Interaction of Se Biogeochemistry With Foodchain Disruption In Full-Scale Evaporation Basins and Pilot-Scale Drain water Systems

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ABSTRACT:

The purpose of this project is to evaluate the Se bioremediation potential (via reduction of ecotoxic risk) of combined foodchain disruption and Se volatilization in full-scale TLDD evaporation basins and pilot-scale drain water systems. Our approach has been to assess the influence of ongoing brine shrimp harvest and drainage water manipulation on water chemistry, water and biota Se status, Se volatilization activities, and algal community. Analyses have been conducted for monthly water and brine shrimp samples from selected saline basin cells, both harvested and non-harvested, as well as for the annual collections of water and macroinvertebrate samples from both saline and less saline cells. Brine shrimp harvesting was shown to reduce the efficiency of Se transfer from algae to invertebrates, enhance algal growth, and reduce Se incorporation into benthic organisms. Salinity was shown to be an important determinant to Se volatilization rates.

The compilation of data collected over several years of this type of monitoring revealed that the microalgal communities in the basins are serving a variety of functions relative to Se biochemistry. Whether these communities tend to accumulate or volatilize Se and whether they provide food for brine shrimp has a measurable influence on the amount and compartmentalization of Se in the system. As these communities were better functionally described in this work, they are also being quantitatively described using a newly developed procedure for tracking populations of *Synechococcus* sp.

PURPOSE:

The purpose of this project is to evaluate the Se bioremediation potential (via reduction of ecotoxic risk) of combined foodchain disruption and Se volatilization in full-scale TLDD evaporation basins and pilot-scale drain water systems.

Preliminary investigation in hypersaline ponds of TLDD indicates that Se volatilization may be combined with brine shrimp harvest to reduce ecotoxic Se load in waters and biota. In addition, it appears that both processes could be enhanced by manipulating the water chemistry to increase a microphyte population that functions to dissipate Se by volatilization and/or as food for brine shrimp. If mechanistically understood, this coupled process should prove to be a highly economical and flexible option for remediating Se ecotoxic risk in agricultural drainage systems. These advantages are in part due to a market demand for brine shrimp and the practicality of implementing the option together with other drainage mitigation plans that produce brine such as IFDM and reverse osmosis.

INTRODUCTION:

OBJECTIVES

Our objectives are to investigate and understand the effect of brine shrimp harvest on Se biogeochemistry and to uncover conditions that simultaneously favor Se volatilization and brine shrimp production while minimizing the accumulation of Se ecotoxic indicators. We approach these objectives by both full-scale monitoring and pilot-scale studies as follows:

1. Change in Se status in TLDD hypersaline ponds (Hacienda A4 in particular since we have data on its Se status before harvest began) elicited by brine shrimp harvest;

2. Effects of brine shrimp harvest on selenium status of microalgae and brine shirmp, microalgal community, as well as waterborne Se status in TLDD hypersaline ponds.

3. Establish pilot-scale drain water system at the Red Rock Ranch to better control the water chemistry (which in turn regulates microalgal populations and community) to optimize Se volatilization and brine shrimp harvest.

APPROACH

Rates of brine shrimp harvest in each of the TLDD evaporation basins have been compiled from daily records provided by Novlek. On a monthly basis, water, microalgae, and brine shrimp samples have been collected from TLDD evaporation basins, processed, and analyzed for total Se and/or Se speciation into proteins. The microalgal community has also been profiled using 16S cDNA in combination with Denaturing Gradient Gel Electrophoresis (DGGE). In July, 2004, extensive field sampling was conducted at TLDD basins to collect water column and benthic macroinvertebrates, with the assistance of Julie Vance from Dept. of Water Resources, Fresno. Selenium status of these samples should indicate the distribution of Se in the ecological niches of the basins. In situ Se volatilization measurements were also made at selected TLDD basin cells.

RESULTS:

CHANGES IN TLDD WATER MANAGEMENT

Water management in 2005 was similar to that in 2004. As it was last year, harvest from Cell A4 of the Hacienda basins (HAC A4) was minimal and the un-harvested C4 comparison basin was not monitored due to low water supplied to those basins. The rise in salinity seen in HAC A4 coincided with the water low volume time period beginning in early June. At the South basins, water continued to be drawn from SEB 8 to both SEB 9 and SEB 10. This had the desired effect of stabilizing the water level and salinity in the three ponds and made salinity comparable in all of them. Shrimp harvest was greatest in SEB 9 and also substantial in SEB 10, with production in SEB 9 about twice that in SEB 10. Of the regularly monitored basins, the most saline basin (HAC A4) continues to be the most Se volatilizing (cf. Fig. 16), though it was harvested only lightly for brine shrimp. The basin with the highest brine shrimp yields actually had the second highest rate of Se volatilization, of the four monitored basins

WATER CHEMISTRY AND SE STATUS IN TLDD MICROALGAE AND MACROINVERTEBRATES

Water, algae, and invertebrate samples were collected monthly from TLDD basin cells and analyzed for *chlorophyll a* (*chl a*) fluorescence, total Se of water, algae, and brine shrimp, as well as protein Se content of algae and brine shrimp. Figure 1 shows the monthly trend of in vivo *chl a* fluorescence of the 4 monitored basin cells. Variations in fluorescence or algal population in the harvested ponds can be compared with the corresponding cumulative brine shrimp harvest, as shown in Figure 2. In general, the declining algae population at the end of the year (September and October) was met with tailing off of shrimp harvest in both active ponds (SEB 9 and 10). In SEB9, where shrimp harvest was most active (Fig. 2), the *chl a* fluorescence was consistently low year-round (Fig. 1). This may be attributed to the grazing of the consistently high population of brine shrimp in SEB 9. In SEB10, *chl a* fluorescence readings are seen to peak when harvest slowed in June and fall again when harvest activity resumes in August. hese observations are consistent with the notion that brine shrimp grazing regulates microalgal population in these hypersaline basin cells. Interestingly, in 2004, the chl a fluorescence readings were similar in the moderately harvested pond SEB 10 and the significantly less harvested basin HAC A4. Although it was only harvested once in 2004, the long history of brine shrimp harvest in HAC A4 may still be influencing the algal population there.

In addition to *chl a* fluorescence, salinity and water Se concentration ([Se]) also exhibited seasonal changes and differences among the basin cells (as observed in previous years), which is shown in Figures 3-6. As mentioned above, the salinity (in parts per thousand or ppth) of South Basins 8, 9, and 10 (Fig. 3) was relatively low, compared with previous years, and stable over time. However, the salinity of HAC A4 was more variable and peaked higher than that observed previously. As stated above, this was due to the differences in TLDD water management between the two systems. As observed previously, salinity and water Se concentration did not correlate, as would be expected from simple evaporite chemistry (Figs. 3 and 5). The pH was similar and stable over time in all cells (Fig. 4). The water Se concentration of all cells was comparable all year round, with the exception of the uniformly higher Se concentrations measured in SEB 10 (Fig. 5). Though the source water (SEB 8) and the shrimp harvest patterns, though not in magnitude, are comparable to those of SEB 9, the waterborne Se concentrations of the latter are similar to the two unharvested basins (HAC A4 and SEB 8).

The effect of brine shrimp harvest on algal Se status is shown in Figure 6 (algae Se concentrations), Figure 7, (Se bioconcentration factor or BCF based on dry mass), and Figure 8 (protein Se concentrations) in the TLDD basins. The algal Se body burden data of the three ponds for which data are most complete (HAC A4 and SEB 8 and 10) follow the same patterns over time, peaking at the beginning of the year when conditions favor algal growth and declining towards the end of the year, though algal Se returned to early season levels in HAC A4 in November, 2004 (Fig. 6). The heavily harvested basin SEB 9 had so little algal biomass that samples were usually not available for analysis of algal Se body burden and the few data points presented are difficult to interpret (Fig. 6). Also noted is that the algal Se burden of the historically harvested cells (SEB10 and HEB A4) was lower at the start of the harvest season (April, Fig. 2) than that of the unharvested cell (SEB 8), similar to trends reported previously. The concentrations of Se in the protein of algae were similar in all ponds and generally followed the trend of the total Se body burden but unrelated to harvest activity. The Se BCF in algae varied similarly over time among the South Basins (SEB 8 and 10) while it declined steadily throughout the year in the Hacienda basin, HAC A4 (Fig. 7). Again, data are scarce for SEB 9 due to low recovery of algal biomass. Measurements of concentrations of Se associated with protein in algae represent Se that is readily bioavailable to the next tropic level (invertebrates). Protein Se in algae was similar in the historically harvested HAC A4 and SEB 10 basins, declining over the harvest season and increasing slightly at the end of the year (Fig. 8). Again, data are difficult to interpret for SEB 9 because there were only four samples collected for the year. Wide variation was seen in the protein Se content of algae in the unharvested basin, SEB 8, showing a cyclic trend similar to that noted in the algal density (Fig. 1).

Considering the Se status in brine shrimp, the one effect of harvesting appears to be a disruption of Se transfer from microalgae to the macroinvertebrates, as noted in previous years. For example, the negative correlation between water Se concentration and brine shrimp BCF is more scattered in the harvested basins than in those that were not harvested (Fig. 9). Also, while the lowest water-borne Se concentrations were seen in un-harvested basins, the highest shrimp BCF were also seen there. This observation indicates a greater efficiency in the un-harvested basins at transferring Se from the water column into the invertebrates, relative to harvested basins.

In addition to monthly sampling of algae and brine shrimp in hypersaline cells, a composite each of water column and benthic organisms was collected from TLDD basin cells of low to high salinity on July 19 and 20, 2004. The Se status of these samples along with the water [Se] is shown in Figure 10 (total Se) and 11 (protein Se). As in the monthly monitoring, the highest waterborne Se concentration measured was in the most heavily harvested basin, SEB 9. The algae of this basin also had the lowest Se body burden of any measured, with low Se concentrations in invertebrates as well (Fig. 10). In SEB 10 the Se body burden of the water column macroinvertebrates was also consistently higher than that in the algae or in the benthic organisms. The total Se concentration measured in biota from the unharvested basins (SEB 1, SEB 8, and HAC A3, HAC C1, HAC C3) exhibited no trend related to taxa. Exhaustive harvest of the brine shrimp in SEB 9 have helped to funnel Se to the water column organisms via enhancing algal growth, while reducing Se incorporation into benthic organisms by limiting detrital deposition and, thus, growth and consumption of Se by benthic organisms. A similar trend was observed for the proteinaceous Se burden of algae, water column and benthic macroinvertebrates composites (Fig. 11). In fact, total and proteinaceous Se concentrations were well correlated in the annual survey samples (data not shown). As observed in previous years, no clear correlation was discerned from the water [Se] to the Se burden of algae, water column or benthic invertebrates and from salinity (cf. Fig. 10) to water [Se]; the former indicates that water [Se] is not a good predictor of Se accumulation in aquatic biota. This is presumably due to the influence of complex Se biogeochemistry on Se bioconcentration. The lack of water Se buildup with increasing salinity is consistent with Se removal via volatilization and/or brine shrimp harvest (see Se volatilization by Microalgae).

SE VOLATILIZATION BY MICROALGAE

As in previous years, measurements of volatile Se of water collected at the upwind and downwind corners of the basins in both morning and afternoon were made on site during the annual field sampling trip (July 19, 2004). Figure 12 shows the volatile Se content of TLDD basin waters in downwind locations (collected from the south shores) in the morning and in the afternoon in comparison with basin salinity. Diurnal differences were not consistent, as they have been previously, with the terminal ponds for the two Hacienda series basins (C3 and A4) showing a different pattern (higher in the afternoon) than the other ponds. As seen previously, there appears to be a rough relationship between salinity and volatile Se production. The least saline ponds (SEB 1 and HAC C1) had non-detectible Se volatilization and, of the regularly monitored ponds, the most saline (HAC A4) had the highest rate of Se volatilization. The other terminal pond in the Hacienda system (HAC C3) had the highest total Se volatilization, though at the time of testing salinity was not as high there as in HAC A4.

Figure 13 shows the average downwind volatile Se content of TLDD basin waters in comparison with the bioconcentration factor (BCF, on a dry wt basis) of Se by microalgae for the 2004 field measurement. As noted above, of the basins which are regularly monitored, the basin with the highest Se volatilization was HAC A4. This basin also tended to accumulate the very little Se (Fig. 13). This basin was only occasionally harvested in 2004, but has been extensively harvested in past years. It also had the highest average salinity for 2004. The other terminal basin in the Hacienda series (HAC C3) also had high Se volatilization, but the algal BCF was not among the lower measured values. Basins SEB 9 and SEB 10, which were most heavily harvested, show mid-level Se volatilization, but one had very low algal BCF (SEB 10) while the other had high algal BCF (SEB 9). The un-harvested basins, SEB 1 and SEB 8 show negligible Se volatilization and low algal BCF. Un-harvested basin HAC C1 shows negligible volatile Se and highest measured algal BCF, which has been repeatedly observed in less saline un-harvested basins in previous years. These results indicate that high salinity is an important factor in modulating Se volatilization.

MULTI-YEAR TRENDS

The chl a fluorescence and harvest activity observations described above and compiled for the multiple years of this study suggest relationships between harvest activity and algae yields . However, algae yields obtained in this study are calculated from monthly sampling and brine shrimp harvest data is monitored on a daily basis. Furthermore, the timing of the monthly sampling is not explicitly linked to harvest activity such that the day of sampling may not be representative of the harvest that month. Also the effects of harvest activity will not be translated onto the algal community within a 24 hour period. Therefore, the algae yield was related to a period of harvest activity longer than one day to develop a connection to shrimp harvest. A period of 7 days was chosen such that the net effects of brine shrimp harvest on the algal population at the beginning and end of the time frame are representative of the entire time frame. High salinity and the presence of corixids may also interfere with algae yield calculation. Data obviously impacted by these conditions were excluded from analysis of harvest activity versus algae yield.

Consistently, when compiled over multiple years, reliable algae yield data and significant shrimp harvest were attained in South Basins S9 and S10. Figure 14 shows a plot of a cumulative 7 day brine shrimp harvest versus algae yield for basin S9 in years 2001, 2002 and 2004. Figure 15 shows a plot of cumulative 7 day brine shrimp harvest versus algae yield for basin S10 in years 2001, 2002 and 2004. The data from basins S9 & S10 in 2001 and 2004 show the algae yield decreasing as brine shrimp harvest increases in both basins, supporting the hypothesis of algal consumption by brine shrimp production drawn from the chl a fluorescence and harvest activity data. However, in 2002 the algae yield in basin S9 and S10 increased with increasing brine shrimp. This suggests that there was a large portion of the algal population that was not consumed by brine shrimp and continued to grow despite the grazing of brine shrimp.

The algal communities in each basin can be categorized as Se accumulating or Se volatilizing communities based on data collected from yearly sampling (Figure 16). When the algal bioconcentration factor (BCF) is large relative to the Se volatilization potential, the community can be considered accumulating, while the opposite relationship characterizes a volatilizing community. These assignments along with the fact of shrimp harvest as a net consumption of algae or not (figures 14 and 15) can be used to understand the movement of Se in each basin.

The algal community in basin S9 year 2001 can be designated as an accumulating community (figure 16) being consumed by brine shrimp (figure 14). The same can be said of the algal community in basin S10 in 2001 and S9 in 2004. In these basins and years the whole water [Se] remains very close to the filtered water [Se] as algae yield increases (figure 17 a and c). These observations support the notion that the portion of the algal community accumulating Se is being eaten by and accumulating in the brine shrimp.

The algal community in S9 year 2002 can be designated as a volatilizing community (figure 16) with a larger portion of the community not being consumed by brine shrimp (figure 14). As in 2001 and 2004 the whole water [Se] remains very close to the filtered water [Se] as algae yield increases in basin S9 (figure 17 b). Although less algae is being removed from the system, Se is not accumulating in the algae. This suggests that Se is being removed from the algal compartment through volatilization with less accumulation.

The algal community in S10 year 2002, although the BCF is low relative to other years, can be designated as a primarily accumulating community (figure 16) with a large portion of the community not consumed by brine shrimp (figure 15). The difference between whole water [Se] and filtered water [Se] increases with increasing algae yield in this basin (figure 17 b). Low removal of algae through consumption, low removal of Se from the basin through volatilization, and an accumulation of Se in the algae compartment as the algae yield increases supports these observations. In 2004 the algal community in basin S10 is primarily, although low, a volatilizing community (figure 16) that is being consumed by brine shrimp (figure 15). The difference between the whole water [Se] and filtered water [Se] increases with increasing algae yield (figure 17 c). This supports the idea that the volatilizing algae are being removed from the system through consumption by brine shrimp and Se is accumulating in the portion of the algal community left behind.

Although, these relationships illustrate the movement of Se in the evaporation basins at TLDD, they also demonstrate the importance of continued study of algal typing and Se loads, together with shrimp harvest, invertebrate loads, and Se volatilization in these basins.

MICROALGAL COMMUNITY ANALYSES

Monthly microalgal composite samples were extracted for total DNA, amplified with cyanobacterial 16S rDNA primers, and the resulting Polymerase Chain Reaction (PCR) products analyzed by Denaturing Gradient Gel Electrophoresis (DGGE). Figure 18 shows the DGGE gel patterns for environmental algae samples collected during 2003 from TLDD basins SEB 10, SEB 9, SEB 8, and HAC A4, along with two isolated monocultures. Our effort was to identify the dominant DGGE bands by DNA sequencing and thus reveal the microalgal community composition. Bands indicated with arrows (#1-9, Fig. 18) were cut, re-amplified, run on agarose gel, and purified with Quiagen kit before sending for sequencing. Based on sequencing comparison with the available 16S rRNA data base (Gene Bank and RDP) we identified the following algal species: *Chlorella mirabilis* (band # 1, 3, 6, and #8 –pure culture), *Koliella spiculiformis* (band # 2, #5), *Synechococcus* sp. (band # 4, band #9), and *Oscillatoria neglecta* (# 7, pure culture filamentous alga). Band #10 (Fig. 19) is most similar to uncultured phototophic eukaryotic clone from hypersaline Mono Lake, CA, and is dominant band in all samples from year 2004 .

Our previous observations of the gel patterns of algae collected during 2002-2003 were that a number of the samples had the same 2 gel bands (#2 and 3), now identified as the green algae *Chlorella* and *Koliella* sp. For example, the appearance of these 2 species for the HEB A4 samples seemed to relate to the strength of the harvest activity. The 2 green algae were present starting 4/4/02 and persisted until 7/19/02, after which they were not detected (cf. Fan's report, 2003). The opposite is true for the *Synechococcus* sp. (band # 4), which was not present during this intensive harvest period (4/4/02 to 7/19/02), but was very persistent after 7/19/02 and during the 2003 sampling. It is likely that the green algae *Chlorella* and *Koliella* sp. promote brine shrimp growth, while

Synechococcus sp. was left behind by the brine shrimp. It should also be noted that *Synechococcus* sp. is very active in Se volatilization (cf. Fan's report, 2003).

Significant change in microalgal community composition was observed in 2004 samples in comparison with the previous three years, probably due to changes in management operation (e.g. due to differences in water availability) of the TLDD saline basin cells (Figure 19).

To follow up on studying the ecological role and significance in Se volatilization of *Synechococcus* sp., we utilized quantitative PCR approach to estimate the cell densities of *Synechococcus sp.* in relationship with total micro-eukaryotic cell densities present in the ponds. During the past year we optimized PCR conditions and generated standards by cloning rDNA from *Synechococcus sp.* and rDNA from *Sacchromyces cerevizae* pure cultures. Figure 20 shows the standard curves used for quantification of rDNA copy numbers of *Synechococcus* and total micro-eukaryotes in environmental samples. For year 2002 monthly samples we determined the relative abundance of *Synechococcus* sp. at four different ponds. In general, PCR quantified total micro-eukaryote's copy numbers, are in the range of 10^7 to 10^8 copies per µg extracted DNA per mg dry algal biomass in all four ponds, indicating that the same size of population is present at this hypersaline environment (Figure 21). Additionally we observed that *Synechococcus* sp. are representing significant portion (more than 90 %) of algal community during the summer months (July to September) in S10 and S8 ponds. Also the population size of *Synechococcus* was larger in S8 pond (July to December) ranging from 10^5 to 10^7 copies/µg DNA/ mg dry biomass in comparison with the other three ponds (Fig. 21). Note that, for these samples, S8 and S10 are the less saline and harvested cells than the rest (cf. Fan et al., 2001), although that may not be generally true.
We are currently in the process of quantifying by qPCR the eukaryotes and *Synechococcus* populations for years 2001 and 2003 samples.

CONCLUSION:

The data acquired in 2004 indicate that waterborne Se, algal Se, and invertebrate Se did not increase as a result of increasing salinity, as repeatedly reported for previous years. Biotic and abiotic dynamics of Se that were observed in the TLDD evaporation basin system during the 2004 monitoring effort included:

- Brine shrimp grazing had a measurable effect on microalgal population density
- Brine shrimp harvesting reduced the efficiency of Se transfer from algae to invertebrates as determined by comparison of shrimp BCF in harvested and un-harvested basins.
- Brine shrimp harvest, while enhancing algal growth, reduced Se incorporation by benthic organisms in some basins.
- Salinity was an important determinant in Se volatilization rates by microalgal communities.

Data collected during many years of monitoring have demonstrated the stability with regards to Se water and biota concentrations of the TLDD evaporation basin system in both un-manipulated and brine shrimp producing scenarios. Specific conditions present at certain times in certain basins produced algal communities observed to serve a variety of functions relative to Se biochemistry. They were shown to:

- accumulate Se and provide food for brine shrimp, passing Se up the food chain,
- remove Se from the system via volatilization,
- accumulate Se without supporting brine shrimp growth, and
- volatilize Se and provide food for brine shrimp.

Our recently developed ability to quantify the percent of the algal population represented by a proven Se volatilizer (*Synechococcus* sp.) will further investigations into the exact make-up of microalgal communities exhibiting each of the functionalities observed. Further documentation of changes in microalgal community accompanying the harvest operations and mechanisms for directing community development to support shrimp harvest and/or Se volatilization are the future focus of this project.

PUBLICATION:

2005 Higashi, R.M., Cassel, T.A., Skorupa, J.P., and Fan, T.W-M. Remediation and Bioremediation of Selenium-Contaminated Waters. In: Water Encyclopedia, John Wiley & Sons, Int'l Digital Object Identifier (DOI): 10.1002/047147844X.wq496. Available at: http://www.mrw.interscience.wiley.com/eow/articles/wq496/frame.html

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FIGURES:

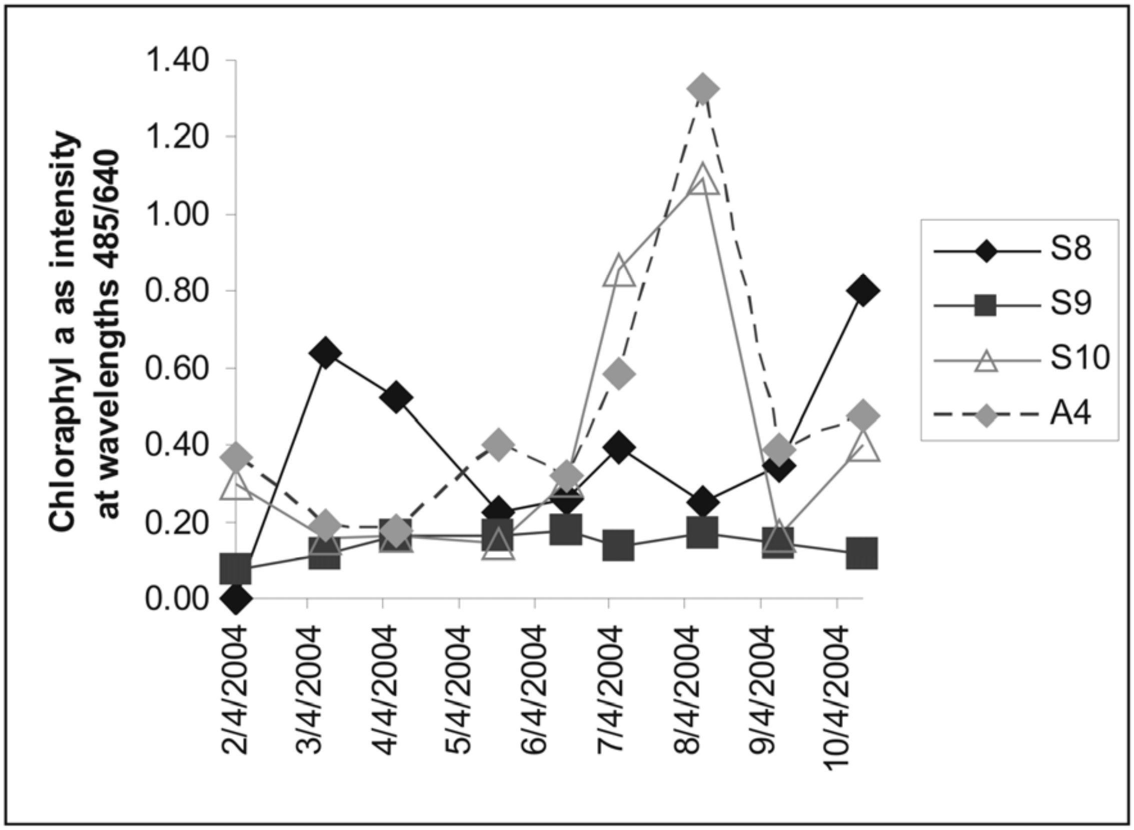


FIGURE 1: Monthly trend of Chl a fluorescence in water samples.

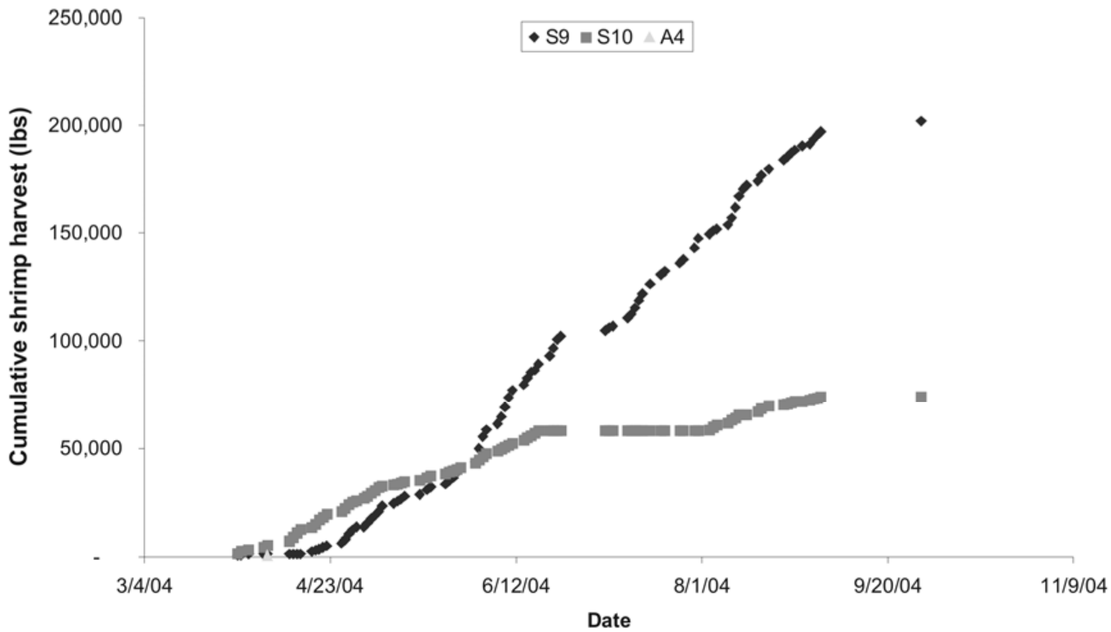


FIGURE 2: Cumulative brine shrimp harvest.

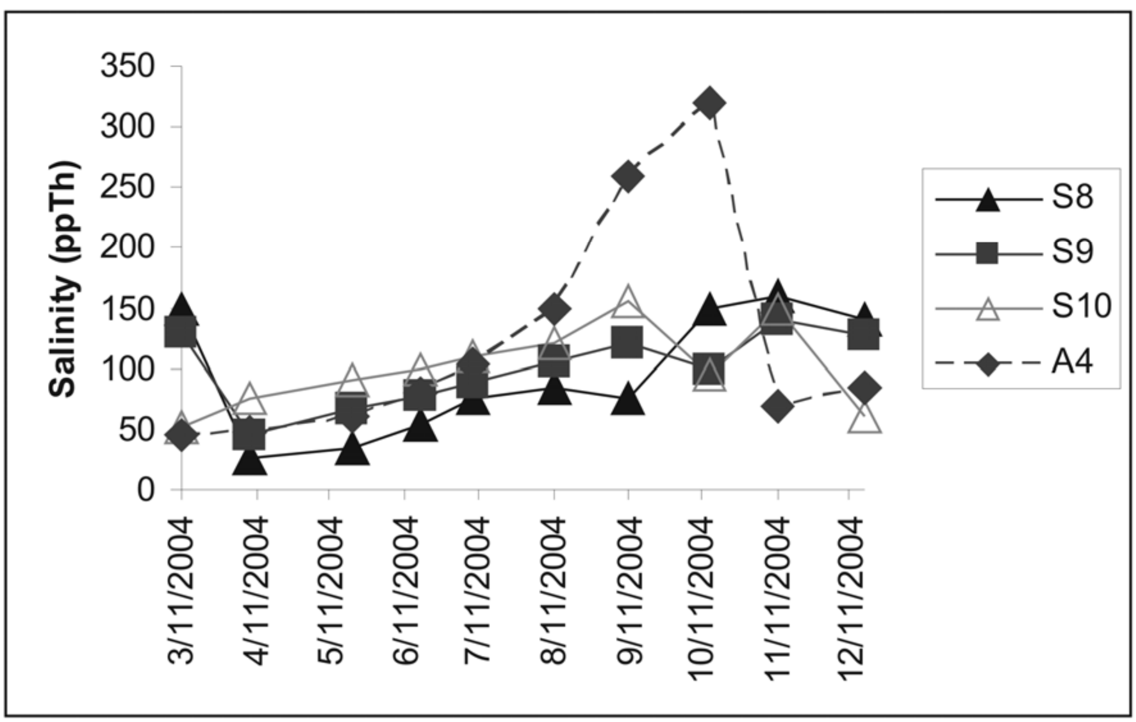


FIGURE 3: Salinity in monthly water samples.

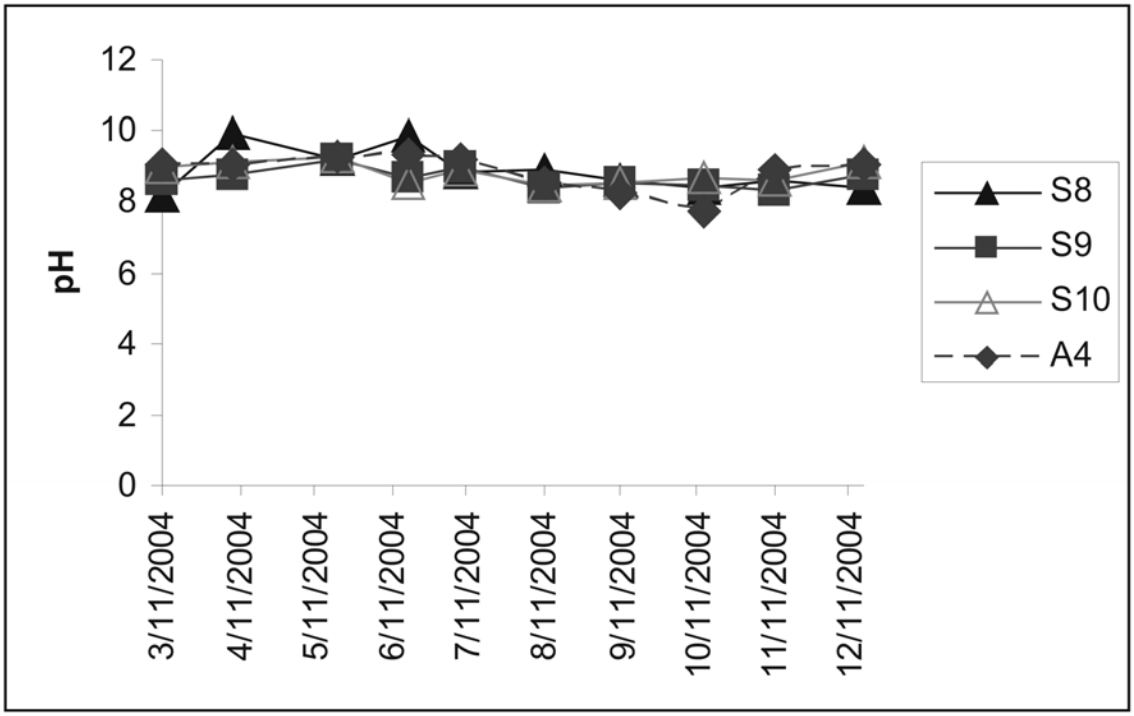


FIGURE 4: pH in monthly water samples.

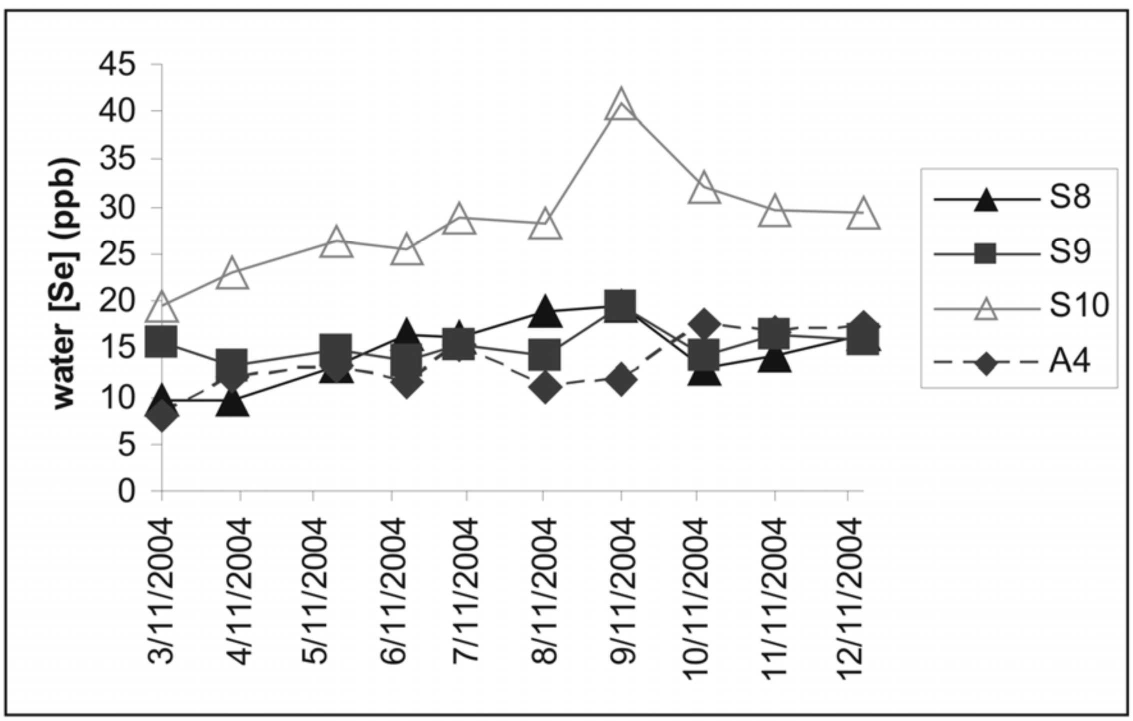


FIGURE 5: Se concentrations in filtered monthly water samples.

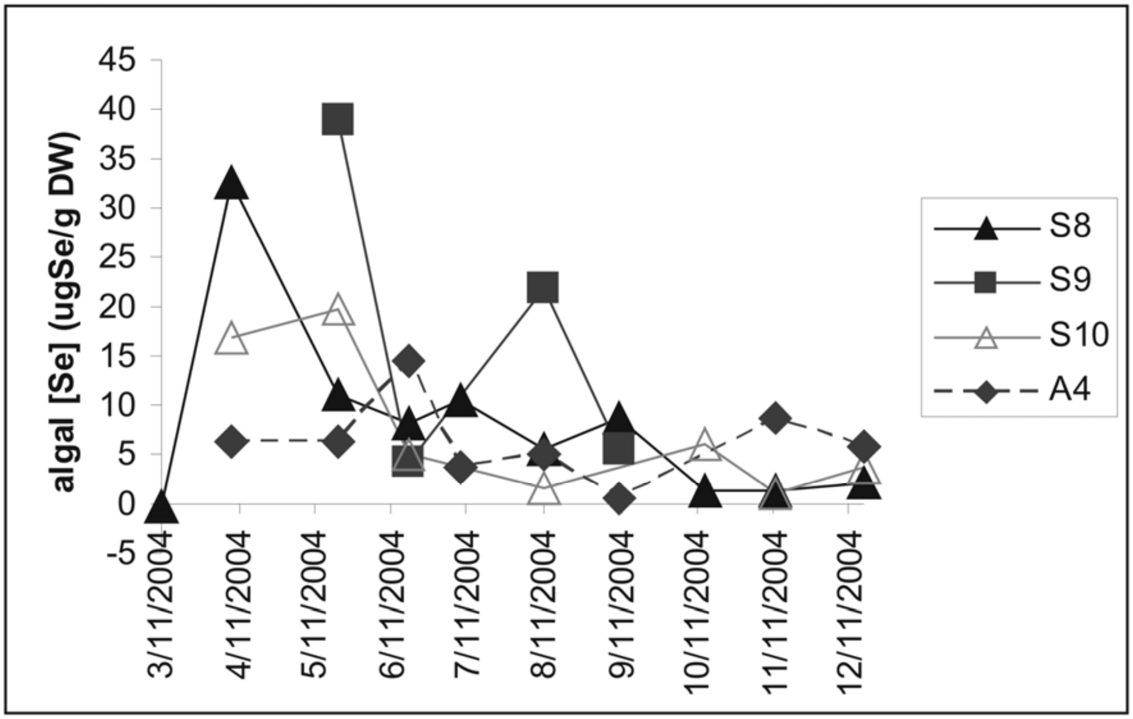


FIGURE 6: Algae Se concentrations.

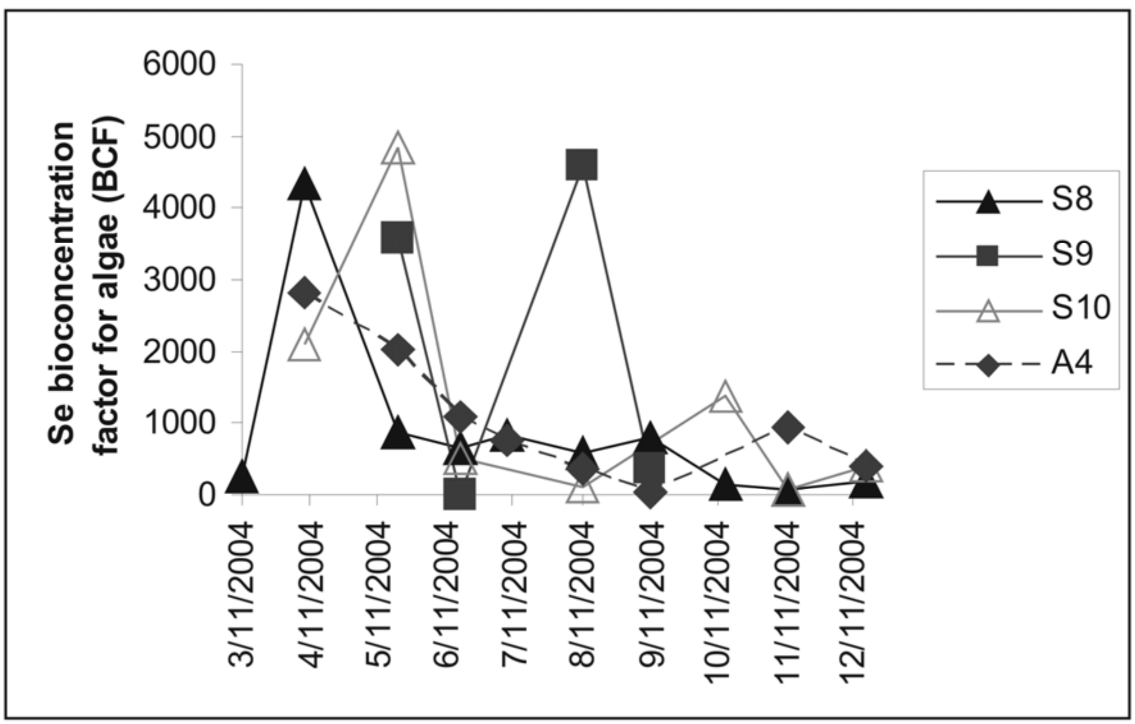


FIGURE 7: Se bioconcentration factor or BCF of algae, based on dry mass.

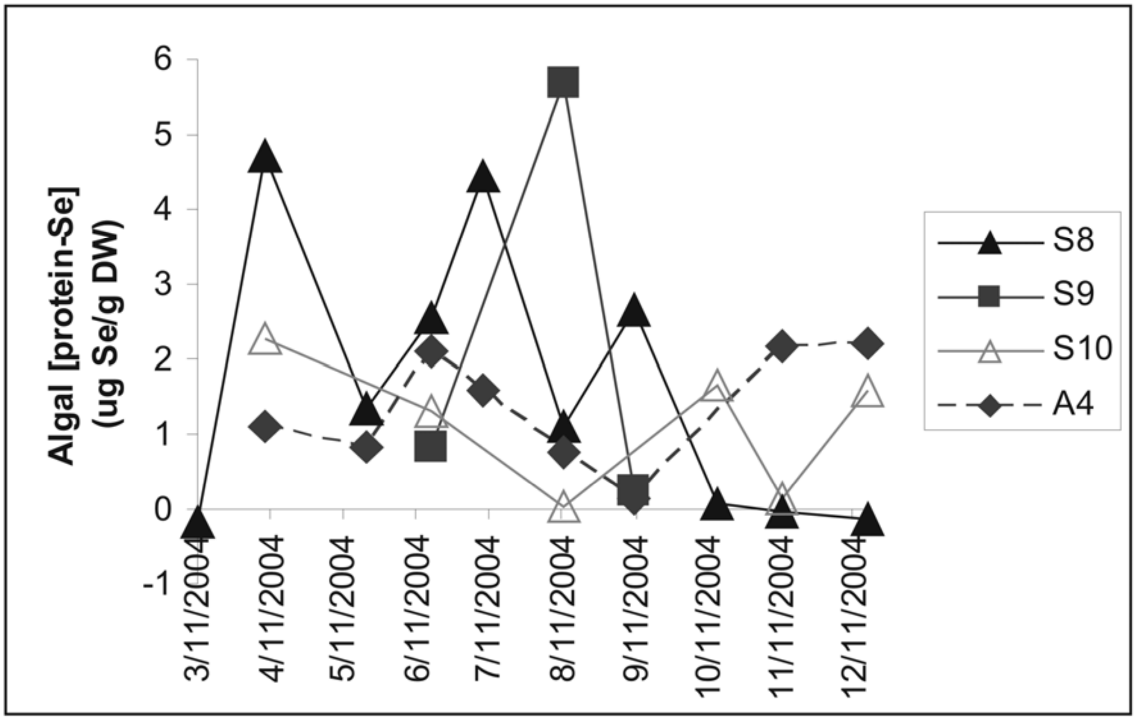


FIGURE 8: Algal protein Se concentrations.

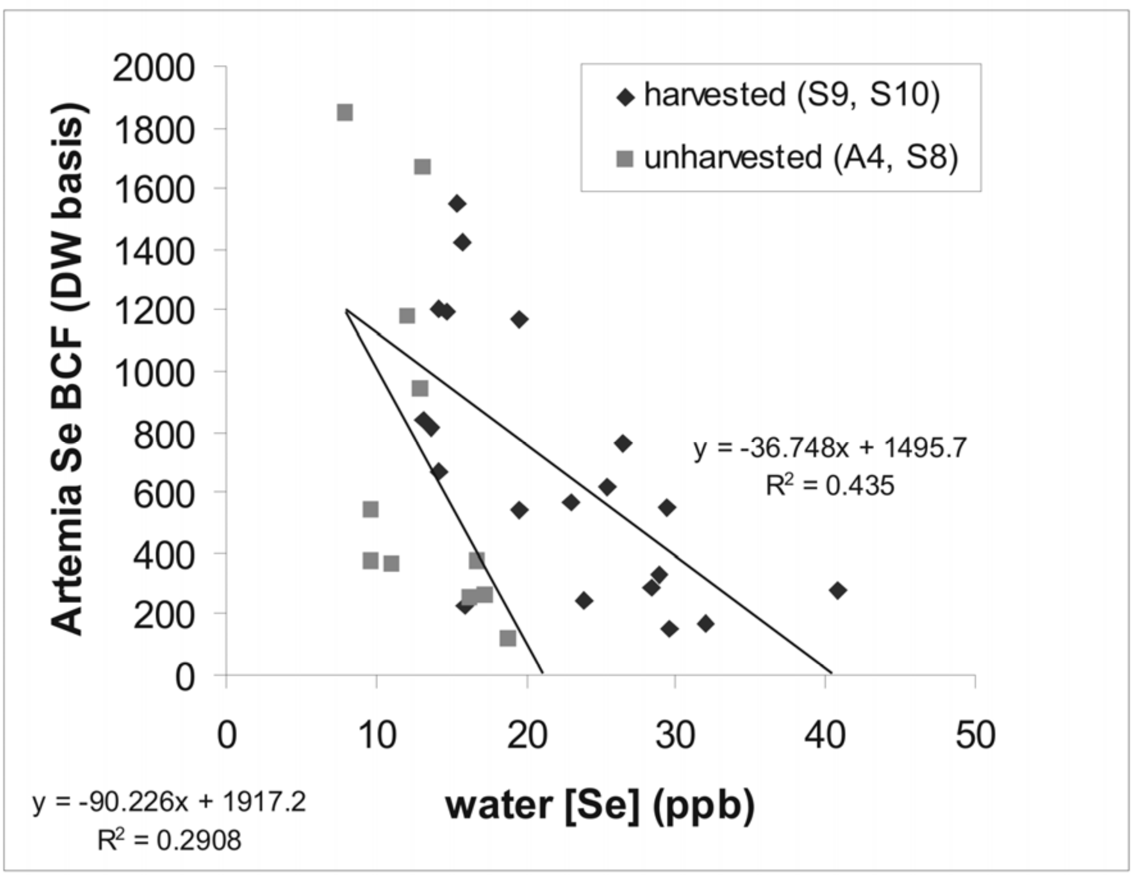


FIGURE 9: Water Se concentration and brine shrimp BCF.

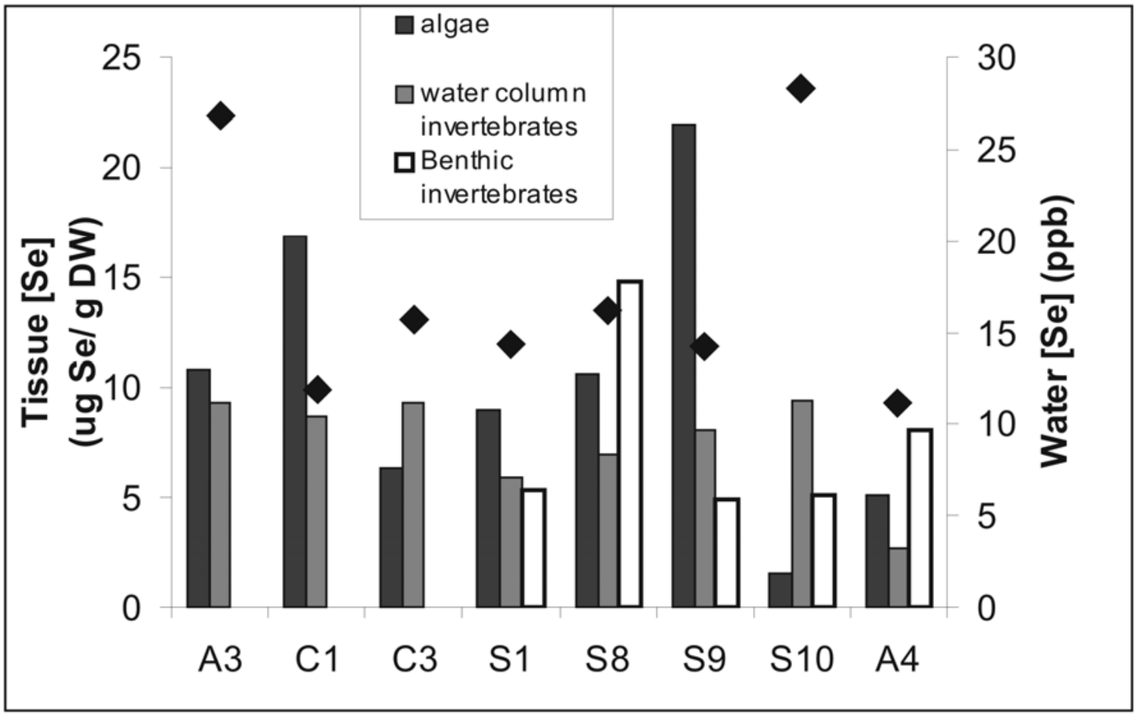


FIGURE 10: Total Se concentrations in algae, water column invertebrates, benthic invertebrates, and water samples collected on July 19, 2004.

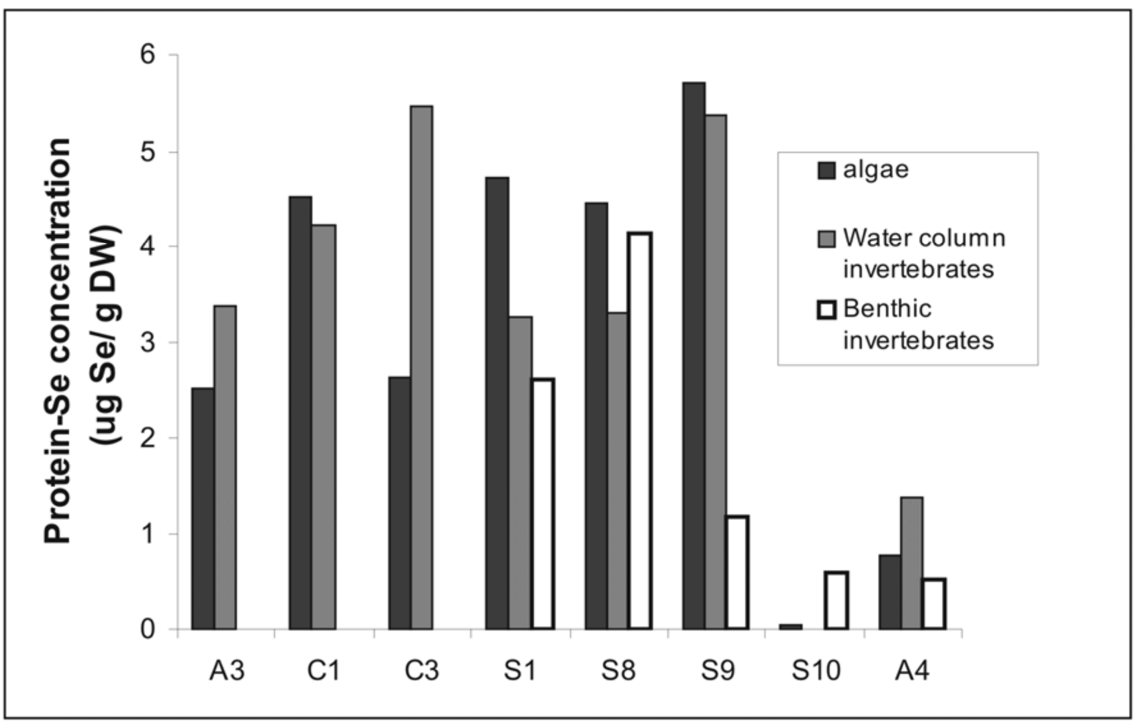


FIGURE 11: Proteinaceous Se concentrations in algae, water column invertebrates, benthic invertebrates, and water samples collected on July 19, 2004.

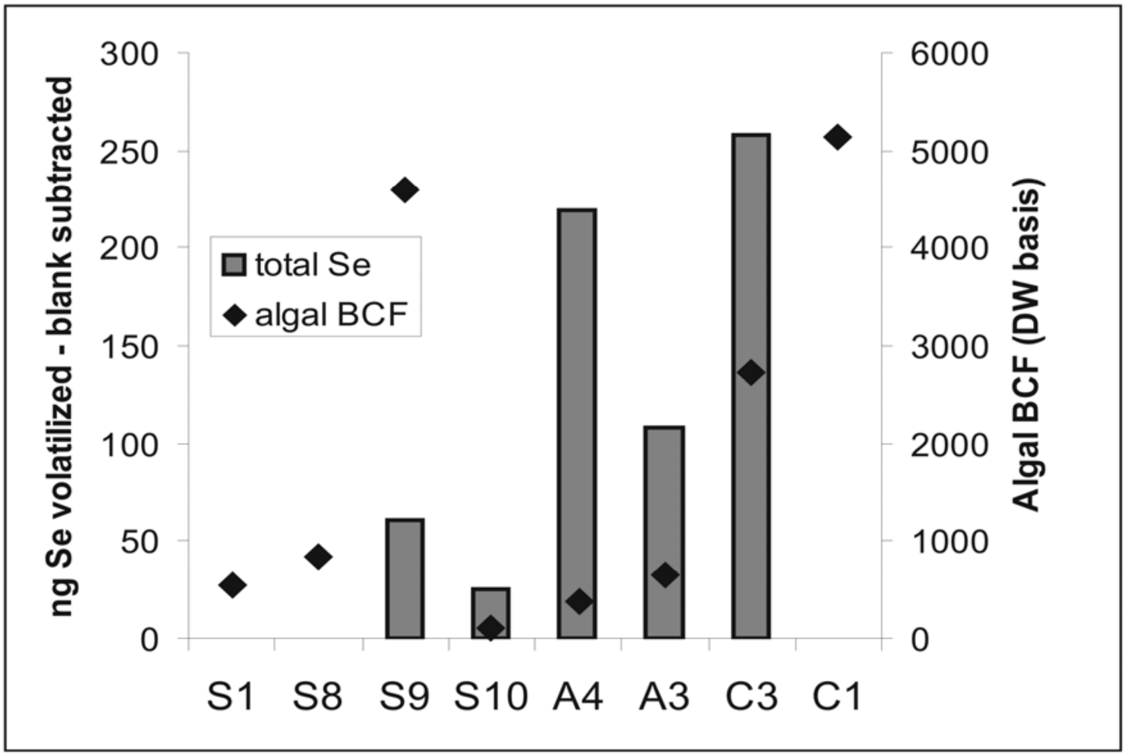


FIGURE 12: Volatile Se content of TLDD basin waters at a downwind location in the morning and in the afternoon in comparison with basin salinity.

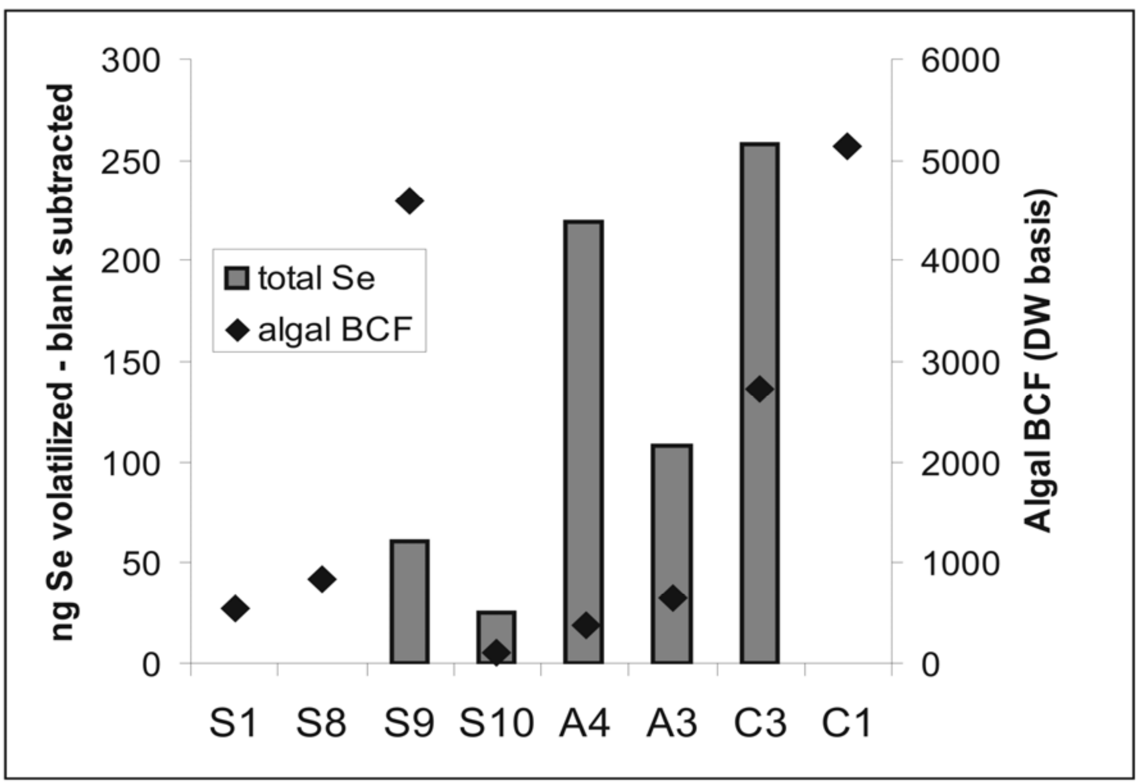


FIGURE 13: Total (am and pm) downwind volatile Se content of TLDD basin waters in comparison with the algal Se bioconcentration factor (BCF, on a dry wt basis).

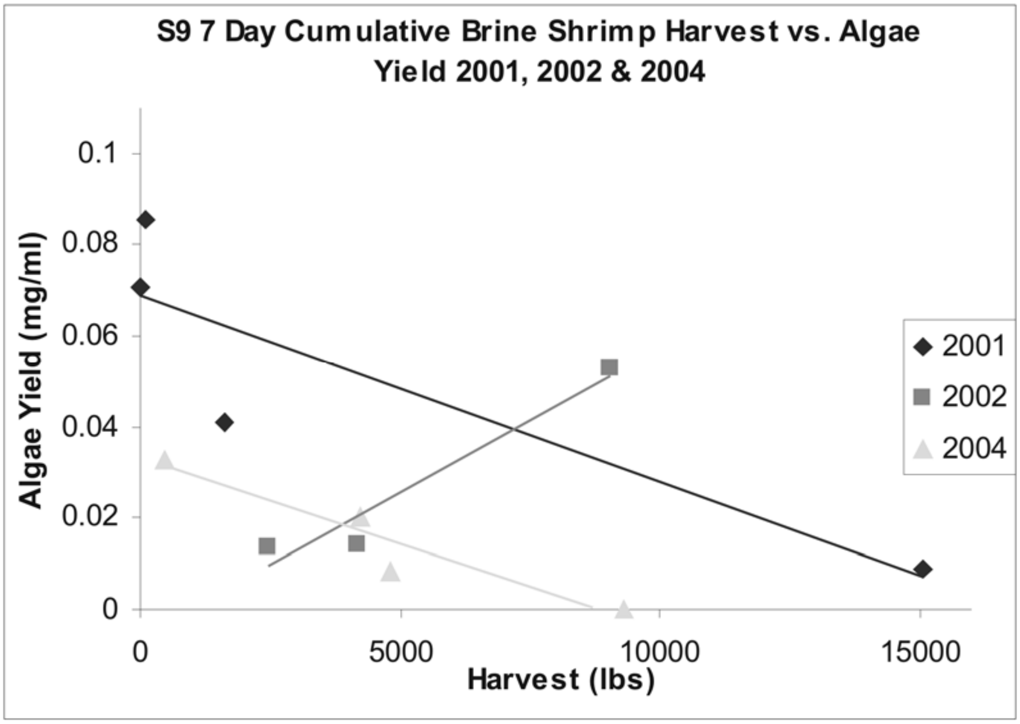


FIGURE 14: Cumulative 7 day brine shrimp harvest versus algae yield for basin S9 in years 2001, 2002 and 2004

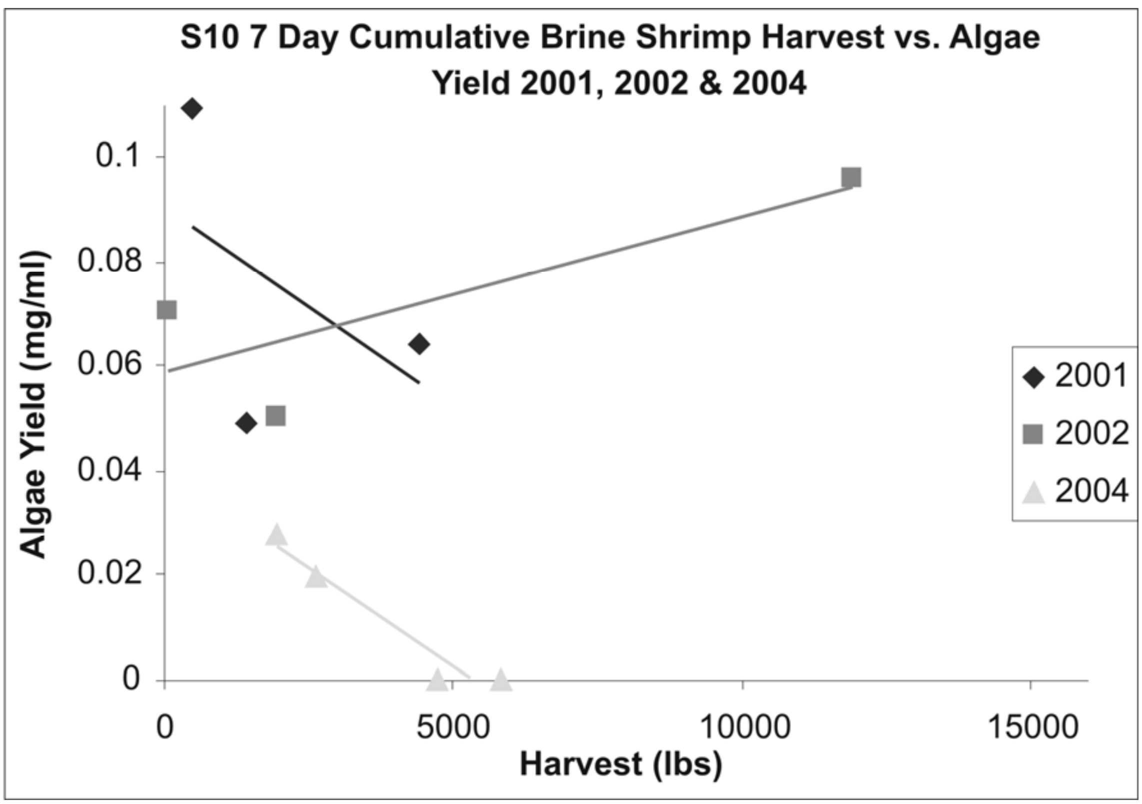


FIGURE 15: Cumulative 7 day brine shrimp harvest versus algae yield for basin S10 in years 2001, 2002 and 2004

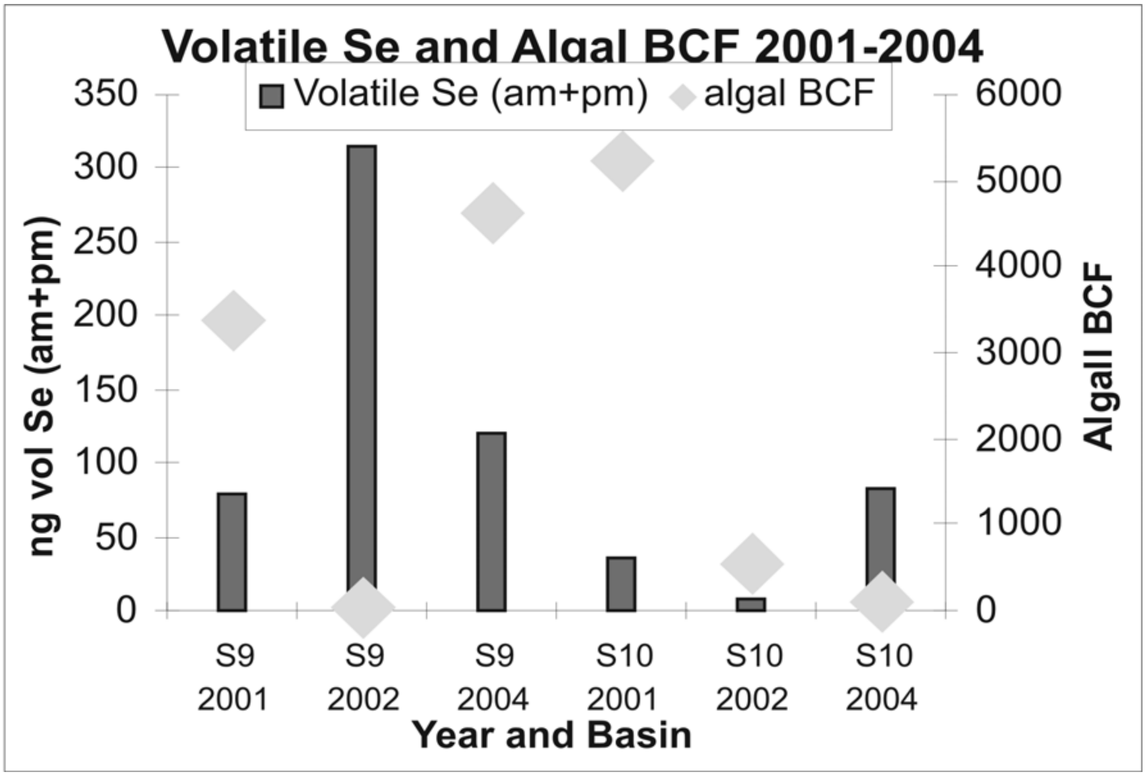
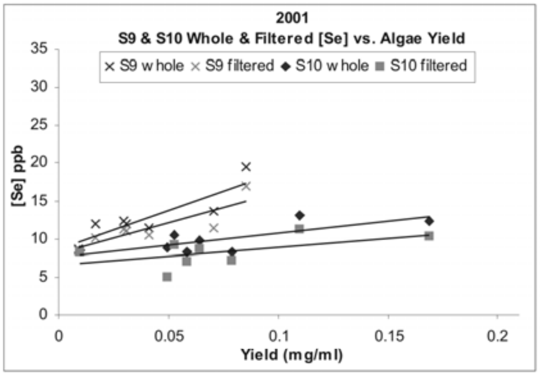
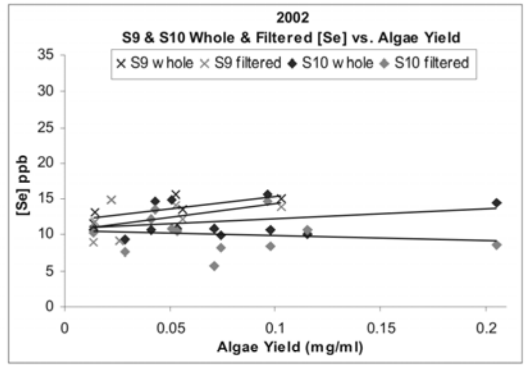


FIGURE 16: Se bioconcentration factors (BCF) for algae and pond water Se volatilization potential derived from annual sampling data for SEB9 and SEB10 in 2001, 2002, and 2004.

A



B



C

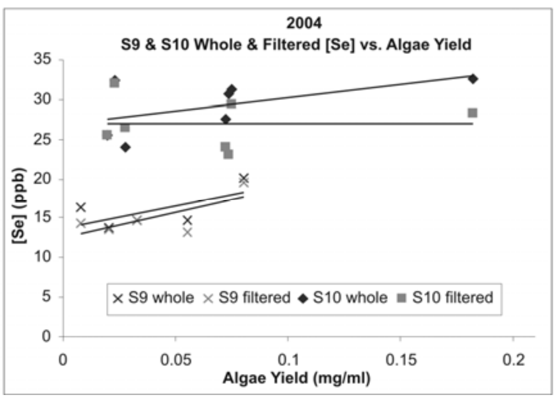


FIGURE 17: Waterborne Se concentrations with (unfiltered) and without (filtered) algae suspended in the water versus algal yield (biomass per volume water).

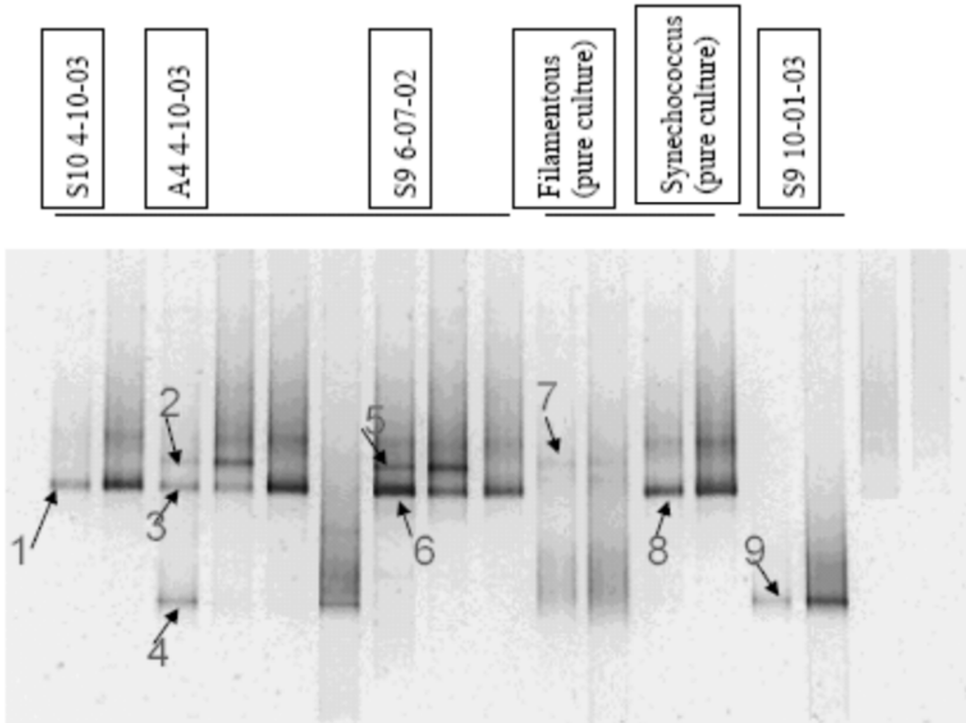


FIGURE 18: DGGE gel used for identification of algal strains through DNA sequencing. Various field and laboratory isolate samples are identified in lanes 1, 3, 7, 10, 12, and 14. Lanes immediately following each of these are further amplified samples of the numbered bands (e.g. band #1 in lane one was further amplified to produce bands in lane 2).

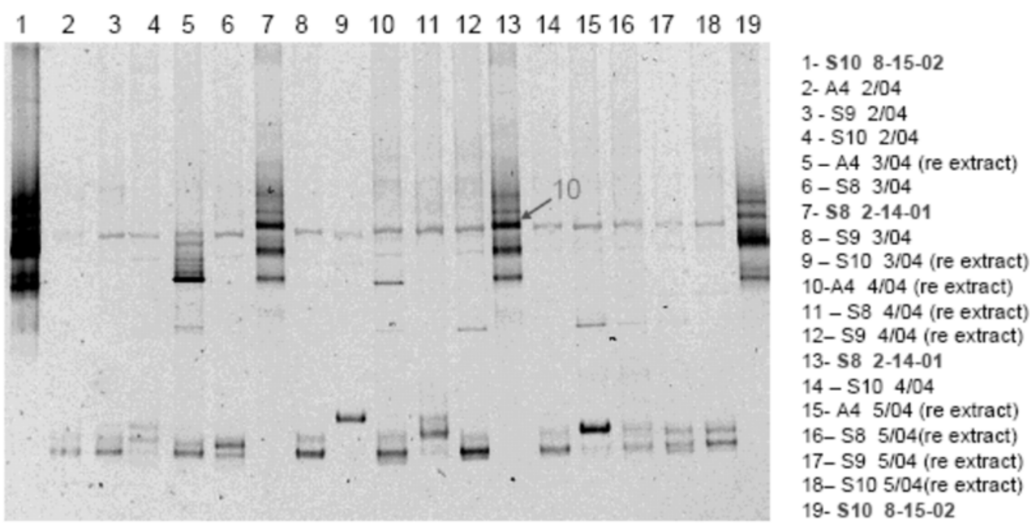
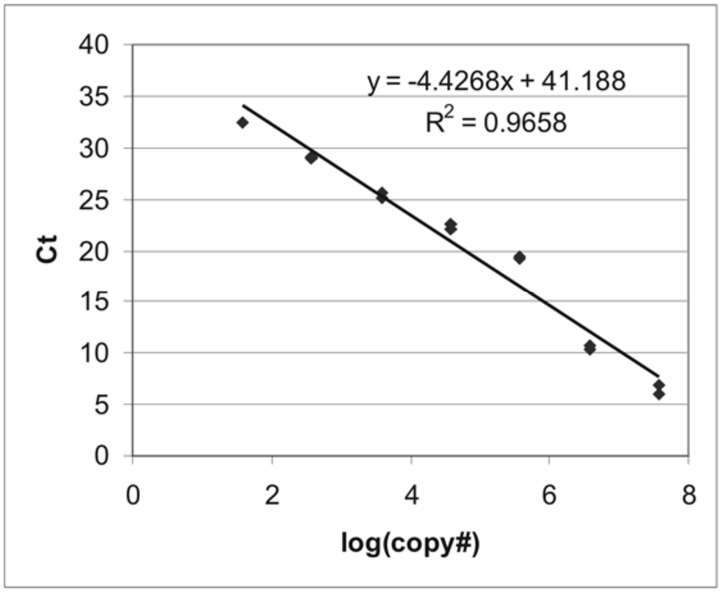


FIGURE 19: DGGE gel of algal microbial community at different TLDD saline ponds sampled 2004 in comparison with samples from previous years.

a)



b)

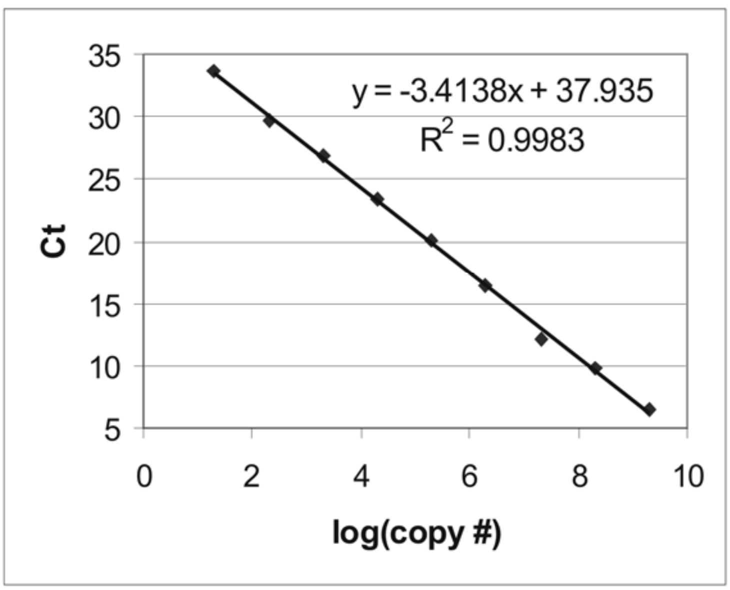


FIGURE 20: Quantitative PCR standard curves for quantification of total micro-eukaryotes (a) and *Synechococcus* algal species (b).

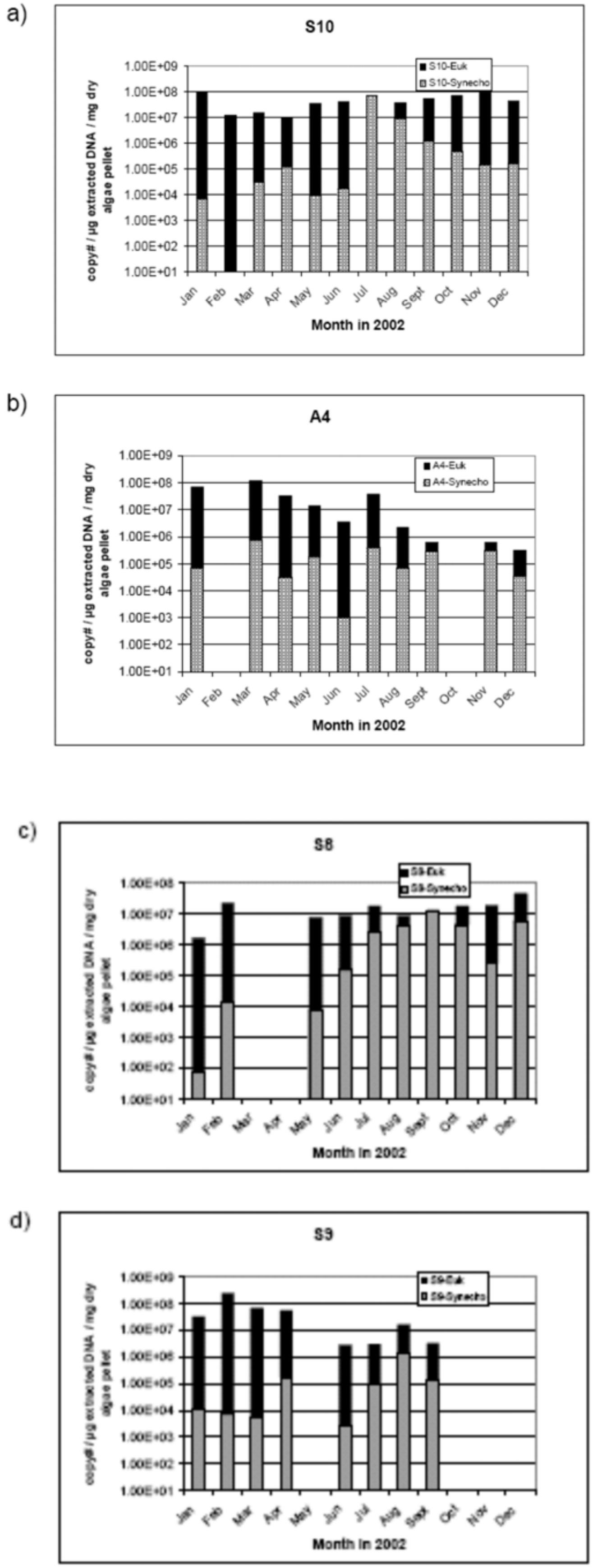


FIGURE 21: PCR quantification of total eukaryotic microbial community and *Synechococcus* algal species at four different hypersaline ponds during year 2002.