Effects of Lanthanum and Lanthanum-modified clay on growth, survival and reproduction of *Daphnia magna*

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Abstract
The novel lanthanum modified clay water treatment technology (Phoslock®) seems very promising in remediation of eutrophicated waters. Phoslock® is highly efficient in stripping dissolved phosphorous from the water column and in intercepting phosphorous released from the sediments. The active phosphorous-sorbens in Phoslock® is the Rare Earth Element lanthanum. A leachate experiment revealed that lanthanum could be released from the clay, but only in minute quantities of 0.13 - 2.13 µg l^{-1} for a worst-case Phoslock® dosage of 250 mg l^{-1}. A life-history experiment with the zooplankton grazer *Daphnia magna* revealed that lanthanum, up to the 1000 µg l^{-1} tested, had no toxic effect on the animals, but only in medium without phosphorous. In the presence of phosphorous, rhabdophane ($LaPO_4 \cdot nH_2O$) formation resulted in significant precipitation of the food algae and consequently affected life-history traits. With increasing amounts of lanthanum, in the presence of phosphate, animals remained smaller, matured later, reproduced less, which resulted in lower population growth rates. Growth rates were not affected at 33 µg La l^{-1}, were 6% and 7% lower at 100 and 330 µg l^{-1}, respectively, and 20% lower at 1000 µg l^{-1}. A juvenile growth assay with Phoslock® tested in the range 0 – 5000 mg l^{-1}, yielded EC_{50} (NOEC) values of 871 (100) and 1557 (500) mg Phoslock® l^{-1} for weight and length based growth rates, respectively. The results of this study show that no major detrimental effects on *Daphnia* are to be expected from Phoslock® or its active ingredient lanthanum when applied in eutrophication control.

Key Words: eutrophication control, lake management, lake restoration, lanthanum, life-hstory, modified clay, Phoslock®

Introduction
Cyanobacterial proliferation and accumulation of biomass in nuisance scum's is an obvious symptom of anthropogenic nutrient overenrichment of surface waters (Fogg 1969; Reynolds, 1987; Reynolds and Walsby 1975; Paerl 1998, 2008).
Such cyanobacterial blooms may cause high turbidity, anoxia, fish kills, bad smell and reflect serious environmental and human health problems, because several cyanobacteria can produce a variety of very potent toxins (Codd et al. 2005; Dittmann and Wiegand 2006; Paerl, 2008; Paerl and Huisman 2008). Climate change is expected even to

aggravate hazardous blooms (Paerl and Huisman 2008), while safe and aesthetically acceptable water is a growing need in a modern society (Steffensen 2008). Hence, water management is faced world-wide with the call for reducing the vulnerability to the threats of harmful cyanobacterial blooms. This means that eutrophication control remains one of the key challenges to global environmental sustainability for the 21st Century (Sharpley and Tunney 2000; Schindler 2006).

Inasmuch as the most important cause of lake eutrophication is phosphorus pollution (Schindler, 1974; 1975; 1977; Correll, 1998), phosphorus control is critical to mitigating eutrophication (Carpenter 2008; Schindler et al. 2008). This requires both input control from point and nonpoint sources as well as the P-removal from the water column and P-retention in the bottom sediments (Welch and Cooke 1995; Carpenter et al. 1998; Søndergaard et al. 2003; Mehner et al 2008).

In the Netherlands, from the early 1980s a variety of restoration techniques have been employed. However, more long-term failures than successes have been recorded that are largely related to insufficient or no in-lake P control (Gulati and Van Donk 2002). As the European Union Water Framework Directive (2000/60/EC) aims to restore all waters to a good ecological status or potential by 2015, it is obvious that additional remedial measures are needed to reduce in-lake P concentrations to low levels and to overcome P release from the P-rich bottom sediments (Gulati and Van Donk, 2002). Here, the novel lanthanum modified clay water treatment technology (Phoslock®) developed by CSIRO (Australia) seems very promising in remediation of degraded water. Phoslock® is highly efficient in stripping dissolved P from the water column and in intercepting P released from the sediments (Robb et al. 2003; Akhurst et al. 2004; Ross et al. 2008).

The active P-sorbens in Phoslock® is the Rare Earth Element lanthanum. This compound might be released from the bentonite when brought in the water and La^{3+}-ions could be toxic to some aquatic organisms, particularly cladocerans such as *Daphnia* (Barry and Meehan 2000; NICNAS 2001). Hence, the potential liberation of La^{3+}-ions from the bentonite could mean a significant environmental risk (Akhurst et al. 2004), but Phoslock® has been classified as not hazardous (Martin and Hickey 2004). It should be noted, however, that there is a no consistency in the results of the few studies on the effects of lanthanum on cladocerans (Barry and Meehan 2000; Sneller et al 2000; NICNAS 2001; Martin and Hickey 2004). In addition, effects of Phoslock® have not been tested as such, but a so-called Toxic Characteristic Leachate Procedure has been employed (NICNAS 2001; Martin and Hickey 2004). The purpose of this study was: 1) to establish a dose response relationship between Phoslock® and the growth of *Daphnia magna*, 2) to determine the amount of lanthanum released from Phoslock®, and 3) to test the effects of lanthanum on life history characteristics of *Daphnia magna* in artificial P-free and P-containing medium. Based on the very strong binding of lanthanum to oxy-anions and especially phosphates (e.g. Haghseresht 2005a; Biswas et al. 2007; Ross et al. 2008), we hypothesize that in the presence of phosphate the formation of the insoluble mineral rhabdophane will dramatically mitigate toxicity of lanthanum.

Materials and methods

Test organisms

Experiments were carried out with the cladoceran *Daphnia magna* Straus that has been isolated from Lake Zwemlust (The Netherlands) and has been maintained for more than 10 years in our laboratory. Here the *Daphnia*'s are kept at 20°C in 1 l jars containing 800 ml artificial RT-medium with a pH of 7.6, a conductivity of 270 µS cm^{-1} and a total hardness of 88 mg $CaCO_3$ l^{-1} (Tollrian 1993). The animals are fed three times a week with the green alga *Scenedesmus obliquus* (Turpin) Kützing (~ 4 mg C l^{-1}). *S. obliquus* SAG 276/3a originated from the culture collection of the University of Göttingen (Germany). *S. obliquus* was maintained in 1.0-l chemostat systems in continuous light of 120-µmol quanta $m^{-2}s^{-1}$ at 20°C on a slightly modified WC medium (Lürling and Beekman 1999) and with a dilution rate of 1.1 d^{-1}.

Phoslock® leachate experiment
Two batches (25 kg each) were obtained from Phoslock® Water Solutions Ltd. (Australia). On average 0.5 g Phoslock® was brought into Erlenmeyer flasks that contained 100 ml nanopure water. Each batch was tested in triplicate (0.5033 ± 0.004 g of batch 1 and 0.5022 ± 0.002 g of batch 2). Three additional Erlenmeyers contained only 100 ml nanopure water. The Erlenmeyers were closed with Parafilm and placed for 48 hours in an incubator in darkness, at 22 °C and continuous orbital shaking (200 rpm). After this the material was centrifuged for 5 min at 3000 rpm, followed by filtration over a 0.45 µm membrane filter. Filtrates were analyzed for metals (Al, Cd, Cu, Hg, La, Pb, Zn) using AAS (Hg) and ICP-MS (the other metals).

Effect of lanthanum on life-history traits of D. magna
Juvenile *Daphnia* born on the same day were collected from the stock cultures and placed individually in separate 125 ml test tubes containing 100 ml of *Scenedesmus* food suspension with a concentration of 5 mm^3 l^{-1} (equivalent to ~2.5 mg C l^{-1}). These *Daphnia* were transferred daily to new tubes with fresh food and newborns from the third broods were used as experimental animals. The newborns were joined in a 500-ml beaker with RT-medium. For each treatment ten neonates were randomly selected and transferred individually into 125-ml test tubes containing 100 ml of a food suspension (in RT-medium) at different concentrations of lanthanum. Stock solutions of lanthanum were made from $La(NO_3)_3 \cdot 6H_2O$ at 3.3 mg La l^{-1}, 10 mg La l^{-1}, 33 mg La l^{-1} and 100 mg La l^{-1} in nanopure water. Concentrations of La in the water were measured by inductively coupled plasma mass spectrophotometry (ICP-MS). Lanthanum was tested at the following nominal concentrations: 0, 33, 100, 330 and 1000 µg l^{-1} in the absence and presence of phosphate (330 µg l^{-1}), yielding 5 La concentrations × 10 replicates × 2 phosphate levels = 100 experimental units.

Each test tube contained only one experimental animal to avoid density effects (Martínez-Jerónimo et al. 2000). The test tubes were incubated in a temperature-controlled room at 20°C. The animals were transferred daily to clean tubes with fresh food suspensions and lanthanum. Before *Daphnia* were transferred into these new tubes their body length was measured using a stereo-binocular microscope. The number of survivors, time to reproduction, and number of new-borns were recorded. Growth and reproduction were followed until animals had reached the fourth adult instar and consequently released their third brood, because the first three broods largely determine population growth rate (Vanni and Lampert 1992). The instantaneous rates of population

increase (*r*) were estimated from abbreviated life-tables (three broods) using the equation:

$r \cong \frac{\ln \sum_{x=0}^{\infty} l_x m_x}{T}$, where *r* = rate of population increase (d^{-1}), x =age class (0...N), l_x = probability of surviving to age x, m_x = fecundity at age x, and T the generation time. A Jack-knifing method was used to calculate standard errors of *r* (Meyer et al. 1986).

The increase in body-size over time for the different treatments was statistically analyzed running repeated measures ANOVAs in the tool pack SPSS version 16.0.1. When the ANOVA indicated significant differences a Tukey post-hoc comparison test was run to distinguish means that were significantly different ($P < 0.05$). Age and size at first reproduction and brood sizes were compared running one-way ANOVA.

Effect of Lanthanum on Scenedesmus

Because precipitation was observed in medium containing P and lanthanum, the effect of lanthanum on the food availability was examined in a short-term assay. Separate 125 ml test tubes were filled with 100 ml suspensions of *Scenedesmus* (with a concentration of 5 $mm^3\ l^{-1}$) in P-free or P-containing (330 µg l^{-1}) RT-medium. Lanthanum (1000 µg l^{-1}) was added to three tubes with P-free medium and to three with P-containing medium, while for each medium three replicate flasks served as controls (no lanthanum added). Initially and after 2, 18 and 25 hours, biovolume was determined using an electronic cell counter (CASY, Schärfe System Gmbh., Reutlingen, Germany). Biovolumes in the different treatments, measured after 2, 18 and 25 h, were statistically compared running one-way ANOVAs and were followed by Tukey post-hoc comparison test ($P < 0.05$).

Effect of Phoslock® on growth of Daphnia

A 5 d juvenile growth experiment was conducted with third-clutch juveniles (<24 h) of *D. magna*. The experiment was carried out in glass jars with 100 ml artificial RT-medium (P-free) in which Phoslock® was tested at 0, 5, 50, 100, 500 and 5000 mg l^{-1}. These concentrations are centered on 100 mg l^{-1}. Based on a recommended Phoslock® dose of 230 mg per mg of filterable reactive phosphorous FRP at neutral pH (Haghseresht 2005b; Ross and Cloete 2006), this would reflect an application dosage to water with 435 µg FRP l^{-1}. Each treatment consisted of three replicates with three animals per beaker. All beakers received *Scenedesmus* (at a concentration of 10 $mm^3\ l^{-1}$, which is equivalent to ~5 mg C l^{-1}) as food to the animals. The beakers were incubated in a temperature-controlled room at 20°C in darkness. At the start of the experiment the body lengths of 15 newborns were measured using a stereo-binocular microscope. Body length is defined as the distance from the most posterior point on the eye to the base of the junction of the tail spine with the carapace. Five groups of three specimens were transferred in small pre-weighed aluminium boats, dried at 105°C for 24 h, and weighed on an electronic balance (Mettler UMT 2; ± 0.1 µg). After 5 days of incubation, experimental animals were collected from the beakers, rinsed in RT-medium after which their body-lengths and dry-weights were determined. The juvenile somatic growth rates (g) were determined as the increase in dry mass (W) and body-length (BL) from the beginning of the experiment (X_0) to day 5 (Xt) using the equation:

$g_w = (\ln X_t - \ln X_0)/t$

For both endpoints growth rates were statistically compared running one-way ANOVA in the statistical tool-pack SPSS Release 16.0.1. Differences between means were distinguished by Tukey's post-hoc comparison ($P < 0.05$). EC_{50} values (i.e. Phoslock® concentration causing a 50% inhibition of growth) were determined using non-linear regression by fitting a 3 parameter sigmoidal function in the toolpack SigmaPlot 2000, version 6.00 (Cleuvers 2003).

Results

Phoslock® leachate experiment

Aluminium, lanthanum and zinc were detected in the filtrates from the Phoslock® suspensions, as were small amounts of copper (Table 1). Mercury and cadmium were not detected and in one batch a trace of lead was found (Table 1). The oldest batch 1 (obtained in August 2006) seemed to release more metals than the younger batch 2 (obtained in April 2008). For example, in the first batch 69 – 360 µg Al l^{-1} was liberated, while in the second batch this was 15 – 19 µg l^{-1}. A similar pattern was observed for La: 12 – 43 µg l^{-1} was measured in filtrate of suspension from the first batch, while La was considerable lower in filtrates from the second batch: 2.5 – 4.1 µg l^{-1} (Table 1).

Effect of lanthanum on life-history traits of D. magna

Measured lanthanum concentrations deviated from the nominal concentrations for the 33 and the 330 µg La l^{-1} treatments. Measured concentrations in P-free water were 0, 12.1, 94.1, 98.9 and 1001 µg La l^{-1} for the controls and nominal concentrations of 33, 100, 330 and 1000 µg La l^{-1}, respectively.

In the absence of phosphate, somatic growth of *D. magna* expressed as the increase of the body-length over time was influenced marginally by lanthanum (Fig. 1A). A repeated measures ANOVA indicated significant differences in body-length over time ($F_{10,420} = 2728$; $P < 0.001$) and a significant lanthanum treatment effect ($F_{4,42} = 6.47$; $P < 0.001$). The differences among treatments were, however, small and a Tukey post-hoc comparison test revealed two homogenous groups: 1) 0, 33, 100, 330 µg La l^{-1} and 2) 0, 33 and 1000 µg La l^{-1}. Animals in the 100, 330 µg La l^{-1} treatments were significantly larger than those in the 1000 µg La l^{-1} treatment, but none of the treatments differed from controls (Fig. 1A).

By contrast, in P-containing medium a pronounced effect of lanthanum on the somatic growth of *D. magna* was observed (Fig. 1B). The repeated measures ANOVA indicated a significant lanthanum treatment effect ($F_{4,39} = 116.1$; $P < 0.001$) and Tukey's test revealed four homogenous groups that were in order from the largest to the smallest animals: 1) 0 and 33 µg La l^{-1}, 2) 100 µg La l^{-1}, 3) 330 µg La l^{-1} and 4) 1000 µg La l^{-1}.

Comparisons of somatic growth of animals in P-free or P-containing medium at similar La exposures revealed that *D. magna* in 0 and 33 µg La l^{-1} were equally sized (Table 2). However, for 100, 330 and 1000 µg La l^{-1} exposures *D. magna* were significantly smaller in medium with P than those reared in P-free medium (Table 2).

In P-free medium, age at first reproduction was similar in controls and treatments ($F_{4,42} = 0.56$; $P = 0.694$), which was also the case for size at first reproduction ($F_{4,42} = 2.40$; $P = 0.065$). Survival was ≥ 90% in controls and treatments (Table 3). In P-containing medium, age at first reproduction was similar ($F_{4,42} = 2.45$; $P = 0.061$), but

size at first reproduction appeared significantly different ($F_{4,42}$ = 49.5; P < 0.001) with animals being the biggest in controls and the smallest in the highest dosage of lanthanum (Table 3). Survival was ≥ 80% in controls and treatments (Table 3).

In P-free medium, the number of offspring in the first brood ($F_{4,42}$ = 1.44; P = 0.240) and second brood ($F_{4,42}$ = 1.02; P = 0.410) was similar among controls and treatments (Fig. 2A). In the third brood, however, significantly less offspring was produced in the 1000 µg La l^{-1} treatment ($F_{4,42}$ = 15.0; P < 0.001). Also the total number of offspring produced per female was significantly ($F_{4,42}$ = 6.16; P < 0.001) lower in the 1000 µg La l^{-1} treatment (Fig. 2A). When grown in P-containing medium and in the highest La treatment, *D. magna* produced significantly less offspring in all three broods (1st brood $F_{4,42}$ = 17.6; P < 0.001; 2nd brood $F_{4,41}$ = 41.3; P < 0.001; 3rd brood $F_{4,41}$ = 34.6; P < 0.001; Fig. 2B).

Intrinsic rates of population increase were excellent for animals reared in P-free medium in all treatments and for animals raised in P-containing medium in controls and 33 µg La l^{-1} treatments (Table 3). However, in P-containing medium compared to P-free medium intrinsic rates of population increase were 6% and 7% lower in 100 and 330 µg La l^{-1}, respectively, while in the highest dosage of 1000 µg La l^{-1} it was 20% lower (Table 3). A significantly reduced reproduction in the P containing medium caused the lower intrinsic rates of population increase at the highest dose of 1000 µg La l^{-1}.

Effect of Lanthanum on Scenedesmus

Because precipitation was observed in medium containing P and lanthanum, the effect of this on the food availability was examined in a short assay (Fig. 3). A one-way ANOVA indicated that biovolumes after two hours were similar in all treatments ($F_{3,8}$ = 1.23; P = 0.362). However, after 18 and 25 hours significant differences were observed ($F_{3,8}$ = 371.4; P < 0.001 and $F_{3,8}$ = 371.4; P < 0.001, respectively), and where the biovolumes were similar in the La-free P-containing medium and in the P-free treatments (both with and without La), biovolumes were significantly lower in the La treatment in P-containing medium (Fig. 3).

Effect of Phoslock® on growth of Daphnia

The juveniles at the start of the experiment had a body-length of 0.91 (± 0.05) mm and a body-weight of 12.3 (± 1.1) µg (N = 15). All animals survived during the experimental period in controls and treatments up to 100 mg Phoslock® l^{-1}; in 500 mg Phoslock® l^{-1} 89% of the animals survived and in the highest dosage (5000 mg Phoslock® l^{-1}) all animals had died. After 5 days, animals reached 2.59 (± 0.25) mm in controls, were slightly smaller in the 500 mg Phoslock® l^{-1} treatment (2.28 ± 0.17) mm, but had not grown in the highest dosage of 5000 mg Phoslock® l^{-1} (Fig. 4). Here animals were 0.91 (± 0.13) mm. Growth rates, based on the increase in body-length over time, were similar in controls and Phoslock® concentrations up to 500 mg l^{-1}, but were significantly lower ($F_{5,12}$ = 128.7; P < 0.001) in the 5000 mg Phoslock® l^{-1} treatment (Fig. 4). The animals reached a body weight of 144 (± 12) µg in controls. They were lighter in the 100 mg Phoslock® l^{-1} treatment (126 ± 4 µg), significantly ($F_{5,12}$ = 189.4; P < 0.001) lighter in the 500 mg Phoslock® l^{-1} treatment (87 ± 8 µg) and with a body weight of 5 ± 2 µg they had lost weight in the 5000 mg Phoslock® l^{-1} treatment (Fig. 4). Estimated EC_{50} values were 871 and 1557 mg Phoslock® l^{-1} for weight and length based growth rates, respectively.

Discussion

Phoslock® leachate

Phoslock® is applied to the degraded waters as slurry through a spray manifold. Settling through the water column it will permanently bind orthophosphates and settled on the sediment, Phoslock® will intercept efficiently P released from the sediments (Robb et al. 2003; Akhurst et al. 2004; Ross et al. 2008). The main carrier of the product is bentonite clay, which may release metals during the application (Guy and Chakrabarti 1976). Indeed, some metals were detected in the Phoslock® leachate. A small amount of copper was found. Based on a worst-case scenario, dosage of 0.25 g Phoslock® l^{-1} (Haghseresht, 2006) would equal 0 – 0.8 % of the Maximum Allowable Concentration (MTR) of 1.5 µg l^{-1} for dissolved copper (RIVM, 2008). For Zinc it fluctuates between 0.5 and 22% of the MTR (9.4 µg l^{-1}) and for lead between 0 and 0.01% of the MTR (11 µg l^{-1}). For aluminium an *ad hoc* MTR of 45 µg l^{-1} exists (Van de Plassche 2002) meaning that leakage during a worst case dosing equals 1.5 – 40 % of this MTR. However, the MTR is based on soluble aluminium, which is strongly pH dependent; between pH 5.2 and 8.8 the solid $Al(OH)_3$ predominates (Martel & Motekaitis 1989; Driscoll and Letterman 1995). Hence, no environmental risks are expected from metals leaking from Phoslock® during an application.

During the preparation of Phoslock®, Rare Earth lanthanum ions are exchanged with surface adsorbed exchangeable cations in the bentonite. However, not all lanthanum is locked permanently into the clay and materials may disperse in the water. Here about 0.001% of the lanthanum in the clay was released from it, which is in the same order of magnitude as the 0.02% reported by NICNAS (2001). Although the amount of free lanthanum released from the clay is only a very small fraction of the total lanthanum contained in the clay, the potential leakage is of importance in The Netherlands, because the maximum permissible concentration of lanthanum in Dutch surface water is 10.1 µg l^{-1} (Sneller et al. 2000). A worst-case scenario dosage of 0.25 g Phoslock® l^{-1} (Haghseresht, 2006), yields dissolved lanthanum concentrations between 0.13 and 2.13 µg l^{-1} based on the results of this study. Lanthanum ion concentrations measured after various Phoslock® applications in Australia were 12 µg l^{-1} in two waters (Edith Cowan University, after 24h; and Queyanbeyan STP Lagoon, after 48 h), but dropped rapidly to below 1 µg l^{-1} (Haghseresht, 2006). In four other Australian waters, the La^{3+} concentrations were below 10 µg l^{-1} (Flapper 2003; Haghseresht, 2006). Although this implies a short-term, minor exceeding of the Dutch lanthanum standard, total lanthanum concentrations in the water might reach as high as 400 µg l^{-1} straight after an application (Flapper 2003; McIntosh 2007; Anonymous 2008a,b). Undoubtedly, a great portion of this lanthanum will be imbedded in suspended clay particles, or biologically unavailable through rhabdophane formation, but these values are exceeding the NOEC of 100 µg La l^{-1} found in a 21 d *Daphnia* reproduction test that forms the basis for the Dutch standard (Sneller et al. 2000).

Effect of lanthanum on life-history traits of D. magna

The results of this study are not in favour of our hypothesis that in the presence of phosphate the toxicity of lanthanum to *Daphnia* would be mitigated through the formation of the insoluble mineral rhabdophane. In fact, in P-free medium no evidence

for lanthanum toxicity to *Daphnia* was found. When grown in P-free medium, somatic growth in all lanthanum treatments was similar to the controls; age and size at first reproduction were similar in controls and treatments, and survival was ≥ 90% in controls and treatments. Only in the highest dosage of 1000 µg La l^{-1} animals produced less offspring in the third brood and also the overall reproduction was significantly lower in these treatments. The measured lanthanum concentration in the (nominal concentration) 330 µg l^{-1} treatments was nearly 100 µg l^{-1}. Based on reproduction this implies a NOEC of 100 µg l^{-1}, which is similar to the result found in a 21 d *Daphnia* reproduction test (Sneller et al. 2000). It should be noted, however, that the reduction in reproduction was only 9% compared to controls and that it hardly influenced the intrinsic rate of population increase, which was only 2.2% lower in the highest La treatments. The per capita rate of increase has often been used as a measure of *Daphnia* fitness (e.g. McCauley et al. 1990; Lurling 2003) and represents a relevant measure of ecological consequences of stressors (Forbes and Calow 1999). Here, population growth rates were ≥ 0.36 d^{-1} that reflect excellent growth of *D. magna* (Lurling and Beekman 2006) and do not support the view that lanthanum is toxic to particularly *Daphnia* in both acute and chronic tests (Akhurst et al. 2004).

In P-containing medium, significant effects of lanthanum emerged. With increasing amounts of lanthanum, animals grew less; matured at a smaller size, showed a tendency to mature slightly later and reproduced less. Consequently, population growth rates were reduced. Precipitation was observed in the P-containing medium dosed with ≥ 100 µg La l^{-1}. Unexpectedly, this precipitation also drastically reduced the amount of food algae. For example, after 25 h in the highest La dosage sedimentation reduced the amount of suspended food particles to 7% of the initial concentration, while in the absence of La food amounts remained at 93% of the initial value. This reduction in food availability at elevated La concentration in the P-containing medium provides a very likely explanation for the reduced growth and reproduction of the daphnids (e.g. Lurling 2003). A direct toxic effect of La on the food alga is not expected as La can promote growth of phytoplankton (Yang et al. 1999; Zhou et al. 2004), or is toxic only at high concentrations (Jin et al. 2008).

There exists a huge variation in the lanthanum toxicity data. When we exposed *D. magna* to lanthanum in RT-medium a LC_{50} of 14 mg l^{-1} was found (*unpublished data*), which is lower than the 282 mg l^{-1} reported by USEPA (1991). Acute assays have revealed EC_{50} values between 40 and 103000 µg l^{-1} (Table 4), where the lowest values were obtained running assays in deionised water or tap water thereby including additional stress to the animals as indicated by high mortality in controls (Barry and Meehan 2000; NICNAS 2001). The use of different media, container size, modified protocols and organisms makes it rather difficult to delineate causality. However, also within studies some discrepancies appeared. For example, where a 7d *Ceriodaphnia* reproduction assay yielded EC_{50} values of 820 µg La l^{-1} and 4300 µg La l^{-1} in elutriate from Phoslock® and in lanthanum exposure, respectively, while the corresponding acute 48 h toxicity assay gave an EC_{50} of only 80 µg La l^{-1} (NICNAS, 2001). In the latter study, a so-called Toxicity Characteristic Leach Protocol had been employed (NICNAS 2001). A suspension of 50 g Phoslock® l^{-1} was filtered through a 45 µm filter and different proportions were tested on *Ceriodaphnia dubia*. A similar procedure was followed by NIWA where 50 g Phoslock® l^{-1} was filtered through a 40 µm filter, but where *Daphnia*

magna was used (Martin and Hickey 2004). The results were extremely different with *Daphnia* being unaffected and *Ceriodaphnia* influenced in general by leachate of ≥ 25% (NICNAS 2001; Martin and Hickey 2004). A possible explanation here might be the sensitivity to fine suspended clay particles, because *Ceriodaphnia* is much more sensitive than *Daphnia* (Kirk and Gilbert 1990).

Another explanation for the huge differences in results between studies might be caused by factors affecting the availability of lanthanum. Glass containers might absorb up to 25% of the total La to the glass (Weltje et al. 2002). However, employing every day renewal of the medium probably makes this absorption loss much less. In many studies, lanthanum rapidly precipitated with oxyanions, such as orthophosphate, due to its extreme low solubility product $K_{sp} = 10^{-24.7}$to $10^{-25.7}$ mol^2 l^{-2} at 25°C and infinite dilution (Johanneson and Lyons 1994; Liu and Byrne 1997). Also in the current study, in the presence of phosphate clear precipitation was observed. Finally, it has been shown that organic ligands, which can form Rare Earth Elements - Organic complex species led to a great reduction of the REE bioconcentration in algae (Sun et al. 1997). In natural waters, humic substances are typically non-specific complexing ligands and maybe found in concentrations from less than 1 mg l^{-1} to hundreds of mg l^{-1} (Steinberg et al. 2006). The artificial RT-medium used in this study contains 5 mg Na_2-EDTA l^{-1} (Tollrian 1993), which will influence the bioavailability as the EDTA is supposed to compete with cell-membrane ligands (Sun et al. 1997). Moreover, the La^{3+}-ion is a substitute or antagonist of Ca^{2+} in biological systems and it has been postulated that La^{3+} can replace Ca^{2+} at well-defined tissue loci or sites (Weiss 1974). Inasmuch as *Daphnia* actively absorbs Ca^{2+} from the water after moulting for hardening the carapace (Porcella et al.1969), La binding to the carapace might be another way of reducing the concentrations in the water and might explain the high concentrations of La in *Daphnia* after exposure (Yang et al. 1999).

Effect of Phoslock® on growth of Daphnia

Growth of juvenile *Daphnia* was not affected up to 100 mg Phoslock® l^{-1}. Nonetheless, at this concentration animals showed a tendency of being lighter (7 – 13%) than their conspecifics in controls and treatments up to 50 mg Phoslock® l^{-1}. Probably feeding inhibition by suspended clay particles had reduced the feeding rate of the animals (Kirk 1991). The NOEC concentration of 100 mg Phoslock® l^{-1} is close to the average application dosage. Data on four different applications yield an estimated average application dose of 84 (± 24) mg Phoslock® l^{-1} (Anonymous 2008a; Ross and Cloete 2006). Application usually takes places through a spray manifold where the slurry is brought into the upper water layer (Robb et al. 2003). Consequently, Phoslock® concentrations in these moments in the top layers will be much higher than the ones calculated over the whole water body. However, rapid sedimentation and dispersal of the clay particles is expected lowering the turbidity to normal values within a few days (Haghseresht F. 2005a; Ross and Cloete 2006).

In this study, a juvenile growth assay was employed without refreshment of water to avoid continuous exposure of the animals to high concentrations of suspended material and without adding additional food to mimic the presumed phytoplankton growth reduction through P-depletion. The relationship between juvenile growth rates and population growth rates, either calculated from individual biomass or length at successive

times, is highly significant in a wide range of natural and stress conditions (Lampert and Trubetskova, 1996; Hanazato, 1998).

The Phoslock® application is developed for restoring degraded or eutrophicated systems by immobilizing phosphate and thereby reducing blooms of nuisance cyanobacteria. It is generally accepted that cyanobacteria may cause major disruptions of the aquatic ecosystem and that cyanobacteria have strong negative effects on *Daphnia* (Lampert 1987; Christoffersen 1996; DeMott 1999; Lurling 2003). Hence, improving water quality and reducing the vulnerability to the threats of harmful cyanobacterial blooms is a key issue. Despite possible short-term reductions in juvenile *Daphnia* survival and growth by higher clay concentrations (Kirk and Gilbert 1990), such minor effects will be outweighed by significant reductions in the amount of cyanobacteria. Although this study did not reveal any major detrimental effects on *Daphnia* when using Phoslock® or its active ingredient lanthanum, further studies should be undertaken including monitoring of the zooplankton community, and should preferably focus on bottom-dwelling organisms that might experience the highest exposure to the modified clay.

Acknowledgements
Mr Nigel Traill and Patrick van Goethem are cordially thanked for delivering the batches of Phoslock®.

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Table 1. Metals (in µg l^{-1}, ± 1 SD, N = 3) measured in 0.45 µm filtrates from 5 g l^{-1} suspension of two different batches of Phoslock®.

	Al	Cd	Cu	Hg	La	Pb	Zn
Control	0.7 (1.3)	0	0.17 (0.09)	< 1	0	0	9.9 (2.1)
Batch 1	218.1 (145.5)	0	0.31 (0.09)	< 1	22.9 (17.5)	0.02 (0.02)	30.3 (18.0)
Batch 2	16.2 (2.6)	0	0.21 (0.10)	< 1	3.4 (0.8)	0 (0.01)	26.2 (15.0)

Table 2. Average body-size of the animals during the experimental period (± 1 SD) in P-free medium and P-containing where they were exposed to different concentrations of lanthanum (La), including F and P-values of the between-subject effects (presence/absence of P in medium) of repeated measures ANOVAs.

La (µg l^{-1})	Size (mm) P-free	Size (mm) P-containing	F	P
0	2.94 (0.11)	2.97 (0.03)	0.64	0.434
33	2.95 (0.04)	2.97 (0.03)	2.15	0.160
100	3.03 (0.09)	2.89 (0.04)	19.0	< 0.001
330	3.00 (0.04)	2.81 (0.08)	37.9	< 0.001
1000	2.87 (0.06)	2.47 (0.07)	147.2	< 0.001

Table 3. Life-history characteristics, age at first reproduction (AFR, d), size at first reproduction (SFR, mm), Survival (%) and population growth (d^{-1}) of *Daphnia magna* exposed to different concentrations of lanthanum in P-free and P-containing medium including controls. Different symbols (A,..,E) indicate significant differences at the 95% level (Tukey's post hoc comparison test). Values for AFR and SFR are means ± 1 SD, population growth rates are means ± 1 SE.

P-free medium

Treatment	AFR (d)	SFR (mm)	Survival (%)	Population growth (d^{-1})
Control	7.9 (0.3)^{A}	3.24 (0.08)^{A}	90	0.368 (0.026)
33 µg La l^{-1}	7.9 (0.3)^{A}	3.27 (0.10)^{A}	100	0.380 (0.031)
100 µg La l^{-1}	7.6 (0.5)^{A}	3.30 (0.07)^{A}	100	0.383 (0.039)
330 µg La l^{-1}	7.8 (0.4)^{A}	3.29 (0.07)^{A}	90	0.374 (0.032)
1000 µg La l^{-1}	7.8 (0.5)^{A}	3.19 (0.12)^{A}	90	0.360 (0.027)
		P-containing medium		
Treatment	AFR (d)	SFR (mm)	Survival (%)	Population growth (d^{-1})
Control	7.8 (0.4)^{A}	3.28 (0.09)^{AB}	100	0.376 (0.029)
33 µg La l^{-1}	7.9 (0.3)^{A}	3.25 (0.07)^{BC}	100	0.381 (0.036)
100 µg La l^{-1}	8.0 (0.0)^{A}	3.14 (0.08)^{CD}	80	0.359 (0.027)
330 µg La l^{-1}	8.0 (0.0)^{A}	3.13 (0.12)^{D}	90	0.348 (0.029)
1000 µg La l^{-1}	8.2 (0.4)^{A}	2.71 (0.07)^{E}	80	0.288 (0.035)

Table 4: Summary of acute toxicity assays (following OECD protocol 202) with cladocerans (Dc = *Daphnia carinata*, Dm = *Daphnia magna*, Cd = *Ceriodaphnia dubia*) exposed fro 48 h to lanthanum or leachate from Phoslock®, including hardness of the water used (in mg $CaCO_3$ l^{-1}).
[a] = total lanthanum, [b] = animals exposed to leachate; EC50 corresponding to La in leachate, [c] No lanthanum concentration given. Art. Medium = artificial medium.

Water type	Hardness	Species	EC50 (µg La l-1)	Reference
Tap water	22	Dc	43	Barry & Meehan (2000)
Art. medium	98	Dc	49	Barry & Meehan (2000)
ASTM	160	Dc	1180	Barry & Meehan (2000)
Art. medium	40-48	Cd	5000[a]	NICNAS (2001)
Art. medium	210	Dm	23000	Sneller et al. (2000)
Art. medium	---	Dm	103000	Inst. Nowak (2008)
Art. medium	40-48	Cd	80[b]	NICNAS (2001)
Milli-Q	<10	Cd	40[b]	NICNAS (2001)
Art. medium	40-50	Cd	>50[c] g Phoslock® l^{-1}	Martin & Hickey (2004)

Figure legends

Figure 1. Body-length (mm ± 1 SD) of *Daphnia magna* exposed to different concentrations of lanthanum (nominal: 0, 33, 100, 330 and 1000 µg l^{-1}) in P-free medium (upper panel A) and P-containing medium (phosphate 330 µg l^{-1}, lower panel B) during a 14 d experimental period.

Figure 2. Number of neonates per female (± 1 SD) *Daphnia magna* exposed to different concentrations of lanthanum (nominal: 0, 33, 100, 330 and 1000 µg l^{-1}) in P-free medium (upper panel A) and P-containing medium (phosphate 330 µg l^{-1}, lower panel B) for the first three consecutive broods.

Figure 3. Biovolume of the green alga *Scenedesmus obliquus* (in μm^3 ml^{-1}), used as food for *Daphnia*, in experimental tubes initially and after 2, 18 and 25 hours in P-free and P-containing medium in the absence (No La) and presence of lanthanum (1000 µg l^{-1}). Error bars indicate one standard deviation (N = 3).

Figure 4. Juvenile growth rates of *Daphnia magna*, based on the increase in weight (black bars) and on the increase in body-length (white bars), exposed for 5 days to different concentrations of Phoslock® (0 – 5000 mg l^{-1}). Error bars indicate one standard deviation (N = 3).

Figure 1

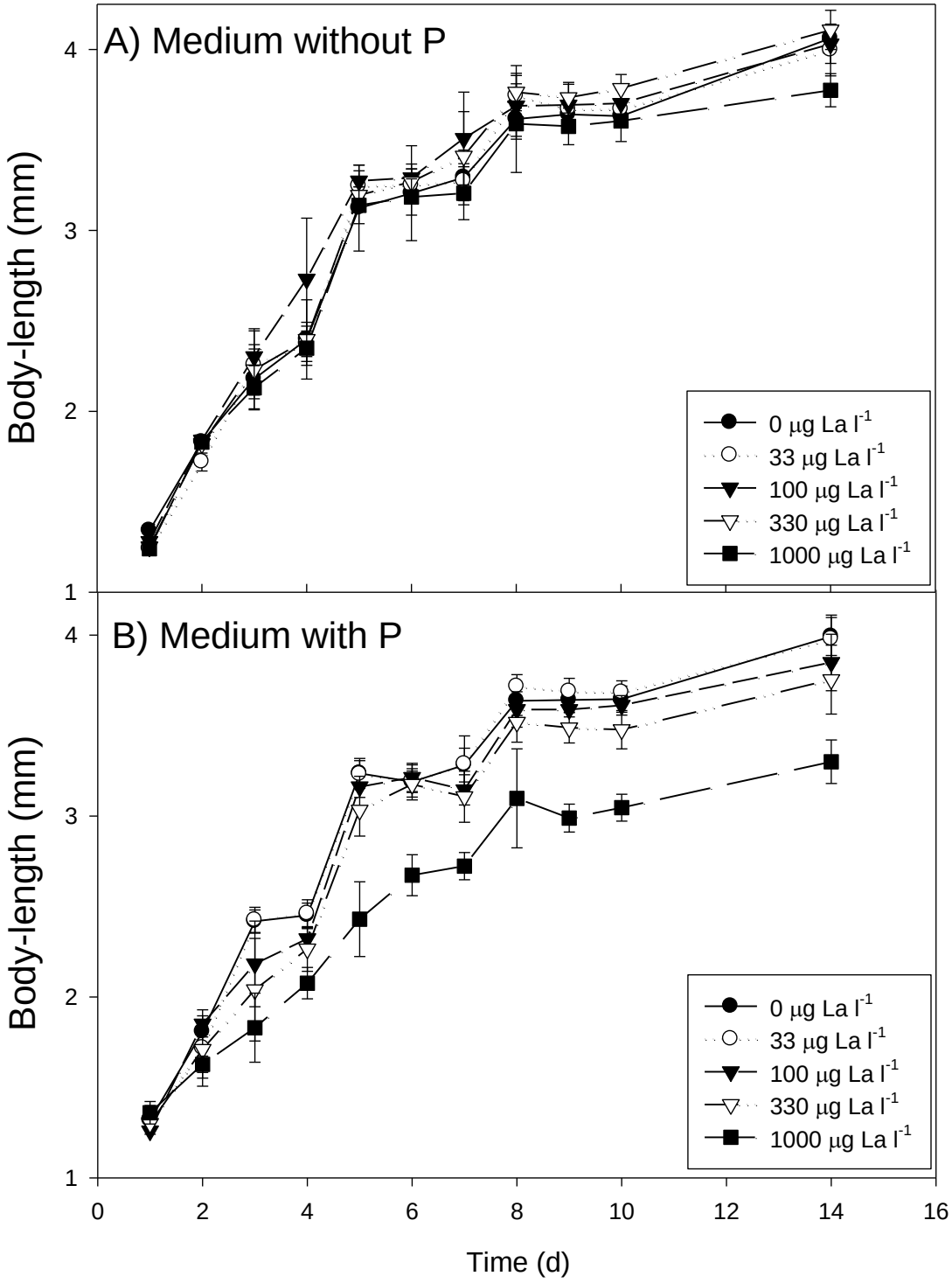
A) Medium without P
B) Medium with P
Body-length (mm)
Time (d)
0 µg La l-1
33 µg La l-1
100 µg La l-1
330 µg La l-1
1000 µg La l-1

Figure 2

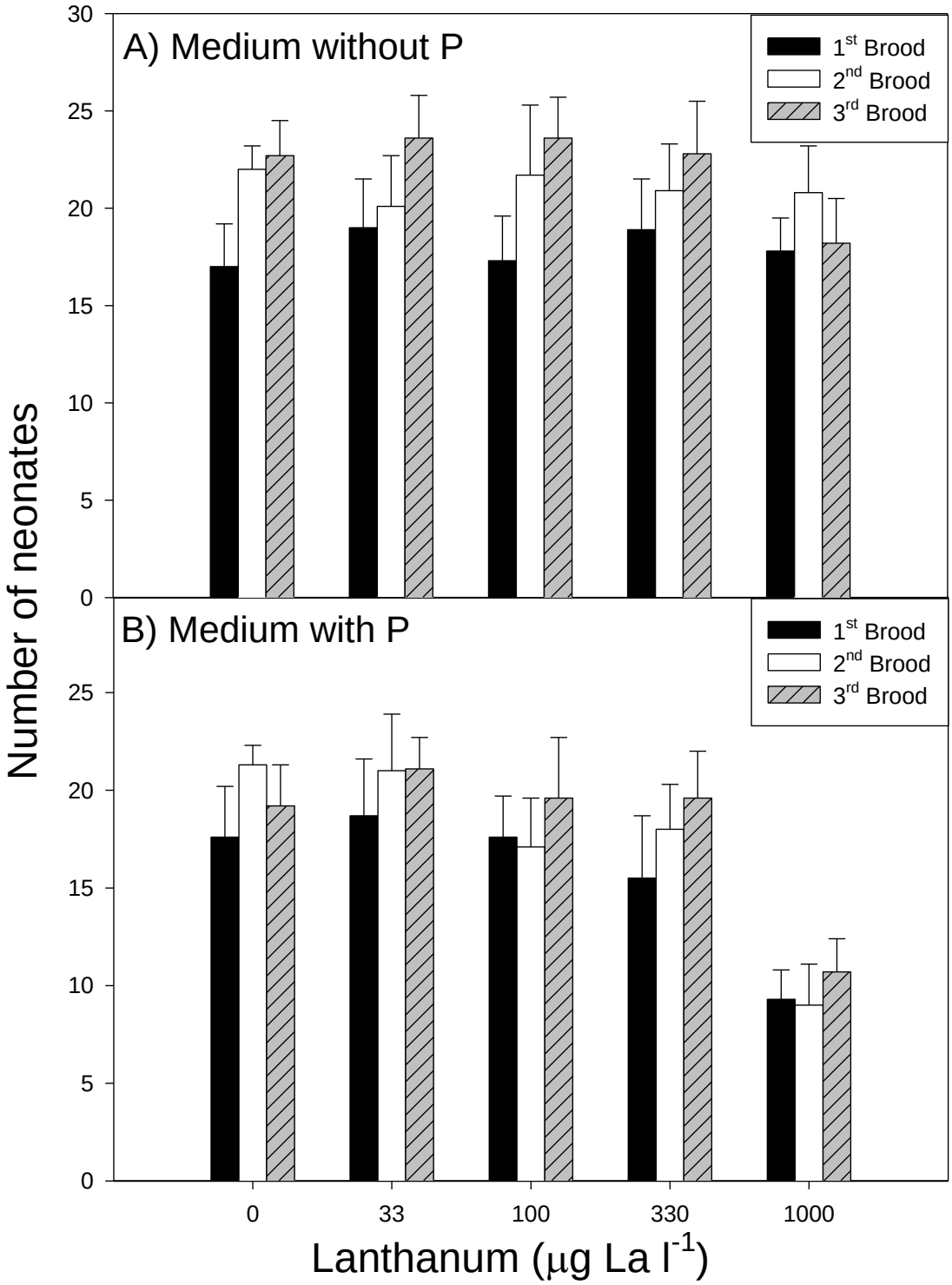
A) Medium without P
B) Medium with P
1st Brood
2nd Brood
3rd Brood
Number of neonates
0
5
10
15
20
25
30
0
33
100
330
1000
Lanthanum (µg La l-1)

Figure 3

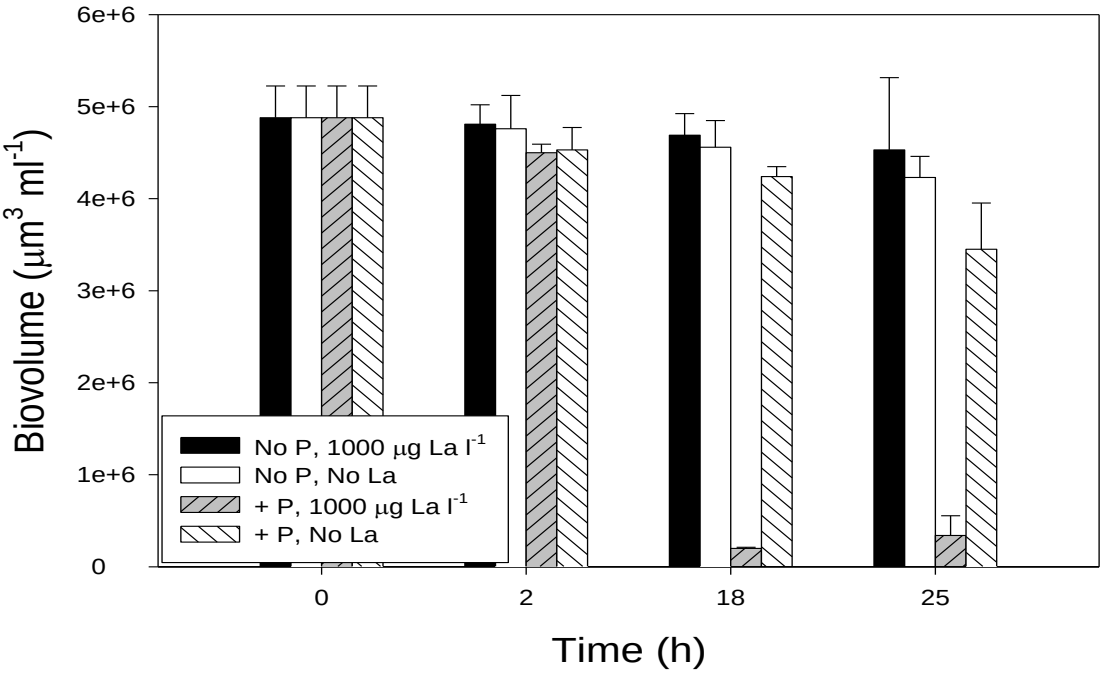

Figure 4