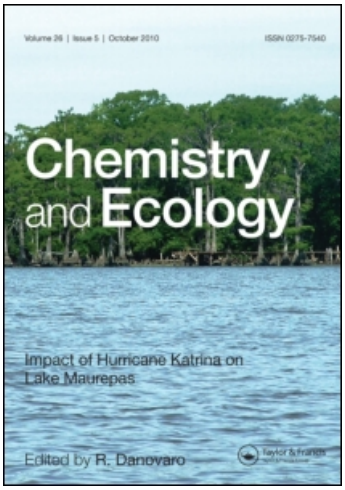

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Effect of the direct discharge of reverse-osmosis effluent on the microbiology of a natural surface-water system

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The abundance of micro-organisms (dinoflagellates and euglena) and the concentrations of sodium, potassium and chloride ions were monitored in the Big Wichita River prior to the opening of a reverse-osmosis (RO) treatment facility. While providing access to surface water sources containing high levels of ions, the facility will directly discharge effluent containing an estimated 5200 mg l^{-1} Cl^- into the river. Natural fluctuations in ion concentrations and the level of micro-organisms in the river were monitored over a 9-week period. To predict the effect on micro-organisms when RO effluent is discharged into the river, water samples were spiked with salts in the laboratory. Increasing K^+ up to ten times its natural level had no effect on dinoflagellate and euglena levels. Increasing Cl^- and Na^+ to 2.3 and 3.1 times the natural concentrations, respectively, led to statistically significant decreases in the concentration of these micro-organisms.

Keywords: Reverse osmosis; Sodium; Potassium; Chloride; Dinoflagellates; Euglena

1. Introduction

According to the United Nations, an estimated 1.1 billion persons worldwide do not have access to sufficient volumes of potable drinking-water from freshwater sources [1]. One factor leading to decreasing levels of available freshwater, particularly on a regional level, is drought. This is the primary limiting factor in the North Texas and Southern Oklahoma regions surrounding Wichita Falls, Texas, USA. Since 1946, the average annual mean stream flow for the Little Wichita River, which feeds the primary drinking-water reservoirs (Lakes Arrowhead and Kickapoo) for the city of Wichita Falls, has been 1.399 m^3 s^{-1}. However, over the 5 yr period from 1998 to 2002, a lower-than-average annual rainfall caused a decrease in the annual mean stream flow to 0.745 m^3 s^{-1}. The 52.3% decrease in stream flow has led to concurrent lowering of water levels in the reservoirs. In 18 out of 57 months from 1998 to 2002, the combined volume in the reservoirs fell below 50% of their capacity (4.54×10^{11} l) and has fallen as low as 34.5%. The city has access to two additional reservoirs on the Big Wichita River, Lakes Kemp and Diversion with an available capacity for drinking-water usage of 3.94×10^{11} l. Like

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the Little Wichita River, a large decrease in average annual stream flow is observed when comparing the historical average (1939–2002) to that of the 5 yr period from 1998 to 2002, 7.257 and 3.806 $m^3 s^{-1}$, respectively. Despite the 49.5% decrease in stream flow for the Big Wichita River, its higher overall flow compared with the Little Wichita River represents a significant water resource. Unfortunately, chloride concentrations in the Kemp/Diversion reservoirs are much higher than in the Arrowhead/Kickapoo reservoirs, 1101–1178 and 58–116 mg l^{-1} Cl^-, respectively [2].

In order to effectively access the higher salinity water from the Big Wichita River, advanced desalination techniques must be employed. The United Nations estimate that there are 12,500 desalination facilities worldwide using either distillation or reverse-osmosis (RO) treatment techniques [1]. Since the first US desalination facility began producing drinking-water in Buckeye, Arizona in 1962, more than 168 such US facilities have opened [3]. Of these facilities, most employ RO treatment methods and are located primarily in Florida or southwestern states [4]. The local publicly owned treatment works is currently constructing a RO treatment facility to access the higher salinity water source, easing concerns about the availability of drinking-water. The completion of the Wichita Falls facility, originally scheduled for July 2002, is currently scheduled for late 2005. Following completion, the facility will process 3.78–5.30 $\times$ 10^7 l d^{-1} of high saline water and discharge 0.78–1.14 $\times$ 10^7 l d^{-1} of effluent containing an estimated 5200 mg l^{-1} Cl^- directly into the Big Wichita River 77.6 km downstream from the initial collection point at Lake Diversion. Discharge of high salinity effluent is expected to increase the salinity of the Big Wichita River at least near the discharge site. A likely consequence of increasing salinity is a decrease in the viability of micro-organisms in the surface water system. Changes in the number and type of micro-organisms have been reported due changes in salinity, both in the laboratory and in natural systems [5–15]. Most of these studies involve the effect of natural changes in salinity due to intrusion of freshwater river systems into marine estuaries on micro-organisms.

In this work, pH and concentrations of chloride, potassium, and sodium ions in the Big Wichita River bracketing the expected effluent discharge site were monitored by potentiometry using ion-selective electrodes (ISE). The application of ISE for environmental monitoring has been successful and cost-effective [16, 17]. The abundance of micro-organisms, in this case dinoflagellates and euglena, was determined by counting under magnification [18]. The effect of increasing concentrations of salts containing chloride, potassium, and sodium ions on dinoflagellates and euglena in the river water was examined in the laboratory as a predictor of the effect of RO effluent discharge on the natural water system. When the RO treatment facility opens, the effect of its discharge on these micro-organisms in the river will be examined.

2. Materials and methods

2.1 *Site selection and sample collection*

Summer months in this region typically have the lowest rainfall leading to the lowest flow rates for the Big Wichita River and the highest demands on the municipal water system. These two trends lead to the largest expected output of RO effluent during the lowest discharge rates for the river. Therefore, the largest changes in salinity would be expected during the interval of this study (May–August). The 179 km lower segment Big Wichita River downstream from the Lake Diversion dam flows primarily west to east ultimately emptying into the Red River. Three sites on the Big Wichita River, located at convenient road bridges, were surveyed. Site 1 was 6.1 km upstream from the proposed effluent discharge site, site 2 was at the discharge location, and site 3 was 8.0 km downstream. Site 3 was split into two channels by a mid-channel bar

(3-north and 3-south). Initially, four sampling points were selected laterally across the river at each site based on the equal discharge increment method (EDI) outlined by the United States Geological Survey [19]. Initial monitoring of ions in the river showed no statistical difference between ion concentrations in the four discharge increments at a single site. Therefore, one location was selected for sample collection at each site for the final 9-week study. The incremental and total discharges were calculated after measuring the river depth and flow rate at 3 foot (0.9 m) intervals across the river for each site. The lateral positions (9.9, 6.1, 5.6, and 2.1 m at sites 1, 2, 3n, and 3s, respectively) corresponding to 50% of the total discharge at each site were selected for sample collection. The process is illustrated for the effluent discharge site (site 2) in figure 1. While the exact discharges varied, the lateral sampling positions at each site did not change significantly over the course of the study. Water samples were collected by lowering Science Source 1.5 l student water samplers (Forestry Suppliers, Inc., Jackson, MI) from bridges at each sampling site at the lateral positions determined above until the samplers were submerged. Following submersion, the trigger mechanism was released, sealing the sample into the chamber. Sampling always took place on the upstream side of the bridge to limit changes in the samples due to turbulent water flow around the bridge. During the final phase of this study, samples were collected from each site within 60 min on 15 separate days.

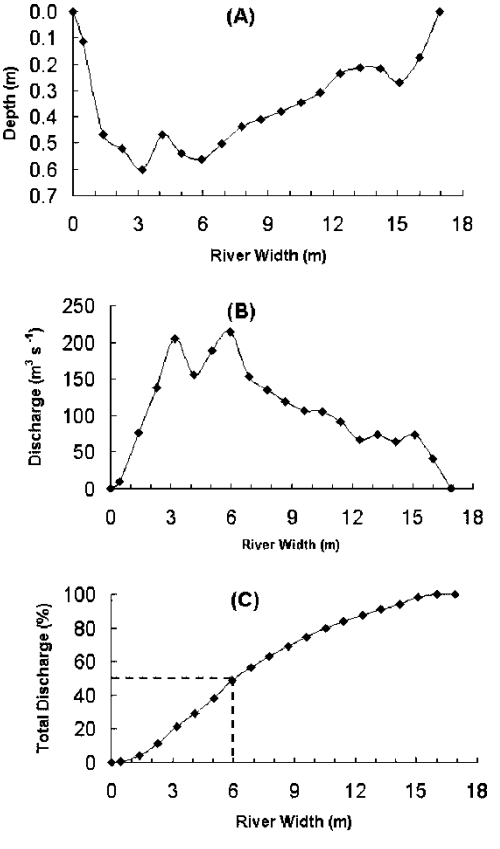

Figure 1. Hydrogeologic data for site 2: the effluent discharge point. (A) River depth profile; (B) discharge; (C) total discharge.

2.2 *Analytical methods*

Following collection, each sample was transferred to a clean, dry, liter polystyrene bottle and returned to the laboratory for analysis. Samples not used for the counting of micro-organisms were refrigerated until chemical analysis was completed. The pH and the concentration of chloride, potassium, and sodium ions in river water samples were determined by potentiometry using ion-selective electrodes connected to an Orion Model 720A pH/ISE meter (Orion Research Inc., Boston, MA). For the measurement of pH, a model 91-02 glass membrane combination electrode (Orion Research Inc.) was employed following calibration with commercial pH 7.00 and pH 10.00 buffers (Ricca Chemical Company, Arlington, TX) in the pH mode. The remaining ISE measurements were completed in the potential mode and compared with standard solutions of the respective ions. Appropriate ionic strength adjustment buffers were added to all standard solutions and river water samples prior to analysis, and all solutions were stirred at a constant rate using a magnetic stirrer. Ion-selective electrodes employed in this work were the chloride (pHoenix Electrode Company, Houston, TX), potassium (Denver Instrument, Denver, CO), and sodium ISEs (Weiss Electrodes, Austin, TX).

2.3 *Micro-organism determination*

The total number of dinoflagellates and euglena in individual samples was determined by counting under 100× magnification using compound microscope (Model 60, American Optical Company). Each sample was shaken to homogenize. A 1.00 ml aliquot was immediately removed and transferred to the well of a gridded (1 mm^2) Sedgewick-Rafter counting slide (model 1801-G20, Wildlife Supply Company, Buffalo, NY). A cover slip was immediately applied to the slide to limit evaporation. Samples were allowed to settle for 10 min prior to counting. To determine the effect of increasing concentrations of Na^+, K^+, and Cl^-, their salts were added to fresh river water samples at different concentrations and micro-organisms were counted after 30 min.

3. Results and discussion

3.1 *Effect of natural fluctuations in ion concentrations*

The concentrations Na^+, and Cl^- were monitored at four equal discharge locations for each site over a 6 month period. Samples were collected at a rate of approximately one site per week. Each site was sampled five times during the initial monitoring. Differences between sites and, particularly, between the lateral sampling locations at each site were compared. Typically, differences of less than 6.5% and 3.8% were observed between lateral sampling locations at each site for the concentration of Cl^- and Na^+, respectively. The most pronounced differences occurred between samples from the north and south channels at site 3. Changes in temperature and pH were less pronounced laterally across the river than changes in Na^+ and Cl^- concentrations. While concentrations of Na^+ and Cl^- had limited variability laterally across the river, they varied much more widely with time. Chloride concentrations ranged from 987 mg l^{-1} Cl^- during the second month of the study to 1913 mg l^{-1} Cl^- near the end of the initial monitoring period at site 1. Similar variations were observed in Na^+ concentrations, ranging from 487 to 1477 mg l^{-1} Na^+ during the same monitoring periods as the chloride, also at site 1.

During the final stage of the study when monitoring the total abundance of dinoflagellates and euglena, the single sampling points described above were monitored at sites 1 and 2 and

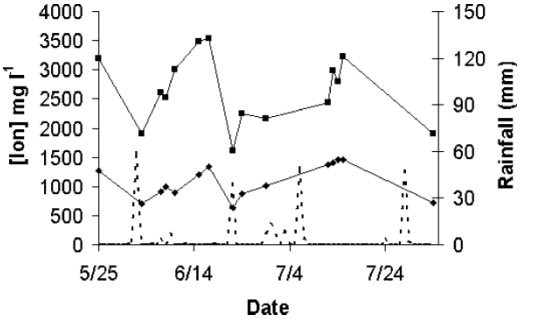

Figure 2. Daily average concentration of Na^+ (♦, mg l^{-1} Na) and Cl^- (■, mg l^{-1} Cl) compared to rainfall (- - -, mm).

in each channel at site 3 (north and south) to facilitate more rapid data analysis. During the final monitoring program, 15 samples were obtained from each site over a 71 d period. The concentrations of Cl^-, Na^+, and K^+, and the pH were determined for each sample. The last seven samples for each site were also used to determine the total number of dinoflagellates and euglena in the water. The chemical analyses for individual samples had less than 1% relative standard deviations. To compare ion concentrations over time, daily averages for each ion in the river were calculated by combining data from each of the four sampling sites. The average daily concentrations for Na^+ and Cl^- are shown in figure 2, while those of K^+ are shown in figure 3. Table 1 demonstrates typical chemical analysis results for one day, in this case, 12 July. The average concentration of each ion at each site over the 9 week period was determined from chemical analyses of the fifteen individual samples from each site (table 2). The grand average of each ion concentration was calculated from all 60 samples. The standard deviations and resulting confidence limits were significantly larger when the average daily ion concentrations in the river and the average ion concentrations for one site over time were calculated.

Variation in pH was limited over the course of the study for single sites, daily river averages, and long-term averages at each site. The average pH for sites over the course of the monitoring period showed no statistically significant differences (table 2), and daily averages ranged from 7.3 to 8.7. As a general trend, the pH increased over the monitoring period, with four of the highest pH values measured during the last five sampling days. The average K^+, Na^+, and Cl^- concentrations for each site were not statistically different over the course of the study (table 2). The average daily sodium and chloride concentrations ranged from 630 to 1450 mg l^{-1} Na^+

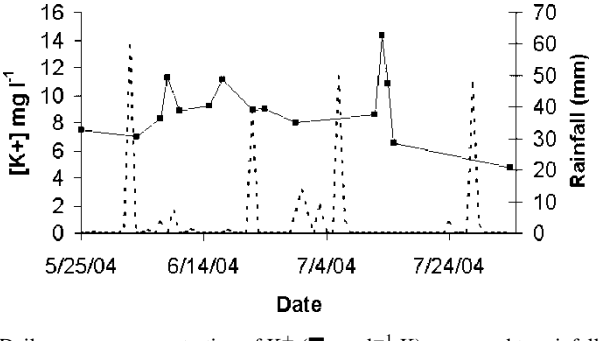

Figure 3. Daily average concentration of K^+ (■, mg l^{-1} K) compared to rainfall (- - -, mm).

Table 1. Results and daily average of chemical and microbial analyses for all sampling sites on 12 July 2004.

Site	pH	$[Cl^-]$ ($mg\,l^{-1}$)	$[Na^+]$ ($mg\,l^{-1}$)	$[K^+]$ ($mg\,l^{-1}$)	[Micro-organism] (micro-organisms ml^{-1})
1	8.24	3037	1476	8.8	2400
2	8.24	2570	1393	8.9	3700
3n	8.46	2044	1333	10.1	3600
3s	8.36	2074	1266	6.7	1900
Average ±95% CL	8.3 ± 0.2	2400 ± 700	1400 ± 100	9 ± 2	3000 ± 1000

Table 2. Results of chemical and microbial analyses at each sampling site over 9 weeks and the grand average of all samples.

Site	pH	$[Cl^-]$ ($mg\,l^{-1}$)	$[Na^+]$ ($mg\,l^{-1}$)	$[K^+]$ ($mg\,l^{-1}$)	[Micro-organism] (micro-organisms ml^{-1})
1	8.1 ± 0.2	2800 ± 300	1140 ± 150	9 ± 2	2300 ± 1100
2	8.1 ± 0.3	2800 ± 300	1130 ± 150	9.0 ± 1.5	2300 ± 1100
3n	8.1 ± 0.3	2500 ± 400	1030 ± 190	8.9 ± 1.5	2000 ± 800
3s	8.0 ± 0.2	2500 ± 400	1040 ± 190	8.4 ± 1.4	1400 ± 300
Grand average ±95% CL	8.1 ± 1.0	2600 ± 1500	1100 ± 700	9 ± 7	2000 ± 3000

and from 1610 to 3540 $mg\,l^{-1}$ Cl^-, respectively. These are summarized and compared with rainfall levels [20] in figure 2. Average daily potassium concentrations ranged from 4.7 to 14.3 $mg\,l^{-1}$ K^+. These are summarized and compared with rainfall levels in figure 3. Changes in chloride and sodium concentration are related to the level of rainfall preceding sampling. The two lowest values were recorded within 24 h following significant rainfall events, while higher values were observed when sampling occurred following several days of dry weather. These trends in sodium and chloride concentrations are expected as rainwater dilutes the high-salinity groundwater that is the primary source of the Big Wichita River. Potassium ion concentrations, however, are not related directly to rainfall, as dilution is not observed following significant rainfall events, and spikes in K^+ concentration occur when no rainfall events were recorded. It is likely that both high-salinity ground water and runoff contribute significantly to K^+ levels in the river.

The average daily concentration of micro-organisms ranged from 840 to 2900 micro-organisms ml^{-1} (figure 4). Generally, the level of micro-organisms appears to follow changes in the concentrations of sodium, potassium, and chloride ions (figure 5). One striking exception, however, is the sample taken on 13 July, where the number of micro-organisms decreases

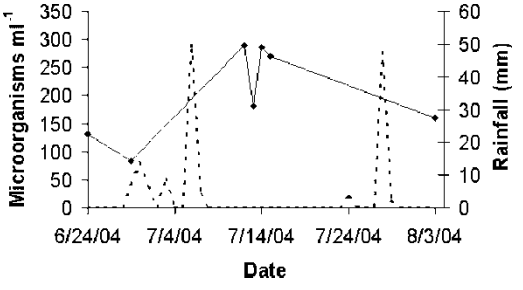

Figure 4. Daily average concentration of dinoflagellates and euglena (♦, microorganisms ml^{-1}) compared to rainfall (- - -, mm).

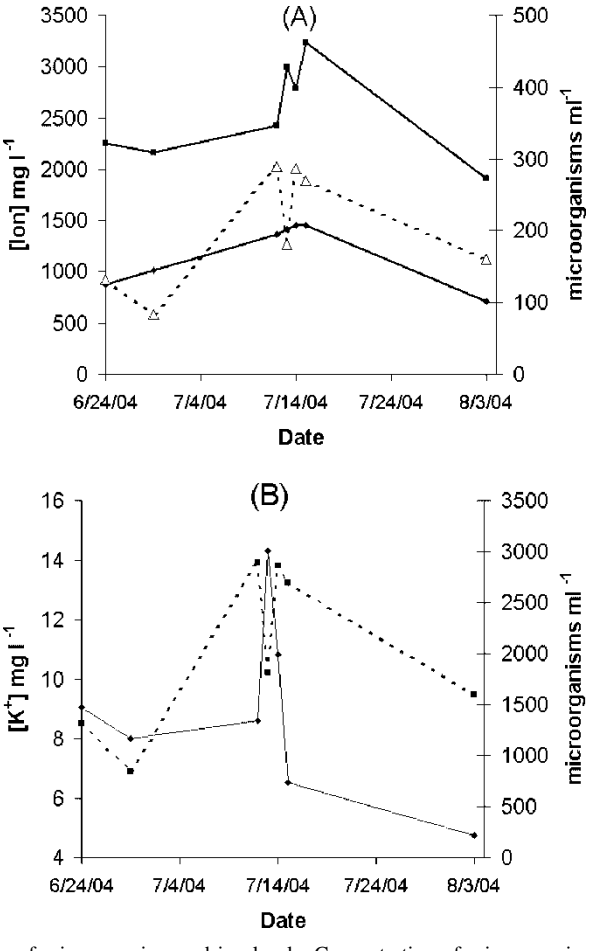

Figure 5. Comparison of microorganism and ion levels. Concentration of microorganisms (△, microorganisms ml^{-1}) compared to (A) Na^+ (♦, mg l^{-1} Na) and Cl^- (■, mg l^{-1} Cl), and (B) K^+ (♦, mg l^{-1} K).

sharply, while chloride and, particularly, potassium ion concentrations increase sharply. However, on the following day, the levels of chloride and potassium remain elevated, while the level of micro-organisms returns to a level similar to preceding samples. The density of dinoflagellates and euglena in the river does not appear to be adversely affected by natural fluctuations in the concentrations of chloride, potassium, or sodium ions. The sharp decrease in the level of dinoflagellates and euglena corresponding to the sharp increase in potassium ion concentration initially appeared relevant. However, further testing of the susceptibility of the naturally occurring dinoflagellates and euglena to changes in potassium concentrations proved that the observed fluctuation was not capable of causing significant changes in the mortality of these micro-organisms.

3.2 *Effect of increasing salt concentrations on micro-organisms in the laboratory*

In order to predict the effect of directly discharging concentrated RO effluent into the river, samples collected from the river were spiked with varying concentrations of NaCl or KCl in the laboratory. First, the combined levels of dinoflagellates and euglena were determined over time without the addition of salts to assure that cell death was not occurring naturally over the course

of the experiment. The river water samples were kept open to the atmosphere, and 1.00 ml aliquots were examined under 100× magnification using a Sedgewick-Rafter counting slide (1 mm × 1 mm grid) every 10 min for 1 h. The dinoflagellates and euglena in five randomly selected grids were counted, and the counts were completed in triplicate for each sample. The micro-organisms ranged from 1870 micro-organisms ml^{-1} at 30 min to 2270 micro-organisms ml^{-1} at 60 min. Differences between the six counts were not statistically significant.

In a second series of experiments, counts of dinoflagellates and euglena were completed every 10 min in river water containing added NaCl (5520 mg l^{-1} Na^+ and 9420 mg l^{-1} Cl^-). Levels of micro-organisms were observed in each aliquot sampled at 10 min intervals and decreased significantly in the first 10 min after addition of salt. The remaining five samples showed no statistical differences. For convenience in counting, all further micro-organism counts were completed just prior to and 30 min after the addition of salts to the natural water samples.

The effect of added salts on dinoflagellates and euglena in the river water is illustrated in figure 6a–c. The first point in each graph represents the initial concentration of dinoflagellates and euglena in water from the Big Wichita River under natural concentrations of the reported ion. Significant changes in micro-organism levels do not occur until the level of Na^+ increased to more than three times the natural level, and the Cl^- concentration increased to more than twice its natural level. The levels of dinoflagellates and euglena did not change significantly in the K^+ range employed in this study (9–90 mg l^{-1}). Based on average stream flow in the Big Wichita River and the expected level of discharge spread throughout a 24 h period, the level of Cl^-, Na^+, and K^+ in the river downstream from the discharge site is predicted to rise by only 75, 32, and 0.3 mg l^{-1}, respectively. The continuous mode of discharge, however, will only be employed during peak summer operating months. During typical operation, effluent will be discharged from a holding tank when the tank exceeds a pre-set storage capacity. This type of discharge will lead to larger volumes of brine rapidly entering the river and higher initial levels of ions near the discharge site. These levels could be as much as ten times greater than ion levels produced during continuous discharge. However, even at these higher levels of ion concentration, little effect on dinoflagellate and euglena levels would be expected. The highest predicted level chloride in the river, based on the maximum measured concentration during river monitoring and the addition of 750 mg l^{-1} Cl^- from the effluent, is 4450 mg l^{-1} Cl^-. In the laboratory, no decrease in the level of dinoflagellates and euglena was observed at 4250 mg l^{-1} Cl^-. For sodium, the addition of 320 mg l^{-1} Na^+ would increase the level to a maximum of only 1880 mg l^{-1}. At levels as high as 2500 mg l^{-1} Na^+, statistically relevant changes in micro-organism levels are not observed. Likewise, no changes in dinoflagellate and euglena levels are observed with increasing K^+ levels as high as ten times the natural level, well above the predicted maximum increase in K^+ concentration to 21 mg l^{-1} K^+.

4. Conclusions

The level of ions and micro-organisms (dinoflagellates and euglena) in a small natural water system was monitored prior to completion of a RO water treatment plant that, once operational, will discharge high-salinity effluent directly into the river. One possible effect of the discharge is damage to the river ecosystem, and the level of micro-organisms in the river may act as a biomarker for such changes. In this work, natural levels of chloride ranged from 850 to 3700 mg l^{-1} and sodium levels ranged from 320 to 1560 mg l^{-1}. Maximum predicted levels in the river following discharge from the RO plant are 4450 mg l^{-1} Cl^- and 1880 mg l^{-1} Na^+. Increasing the river water concentration of Cl^- and Na^+ to these levels in the laboratory causes

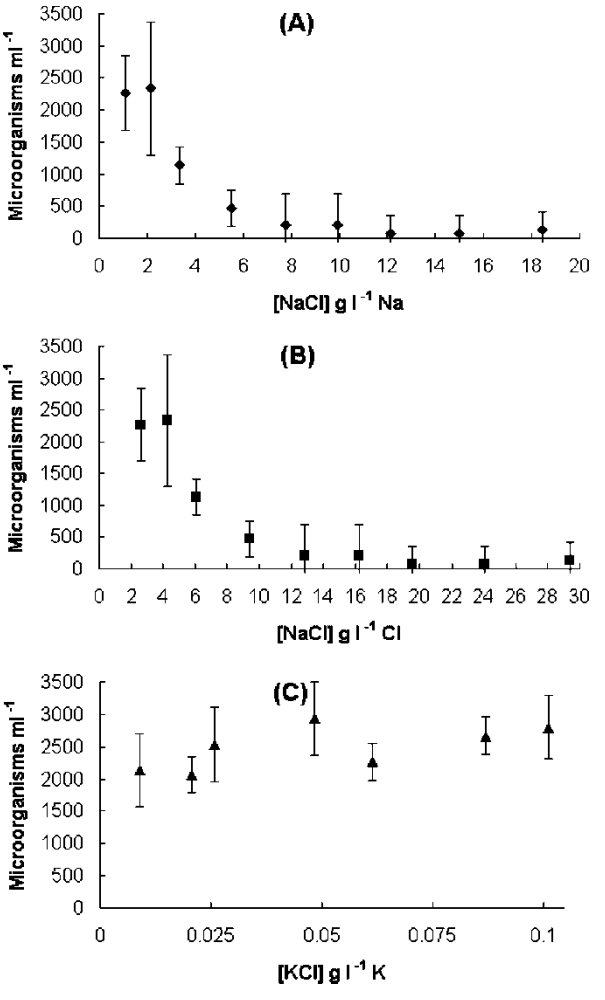

Figure 6. Effect of salt addition on total dinoflagellate and euglena concentrations. Microorganism counts were completed in triplicate for each addition of NaCl after 30 min. (A) Na^+ (♦, g l^{-1} Na); (B) Cl^- (■, g l^{-1} Cl); and (C) K^+ (▲, g l^{-1} K). Ion concentrations are the sum of the original concentration in the river water plus added NaCl (A and B) or KCl (C).

no statistically relevant changes in the concentration of dinoflagellates and euglena. However, raising the levels of chloride and sodium ions slightly beyond the expected increase in the river following discharge of RO effluent could cause significant cell death in micro-organisms and should be closely monitored following the opening of the RO facility.

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