NITROGEN AND PHOSPHORUS UPTAKE BY CULTURED MICROBES COLLECTED FROM THE LAS VEGAS WASH AND ASSOCIATED AREAS

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ABSTRACT

In Las Vegas, effluents from wastewater treatment plants are discharged into Lake Mead via Las Vegas Wash (Wash). Nutrients contained in these effluents, especially nitrate and phosphate, are a concern because they could promote algal growth in Lake Mead, the region's primary water supply. To minimize this potential problem, several constructed wetlands have been established in various segments of the Wash as part of an erosion control program. Although these facilities are a consequence of stabilizing the stream channel, there is an expectation that that plants will grow and take up some of the nutrients. A recent report indicates that wetland plants indeed have a positive effect on water quality. The objective of this study is to determine whether algae play a similar role. Surveys indicate that in shallow parts of the Wash macroscopic algae grow to a considerable density in the summer months. In the laboratory, four cultures of microalgae, with one exception, were found to take up nitrate and phosphate from Wash water. Three bacteria isolated from the Wash, as expected, produce nitrate. These results indicate that algae do take up nutrients from the Wash, but the net benefit of this activity cannot be realized unless algal growth is removed from the system. Without removal, as is now the case, algae would ultimately die and decay, releasing the absorbed nutrients back to the Wash.

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1.0 INTRODUCTION

Algae are primitive photosynthetic organisms that grow in all aquatic habitats (van den Hoek et al., 1995). Ecologically, they play the same essential role as plants do on land. Simply put, they are the basis of the food chain that sustains all animal life. As such, they are a critical component of any healthy aquatic ecosystem.

However, algal overgrowth (i.e. algal blooms), could cause environmental problems. At minimum, algal blooms are a nuisance. Biomass decay can quickly deplete oxygen in the hypolimnion, killing fish and other oxygen-requiring aquatic life (Diego-McGlone et al., 2008; Mhlanga et al., 2006). Worse, some algal blooms are toxic. For example, *Microcystis*, a single-celled freshwater cyanobacterium, produces microcystin, a potent heptatoxin (Nishizawa et al., 2001).

Algal blooms in Lake Mead are of particular concern. The reservoir is not only used for recreational activities, it is also a source of drinking water for Las Vegas and downstream users. Currently, the concern is being managed through Total Maximum Daily Load restrictions for phosphorus in discharges from wastewater treatment facilities. These effluents flow into the Las Vegas Wash (hereafter, "Wash") and, ultimately, into Lake Mead. The rationale is to withhold a critical nutrient from algae, thereby preventing blooms. Despite this control effort, a major algal bloom occurred in parts of the lake (Boulder Basin and Virgin Basin) in the spring of 2001. Fortunately, that bloom was caused primarily by a nontoxic alga, *Pyramichlamys disecta*. *Cylindrospermopsis*, a cyanobacterium that produces the toxin cylindrospermopsin, was also present, but only in small numbers. The 2001 incident indicates that Lake Mead was not entirely safe from algal blooms. Consequently, the wastewater dischargers have since been voluntarily removing phosphorus well beyond regulatory requirements. The fact that Lake Mead has not had any algal blooms in the intervening years may be attributable, at least in part, to the stringent control for phosphate.

Since 1998, many erosion control structures (hereafter, "weir") have been built in the Wash to reduce erosion. As a result, wetlands have been established behind each weir. Constructed wetlands are widely considered natural biological filtering systems that degrade or remove organic pollutants (de-Bashan and Bashan 2004). They are also expected to remove inorganic nutrients, such as phosphorus. Therefore, it is reasonable to hypothesize that similarly beneficial functions are performed by the wetlands associated with the Wash. Indeed, these constructed wetlands have by now developed into fully functional ecosystems, consisting of a host of aquatic plants and animal species. Research indicates that wetland plants have a positive influence on water quality (Acharya and Adhikari, 2010).

The objective of this project is to investigate whether algae that live in the Wash and associated wetlands have a similar positive influence on water quality. This study will focus on the extent that the algae could absorb nitrate and phosphorus. Heterotrophic bacteria were also included in the study. Bacterial activity may negate the benefit from the plants and algae, because decomposition could release the absorbed nutrients back into the Wash.

2.0 METHODS.

From September to December 2009, monthly visits were made to the Flamingo Wash near the Desert Research Institute campus, the Wash at Pabco Road Weir, and site 10.75 just below Vegas Valley Drive (Figure 1). At each location, benthic and water samples were collected as grab samples and transported immediately to the laboratory for processing. In the laboratory, they were examined under a microscope, quantified for algal biomass, and cultured on BG-11 and diatom medium (Table 1). Algae that

Table 1. Compositions of algal growth media used in the study.

BG-11 Medium	
$NaNO_3$	150 g/L
K_2HPO_4	4.0 g/L
$MgSO_4.7H_2O$	0.75 g/L
$CaCl_2.2H_2O$	3.6 g/L
Citric acid.H_2O	0.6 g/L
Ammonium Ferric Citrate	0.6 g/L
$Na_2EDTA.2H_2O$	0.1 g/L
Na_2CO_3	2.0 g/L
H_3BO_3	2.86 g/L
$MnCl_2.4H_2O$	1.81 g/L
$ZnSO_4.7H_2O$	0.22 g/L
$Na_2MoO_4.2H_2O$	0.39 g/L
$CuSO_4.5H_2O$	0.079 g/L
$Co(NO_3)_2.6H_2O$	0.0494 g/L
Diatom Medium	
$Ca(NO_3)_2.4H_2O$	20.0 g/L
KH_2PO4	12.40 g/L
$MgSO_4.7H_2$	25.0 g/L
$NaHCO_3$	15.9 g/L
EDTAFeNa	2.25 g/L
$EDTANa_2$	2.25 g/L
H_3BO_3	2.48 g/L
$MnCl_2.4H_2O$	1.39 g/L
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.00 g/L
Cyanocobalamin	0.04 g/L
Thiamine HCl	0.04 g/L
Biotin	0.04 g/L
$NaSiO_3.9H_2O$	57 g/L
Adjust to pH 6.9 with 1M HCl	

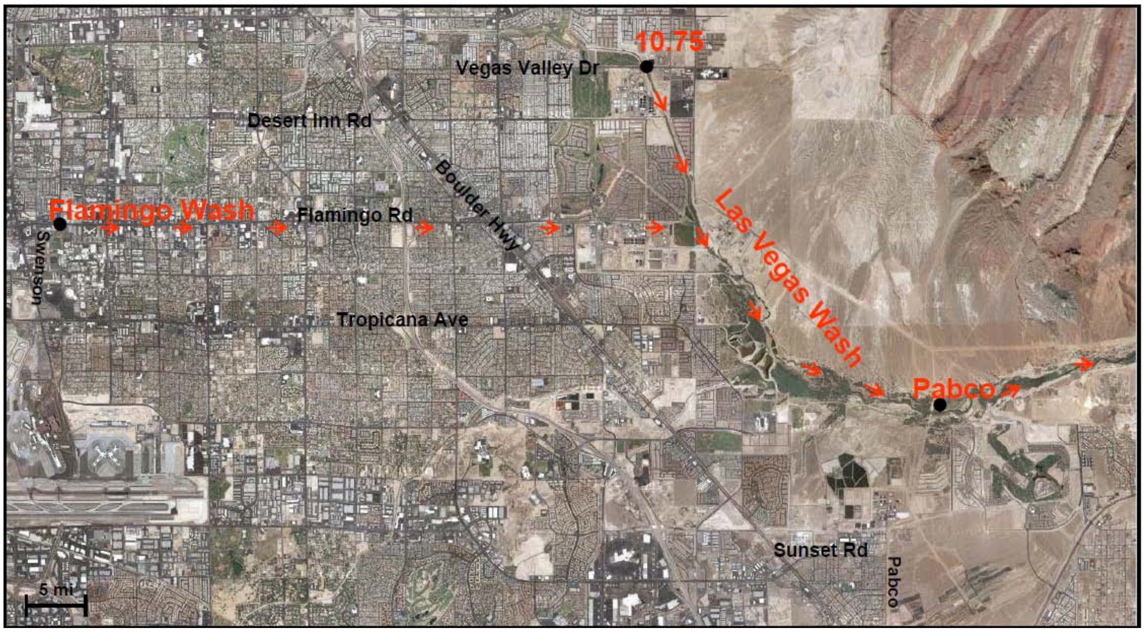

Figure 1. Map showing study locations (black dots) along Flamingo Wash and Las Vegas Wash.

appeared in our cultures were purified to the extent that they were free of bacteria. A few bacteria were also isolated into pure culture. The uptake of nitrate and phosphate from Wash water by these organisms was determined.

Algae in water samples were enumerated under a light microscope using a hemocytometer. Sixteen grids were counted, and the average cell count was calculated. In Flamingo Wash, where the alga was macroscopic, growth from a measured area was collected, air-dried, and weighed.

Nitrate-N was determined by the use of the Hach Nitraver® 5 Nitrate Reagent. The content of one kit was dissolved in 10 mL of Nanopure® water. One milliliter (mL) of the reagent solution was combined with one mL of water sample. After letting it stand for ten minutes, its absorbance at the wavelength of 423 nm was determined in a spectrophotometer. As shown in Figure 2, the relationship between nitrate-N and absorbance is linear in the range of nitrate concentrations from 0 to 20 mg N per Liter.

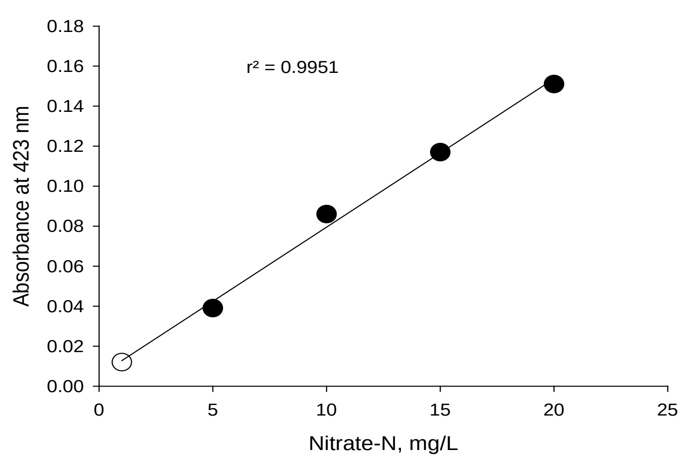

Figure 2. Calibration curve for nitrate-N assay.

3.0 RESULTS AND DISCUSSION

3.1 Algae Survey

Flamingo Wash. In September, 2010, this tributary was taken over by the macroscopic green alga *Cladophora* sp. (Figure 3). This organism is cosmopolitan. Its dominance in Flamingo Wash may be due to a combination of slow flow and shallow water depth.

Figure 3. Light micrograph of *Cladophora* sp. from Flamingo Wash. Filament is about 8 microns wide.

To evaluate standing biomass of *Cladophora* sp., material was harvested from an area of 9.29 m^2 (100 ft^2) of the Wash (Figure 4). This yielded a total of 9 kg (20 lb) dry algal biomass. The cleared area appeared to recover to its previous extent in two weeks. This results in an anecdotal growth rate of about 69 grams (2.4 ounces) of dry weight per square meter per day.

Las Vegas Wash. The number of algae in the center of the flow was below detection. No algae were found even after the sample was concentrated one thousand times by centrifugation. Thus, planktonic algae, if present, are below one cell per liter.

Benthic algae were observed on the edge of the Wash where the water was stagnant (Figure 5, green arrow). Thick films of filamentous diatoms were also observed on the Pabco Road Weir (Figure 5, red arrow). The algae observed from these habitats are listed in Table 2. The dominant diatom species on the weir is *Stephanodiscus* sp. A scanning electron micrograph of this organism is shown in Figure 6.

Figure 4. Top: *Cladophora* sp. bloom in Flamingo Wash. Bottom: quantifying algal growth rate.

Table 2. Observed benthic algae represented in Las Vegas Wash at Pabco Road Weir and an estimate of their relative abundance.

Organisms	Abundance*
Green Algae	
Chlorella sp.	+
Dictyococcus varians	+
Rhizoclonium hieroglyphicum	++
Spirogyra sp.	+++
Cyanobacteria	
Oscillatoria sp.	+++
Diatoms	
Gomphonema sp.	++
Stephanodiscus sp.	+++

*Note: +++ dominant, ++ intermediate abundance, + relative rare

Figure 5. Las Vegas Wash at Pabco Road Weir, showing benthic algae growing on concrete bottom near shore (green arrow) and on weir (red arrow).

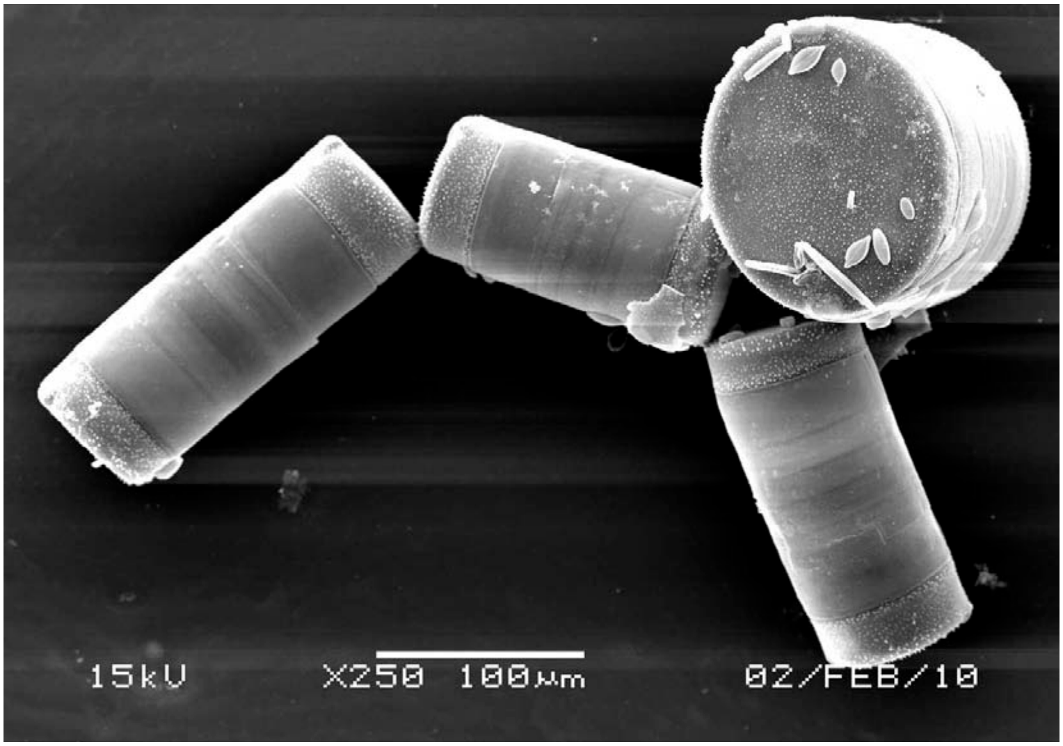

Figure 6. Scanning electron micrograph of diatom *Stephanodiscus* sp. from Pabco Road Weir shown in Figure 5.

LW10.75. Water here, like water further downstream, is essentially free of planktonic algae. Algae grow mainly on the concrete bottom of the Wash in the form of benthic biofilm. Microscopic examination revealed a mixture of single celled green algae and diatoms (Figure 7 and Table 3).

Figure 7. Las Vegas Wash at site 10.75. A mixture of diatoms and green algae grow on concrete bottom.

Table 3. Observed algae represented at site 10.75 and an estimate of their relative abundance.

Organisms	Abundance*
Cyanobacteria	
Chlorogloea epiphytica	++
Chroococcus turgidus	++
Merismopedia glauca	+++
Oscillatoria sp.	+
Diatoms	
Fragilaria crotonensis	+
Gomphonema sp.	+
Unidentified diatom	+

*Note: +++ dominant, ++ intermediate abundance, + relative rare

3.2 Algae Cultured

Only some of the algae observed in the study sites could be cultured (Figure 8). These, listed below (Table 4), were used in nutrient uptake experiments.

Table 4. Algae cultured from the Wash and associated wetlands. These were used to assess nitrate and phosphate availability of Las Vegas Wash water.

Green Algae
Chlorella sp.
Cyanobacteria
Oscillatoria sp.
Diatoms
Fragilaria crotonensis
Gomphonema sp.
Unidentified diatom

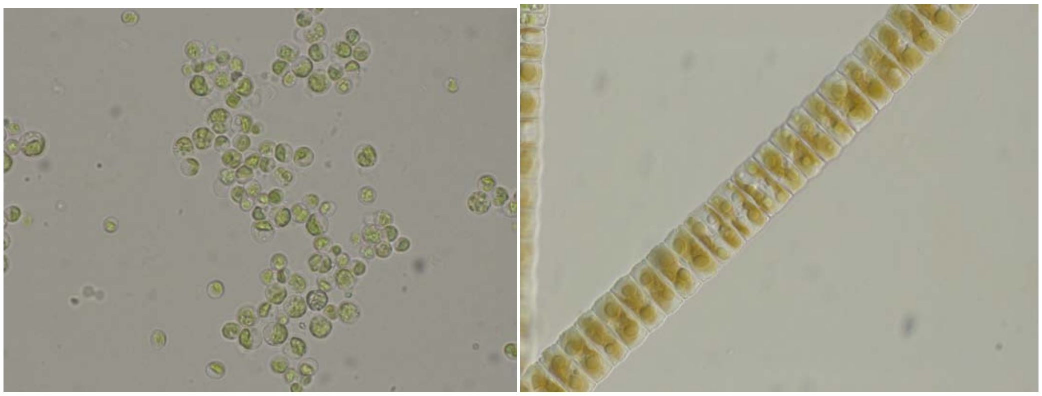

Figure 8. Light micrographs of *Chlorella* sp. (left) and of an unidentified diatom (right) cultured from Las Vegas Wash and associated wetlands. *Chlorella* cells are about 5 microns in diameter. Diatom filament is 8 microns wide.

3.3 Nitrate Bioavailability

Four of the algae listed in Table 4 were studied to see if they can take up nitrate from Wash water. Three bacteria were studied to see if they produce nitrate by oxidizing ammonia available in Wash water. Briefly, the study organism was raised in appropriate medium. The population was collected by centrifugation, washed in sterile Wash water, and suspended in sterile Wash water. Wash water, collected at the Pabco Road Weir, was sterilized either by filtering or autoclaving. The latter resulted in a slight loss of nitrate-N. The culture was incubated with continuous shaking. For algae, illumination was provided with fluorescent lights at an intensity of about 1,000 lux or 13.5 mole m^{-2} s^{-2} (daily solar radiation maximum in Las Vegas is about 75,000 lux). The bacteria were incubated in the dark. At regular time intervals, the cultures were sampled. The samples were centrifuged to remove cells and particulates. The amount of nitrate-N remaining in Wash water was determined.

The results for the algae experiments are shown in Figure 9. In the presence of the algae the amount of nitrate-N in the Wash water medium decreased. In the case of *Gomphonema* sp., it decreased from 10 mg/L to 1 mg/L. In the case of the unidentified diatom and *Fragilaria crotonensis*, nitrate-N decreased from 18 mg/L to 7 mg/L (ppm). After reaching these values, no further decline was observed. The absorbed nitrate may or may not stay absorbed. In diatom *Fragilaria crotonensis*, the level of nitrate-N stayed at 7 mg/L (ppm). In diatom *Gomphonema* sp. and the unidentified diatom, further incubation resulted in nitrate-N release, almost restoring its concentration to the initial level. This is presumably due to cell death and lysis, something that inevitably occurs to algae whether they grow in culture or nature.

The result for the experiment with the cyanobacterium *Oscillatoria* sp. is shown in Figure 10. This result is similar to those for *Gomphonema* sp. and for the unidentified diatom. Initially, there was rapid nitrate uptake. This was followed by slow, gradual release of nitrate.

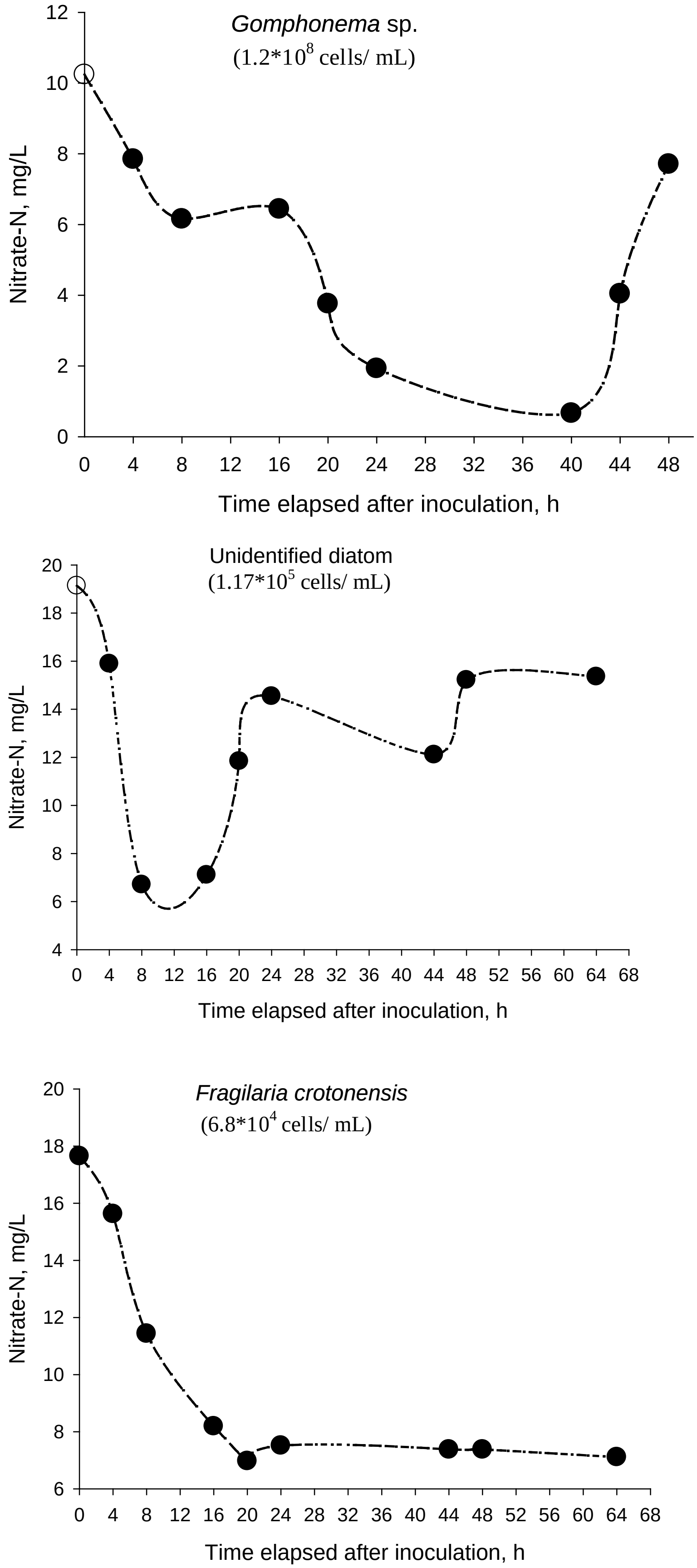

Figure 9. Wash water nitrate dynamics in presence of diatoms.

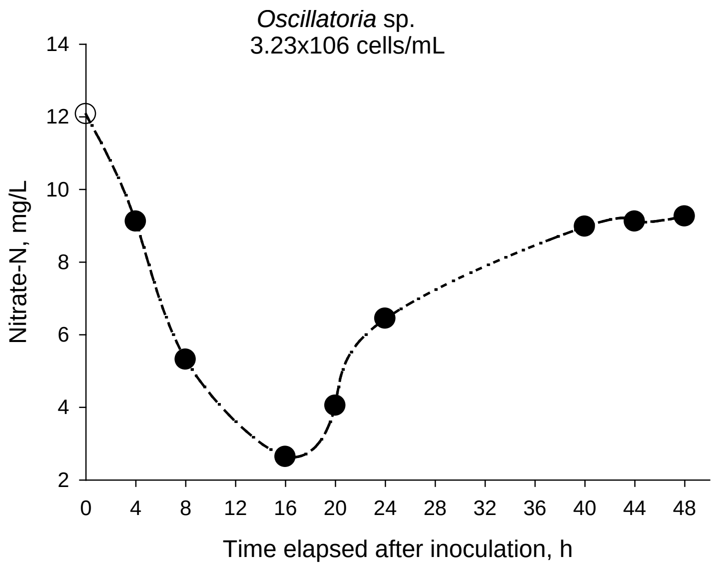

Figure 10. Wash water nitrate dynamics in presence of cyanobacterium *Oscillatoria* sp.

The bacteria were studied to determine how their activities would change nitrate concentrations in Wash water. The results for those experiments are shown in Figure 11. In the cultures that contained bacterium #1 and #2, the concentration of nitrate increased, from 14 mg/L to around 45 mg/L. This is probably evidence for nitrification activities. Presumably, the bacteria take up organic nitrogen, deaminate it (removing the amine group), and oxidize the carbon skeleton for energy. Ammonia, the product of deamination, is oxidized to nitrate.

Nitrate in the culture of bacterium #3 decreased, not increased. This is not entirely unexpected. The range of organic compounds utilized by bacteria can vary widely from species to species. Bacterium #3 may be growing on carbohydrates. Because carbohydrates do not contain nitrogen, nitrate would be taken up as a nitrogen source.

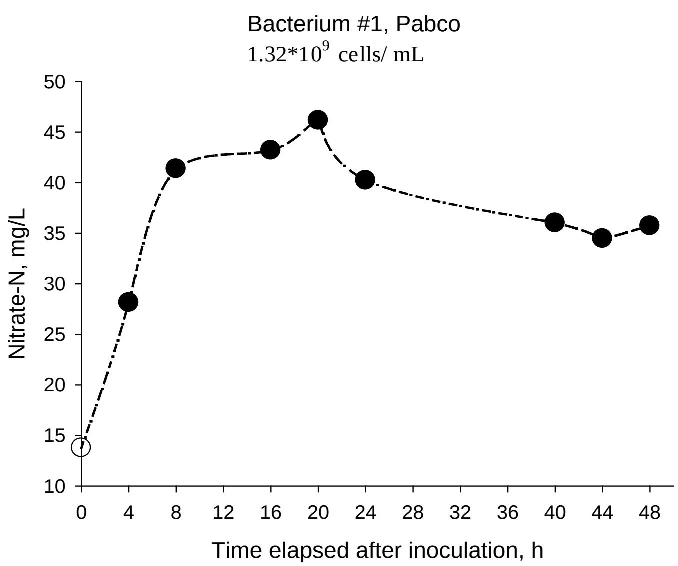

Figure 11. Wash water nitrate dynamics in presence of bacteria (continued on page 12).

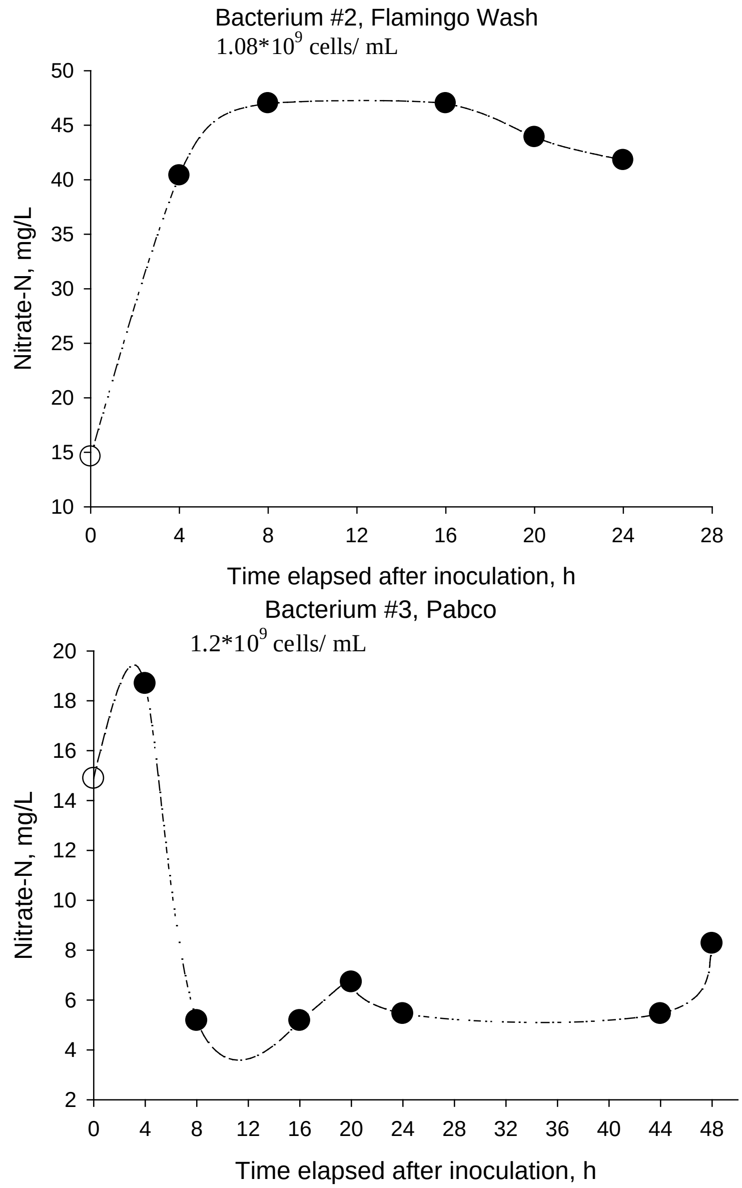

Figure 11. Wash water nitrate dynamics in presence of bacteria.

3.4 Phosphate Bioavailability

The fact that algae grow in the Wash suggests the presence of algae-available phosphate. However, an uptake study similar to those done with nitrate is technically more challenging. Because there were limited resources available to complete this study, there were no routine phosphate assays that were of sufficient resolution (sub-ppm).

To determine if the phosphate level is limiting, we took the green algal isolate *Chlorella* sp. and grew it in Wash water supplemented with various amounts of phosphate. The results are shown in Figure 12. It is evident that the amount of phosphate in Wash water is growth-limiting. With increases in available phosphate, the specific growth rate steadily increased until reaching a saturating concentration at 20 ppm. To the extent the study alga is representative of those in Lake Mead, this result suggests that the emission standard for wastewater treatment facilities with respect to phosphate in their effluents is a sound strategy for preventing algal blooms in Lake Mead.

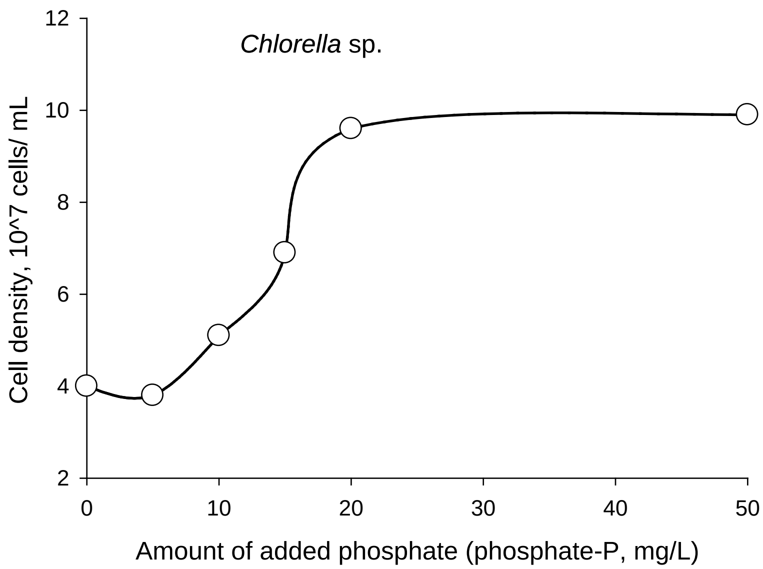

Figure 12. Algal growth rate as a function of phosphate supplement in Wash water.

4.0 RECOMMENDATIONS

Given the limited scope and preliminary nature of the study, caution should be exercised in basing management policy decisions on the data presented here. Nevertheless, some general conclusions are warranted. First, algae growing in the Wash and associated wetlands apparently have the potential to influence water quality. By absorbing the nutrients upstream, they could potentially reduce nutrient flux to Lake Mead. However, this benefit is realized only under certain conditions. For instance, the shallow, calm stream in the Flamingo Wash is ideal for abundant algal growth. In contrast, the deep, fast flow of the Wash is not suitable for algae except in localized niches. We don't know the factors responsible for the general absence of algae in the Wash.

Second, even in the Flamingo Wash, algae may not have a net benefit unless the biomass is removed from the system. Loss of nitrate-N can occur through denitrification activity. But phosphorus has no volatile forms. The only way for it to leave the system is physical removal. If, as currently is the case, algae are left in the Wash, they ultimately decompose, releasing the absorbed nutrients back to the environment. The situation is similar to wetland plants. If not harvested, they would ultimately decay, becoming a source of nutrients. There are several ways to create incentives for harvesting algae from the Flamingo Wash and possibly from the Wash associated wetlands. Algal biomass is a material in demand for biofuel production (Demirbas, 2008). Future studies should look into the biofuel potential of these algae. Another way to utilize algae is as organic fertilizers for horticulture and agriculture. Soils fertilized with composts are less susceptible to leaching than soils fertilized with chemicals (Steiner et al., 2008), which would reduce nutrient input to Lake Mead from non-point sources. Such waste-recycling practices could contribute to a sustainable economy in southern Nevada if they were conducted at a substantial scale.

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