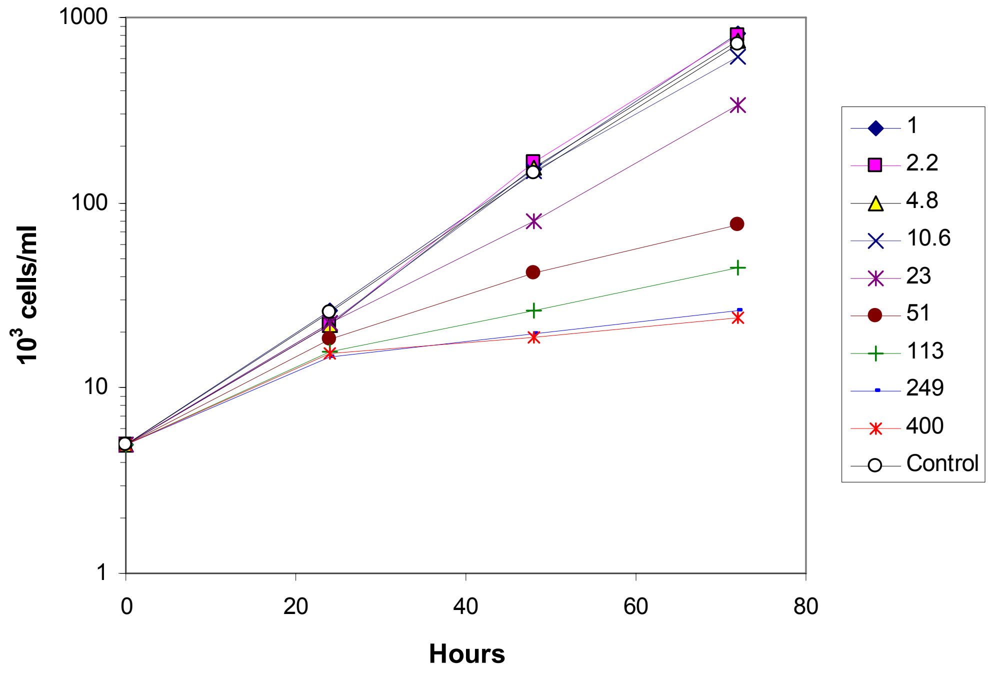

Figure 25. Growth curves for test of Cd in Lake Maridalsvann with EDTA. Hardness 40+.

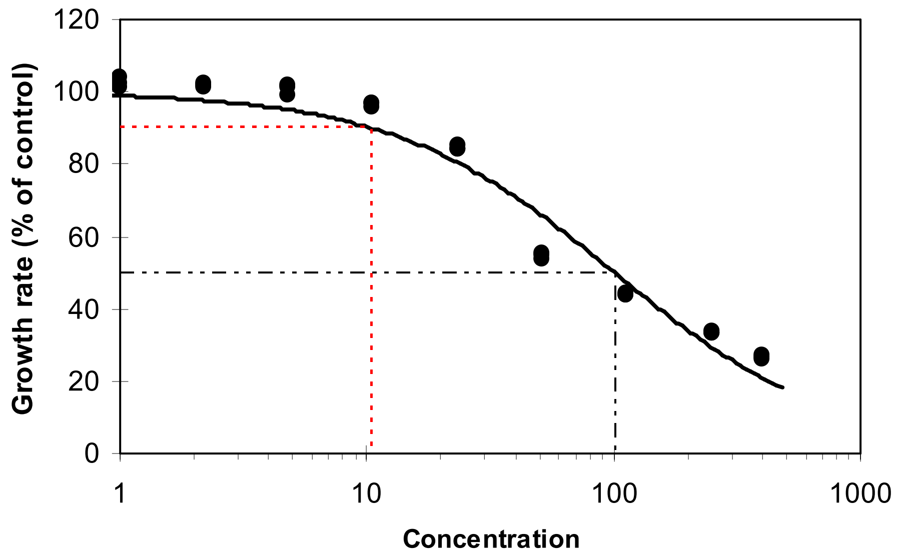

Figure 26. Effect of Cd on growth rate of *P. subcapitata* in Lake Maridalsvann. Hardness 40+.

Table 10. Effect concentrations calculated for tests in Lake Maridalsvann with EDTA. (Nominal concentrations, µg Cd/l).

Hardness	**EC_{10}**	**95 % c.i.**	**EC_{20}**	**95 % c.i.**	**EC_{50}**	**95 % c.i.**
8.1 mg $CaCO_3$/l	9.5	7.5 - 12	17	15 – 20	48	43 – 52
18.1 mg $CaCO_3$/l	12	9.8 - 15	24	20 – 28	76	68 – 83
48.1 mg $CaCO_3$/l	10	8.5 - 15	28	22 – 33	111	92 – 123

3.5.4 Lake Byglandsfjorden with EDTA

Test nr. 14. Lake Byglandsfjorden with EDTA. Hardness 0+

More than 10 % reduction of the average growth rate (0-72 h) as compared to the control was observed at 10.6 and higher Cd concentrations. At 51 µg/l and higher concentrations the growth was completely inhibited after 24 hours.

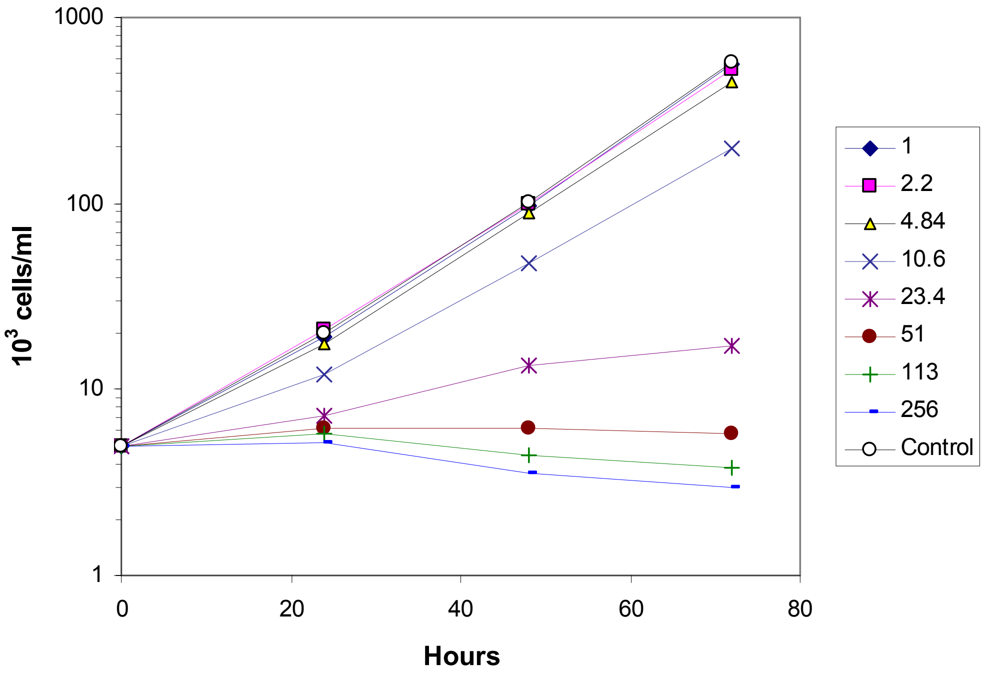

Figure 27. Growth curves for test of Cd in Lake Byglandsfjorden with EDTA. Hardness 0+.

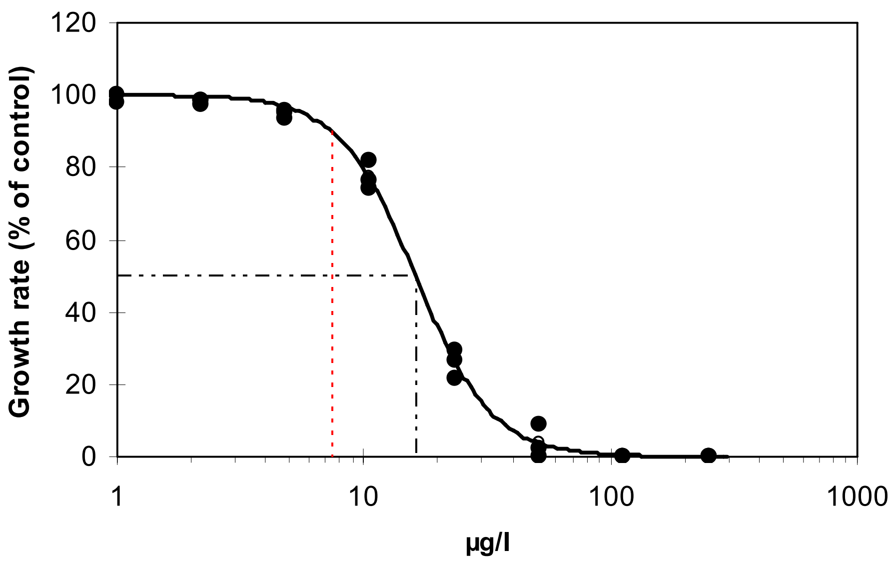

Figure 28. Effect of Cd on growth rate of *P. subcapitata* in Lake Byglandsfjorden with EDTA. Hardness 0+.

Test nr. 15. Lake Byglandsfjorden with EDTA. Hardness 10+.

More than 10 % reduction of the average growth rate (0-72 h) as compared to the control was observed at 51 and higher concentrations of Cd. The growth inhibition increased slightly with time at 51 and 113 µg/l.

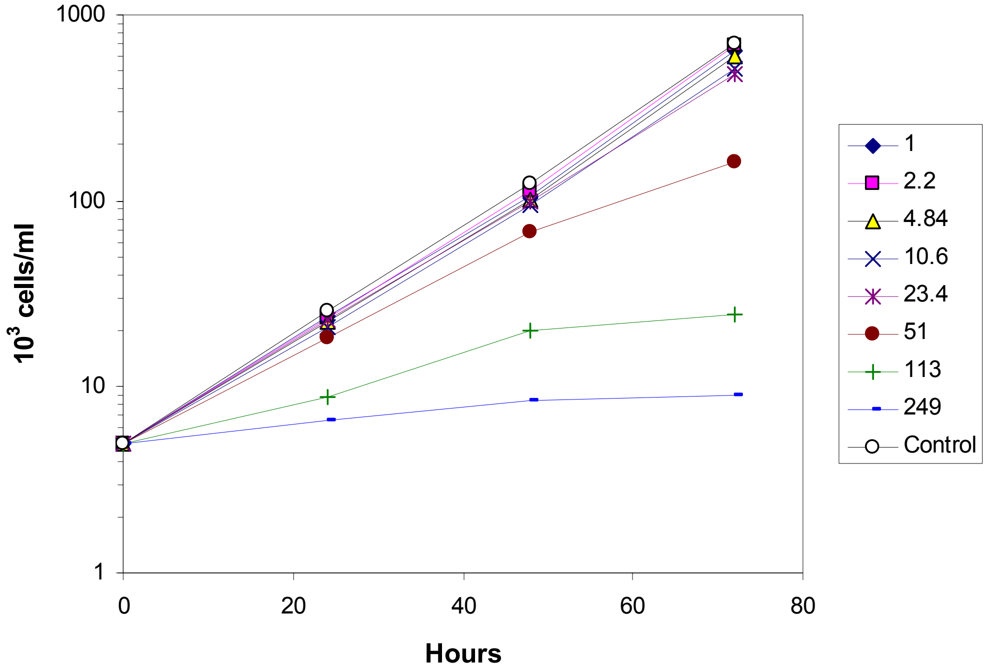

Figure 29. Growth curves for test of Cd in Lake Byglandsfjorden with EDTA. Hardness 10+.

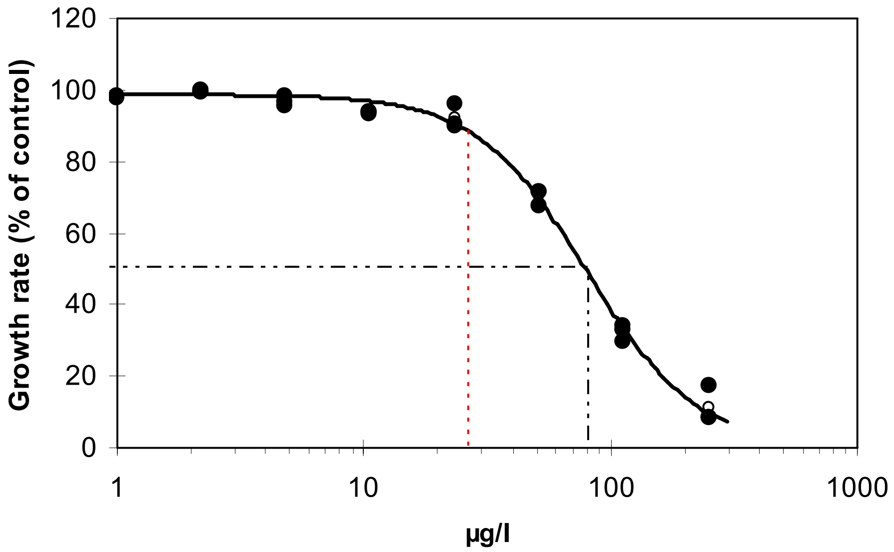

Figure 30. Effect of Cd on growth rate of *P. subcapitata* in Lake Byglandsfjorden with EDTA. Hardness 10+.

Test nr. 16. Lake Byglandsfjorden with EDTA. Hardness 40+.

More than 10 % reduction of the average growth rate (0-72 h) as compared to the control was observed at 51 µg/l and higher concentrations of Cd. The growth rate inhibition at the highest concentration (400 µg/l) was approximately 70 %.

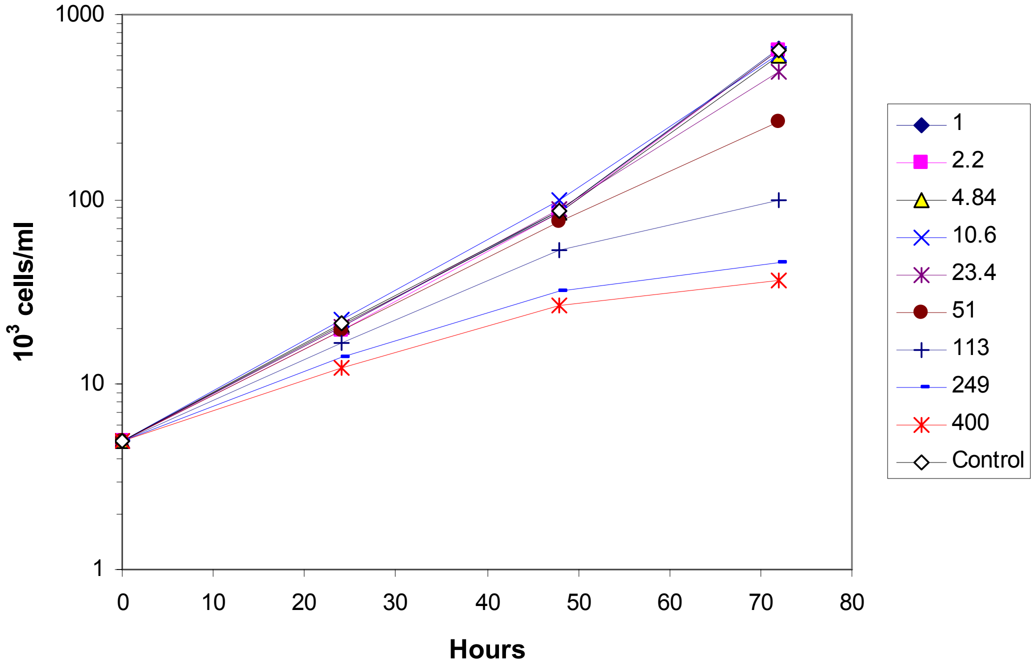

Figure 31. Growth curves for test of Cd in Lake Byglandsfjorden with EDTA. Hardness 40+.

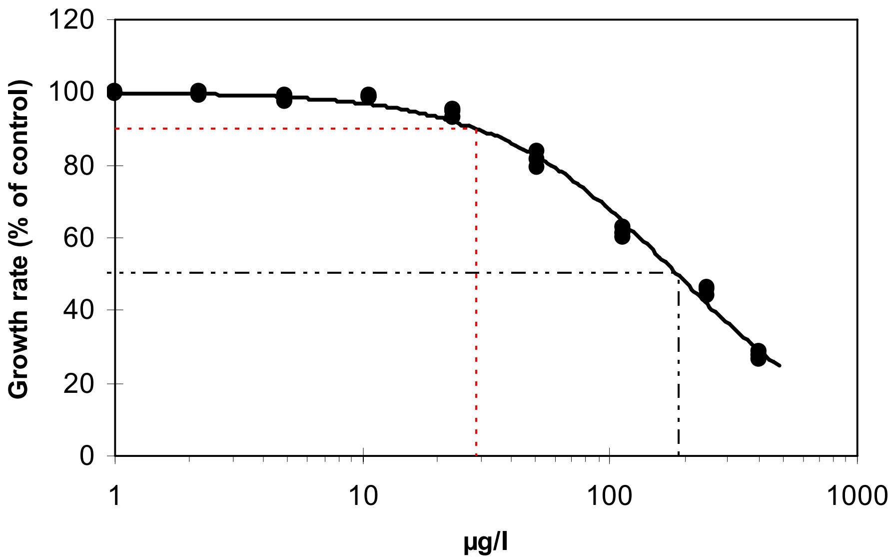

Figure 32. Effect of Cd on growth rate of *P. subcapitata* in Lake Byglandsfjorden with EDTA. Hardness 40+.

Table 11. Effect concentrations calculated for tests in Lake Byglandsfjorden with EDTA. (Nominal concentrations, µg Cd/l).

Hardness	**EC_{10}**	**95 % c.i.**	**EC_{20}**	**95 % c.i.**	**EC_{50}**	**95 % c.i.**
3.4 mg $CaCO_3$/l	7.3	6.8 – 7.9	9.7	9.3 – 10.3	16	15.5 – 16.6
13.4 mg $CaCO_3$/l	26	24 - 29	40	30 – 36	80	77 – 84
43.4 mg $CaCO_3$/l	29	26 - 33	58	54 - 63	188	111 – 126

3.5.5 Lake Byglandsfjord without EDTA

Test nr. 17. Lake Byglandsfjord without EDTA

The average growth rate (0-72 h) was more than 10 % lower than the control at 2.5 µg /l and higher concentrations of Cd. The growth was slightly irregular, with slightly increasing growth rate during the last day of exposure at intermediate exposure levels (4-10 µg/l). Complete growth inhibition was observed at 40 µg/l.

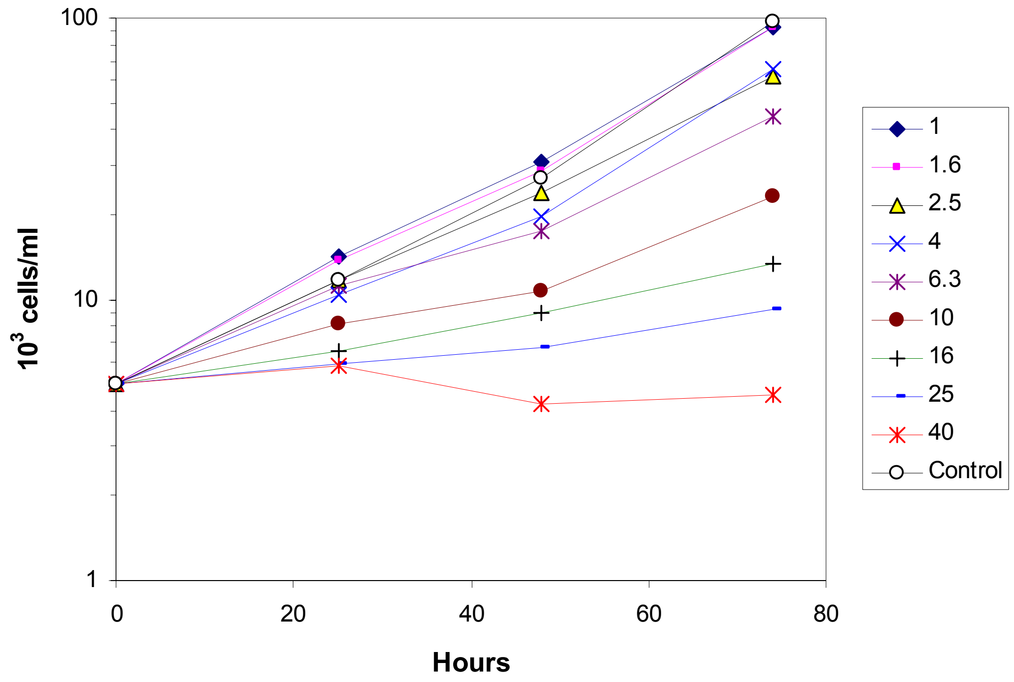

Figure 33. Growth curves for test of Cd in Lake Byglandsfjorden without EDTA. Hardness 0+.

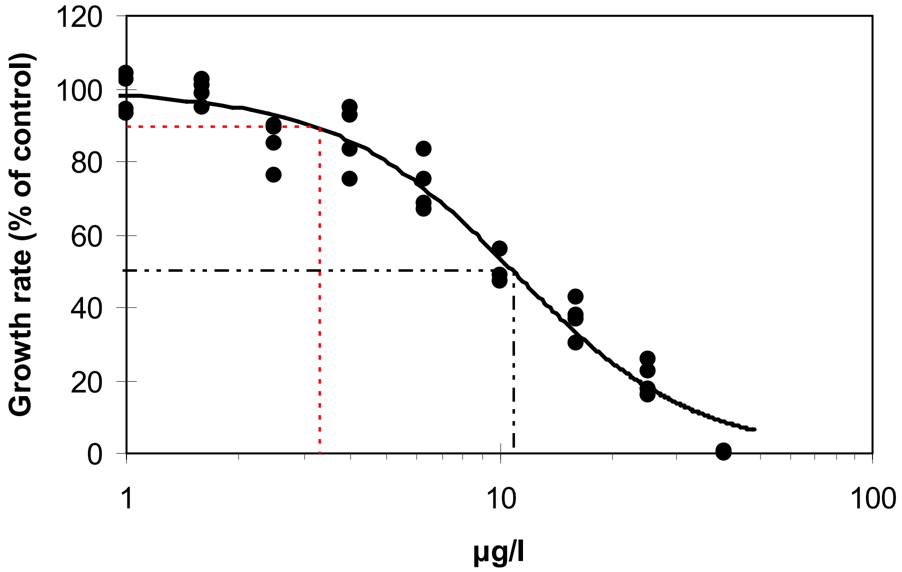

Figure 34. Effect of Cd on growth rate of *P. subcapitata* in Lake Byglandsfjorden without EDTA. Hardness 0+.

Table 12. Effect concentrations calculated for tests in Lake Byglandsfjorden without EDTA . (Nominal concentrations, µg Cd/l).

Hardness	EC_{10}	95 % c.i.	EC_{20}	95 % c.i.	EC_{50}	95 % c.i.
3.4 mg $CaCO_3$/l	3.3	2.6 - 4.1	5.1	4.3 – 6.0	11	9.9 – 11.8

4. Discussion

The analyses of freshly prepared test solutions showed that the concentrations of Cd were on average 85 % of the nominal. Since the same stock solution of Cd was used for all the toxicity tests it can be assumed that all exposure concentrations were 15% lower than the nominal concentrations. The effect concentrations derived from the tests should therefore be corrected for the deviation in nominal and actual exposure concentrations. A summary of effect concentrations for all the tests, expressed as initial, measured concentrations is presented in table 13.

In each of the five basal growth media where simultaneous tests were performed at three hardness levels, a reduction of the steepness of the response curve with increasing hardness level was observed. This is exemplified with a plot of response curves for tests in Lake Byglandsfjorden with EDTA (See figure 35). The fact the hardness changed the steepness of the curve, rather than merely displace the curves, means that the effect of hardness on the Cd effect concentrations was higher for EC_{50} than for EC_{20} and EC_{10}. This means that in the risk assessment context, where NOEC or EC_{10} is treated as a "chronic toxicity value" and EC_{50} as an "acute toxicity value", the effect of hardness is more pronounced for acute toxicity than for chronic toxicity.

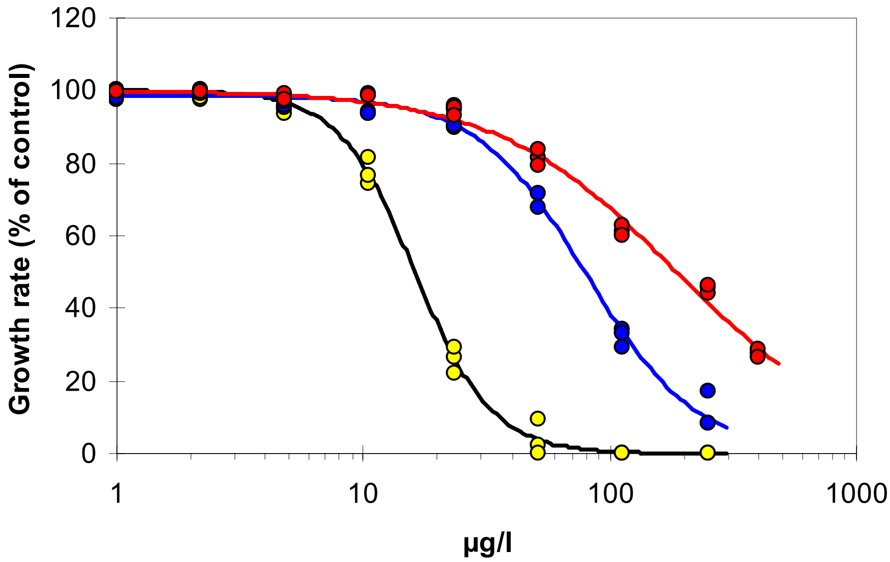

Figure 35. Response curves for the growth inhibition of Cd on *P. subcapitata* at three different hardness levels in Lake Byglandsfjorden with EDTA. (Black/yellow: 3.4 mg $CaCO_3$/l, blue: 13.4 mg $CaCO_3$/l and red: 43.4 mg $CaCO_3$/l).

Table 13. Summary of effect concentrations for inhibition of growth rate of *P. subcapitata* in different media (µg Cd/l, measured concentrations).

Artificial medium

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
3.5 mg $CaCO_3$/l	**11.9**	*9.4*	*13.6*	**16.2**	*14.5*	*18.7*	**31**	*28*	*32*
13.5 mg $CaCO_3$/l	**17.9**	*15.3*	*22.1*	**29**	*25*	*32*	**62**	*56*	*67*
43.5 mg $CaCO_3$/l	**22.1**	*15.3*	*28.9*	**43**	*34*	*51*	**131**	*119*	*153*

Lake Store Sandungen with EDTA

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
6.2 mg $CaCO_3$/l	**17.9**	*16.2*	*20.4*	**25.5**	*23.0*	*28.1*	**46**	*43*	*48*
16.2 mg $CaCO_3$/l	**15.3**	*12.8*	*17.9*	**28.1**	*25.5*	*30.6*	**77**	*74*	*82*
46.2 mg $CaCO_3$/l	**13.6**	*11.1*	*17.0*	**32.3**	*27.2*	*36.6*	**138**	*126*	*149*
6.2 mg CaCO3/l*	**11.1**	*9.4*	*13.6*	**17.9**	*15.3*	*20.4*	**40**	*37*	*43*

* Inoculated 1 day after preparation of test solutions

Lake Store Sandungen without EDTA

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
6.2 mg $CaCO_3$/l	**7.5**	*5.5*	*10.2*	**12.8**	*10.2*	*15.3*	**29**	*26*	*33*
16.2 mg $CaCO_3$/l	**8.5**	*7.1*	*10.2*	**16.2**	*13.6*	*17.9*	**43**	*37*	*47*
46.2 mg $CaCO_3$/l	**6.0**	*3.5*	*11.6*	**22**	*15*	*33*	**199**	*158*	*265*

Lake Maridalsvann with EDTA

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
8.1 mg $CaCO_3$/l	**8.1**	*6.4*	*10.2*	**14.5**	*12.8*	*17*	**41**	*37*	*44*
18.1 mg $CaCO_3$/l	**10.2**	*8.3*	*12.8*	**20.4**	*17*	*24*	**65**	*58*	*71*
48.1 mg $CaCO_3$/l	**8.5**	*7.2*	*12.8*	**23.8**	*18.7*	*28*	**94**	*78*	*105*

Lake Byglandsfjorden with EDTA

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
3.4 mg $CaCO_3$/l	**6.2**	*5.8*	*6.7*	**8.2**	*7.9*	*8.8*	**13.6**	*13.2*	*14*
13.4 mg $CaCO_3$/l	**22.1**	*20.4*	*24.7*	**34.0**	*31.5*	*36.6*	**68**	*65.5*	*71*
43.4 mg $CaCO_3$/l	**24.7**	*22.1*	*28.1*	**49.3**	*45.9*	*53.6*	**160**	*153*	*167*

Lake Byglandsfjorden without EDTA

Hardness	EC_{10}	95 % c.i.		EC_{20}	95 % c.i.		EC_{50}	95 % c.i.	
3.4 mg $CaCO_3$/l	**2.8**	*2.2*	*3.5*	**4.3**	3.7	5.1	**9.4**	*8.4*	*10*

A plot of the EC_{50}-values at different hardness levels in media with EDTA is shown in figure 36. In all media the effect of hardness on the toxicity was highest in the low hardness range. The steepness of the curves differs between the media, with the highest effect of hardness in Lake Byglandsfjorden. In Lake Store Sandungen and Lake Maridalsvann, the effect of hardness is less pronounced than for Lake Byglandsfjorden and the artificial medium. This may be due to the higher concentration of TOC in

Lake Store Sandungen and Lake Maridalsvann. The fact that The EC_{50} at the highest hardness level (40+) was higher in Lake Byglandsfjorden than in the more humic lakes is, however, surprising. A similar phenomenon appears when the results of the tests in Lake Store Sandungen with and without EDTA are compared. At the two lowest hardness levels (6.2 and 16.2 mg $CaCO_3$/l) the EC_{50} was lower in the medium without EDTA than with EDTA as would be expected. At the highest hardness (46 mg $CaCO_3$/l), however, the EC_{50} was highest in the medium without EDTA.

The effect of hardness on the EC_{10} is less obvious than for EC_{50}. Especially in the higher hardness range tested (10+ - 40+), the EC_{10} is rather independent on the hardness. In Lakes Maridalsvann and Store Sandungen the EC_{10} was slightly lower at hardness 40+ than at 10+, while the opposite was observed in Lake Byglandsfjorden and in the artificial medium. In the low hardness range tested (0+ - 10+), EC_{10} increased strongly with hardness in Lake Byglandsfjorden but less so in Lake Maridalsvann and the artificial medium. In Lake Store Sandungen the EC_{10} decreased with hardness in the whole range tested. In table 14, the maximum and mean slopes of the EC_{10}/hardness and EC_{50}/hardness plots in figures 36 and 37 are shown. The slopes have been calculated for the two ranges of hardness tested. Because of the different background hardness in the lake waters these ranges have been defined as approximately 3.4-15 and 15-45 mg $CaCO_3$/l.

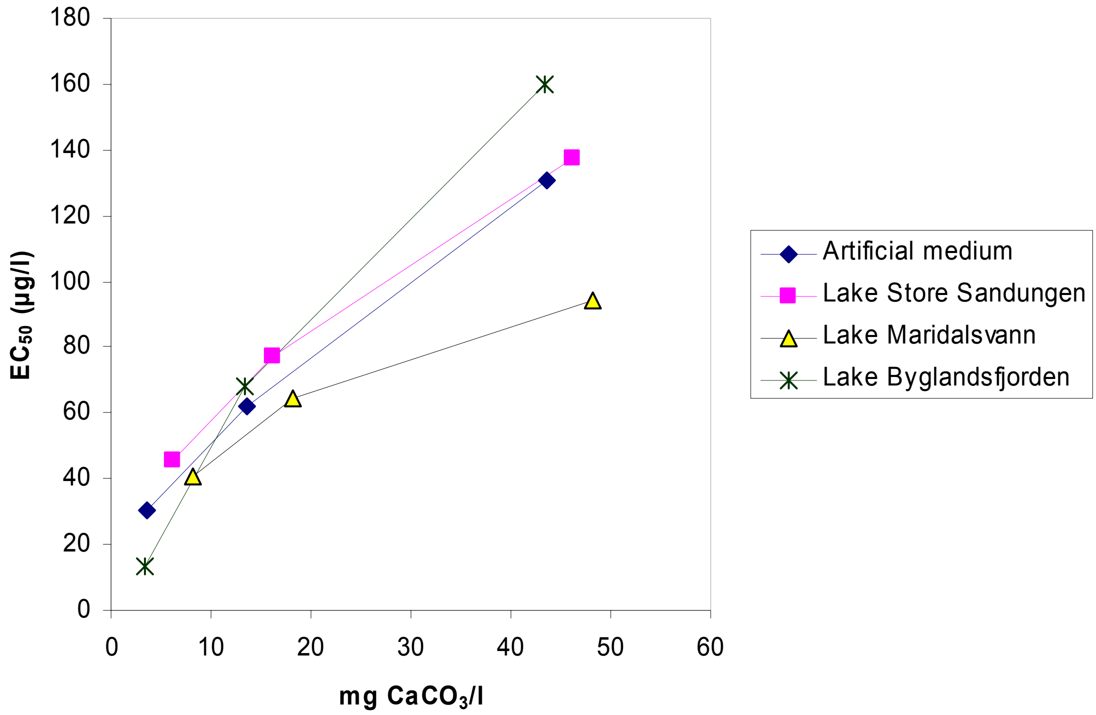

Figure 36. Effect of hardness on EC_{50} for Cd in different growth media with EDTA.

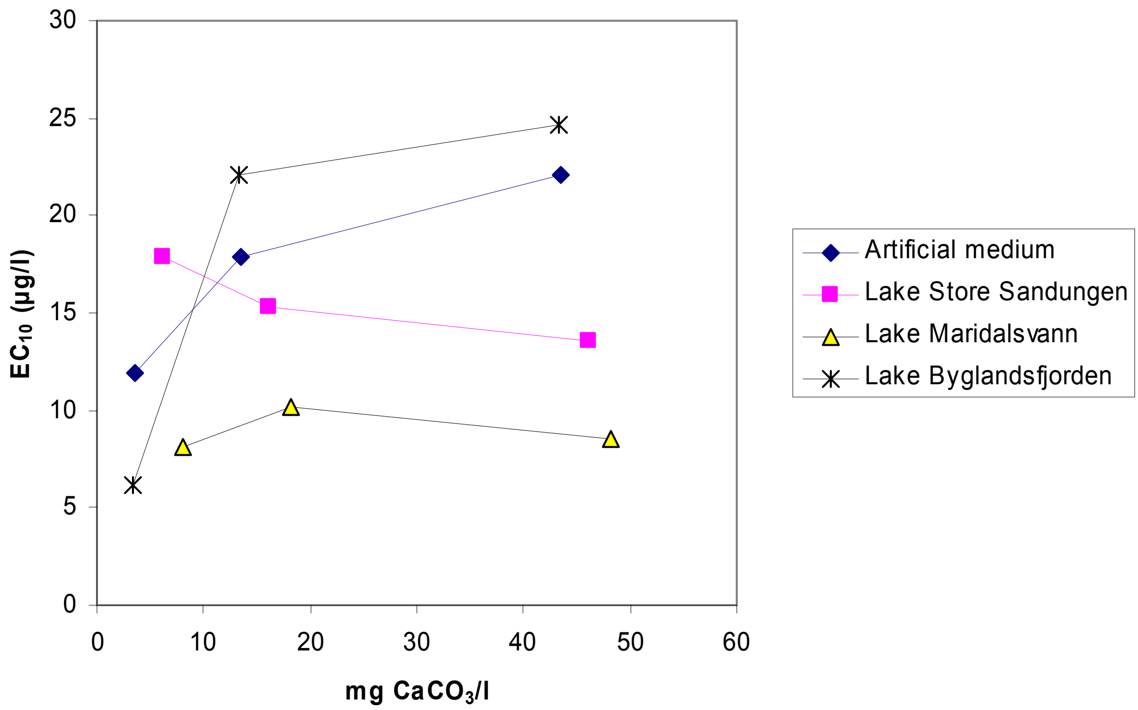

Figure 37. Effect of hardness on EC_{10} for Cd in different growth media with EDTA.

Table 14. Maximum and mean values for the slope (µg Cd/mg $CaCO_3$) of effect concentrations versus hardness in media with EDTA. The slopes are calculated for two approximate ranges of hardness (3.4-15 mg and 15-45 mg $CaCO_3$/l).

	Slope (µg Cd/mg $CaCO_3$)			
	Hardness 3.4 -15 mg $CaCO_3$/l		**Hardness 15 - 45 mg $CaCO_3$/l**	
Effect concentration	**Max**	**Mean**	**Max**	**Mean**
EC_{10}	1.59	0.54	0.14	0.03
EC_{50}	5.44	3.53	3.06	2.09

The test performed water from Lakes Store Sandungen and Byglandsfjorden without EDTA showed lower growth rates than the corresponding tests with EDTA. This indicates that the growth was limited by the supply of trace metals, presumably primarily iron. In Lake Store Sandungen the control growth rate decreased with increasing hardness, which indicates that the availability of iron was affected by the added calcium. Irregular growth and larger variation between replicates accompanied the reduction of growth rates in media without EDTA and made the results of these tests less reliable. Still it may be concluded, that in both lake waters without spiking with Ca, EDTA reduced the toxic effects as would be expected. The presence of 0.27 µM EDTA caused an increase of approximately a factor 1.5 on the EC_{50} and 2.4 on the EC_{10}.

The test in Lake Byglandsfjorden without EDTA was performed to determine effect concentrations for Cd in a typical low hardness and low organic carbon type lake. This test was performed with denser spacing of the concentration levels to achieve better precision of the EC estimates and allow calculation of a NOEC. This test yielded an EC_{50} of 9.4 µg/l, an EC_{10} of 2.8 µg/l and a NOEC of 1.36 µg/l for growth rate inhibition of *P. subcapitata* (measured concentrations). This is lower than the previously reported toxicity values for this species according to the draft RAR. It should be noted, that the hardness of Lake Byglandsfjorden is not extremely low, in fact it is near the 50 percentile for Norwegian lakes. The steep slope of the EC_{10}/hardness-slope that was found in the low hardness range for Lake Byglandsfjorden (See fig. 37) indicates that growth inhibition of algae may occur at considerably lower concentrations than what was observed in Lake Byglandsfjorden in those areas where water hardness is even lower.

The initial pH-values in different media were in the range 6.5 (Lake Byglandsfjorden) to 7.0 (Artificial medium). No organic buffer was used to control the pH in the test solutions during the test and therefore an increase in pH, caused by photosynthetic assimilation of CO_2 was observed in cultures with significant growth. (See data in appendix.) In algal growth inhibition tests such pH-increases typically happen abruptly, when the assimilation of CO_2 by the increasing algal biomass exceeds the mass transport of CO_2 from the atmosphere into the culture. Under the conditions used in the present study, such pH increases normally occur only during the last day of a 72 hours test. Increased pH-values are expected to increase the toxic effects of Cd because of competition between H^+ and Cd^{2+} at the cellular membrane. Thus, theoretically, one would expect a tendency of increasing growth inhibition during the last day of exposure in cultures exposed to slightly inhibiting concentrations of Cd. In fact, an increased inhibition with time <u>was</u> observed at intermediate Cd concentrations in most of the tests performed in this study. However, this phenomenon was most pronounced at concentrations causing a rather high growth inhibition (typically 51-113 µg/l) and in these cultures the pH increase was minor. This can be seen e.g. in the test in Lake Maridalsvann at hardness 10+ (Figure 23). At 51 and 113 µg Cd/l, the growth rate declined gradually for each day, indicating an increasing toxic effect even though pH was constant. At 23.4 µg Cd/l, the growth rate was constant, approximately 20% lower than the control throughout the test in spite of a 0.3 unit pH increase. At the lowest Cd concentrations (1.0-10.6 µg/l) the pH increased with approximately one unit, but the growth curves did not indicate increasing toxic effects with time at these concentrations of Cd.

From the discussion above it follows that the increasing growth inhibiting effect that was observed at intermediate Cd concentrations was not caused by pH-variations in the medium. Rather, it appears that there is a delayed effect of the exposure, indicating that the cells are gradually more affected by the exposure to Cd.

Another factor, which may influence the sensitivity of an algal batch test system, is adsorption of the test material on the algal biomass. This may cause a decrease of the internal concentration of the toxic substance as the biomass density is increasing, with the effect that the growth inhibition is declining during the exposure period. The experiment that was performed in order to measure the adsorption of Cd to algal biomass resulted in different K_d at two levels of biomass density. The reason for this is not known. In order to assess the possible effect of adsorption of Cd on the exposure concentration, the highest K_d value obtained (12500 l/kg) will be used. The maximum cell density that was obtained in non-inhibited cultures at the end of the tests was approximately 900×10^6 cells/l which corresponds to a biomass density of 27 mg/l. With a K_d=12500 for adsorption to algal biomass approximately 25 % of the Cd will be adsorbed to algae and 75 % in solution.

The same calculation for the maximum biomass density after 48 hours (150×10^6 cells/l or 4.5 mg/l) shows that approximately 95 % of the Cd will be in solution after 48 hours. At the EC_{50}, where the growth rate is 50% of the control growth rate, the final cell density is less than 70×10^6, and more than 97 % of the Cd is in solution. Thus, it may be concluded that adsorption to the algal biomass probably has not influenced the observed toxicity of Cd in the experiments significantly.

The effect of an equilibration period for the test solution before inoculation with algae was only studied with water from Lake Store Sandungen with EDTA and at the lowest hardness level (0+). This test was performed to see whether an equilibration period may reduce the toxicity of Cd because of a time depending formation of complexes. The result showed, however, slightly lower EC-values for the test in equilibrated solutions than in the solution inoculated immediately after preparation.

5. Conclusions

- The experiments performed with *P. subcapitata* in artificial media and nutrient spiked lake waters showed a decreasing growth inhibiting effect of Cd with increasing hardness in the range 3.4-48 mg $CaCO_3$/l.
- The effect of hardness on Cd toxicity was higher in the low range of hardness tested (approximately 3.5-15 mg $CaCO_3$/l) than in the high range (15-45 mg $CaCO_3$/l).
- Increasing hardness caused a decrease of the slope of the concentration/response curves. Because of this the low effect concentrations (e.g. EC_{10}) were less dependent on the hardness than the EC_{50}.
- Highest toxicity of Cd was observed in water from Lake Byglandsfjorden with a hardness of 3.5 mg $CaCO_3$/l and 1.8 mg/l total organic carbon. In this water, the following effect concentrations were determined (NOEC: 1.36 µg/l, EC_{10}: 2.8 µg/l and EC_{50}: 9.4 µg/l.
- The high initial slope of the toxicity/hardness slope indicates that significantly lower effect concentrations of Cd than those observed in Lake Byglandsfjord may be found in waters with lower hardness and similar or lower content of organic carbon.

6. References

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Appendix

Cell density, pH and growth rate in growth inhibition tests

Test 1. Artificial medium with EDTA, Hardness 0+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
2.24		5			876		1.75
2.24		5			625		1.63
2.24		5			630		1.63
4.84		5			623		1.63
4.84		5			650		1.65
4.84		5			764		1.70
10.6		5			485		1.55
10.6		5			433		1.51
10.6		5			383		1.47
23.4		5			222		1.28
23.4		5			204		1.25
23.4		5			203		1.25
51		5			22		0.50
51		5			20		0.47
51		5			16		0.39
113		5			8		0.16
113		5			7		0.11
113		5			7		0.11
249	7.03	5			8	7.10	0.16
249		5			8		0.16
249		5			7		0.11
Control	7.03	5			506	7.32	1.56
Control		5			869		1.74
Control		5			899		1.75

Test 2. Artificial medium with EDTA, Hardness 10+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
2.24		5			1124		1.83
2.24		5			1112		1.83
2.24		5			1032		1.80
4.84		5			890		1.75
4.84		5			602		1.62
4.84		5			990		1.79
10.6		5			818		1.72
10.6		5			942		1.77
10.6		5			1007		1.79
23.4		5			536		1.58
23.4		5			267		1.34
23.4		5			325		1.41
51		5			201		1.25
51		5			144		1.14
51		5			186		1.22
113		5			26		0.56
113		5			25		0.54
113		5			35		0.66
249	7.04	5			8.5	7.08	0.18
249		5			5.1		0.01
249		5			6.7		0.10
Control	7.04	5			995	7.29	1.79
Control		5			788		1.71
Control		5			973		1.78

Test 3. Artificial medium with EDTA, Hardness 40+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
2.24		5			841		1.73
2.24		5			704		1.67
2.24		5			813		1.72
4.84		5			711		1.68
4.84		5			539		1.58
4.84		5			573		1.60
10.6		5			359		1.44
10.6		5			632		1.64
10.6		5			606		1.62
23.4		5			592		1.61
23.4		5			209		1.26
23.4		5			529		1.58
51		5			380		1.46
51		5			349		1.44
51		5			404		1.48
113		5			94		0.99
113		5			127		1.09
113		5			134		1.11
249	7.03	5			31	7.05	0.62
249		5			28		0.58
249		5			32		0.63
Control	7.03	5			819	7.34	1.72
Control		5			866		1.74
Control		5			635		1.64

Test 4. Lake Store Sandungen with EDTA, Hardness 0+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	24.0	123.0	704		1.65
1.0	6.8	5	28.0	128.0	792	7.35	1.69
1.0		5	23.0	133.0	820		1.70
2.24		5	23.0	131.0	710		1.65
2.24	6.8	5	20.0	132.0	774	7.37	1.68
2.24		5	14.4	131.0	721		1.66
4.84		5	21.0	137.0	786		1.69
4.84	6.8	5	22.0	130.0	786	7.38	1.69
4.84		5	21.0	131.0	654		1.62
10.6		5	22.0	120.0	658		1.63
10.6	6.8	5	22.0	125.0	735	7.30	1.66
10.6		5	21.0	119.0	483		1.52
23.4		5	21.0	109.0	549		1.57
23.4	6.8	5	19.0	87.0	530	7.15	1.55
23.4		5	19.0	86.0	421		1.48
51		5	13.6	39.0	63		0.84
51	6.8	5	13.4	34.0	58	6.81	0.82
51		5	11.8	29.0	63		0.84
113		5	7.2	14.5	15		0.37
113	6.8	5	6.9	10.9	13	6.72	0.31
113		5	6.1	9.4	12		0.29
249		5	5.5	5.6	4.7		-0.02
249	6.8	5	5.1	4.4	4.5	6.70	-0.04
249		5	5.8	4.9	4.8		-0.01
Control		5	26.0	139.0	821		1.70
Control		5	23.0	136.0	766		1.68
Control	6.8	5	20.0	134.0	754	7.39	1.67
Control		5	23.0	156.0	888		1.73
Control		5	24.0	153.0	813		1.70
Control		5	19.0	147.0	810		1.70

Test 5. Lake Store Sandungen with EDTA, Hardness 10+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	22.0	152	864		1.72
1.0	6.7	5	24.0	142	822	7.5	1.70
1.0		5	22.0	137	821		1.70
2.24		5	23.0	129	761		1.68
2.24	6.7	5	23.0	134	810	7.4	1.70
2.24		5	21.0	115	855		1.71
4.84		5	18.4	133	842		1.71
4.84	6.7	5	20.6	126	755	7.3	1.67
4.84		5	19.4	127	807		1.69
10.6		5	17.6	123	762		1.68
10.6	6.7	5	18.8	127	654	7.3	1.62
10.6		5	19.8	113	710		1.65
23.4		5	22.0	114	548		1.57
23.4	6.7	5	22.0	112	560	7.2	1.57
23.4		5	21.0	95	535		1.56
51		5	17.1	71	146		1.12
51	6.7	5	19.7	62	123	6.9	1.07
51		5	18.8	77	160		1.16
113		5	14.6	41	65		0.85
113	6.7	5	13.5	33	59	6.7	0.82
113		5	13.9	28	51		0.77
249		5	7.2	10	15		0.37
249	6.7	5	6.5	9.9	14	6.7	0.34
249		5	7.1	8.5	11		0.26
Control		5	21.0	130.0	831		1.70
Control		5	24.0	135	842		1.71
Control	6.7	5	23.0	151	959	7.5	1.75
Control		5	25.0	154	1041		1.78
Control		5	23.0	147	903		1.73
Control		5	23.0	134	913		1.74

Test 6. Lake Store Sandungen with EDTA, Hardness 40

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	20.0	122	873		1.72
1.0	6.7	5	18.3	130	910	7.5	1.73
1.0		5	15.4	120	934		1.74
2.24		5	11.9	81	699		1.65
2.24	6.7	5	19.1	109	843	7.5	1.71
2.24		5	19.0	107	771		1.68
4.84		5	21.0	107	765		1.68
4.84	6.7	5	21.0	135	828	7.5	1.70
4.84		5	18.9	114	820		1.70
10.6		5	18.1	126	765		1.68
10.6	6.7	5	16.3	112	741	7.5	1.67
10.6		5	20.0	126	769		1.68
23.4		5	18.5	91	583		1.59
23.4	6.7	5	21.6	105	557	7.3	1.57
23.4		5	20.9	110	615		1.60
51		5	13.3	80	204		1.24
51	6.7	5	19.6	83	182	7.0	1.20
51		5	17.0	80	198		1.23
113		5	20.7	60	90		0.96
113	6.7	5	15.4	43	63	6.8	0.84
113		5	16.6	52	85		0.94
249		5	9.9	23	38		0.68
249	6.7	5	13.4	28	45	6.8	0.73
249		5	7.4	23	41		0.70
400		5	12.6	17.7	29		0.59
400		5	9.0	15.0	28	6.7	0.57
400		5	9.5	17.5	26		0.55
Control		5	13.6	136	866		1.72
Control		5	20.6	125	889		1.73
Control	6.7	5	20.5	141	905	7.5	1.73
Control		5	17.1	135	911		1.74
Control		5	23.2	146	928		1.74
Control		5	14.5	118	940		1.75

Test 7. Lake Store Sandungen with EDTA, Hardness 0+. Incubated 24 hours after preparation of test solutions.

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	22	127	705		1.65
1.0	6.75	5	21	118	700	7.18	1.65
1.0		5	23	129	791		1.69
2.24		5	19	99	561		1.57
2.24	6.75	5	20	105	582	7.26	1.59
2.24		5	21	113	657		1.63
4.84		5	24	139	827		1.70
4.84	6.75	5	24	134	752	7.44	1.67
4.84		5	23	127	765		1.68
10.6		5	19	120	869		1.72
10.6	6.75	5	24	96	466	6.9	1.51
10.6		5	18	91	438		1.49
23.4		5	16	67	271		1.33
23.4	6.75	5	15	62	226	6.95	1.27
23.4		5	12	61	215		1.25
51		5	16.2	44	98		0.99
51	6.75	5	13.7	31	62	7.08	0.84
51		5	14.8	33	77		0.91
113		5	7.9	12.0	16		0.39
113	6.75	5	8.3	14.7	13	6.79	0.31
113		5	8.4	12.3	11		0.25
249		5	7	6.9	5		-0.04
249	6.75	5	7.8	5.9	6	6.8	0.03
249		5	6.3	5.1	6		0.03
Control		5	25	137	861		1.72
Control		5	25	144	925		1.74
Control	6.75	5	25	142	842	7.1	1.71
Control		5	29	143	926		1.74
Control		5	32	141	768		1.68
Control		5	28	137	683		1.64

Test 8. Lake Store Sandungen without EDTA, Hardness 0+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	24.0	85	482		1.52
1.0	6.85	5	27.0	96	526	7.37	1.55
1.0		5	25.0	115	679		1.64
2.24		5	21.0	97	581		1.59
2.24	6.85	5	25.0	132	689	7.56	1.64
2.24		5	26.0	114	671		1.63
4.84		5	26.0	95	513		1.54
4.84	6.85	5	28.0	114	608	7.45	1.60
4.84		5	20.0	65	263		1.32
10.6		5	29.0	79	411		1.47
10.6	6.85	5	28.0	77	349	7.34	1.42
10.6		5	27.0	75	348		1.41
23.4		5	19.0	56	95		0.98
23.4	6.85	5	21.0	60	141	7.11	1.11
23.4		5	21.0	55	123		1.07
51		5	13.3	27	37		0.67
51	6.85	5	12.4	28	39	6.99	0.68
51		5	11.5	26	34		0.64
113		5	9.7	15.2	16		0.39
113	6.85	5	10.0	16.9	16	6.88	0.39
113		5	10.3	15.7	17		0.41
249		5	7.5	9.9	9		0.19
249	6.85	5	7.6	7.8	7	6.88	0.12
249		5	7.3	7.8	8		0.14
Control		5	32.0	124	657		1.63
Control		5	29.0	105	617		1.61
Control	6.85	5	31.0	130	790	7.41	1.69
Control		5	28.0	119	600		1.60
Control		5	28.0	125	701		1.65
Control		5	25.0	114	626		1.61

Test 9. Lake Store Sandungen without EDTA, Hardness 10+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	22.0	77	369		1.43
1.0	6.74	5	24.0	75	349	7.2	1.42
1.0		5	24.0	77	410		1.47
2.24		5	26.0	69	267		1.33
2.24	6.74	5	25.0	69	381	7.2	1.44
2.24		5	26.0	76	366		1.43
4.84		5	23.0	70	279		1.34
4.84	6.74	5	26.0	86	509	7.2	1.54
4.84		5	27.0	73	352		1.42
10.6		5	23.0	62	187		1.21
10.6	6.74	5	26.0	63	266	7.2	1.32
10.6		5	27.0	73	276		1.34
23.4		5	26.0	65	159		1.15
23.4	6.74	5	21.0	58	102	7.1	1.01
23.4		5	19.0	47	132		1.09
51		5	20.0	57	76		0.91
51	6.74	5	18.7	57	75	7.0	0.90
51		5	20.0	52	73		0.89
113		5	13.0	27	29		0.59
113	6.74	5	12.8	27	32	6.9	0.62
113		5	12.6	26	29		0.59
249		5	9.1	18.4	19		0.44
249	6.74	5	9.8	16.5	15	6.9	0.37
249		5	9.3	15.4	16		0.38
Control		5	30.0	107	600		1.60
Control		5	35.0	96	430		1.48
Control	6.74	5	33.0	96	545	7.2	1.56
Control		5	32.0	102	544		1.56
Control		5	32.0	96	377		1.44
Control		5	31.0	84	290		1.35

Test 10. Lake Store Sandungen without EDTA, Hardness 40+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	20	63	139		1.11
1.0	6.65	5	18	71	188	6.9	1.21
1.0		5	20	62	208		1.24
2.24		5	28	80	432		1.49
2.24	6.65	5	26	58	138	7.0	1.11
2.24		5	28	58	171		1.18
4.84		5	28	73	395		1.46
4.84	6.65	5	26	57	155	7.1	1.14
4.84		5	20	64	147		1.13
10.6		5	28	68	304		1.37
10.6	6.65	5	29	63	170	7.1	1.18
10.6		5	29	60	152		1.14
23.4		5	25	57	128		1.08
23.4	6.65	5	24	57	109	7.0	1.03
23.4		5	24	58	109		1.03
51		5	22	53	73		0.89
51	6.65	5	24	51	76	7.0	0.91
51		5	21	52	75		0.90
113		5	20	42	55		0.80
113	6.65	5	21	44	62	6.9	0.84
113		5	20	45	58		0.82
249		5	16.1	32	36		0.66
249	6.65	5	17.0	20	35	6.9	0.65
249		5	16.4	31	36		0.66
400		5	11.7	23	27		0.56
400	6.65	5	11.9	23	25	6.8	0.54
400		5	11.1	24	26		0.55
Control		5	31.0	68	321		1.39
Control		5	32.0	65	169		1.17
Control	6.65	5	32.0	68	204	7.0	1.24
Control		5	31.0	74	394		1.46
Control		5	33.0	83	379		1.44
Control		5	33.0	69	180		1.19

Test 11. Lake Maridalsvann with EDTA, Hardness 0+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	35	133	667		1.63
1.0	6.98	5	27	134	722	7.21	1.66
1.0		5	31	142	784		1.68
2.24		5	27	149	728		1.66
2.24	6.98	5	30	153	763	7.35	1.68
2.24		5	27	157	761		1.68
4.84		5	28	160	688		1.64
4.84	6.98	5	28	162	717	7.45	1.66
4.84		5	28	168	723		1.66
10.6		5	28	142	631		1.61
10.6	6.98	5	27	143	686	7.43	1.64
10.6		5	26	144	568		1.58
23.4		5	23	68	246		1.30
23.4	6.98	5	20	66	212	6.96	1.25
23.4		5	21	65	224		1.27
51		5	13.6	28	38		0.68
51	6.98	5	14.6	29	40	6.81	0.69
51		5	14.2	27	40		0.69
113		5	11.0	16.9	22		0.49
113	6.98	5	11.6	16.7	20	6.71	0.46
113		5	11.6	16.1	19		0.44
249		5	8.0	8.7	12		0.28
249	6.98	5	7.7	8.5	9.4	6.69	0.21
249		5	8.1	8.4	8.8		0.19
Control		5	29	184	799		1.69
Control		5	30	175	777		1.68
Control	6.98	5	31	194	913	7.72	1.74
Control		5	29	172	821		1.70
Control		5	31	199	928		1.74
Control		5	32	192	908		1.73

Test 12. Lake Maridalsvann with EDTA, Hardness 10+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	27.0	171.0	810		1.70
1.0	6.9	5	25.0	170.0	784	8.1	1.68
1.0		5	28.0	209.0	828		1.70
2.24		5	27.0	166.0	783		1.68
2.24	6.9	5	27.0	174.0	771	8.0	1.68
2.24		5	26.0	161.0	750		1.67
4.84		5	25.0	151.0	685		1.64
4.84	6.9	5	22.0	149.0	705	7.9	1.65
4.84		5	25.0	151.0	665		1.63
10.6		5	26.0	144.0	660		1.63
10.6	6.9	5	27.0	144.0	643	7.8	1.62
10.6		5	27.0	149.0	682		1.64
23.4		5	20.1	82.0	308		1.37
23.4	6.9	5	23.2	84.0	321	7.2	1.39
23.4		5	22.8	81.0	306		1.37
51		5	20.0	42.0	63		0.84
51	6.9	5	19.5	42.0	64	6.9	0.85
51		5	16.9	40.0	67		0.87
113		5	12.7	20.9	33		0.63
113	6.9	5	13.9	25.1	32	6.8	0.62
113		5	12.9	22.2	32		0.62
249		5	10.0	13.8	18		0.43
249	6.9	5	8.7	13.4	17	6.8	0.40
249		5	9.3	15.0	18		0.43
Control		5	25.0	156.0	683		1.64
Control		5	26.0	156.0	786		1.69
Control	6.9	5	26.0	153.0	774	7.8	1.68
Control		5	26.0	157.0	772		1.68
Control		5	28.0	151.0	819		1.70
Control		5	29.0	154.0	769		1.68

Test 13. Lake Maridalsvann with EDTA, Hardness 40+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	27.0	145.0	779		1.68
1.0	6.9	5	25.0	151.0	812	7.3	1.70
1.0		5	26.0	162.0	879		1.72
2.24		5	25.0	163.0	813		1.70
2.24	6.9	5	23.0	169.0	798	7.4	1.69
2.24		5	18.0	167.0	773		1.68
4.84		5	19.0	133.0	698		1.65
4.84	6.9	5	20.0	169.0	769	7.4	1.68
4.84		5	27.0	163.0	794		1.69
10.6		5	16.6	157.0	629		1.61
10.6	6.9	5	26.0	139.0	589	7.3	1.59
10.6		5	25.0	144.0	614		1.60
23.4		5	26.6	85.0	327		1.39
23.4	6.9	5	21.7	76.0	352	7.2	1.42
23.4		5	19.2	80.0	325		1.39
51		5	20.2	43.0	73		0.89
51	6.9	5	16.8	41.0	78	7.1	0.92
51		5	17.7	40.0	77		0.91
113		5	16.9	26.3	44		0.72
113	6.9	5	16.7	26.4	44	7.0	0.72
113		5	13.9	25.6	45		0.73
249		5	15.2	18.9	27		0.56
249	6.9	5	14.5	19.5	26	7.0	0.55
249		5	14.3	19.8	26		0.55
400		5	12.3	16.4	20		0.45
400	6.9	5	13.0	15.8	19		0.44
400		5	12.0	16.5	19		0.44
Control		5	25.8	144.0	764		1.68
Control		5	26.3	140.0	736		1.66
Control	6.9	5	24.9	140.0	716	7.2	1.65
Control		5	23.4	142.0	719		1.66
Control		5	26.2	148.0	701		1.65
Control		5	26.4	147.0	740		1.67

Test 14. Lake Byglandsfjorden with EDTA, Hardness 0+

Cd (µg/l)	pH start	10³ cells/ml 0	24 h	48 h	72 h	pH 72 h	µ (d^{-1})
1.0		5	19.1	92	521		1.55
1.0	6.7	5	18.9	101	572	7.44	1.58
1.0		5	19.3	100	585		1.59
2.24		5	21.0	99	512		1.54
2.24	6.7	5	22.5	101	541	7.53	1.56
2.24		5	20.1	98	528		1.55
4.84		5	18.9	89	452		1.50
4.84	6.7	5	15.2	86	426	7.53	1.48
4.84		5	18.4	94	465		1.51
10.6		5	11.0	39	168		1.17
10.6	6.7	5	13.9	58	241	7.19	1.29
10.6		5	11.3	46	187		1.21
23.4		5	7.2	14.3	17.7		0.42
23.4	6.7	5	7.0	12.1	14.1	6.86	0.35
23.4		5	7.3	14.2	19.9		0.46
51		5	6.4	6.5	5.6		0.04
51	6.7	5	6.3	7.5	7.7	6.79	0.14
51		5	5.8	4.7	4.0		-0.07
113		5	6.0	4.4	4.1		-0.07
113	6.7	5	5.7	4.5	3.4	6.75	-0.13
113		5	5.5	4.4	3.9		-0.08
249		5	5.0	3.2	2.7		-0.21
249	6.7	5	5.5	3.7	3.3	6.77	-0.14
249		5	4.9	3.8	3.0		-0.17
Control		5	16.9	87	572		1.58
Control		5	20.4	105	580		1.58
Control	6.7	5	17.7	95	560	7.36	1.57
Control		5	20.7	106	550		1.57
Control		5	23.7	103	579		1.58
Control		5	22.1	108	616		1.60

Test 15. Lake Byglandsfjorden with EDTA, Hardness 10+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	23.8	112	652		1.62
1.0	6.6	5	24.7	109	622	7.5	1.61
1.0		5	23.2	98	629		1.61
2.24		5	25.0	118	705		1.65
2.24	6.6	5	23.5	112	677	7.5	1.64
2.24		5	22.5	107	689		1.64
4.84		5	21.6	102	567		1.58
4.84	6.6	5	23.6	107	644	7.5	1.62
4.84		5	21.3	97	597		1.59
10.6		5	22.9	104	517		1.55
10.6	6.6	5	19.7	90	519	7.4	1.55
10.6		5	21.0	91	506		1.54
23.4		5	22.0	93	428		1.48
23.4	6.6	5	24.2	111	571	7.4	1.58
23.4		5	22.5	94	438		1.49
51		5	16.7	77	173		1.18
51	6.6	5	19.9	70	170	7.0	1.18
51		5	17.9	56	143		1.12
113		5	9.4	23	27		0.56
113	6.6	5	8.9	18.3	21	6.8	0.48
113		5	8.4	19.5	25		0.54
249		5	6.2	7.5	8		0.14
249	6.6	5	7.1	11.1	12	6.8	0.28
249		5	6.6	6.5	8		0.14
Control		5	25.0	120	698		1.65
Control		5	24.7	122	696		1.65
Control	6.6	5	25.7	120	764	7.5	1.68
Control		5	26.6	127	670		1.63
Control		5	26.6	126	658		1.63
Control		5	24.2	123	739		1.67

Test 16. Lake Byglandsfjorden with EDTA, Hardness 40+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	20.2	89	659		1.63
1.0	6.5	5	21.4	89	650	7.4	1.62
1.0		5	20.0	85	642		1.62
2.24		5	18.4	79	617		1.61
2.24	6.5	5	19.0	81	651	7.5	1.62
2.24		5	21.7	96	636		1.62
4.84		5	21.4	79	607		1.60
4.84	6.5	5	20.4	87	627	7.5	1.61
4.84		5	21.6	88	574		1.58
10.6		5	22.3	104	619		1.61
10.6	6.5	5	23.4	98	604	7.5	1.60
10.6		5	21.5	93	604		1.60
23.4		5	19.6	78	510		1.54
23.4	6.5	5	22.4	101	516	7.5	1.55
23.4		5	19.5	89	459		1.51
51		5	21.7	80	268		1.33
51	6.5	5	18.8	82	291	7.1	1.35
51		5	17.7	66	237		1.29
113		5	17.9	48	98		0.99
113	6.5	5	16.8	58	105	6.9	1.01
113		5	16.1	54	92		0.97
249		5	13.7	31	46		0.74
249	6.5	5	13.6	30	43	6.8	0.72
249		5	15.1	34.0	48		0.75
400		5	7.9	15.6	19		0.45
400	6.5	5	9.1	16.3	20	6.8	0.46
400		5	8.3	16.5	18		0.43
Control		5	22.2	71	665		1.63
Control		5	23.2	99	654		1.62
Control	6.5	5	21.9	84	683	7.4	1.64
Control		5	21.5	89	645		1.62
Control		5	22.0	86	645		1.62
Control		5	20.8	91	627		1.61

Test 17. Lake Byglandsfjorden without EDTA, Hardness 0+

		10^3 cells/ml					
Cd (µg/l)	**pH start**	**0**	**24 h**	**48 h**	**72 h**	**pH 72 h**	**µ (d^{-1})**
1.0		5	13.5	33.0	103		0.98
1.0	6.7	5	11.9	32.0	78	6.9	0.89
1.0		5	15.9	25.0	81		0.90
1.0		5	15.6	34.0	107		0.99
1.6		5	13.6	30.0	103		0.98
1.6	6.7	5	13.8	21.0	82	6.9	0.91
1.6		5	15.9	34.0	98		0.97
1.6		5	11.8	30.0	91		0.94
2.5		5	11.8	24.0	70		0.86
2.5	6.7	5	16.0	29.0	71	6.9	0.86
2.5		5	7.9	19.0	47		0.73
2.5		5	11.3	23.0	61		0.81
4.0		5	9.1	18.0	46		0.72
4.0	6.7	5	11.2	19.0	58	6.8	0.79
4.0		5	10.7	23.0	82		0.91
4.0		5	10.4	19.0	77		0.89
6.3		5	11.5	21.0	58		0.79
6.3	6.7	5	11.3	16.0	46	6.8	0.72
6.3		5	10.0	15.0	36		0.64
6.3		5	11.7	18.0	38		0.66
10		5	8.5	15.0	26		0.53
10	6.7	5	7.4	12.0	21	6.8	0.47
10		5	8.5	6.8	20		0.45
10		5	8.2	8.8	26		0.53
16		5	7.5	9.5	15		0.35
16	6.7	5	7.1	11.0	18	6.8	0.41
16		5	7.1	9.2	15		0.36
16		5	5.6	8.5	12		0.29
25		5	5.5	6.5	8		0.15
25	6.7	5	6.0	7.0	11	6.7	0.25
25		5	6.7	7.5	10		0.21
25		5	5.5	6.0	8		0.17
40		5	4.7	4.8	5		-0.03
40	6.7	5	6.5	4.7	5	6.7	0.01
40		5	6.6	3.6	4		-0.04
40		5	5.5	3.9	4		-0.06
Control		5	10.5	25.0	74		0.87
Control		5	11.0	32.0	103		0.98
Control		5	12.6	25.0	87		0.93
Control	6.7	5	12.4	26.0	119	6.85	1.03
Control		5	10.2	28.0	108		1.00
Control		5	14.0	31.0	118		1.03
Control		5	11.6	21.0	86		0.92
Control		5	11.7	28.0	80		0.90