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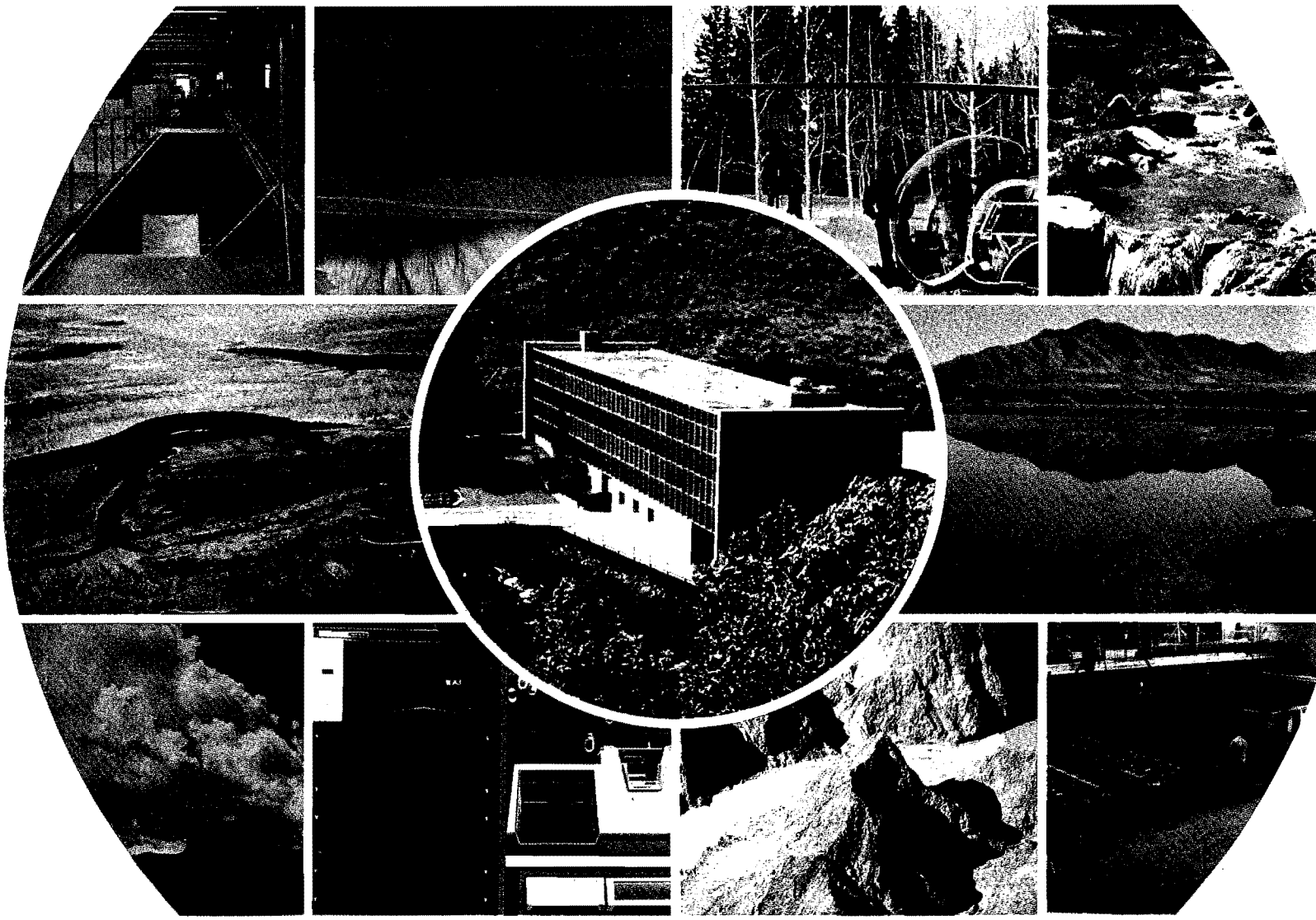

Utah Water Research Laboratory
College of Engineering
Utah State University
Logan, Utah 84322

December 1979

WATER QUALITY SERIES
UWRL/Q-79/05

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ABSTRACT

The effect of oil shale leachate and salinity additions on the productivity of freshwater algae were studied in the laboratory using batch bioassays. These batch bioassays were used to screen variations of ten salts in single and multiple additions of all possible combinations of the ten salts; water extractions of different processed and unprocessed oil shales; and the concentration effects of both the salts from 0.3 N to 0.05 N as NaCl and the oil shale extractions on the growth of standard test algae and indigenous algae from Lake Powell.

The batch bottle bioassays were conducted following the standard algal assay procedure as closely as possible. Variations in the standard algal assay procedure included media variation with the use of indigenous algal species isolated from Lake Powell and the use of three different algal species for test innoculum in the bioassay procedure. The biomass was monitored using optical density, chlorophyll *a* fluorescence, and/or cell counts.

The indigenous algal species were found to be negatively affected but more tolerant to all salinity additions than the standard test alga. The growth of the indigenous algal species (*Scenedesmus bijuga*) was also stimulated by adding oil shale extract at lower concentrations. Higher concentrations of oil shale leachate inhibited the indigenous algal growth.

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INTRODUCTION

Definition of Problem

The development of the oil shale industry will produce large quantities of spent shale and bring to the ground surface large amounts of formerly buried overburden material and raw oil shale strata in the Intermountain West (Pfeffer and Kerr 1974). The raw and spent oil shale and overburden soils all contain high levels of salinity (Colorado State University 1971). Consequently, any mining disturbances which add to the percolation of water through exposed soils, strata, or wastes add to the salinity loading of the streams and rivers (Ward and Reinecke 1972).

Percolation of water could be caused naturally due to precipitation, or artificially as water is used to stabilize the processed shale disposal sites after compaction (Holtz 1977). Even though these disposal sites will be designed as total containment systems (USDI 1973), leachate due to seepage from the bottom of the containment basins or during periods of heavy precipitation could still enter the groundwater or surface drainage system of the area. In the state of Utah, the drainage area that would be primarily affected would be the White River, a tributary to the Colorado River. Along with the addition of salinity, the leaching process through the raw and spent oil shale could potentially load both organic compounds and heavy metals into the contacted drainage system.

Objectives of This Study

1. Utilization of the batch bottle bioassay for toxicity testing

The Algal Assay Procedure: Bottle Test is currently accepted (APHA 1975) for biostimulatory effects of wastes on phytoplankton. This procedure will be extended to study the toxic effects of wastes on phytoplankton such as those that may be caused by the high salinity of oil shale leachate.

2. Effects of salinity on freshwater phytoplankton productivity

Variations in the salinity concentration of an aquatic system could affect the primary productivity by causing osmotic pressure changes within the cells. Variations in the ions comprising the salinity could affect the active transport of nutrients into the algal cells.

3. Evaluation of the U.S. Army Corps of Engineers Standard Elutriate Test

The Standard Elutriate Test was designed by the U.S. Army Corps of Engineers to characterize the pollution potential of dredged material on water quality and aquatic organisms (Keeley and Engler 1974). The applicability of this test to another waste, spent oil shale, will be investigated.

4. Effects of oil shale leachate on freshwater phytoplankton productivity

Many metals are required for the growth of algae, but are toxic in excess of the requirement for growth. Some species of algae can utilize organic compounds directly as an energy source (Stewart 1974) although some organic compounds are toxic to algae. Therefore, a complex waste such as oil shale leachate could either stimulate or depress phytoplankton growth depending on the effective concentration of the waste entering the aquatic system.

5. Application of the bioassay results to the Colorado River System

The data gained from the batch bottle bioassays will be interpreted to estimate possible impacts of leachate release on the phytoplankton of Lake Powell.

LITERATURE REVIEW

Oil Shale Development

The energy contained within oil shale is potentially as large as that in all of the known petroleum energy reserves (Petzrick 1975). However, environmental, economic, legislative, and policy constraints have delayed construction of commercial prototypes (Maugh 1977). The major fraction of oil shale within the United States is contained in the Green River formation beneath northwestern Colorado, northeastern Utah, and southwestern Wyoming. These deposits are estimated to contain 1.8 trillion barrels of oil (Donnell and Blair 1970). Both industrial and governmental activity in oil shale development has increased since the leasing of the federal oil shale tracts in 1974 (Pforzheimer 1974).

Commercial Extraction Operations

Oil shale is actually not a shale, but a marlstone. The composition of a typical oil shale is shown in decreasing detail moving from left to right in Figure 1 (Siggia and Uden 1974). The oil is obtained from the organic matter in the shale, largely from a substance called kerogen. A synthetic crude oil called syncrude is produced with the application of heat in retorting and prerefinement of the retort product (Routson et al. 1979). The retorting processes for extracting oil from the shale are of two major types: The *in situ* process, in which the oil is extracted via pyrolysis within the shale formation; and the above ground retorts. Above ground vertical retorts have been proposed for development of the federal tracts U-a and U-b, within Utah (Figure 2).

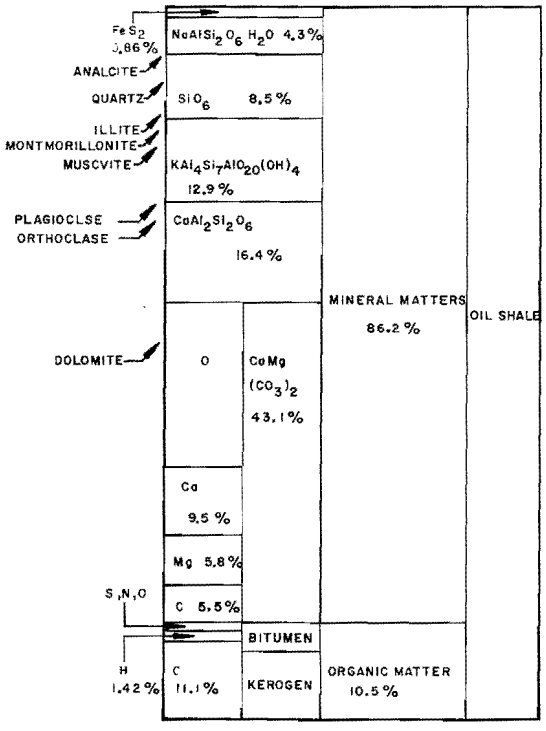


Figure 1. Analysis of a Green River oil shale (Siggia and Uden 1974).

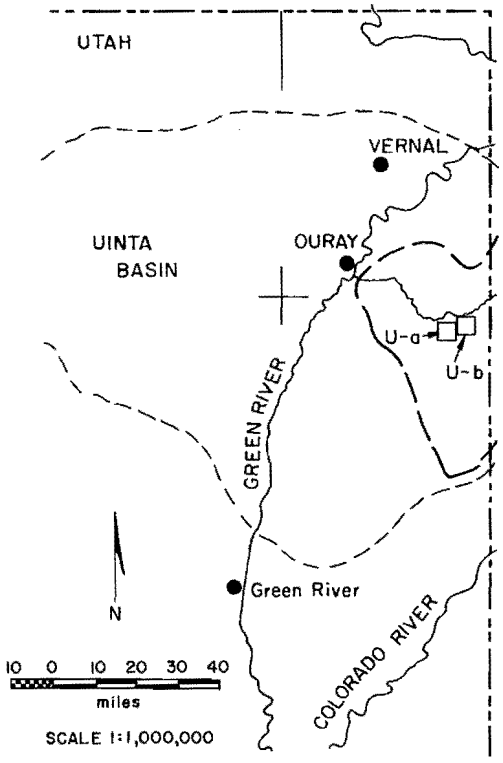


Figure 2. Location of federal oil shale tracts in Utah (USDI 1973).

Two different vertical retorting processes are likely to be used; it has been suggested that about 85 percent of the shale be processed via the Paraho direct heat mode and the other 15 percent representing the crushed fines be processed by the TOSCO II process (Crawford et al. 1977). Although most of these extraction processes are proprietary, they have been generally described and compared (National Petroleum Council 1972). A generalized flow diagram of a 50,000 barrel per day oil shale mine and processing unit is shown in Figure 3 (Conkle et al. 1974).

Mining Operations

Three methods of mining the oil shale have been proposed: Open-pit, which is extraction of the shale by a drag-line after removal of the overburden: room-and-pillar, which is extraction of the shale by loader after selective rubblization leaving pillars of shale for support; and rubblization, which is extraction of the shale by the in situ technique. Since it has been estimated that 1.4×10^5 metric tons per day of oil shale will be required to operate the smallest economical retorting plant, which would produce 105 barrels per day, the mine associated with this development would be larger than any mine currently operating in the United States (Sladek 1975a).

Prerefinement Operations

On site prerefining operations are desirable for two reasons. First, shale oils are usually heavier and more viscous than petroleum, which makes transport without additional refinement difficult. Second, the nitrogen and sulfur compounds contained in the shale oil poison heavy metal catalysts utilized in refinement of oil. Therefore, pretreatment to facilitate transportation is desirable, and the on site prerefining facilities may also be used to remove the nitrogen and sulfur compounds via hydrotreatment as water availability permits (Sladek 1975b).

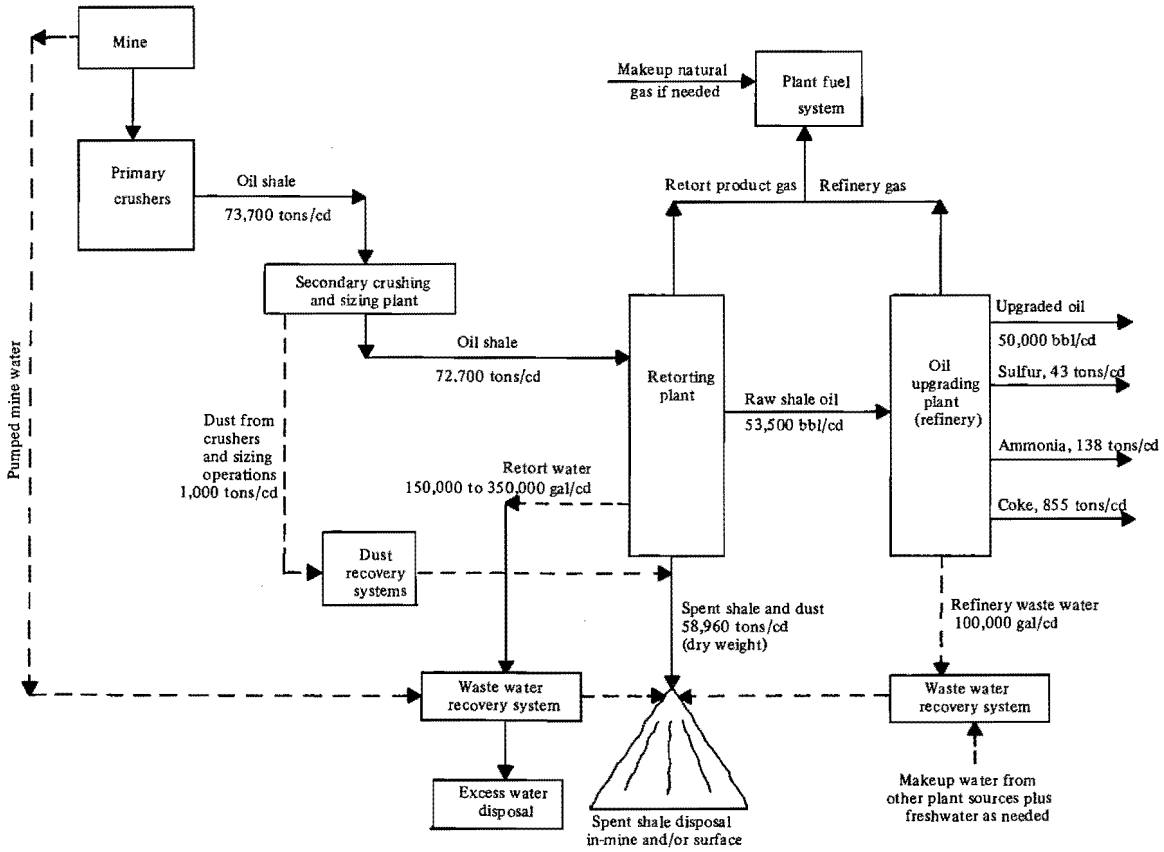


Figure 3. Flow diagram of 50,000-barrel-per-day underground oil shale mine and associated oil shale surface processing units (Conkle et al. 1974).

Pollution Potential of the Oil Shale Industry

Solid Waste Disposal

It has been proposed to dispose of nearly all of the solid and liquid wastes produced from the oil shale industry on the ground surface (Pfeffer and Kerr 1974). The spent oil shale will occupy, even under the greatest compaction, at least 12 percent more space than the in-place raw shale (USDI 1973). This precludes the disposal of all of the spent shale in the location from which it was mined. As a supplemental or alternative disposal site, the spent shale could be disposed of in canyons near retorting operations. For example, waste disposal for tract U-a in Utah is expected to be Southam Canyon. A retention dam would be placed at the northern end of the canyon to prevent contamination of the White River (Crawford et al. 1977). Spent shale from the Paraho process has been evaluated to determine if compaction of this shale can be used to provide an impervious layer for dam and disposal area lining (Holtz 1977). Hand (1969) has estimated that 1.0-1.8 x 10^6 metric tons of spent shale per day would be produced by an above ground retort producing 10^6 barrels of syncrude per day. This is a ratio of 5-6:1 of spent shale to oil on a volume basis. Overall, it is estimated that the total volume of the projected disposal pile in Southam Canyon would reach 727 x 10^6 cubic meters which would occupy approximately 366 hectares (900 acres) with an average depth of 61 meters (200 feet).

Water Use

The water requirements for the oil shale industry have been estimated at 3.7 cubic meters of water per cubic meter of upgraded shale oil (Crawford et al. 1977). The uses of the water are categorized in Figure 4 (Conkle et al. 1974). A number of alternative sources are possible. One proposal suggested the diversion of 36,000 acre feet of the Green River annually. This diversion would deplete 24,000 acre feet annually from the river system and return 12,000 acre feet annually of an unspecified quality return flow to the river. Because most oil shale development plans project total containment of wastes in the spent oil shale disposal sites, the estimate of 12,000 acre feet annually of return flow is probably too high. It has been estimated that this depletion would increase the salinity of the Colorado River at Imperial Dam by about 1.5 mg/l (USBR 1974). The estimated salinity increases at Imperial Dam due to oil shale development are summarized in Table 1 (Siggia and Uden 1974). However, others hypothesized that the salinity of the Colorado River may actually decrease due to the removal of highly saline groundwater contributions diverted into oil shale processing (Crawford et al. 1977). The maintenance of lower salinity in the Colorado River is of interest due to the economic importance of the water to downstream users. It is estimated that the annual economic losses are from $194,000 to $395,000 (1974 dollars) per mg/l increase in salinity at Imperial Dam (USDI 1974). Additional damages occur in Mexico and create

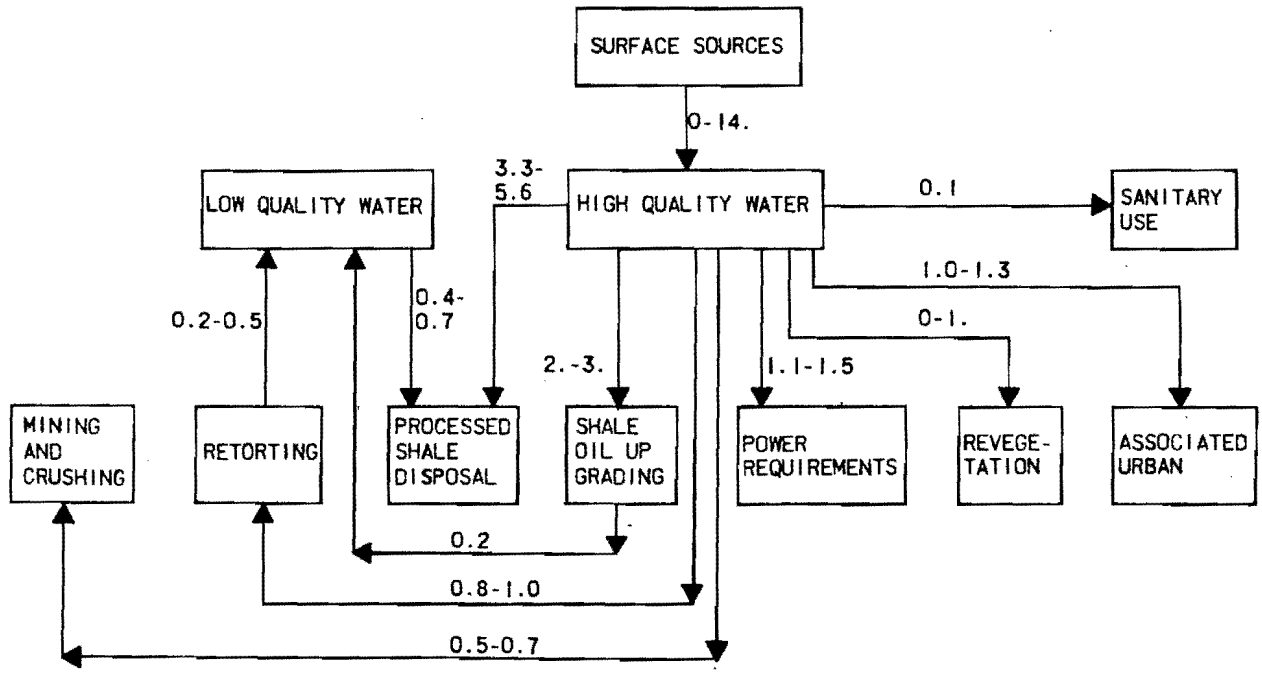


Figure 4. Demand and supply for water: 50,000 barrel per day plant with an underground mine and surface retort at tracts U-a and U-b (cu ft. per sec.) (Conkle et al. 1974).

Table 1. Estimated salinity increases at Imperial Dam (Siggia and Uden 1974).

Level of Development	Year			
	1977	1980	1985	1990
Shale Oil Production (1000 Barrels Per Day)	50	300	1000	1600
Water Use (1000 Acre Feet Per Year)	9	52	155	245
Salt Diverted At 400 mg/l (1000 Tons Per Year)	5	28	84	133
Increased Salinity Concentrations at Imperial Dam (mg/l)				
Resulting from Diversion of Water	0.5	3	9	14
Resulting from Domestic Return Flow		0.1	0.4	0.6
Total	0.5	3.1	9.4	14.6

a somewhat delicate international situation. The salinity load in the Colorado River is also of interest for other reasons. The Colorado River Salinity Control Act of 1974, which provides funding for construction of several desalting and control projects, limits effluents from industrial discharges and authorizes research projects on future salinity problems and programs (Science and Public Policy Program of the University of Oklahoma and Radian Corporation 1977). The salinity of the Colorado River, both past and present, has been discussed in a number of publications (Iorns et al. 1965; Holburt and Valentine 1972; Blackman et al. 1973; UWRL 1975).

In order to contain potential salinity loading from the spent oil shale disposal areas, all of the potential runoff must be contained behind catchment dams (BLM 1975), except for runoff released from the spillway during intense runoff events. Also, large volumes of shale are below the water table and must be dewatered before mining. Since much of the deeper groundwater is saline and cannot be used or discharged to surface waters, it must be treated before release or evaporated in the solid waste disposal areas. It is not uncommon for these groundwaters to have a total dissolved solids concentration exceeding 40,000 mg/l (National Academy of Science 1979).

Liquid Waste Disposal

It is proposed to dispose of excess low quality water at the spent shale disposal sites. It has been estimated that a 50,000 barrel/day operation on tract U-a or U-b in Utah would produce a low quality water waste stream to the process shale disposal site of between 0.4-0.7 ft^3/sec (Conkle et al. 1974). As an additional source of poor quality water, the shale must be leached with good quality water in order to establish vegetation (Bloch and Kilburn 1973). Studies (Colorado State University 1971; Ward and Reinecke 1972) show that the process will leach salinity ions into the water on a continuing basis (Table 2). These studies do not include the other solid and liquid wastes that would comprise approximately 3 percent of the total wastes such as process wastewaters, oily sludges, spent catalysts, shale coke and other prerefinery waste (Crawford et al. 1977).

Trace elements are present within the waste, but of these only boron has been reported in quantities that are toxic to plant growth (Bloch and Kilburn 1973). Also, the presence of trace organic materials which are known carcinogens has been established (Siggia and Uden 1974). These components are polar and heterocyclic, which means they are potentially toxic. Their polar characteristics may increase their solubility and entrance into water systems where bioaccumulation can occur. At critical tolerance levels of key terrestrial and aquatic biota, research is needed for predictive purposes to understand the possible hazards or successional changes and resulting environmental effects of ground disposal of shale oil wastes (Weaver 1974; Routson et al. 1979).

Dissolved Solids and Freshwater Phytoplankton

Total Dissolved Solids

The effects of dissolved solids on phytoplankton has been studied in the lower ranges (0 to 0.5 g/l) of dissolved solids concentrations in order to delineate maintenance media for freshwater phytoplankton. However, little work has been done on the effects of greater salt concentrations on these freshwater organisms. A general literature review of suspended and dissolved solids effects on freshwater biota, conducted by Sorensen et al. (1977), mentioned very few studies of phytoplankton. Specht (1975) reports inhibition of Selenastrum at salinities greater than 9000 parts per million.

It has been shown for lakes in central Alberta that with increase of total dissolved solids (TDS), more nutrients become available. This increases the productive capacity of the water to a certain point. A further increase in the TDS in inland waters tends to inhibit organic production, and so the productivity of the water decreases. In these study lakes, the optimum TDS and alkalinity is about 1400 mg/l and 450

Table 2. Experimental results of the percolation experiment conducted on TOSCO spent oil shale retorting residue (CSU 1971).

Volume of leachate sample (cc)	Total volume of leachate (cc)	Conductance of sample (μmhos/cm @25°C)	Concentration (mg/l) of sample				
			Na^+	Ca^{++}	Mg^{++}	SO_4	Cl^-
254	254	78,100	35,200	3,150	4,720	90,000	3,080
340	594	61,600	26,700	2,145	3,725	70,000	1,900
316	910	43,800	14,900	1,560	2,650	42,500	913
150	1,060	25,100	6,900	900	1,450	21,500	370
260	1,320	13,550	2,530	560	500	8,200	205
125	1,445	9,200	1,210	569	579	5,900	138
155	1,600	7,350	735	585	468	4,520	138
250	1,850	6,825	502	609	536	4,450	80
650	2,500	5,700	-	-	-	-	-
650	3,150	4,800	-	-	-	-	-
650	3,800	4,250	-	-	-	-	-
760	4,560	3,850	-	-	-	-	-
∞*	∞*	1,800	86	64	118	740	11

*These are extrapolated values and were not actually observed. These extrapolated values are probably accurate to within ± 6 percent.

mg/l respectively (Kerekes and Nursall 1966).

In the Sea of Galilee, enrichment of water samples with an inorganic salt medium caused radical changes in the algal composition of the enriched samples. The appearance of a Chrysophycean flagellate, Prymnesium parvum, in the enriched samples caused concern because this alga is known to cause toxic blooms (Rahat and Dor 1968). Gupta (1972) discusses the ability of blue-green algae to withstand high levels of salinity, but it is usually assumed that something other than salinity controls algal growth (Van De Kreeke et al. 1976).

Major Cations

The effect of magnesium on freshwater phytoplankton has been studied more extensively than that of the other ions because magnesium is an essential part of chlorophyll a (Sun and Sauer, 1972; Seitz and Seitz 1973; Bennoun 1974). Magnesium has been found not to inhibit the growth of Selenastrum capricornutum at concentrations less than 92 mg/l (International Association of Theoretical and Applied Limnology 1978). The effects of ratios of calcium to magnesium and monovalent ion to divalent ion on the growth of phytoplankton have been discussed by Provasoli (1958). Different genera have optimum ratios where they dominate communities. For example, diatoms prefer waters with a monovalent ion/divalent ion ratio below 1.5 and have a wide flexibility toward different calcium to magnesium ratios. This wide flexibility seems to narrow with unfavorable total solid concentrations and monovalent ion to divalent ion ratios. The monovalent to divalent ion ratio (Na + K to Ca + Mg) based on the concentration (mg/l) of each of the ions was related to the periodicity of species composition of freshwater phytoplankton by Munawar (1974). Both diatoms and blue-green algae in that study were found to require a monovalent to divalent ion ratio of less than 1.6.

Other Metals

Most of the literature on salinity effects on freshwater phytoplankton deals in terms of specific cations or anions. Cations are often discussed as groups of metals. A study of heavy metal toxicity to algae of the Great Lakes showed that recommended levels of a number of metals for the Great Lakes were toxic to algae that were exposed to these levels of several of these metals simultaneously. The diatom tested displayed a greater sensitivity to heavy metal toxicity than the blue-green and the green algae tested (Wong et al. 1978). The synergistic effect of heavy metal toxicity on photosynthetic activity of freshwater phytoplankton is discussed by Stumm and Baccini (1978). Metal toxicity in mammals has been extensively studied (Luckey and Venugopal 1977).

Many metals which would be common in the leachate from oil shale are also known to be required substances for the growth of phytoplankton. Many of these metals are hormetins, toxic agents that are stimulatory in small doses. For these compounds it is customary to find the zero equivalent point which is the concentration at which the hormetic agent has no effect. The suggested safe concentration is then established at this point. The extent to which a metal is toxic can be predicted in mammals by identifying the group, period, and atomic weight of the metal (Luckey and Venugopal 1977). These types of toxicological studies have not

been widely applied to algae; nevertheless, the concept of hormetins is applicable to algae because essential nutrients for algal growth do become toxic to algae at higher concentrations.

Required Elements for Phytoplankton Growth

The positive effects of specific ions on the productivity of freshwater phytoplankton have also been explored. Stewart (1974) reviewed each of the macroelements required for inorganic nutrition in algae. Macroelements reviewed included: sulfur, potassium, calcium, and magnesium. Also he reviewed each of the microelements essential to all algae: iron, manganese, copper, zinc, molybdenum, chlorine; and also the elements required by only some algae: colbalt, boron, silicon, vanadium, and iodine. Stumm and Morgan (1970) state that the ratios of carbon, nitrogen, and phosphorus necessary for algal growth as 106:15:1. They define algae stoichiometrically as $C_{106}H_{263}O_{110}N_{16}P_1$ (3550 g/mole) with the minor elements being neglected. The carbon can be derived from the aqueous phase (CO_2, bicarbonate, carbonate-inorganic carbon) as illustrated by Goldman et al. (1972, 1974) and Lehman (1978) or it can be supplied as CO_2 from the atmosphere, or from degradation of organics in the water column and the sediments (Mortimer 1971; Schindler and Fee 1978; Rudd and Hamilton 1978; Sonzogni et al. 1977). Schindler (1971) and colleagues have shown that when other nutrients are supplied in adequate or excess amounts, the CO_2 invasion rate from the atmosphere is adequate to provide sufficient carbon for algal blooms.

Osmotic Role of Dissolved Solids

Dissolved solids can also affect the productivity of algae osmotically. Ecological differentiation of algae into marine and freshwater forms is based on definite physiological differences (Stewart 1974). For many algae elevated osmotic pressure inhibits photosynthesis. Positive buoyancy in some algae is also under ionic control and is regulated via the osmotic pressure of the cell (Kahn and Swift 1978).

Osmotic pressure (π) may be calculated in a number of different ways depending on the equation used. The classic equation used to calculate osmotic pressure is stated as (Findlay 1919; Harned and Owen 1950; Moore 1963):

OSMOTIC PRESSURE (atm)

$$\pi = i\,C\,R\,T \qquad (1)$$

where

R = gas constant 0.820575 ℓ atm mol^{-1} K^{-1},

T = temperature K

C = concentration mol ℓ^{-1}, and

i = number of ions from electrolyte dissociation.

Another method for calculating osmotic pressure is based on the equivalent conductance of the solution. The equivalent conductance (Λ) can be calculated with the following equation (Moore 1963):

EQUIVALENT CONDUCTANCE

$$\Lambda = \frac{\kappa}{c} \qquad (2)$$

where

κ = specific conductance μmhos/cm^2, and

c = normal concentration N.

This relationship is extrapolated to a zero concentration using a linear regression to determine the equivalent conductance at infinite dilution.

Using these two variables, the Arrhenius degree of dissociation (α) can be calculated with the following equation:

ARRHENIUS DEGREE OF DISSOCIATION

$$\alpha = \frac{\Lambda}{\Lambda_0} \qquad (3)$$

where

Λ = equivalent conductance ohm^{-1} cm^2 equiv.$^{-1}$, and

Λ_0 = equivalent conductance at infinite dilution ohm^{-1} cm^2 equiv.$^{-1}$.

The van't Hoff factor (i) can then be calculated using the following equation:

VAN'T HOFF FACTOR

$$i = 1 - \alpha + \nu\alpha \qquad (4)$$

where

ν = # of ions that one molecule of solute is capable of dissociating into, and

α = degree of dissociation.

The van't Hoff factor can then be used to calculate the osmotic pressure of the solution shown in Equation 1.

The activity of the solution may also affect productivity. The activity coefficients (γ) can be established via the DeBye-Hückel equation (Barrow 1966):

$$\text{Log} \pm \gamma = \frac{-0.5091/Z^{+}Z^{-}/\sqrt{c}}{1 + A\sqrt{c}} \quad . \quad (5)$$

where

A = 1

c = concentration moles ℓ^{-1}, and

Z = absolute ion charge.

However Stumm and Morgan (1970) recommend the use of the Davies equation for calculation of activity coefficients in solutions with higher ionic strengths. The Davies equation is shown as follows:

$$\text{Log} \pm \gamma = A/Z^{+}Z^{-}/ \left(\frac{\sqrt{I}}{1 - \sqrt{I}} \right) - 0.3I \quad . \quad (6)$$

where

A = 0.509,

Z = absolute ion charge, and

I = ionic strength of the solution.

Osmotic pressure and the activity coefficient are both dependent on the ionic strength of the growth medium for an alga and therefore change as the salinity of the medium changes. Increases in these variables have been shown to cause inhibition of photosynthesis in some freshwater algae (Stewart 1974). Therefore, an increase of these variables can be indicative of a toxic response in freshwater algae. This hypothesis will be tested in this research.

Phytoplankton Effects on Salinity

Not only does the salinity affect the productivity of the phytoplankton, but the phytoplankton directly affects the salinity of the water. As algae photosynthesize, they utilize carbon dioxide. This increases the pH of the water which, in the presence of large quantities of calcium and carbonate ions, could cause the precipitation of calcium carbonate. This precipitation causes a phenomenon in the Great Lakes known as whiting (Strong and Eadie 1978). Also, it has been hypothesized that the electronopaque non-rigid fibrils of approximately 3 to 10 nanometers in diameter that are found abundantly on the surfaces of common lake microbes, free in the water column and free on the surface of the lake bottom, may be the principal component of an organic carrier system for the redistribution of bound but biologically available cations in lakes (Leppard et al. 1977).

Other Potential Leachate Components and Freshwater Phytoplankton

The effects of petroleum products on marine phytoplankton have been studied extensively. Dunstan et al. (1975) projected that the significant environmental effect of oil on marine primary production could be the growth stimulation of particular species by low molecular weight aromatic compounds which would result in an alteration of the natural phytoplankton community structure and its trophic relationships. Other investigators have found oils to have toxic inhibitory effects on algae (Gordon and Prouse 1973; Winters et al. 1976). Kauss and Hutchinson (1975) observed a significant stimulation of algal growth after the toxic compounds of the oil had evaporated. This work was done on a freshwater alga using only the water-soluble components of oil. Actual oil spills in marine environments suggest that phytoplankton are not strongly affected by oil when exposed for short periods (Ignatiades and Mimicos 1977).

Organics

Some algae can utilize organics as a growth substrate, and vitamin requirements have been shown for many algal species (Swift and Taylor 1974). Organic fractions of domestic sewage have been found to stimulate algal growth (Sachdev and Clesceri 1978).

Trace Metals

The trace metals present in oil shale leachate could change the community composition of the phytoplankton. Patrick (1978) discusses the effects of trace metal pollution on diatom communities. In the presence of minor trace metal pollution, a shift in the diatom genera may occur. In the presence of larger amounts of trace metal pollution the diatom community may be replaced by forms of green and blue-green algae which tend to be more tolerant of trace metal contamination than are the diatoms. However, boron, which is abundant in some spent oil shale (Bloch and Kilburn 1973), has been identified as a possible requirement for diatom growth (Thomas and Dodson 1968). Meyer (1978) discusses the changes in algal populations that correlate with trace metals concentrations in a reservoir. This included cyanophytes as well as diatoms. It is difficult to generalize about trace metal toxicity since it has been found to be species dependent and also dependent on the temperature of the environment (Cairns et al. 1978).

Algal Toxicity Tests Using Batch Bottle Bioassays

The algal assay bottle test procedure, which has been extensively applied to biosti-

mulation studies, is presently being applied to toxicity studies. Applying the procedures to toxicity testing has only tentative approval as a Standard Method (APHA 1975). However, the USEPA has included this procedure in its latest protocol of the Selenastrum capricornutum, Printz, algal assay bottle test (Miller et al. 1978). It is assumed that if algal growth remains limited when nutrients are in sufficient supply and the physical conditions for growth exist, a toxicant is present (Payne 1976).

Test Algae

The use of indigenous phytoplankton in these bioassays is not recommended unless there is strong evidence of the presence of persistent sublethal toxicants to which indigenous populations might be tolerant (Greene et al. 1978). However, the use of indigenous species in testing procedures has previously been recommended by others because the testing is presumed to be more rational. Odum (1971) states that to study the microbial activities in low-nutrient, constant-flow environments, the right organisms, or those active under natural low-nutrient condition, must be located. These may not be the "laboratory bugs" that have received the most intensive study. Phytoplankton and other organisms which have evolved in and adapted to physically variable environments would, because of their adaptations, be better able to tolerate any toxic compound (and possibly any perturbation) than would morphologically similar organisms adapted to stable environments (Fisher 1977).

Variations in tolerance within the same taxonomic groups of algae have also been discussed by Rana and Kumar (1974). This variation was noted while studying the tolerance of algae to effluents from a zinc mine and smelter. Additional wide variations in tolerance to this waste was also noted between the algae of differing taxonomic groups.

Lee (1973) stresses the importance of chemical aspects of bioassay techniques for proper evaluations of the environment. The chemical aspects of a bottle bioassay would be dependent on the test alga. For example, blue-green algae produce hydroxamate chelators which appear to act as agents to suppress the growth of other algae by inducing iron deprivation (Murphy et al. 1976). Also the presence of mixed algal cultures could affect the results of the bioassay. The presence of Scenedesmus obliquus and Selenastrum minutum together was found to reduce the algicidal effect of $CuSO_4$ which suggests an involvement of some physiological mechanism in this algal mixture (Dashora and Gupta 1978). Plant polyphenols have also been found to cause inhibition of calcite precipitation in Lake Powell (Reynolds 1978). In this manner the algal assemblage may have a direct effect on the chemistry of carbonate lakes as well as the bioassay flask. However, the detection of algal growth reactions, whether inhibitory or stimulatory, becomes more accurate as detailed background information accumulates on the physiology of a single test species. Also, when using a single algal test species, comparison of algal growth potentials from different water sources is feasible (Miller et al. 1978).

Sources of Variation

Any bioassay whether testing Selenastrum capricornutum, an indigenous alga, or a mixed algal culture can have errors introduced through the biomass analysis. Trees (1978) discussed substantial errors in suspended solids determination in waters with a high dissolved solids content. The error in calculating dissolved solids is magnified when filtering smaller volumes of saline water. The filters should be rinsed with distilled water after filtration to maintain a linear relationship between sample volume and suspended solids content of the water samples.

Problems can also be encountered with biomass estimation via in vivo chlorophyll a measurements. It has been found that the ratio of chlorophyll a concentration to in vivo fluorescence changes in value during the course of bioassays. Tunzi et al. (1974) suggested that a conversion factor to convert in vivo fluorescence to chlorophyll a concentration should be calculated at the beginning and end of each algal bioassay. Kiefer (1973) showed that the chlorophyll a of nitrogen-starved cells fluoresced more strongly than in unstarved cells. Therefore, care must be taken that the differences being measured are of algal biomass and not the cells differential ability to fluoresce.

MATERIALS AND METHODS

The potential effects of the salinity and other constituents of oil shale leachate on phytoplankton productivity were evaluated using both standard and modified algal assay procedures. In general, algal assays consist of monitoring the growth of test algae in separate Erlenmeyer flasks. Each flask is innoculated with an equal amount of cells and incubated under identical physical conditions. Control flasks of the test alga and medium were cultured along with the various treatment flasks which included the same test alga and medium, with the addition of whatever was being tested. The effect of the treatment on the growth of the algae was determined by comparing the growth of the algae in the treated flasks to that of the controls. Standard algal assay procedures were conducted to provide data which would be comparable to other investigations (USEPA 1971; Miller et al. 1978). The modifications to the standard algal assay procedure were made using the general guidelines presented in Standard Methods (APHA 1975). An identification matrix of the bioassays conducted, as listed in Table 3, summarizes both the standard and modified procedures applied to the bioassays during this study.

Algal Isolation and Culture Maintenance

The standard algal assay organisms utilized, Selenastrum capricornutum, Printz, and Anabaena flosaquae (Lyngb) (De Brebisson), were secured from the National Eutrophication Research Program. These algae were maintained in AAM, a synthetic algal nutrient medium (Table 4).

Indigenous algae utilized, Synedra delicatissma var. angustissima and Scenedesmus bijuga, were isolated from samples collected by Bureau of Reclamation personnel, under the supervision of E. G. Bywater, at the Wahweap station on Lake Powell. These algae are abundant in Lake Powell (Stewart and Blinn 1976). These algae were isolated and maintained in Lake Powell Synthetic Medium (TDS = 780 mg/l), which is AAM modified by the addition of the major cations and anions measured in Lake Powell (Table 5). Unialgal cultures of all four of the test algae (hereafter referred to as Selenastrum, Anabaena, Synedra, and Scenedesmus) were maintained via standard algal assay procedures except for the media modification already described.

Regular Bioassay Monitoring Techniques

The bioassay flasks were monitored daily for the first five days of the bioassay and every other day after that period until the algae ceased to grow. Algal growth was measured by a number of different variables: Optical density (absorbance) was determined at 750 nanometers on a Bausch and Lomb Spectronic 70 in a one centimeter cuvette (APHA, A Turner model 111 Fluorometer equipped with a #110-922 (430 nm) excitation and #110-021 (<650 nm) emission filters, a red-sensitive photomultiplier tube, and a high sensitivity door (APHA 1975); 2) A Turner model 430 Spectrofluorometer operated at a band width of 60 nm for both excitation and emission wave lengths of 440 nm and 670 nm respectively. Two auxiliary emission filters were used to block the emission interference, a standard polarizing filter and a Corning #2A glass filter. The procedure for chlorophyll a measurements on the spectrofluorometer is outlined by Turner Associates (1973, 1976).

Cell counts and mean cell volume determinations of unialgal cultures of Selenastrum were conducted on a Coulter Electronic Particle Counter Model B with a Model J Particle Size Distribution Plotter (Coulter Electronics, Inc., no date; Miller et al. 1978). The aperture tube had a 100 micron orifice. Cell counts of the mixed and other algal cultures were conducted via direct microscopic examination in Sedwick-Rafter cells (APHA 1975). Specific conductance was monitored using a Yellow Springs Instrument Company glass conductivity cell Model No. 4303 and wheatstone bridge (APHA 1975).

Limitations were encountered with all of the biomass monitoring techniques utilized. Optical density did not provide good sensitivity, therefore being of questionable value during the first few days of bioassay measurement. Optical density was also prone to interference from precipitates and precipitation is a common problem in samples with high total dissolved solids content especially in batch bioassay systems where it is common to have the pH increase during algal growth. Chlorophyll a fluorescence was more sensitive, therefore being of more value during the first few days of bioassay measurement. It was also less prone to interference from precipitates although this problem was still present. However, chloro-

Table 3. Identification matrix for algal bioassays.

Bioassay Number	Test Algae				Physical Parameters		Biomass Parameters				Medium Perturbations	
	Selenastrum	Synedra	Anabaena	Scenedesmus	Electrical Conductivity	pH	Optical Density	Fluoresence	Cell Count (displacement)	Direct Cell Counts by Genera	Addition Levels	Addition Base (Medium)[a]
I	X					X	X	X	X		g/l 16.000, 14.000, 10.000, 6.585, 4.937, 3.000, 5.826, 7.768, 3.00, 2.00, 1.00, 0.50, 0.25	NaCl, KCl, $MgSO_4$ $CaSO_4$, Na_2SO_4, $MgCl_2$, K_2SO_4, $CaCl_2$, (AAM)
II	X				X	X	X	X	X			NaCl, KCl, $MgSO_4$, $CaSO_4$, Na_2SO_4 K_2SO_4, $CaCl_2$
III	X				X	X	X	X	X		all 0.03N	NaCl, KCl, $MgSO_4$, $CaSO_4$, Na_2SO_4, $MgCl_2$, K_2SO_4, $CaCl_2$, $NaHCO_3$, $KHCO_3$,(AAM)
IV		X			X	X	X	X			0.05N, 0.10N, 0.2N, 0.3N	Same as Above (L.P.S.)
V		X			X	X	X	X			0.05N	All possible two way combinations of the 10 salts. (L.P.S.)
VI		X			X	X	X	X			0.05N	All possible 3 and 4 way combinations of the 10 salts (L.P.S.)
VII		X			X	X	X	X			0.05N	All possible 5, 6, 7, 8, 9, and 10 way combinations of the 10 salts (L.P.S.)
VIII	X (acclimated for 6 months in L.P.S.)				X	X		X			0.30N, 0.10N 0.05N, Shaker Extracted Elutriates	10 salts at each level and shale leachate (AAM)
IX		X	X	X	X	X		X (4λ)		X	0.05N	10 salts and 4 differentials Total = 28 (L.P.S.)
X				X	X	X	X	X			5 ml, 10 ml, 15 ml, 20 ml	Oil shale elutriates and leachate Matching salt solutions (L.P.S.)

[a]Base Media: AAM = Algal assay medium (Table 4); L.P.S. = Lake Powell Synthetic (Table 5).

phyll a fluorescence measurements were more variable than optical density measurements over the course of the bioassay. Cell counts conducted on the Coulter Counter are more sensitive and precise than both the optical density and fluorescence measurement techniques; however, they could only be applied to unialgal cultures of the lunicate alga, Selenastrum. The indigenous algae which have attenuated morphology and clumping tendencies, did not lend themselves to analysis by displacement methods, therefore, direct microscopic counts using a Sedwick-Rafter cell (APHA 1975) were utilized to determine these cell counts.

The cell counts are adjusted for variation in cell sizes by the determination of the mean cell volume. Size variation does occur in algal cultures, with rapidly growing cultures being composed of smaller cells and slow growing populations being composed of larger cells. Size variation in algal cells can also occur with variation in the media. The mean cell volume of the automated cell counts of Selenastrum were determined using a Coulter Cell Size Plotter, Model J. The mean cell volume of the direct microscopic counts were determined with an eyepiece micrometer. This direct microscopic technique is less precise than the automated techniques for cell count and cell volume determinations.

Table 4. The synthetic algal nutrient medium, AAM (USEPA 1971).

Compound	Concentration (mg/l)	Element	Concentration (mg/l)
Macronutrients			
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
$MgCl_2$	5.700	Mg	2.904
$MgSO_4$ $7H_2O$	14.700	S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	C	2.143
$NaHCO_3$	15.000	Ca	1.202
		Na	11.001
		K	0.469
Compound	**Concentration (µg/l)**	**Element**	**Concentration (µg/l)**
Micronutrients			
H_3BO_3	185.520	B	32.460
$MnCl_2$	264.264	Mn	115.374
$ZnCl_2$	32.709	Zn	15.691
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$FeCl_3$	96.000	Fe	33.051
$Na_2EDTA \cdot 2H_2O$	300.000		

Table 5. Salt additions to AAM for Lake Powell synthetic medium (Medine et al. 1977).

Salts	Concentration (mg/l)
$CaCl_2 \cdot 2H_2O$	163.9
$CaSO_4 \cdot 2H_2O$	152.4
$MgSO_4 \cdot 7H_2O$	308.0
Na_2SO_4	108.7
K_2SO_4	9.58
$NaHCO_3$	241.1
$Na_2SiO_3 \cdot 9H_2O$	50.607

Total Ions	mg/l	meq/l
Ca^{++}	80.160	4.0
Mg^{++}	32.225	2.65
Na^{+}	109.355	4.76
K^{+}	4.299	0.1099
Si^{++++}	5.001	0.7123
$SO_4^{=}$	291.15	6.062
Cl^{-}	79.04881	2.2297
HCO_3^{-}	175.1194	2.87

Special Analyses

The chemical analyses performed during the project are summarized in Table 6. The flame photometric and atomic absorption procedures were conducted on a Varian Techtron Atomic Absorption Spectrophotometer, AA6, model 63 (Varian Techtron, 1972; 1975). The automated procedures were conducted on a Technicon Autoanalyzer II system from Technicon Instruments Corporation. The infrared combustion for the total organic carbon analyses was performed on a Oceanography International Corporation 0524B Total Carbon System, with an O.I.C. model 0512 EP electronic printer. The rapid injection technique was utilized as explained by Oceanography International Corporation (no date). The activities of solutions were determined using a Wescor HR-33T Dew Point Microvoltometer equipped with a C-51 sample chamber psychrometer (Wescor, Inc. no date).

The potentiometric method utilized for measuring the total alkalinity of the samples had to be modified due to interference in the test from the high total dissolved solids concentrations of the samples. This modification was the creation of a breakpoint curve based on the samples being analyzed to correct for the precipitation of low solubility compounds present in the samples. This modification is described in Standard Methods (APHA 1975).

Oil Shale Extraction Procedures

Both raw and processed oil shales were extracted via two different elutriation techniques. The first technique is shown in Figure 5. The second technique (Figure 6)

Table 6. An index of the chemical analyses performed.

Analytical Parameters	Unit Sensitivity	Method
Total hardness	1 mg/l; as CaCO3	EDTA Titrimetric; SM p. 202
Total alkalinity	1 mg/l; as CaCO3	Potentiometric; SM p. 278
Carbonate hardness	1 mg/l	Calc. from CaCO3
Bicarbonate hardness	1 mg/l	Calc. from CaCO3
Total dissolved solids	1 mg/l	Gravimetric; SM p. 92
Suspended solids	1 mg/l	Gravimetric; SM p. 94
Calcium, dissolved	mg/l, 2 place	EDTA Titrimetric; SM p. 189
Chloride, dissolved	mg/l, 2 place	Ferricyanide (automated; SM p. 613: Mercuric nitrate method; SM p. 302
Magnesium, dissolved	mg/l, 2 place	calc. from Tot. Hard
Potassium, dissolved	mg/l, 2 place	Flame photometric; SM p. 234
Sodium, dissolved	mg/l, 2 place	Flame photometric; SM p. 250
Sulface, SO4	mg/l, 2 place	Methylthymol blue (automated); SM p. 628: Turbidometric method; SM p. 496
Barium, tot. diss.	mg/l, 2 place	Atomic absorption; SM p. 152
Boron, dissolved	mg/l, 2 place	Carmine; SM p. 290
Cadmium, tot. diss.	mg/l, 3 place	Atomic absorption (flameless); EPA p. 78
Chromium, tot. diss.	mg/l, 3 place	Atomic absorption (flameless); EPA p. 78
Copper, tot. diss.	mg/l, 3 place	Atomic absorption; SM p. 148
Iron, tot. diss.	mg/l, 3 place	Atomic absorption; SM p. 148
Lead, tot. diss.	mg/l, 3 place	Atomic absorption (flameless); EPA p. 78
Manganese, tot. diss.	mg/l, 3 place	Atomic absorption; SM p. 148
Nickel, tot. diss.	mg/l, 3 place	Atomic absorption (flameless); EPA p. 78
Silver, tot. diss.	mg/l, 3 place	Atomic absorption (flameless); EPA p. 78
Zinc, tot. diss.	mg/l, 3 place	Atomic absorption; SM p. 148
Arsenic, tot. diss.	mg/l, 3 place	Atomic absorption (vapor generation); SM p. 159
Selenium, tot. diss.	mg/l, 2 place	Atomic absorption (vapor generation); SM p. 159

SM = Standard Methods for Examination of Water and Wastewater, 14th Ed., APHA, 1975.
EPA = Methods for Chemical Analysis of Water and Wastes, USEPA 1974.

used is the standard technique utilized by the Corps of Engineers for analysis of dredged samples (Keeley and Engler 1974).

Oil shale was also leached in an up-flow column (Figure 7). An up-flow rather than a down-flow column was used to avoid short circuiting of the water through the shale. This is a modification of the technique used by Maase et al. (1975), using gravity flow instead of a pump to force the fluid through the bed of processed oil shale. The shale was air dried to a moisture content of approximately 2 percent and then 2500 grams of the shale was placed in the column without compaction. A sieve analysis of this shale before placement in the column is shown in Figure 8. The sieve analysis showed this shale to have an effective size of 0.098 mm and a uniformity coefficient of 5.63. The flow of distilled water through the column varied slightly at around 1 liter per day. This would yield a velocity in the column of approximately 3×10^{-4} centimeters per second. This velocity was chosen as being approximately the velocity that the water would percolate by gravity flow through spent shale disposal piles. Leachate and elutriate samples were collected and sterilized by filtration through 0.45 micron Millipore filters (Type HA) and placed in sterile containers in the dark under refrigeration until use.

Bioassays

The batch bioassays were conducted to study the effects on algae of large numbers of variations of salts, concentrations, test algae, and oil shale extraction techniques. The initial bioassays were used to establish the salt effects on algal growth. The standard test alga, Selenastrum, was used as the test alga as suggested by the standard

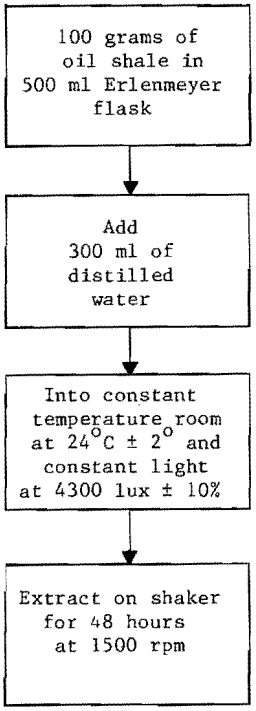


Figure 5. Oil shale elutriation technique number 1.

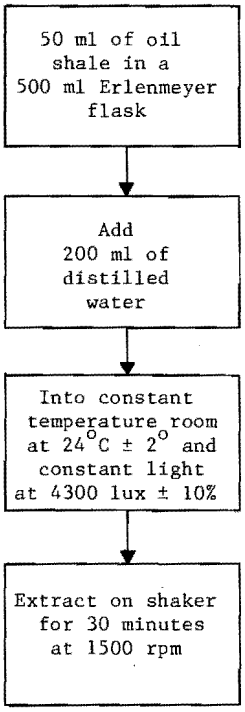


Figure 6. Oil shale elutriation technique number 2 (Keeley and Engler 1974).

algal assay procedure. This series of bioassays was followed by another series of bioassays using an alga which is indigenous to Lake Powell, Synedra, as the test organism. This procedure is also suggested by the APHA (1975), when testing for algal toxicity. After the salt effects on algal growth had been measured, the oil shale extracts were tested. These extracts are high in salinity and so the previous bioassays studying the salt effects on algal growth could be applied to the interpretation of these results. Because changes in water chemistry often cause a shift in the dominant algal species and even the dominant algal phylum present in a body of water, a batch bioassay was conducted using equal numbers of three different algal phyla as the test organisms. This bioassay attempted to identify if the compounds under study would select for a particular algal phyla.

Four bioassays were conducted using the standard test organism, Selenastrum capricornutum, Printz. In the first bioassay, equivalent concentrations of eight different salts were used at the same normality as NaCl. These salt concentrations, from 3 grams per liter to 16 g/l, were added to the bioassay flasks in addition to AAM. A second bioassay was run with the addition of salt concentrations at the same normality as NaCl as follows: 3 g/l, 2 g/l, 1 g/l, 0.5 g/l, and 0.25 g/l. The eight salts tested were: NaCl, KCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, K_2SO_4, $MgSO_4$, and $CaSO_4$. All of the additions on the first two bioassays were single salts only. In the third bioassay, the effects of two more salts were measured: $NaHCO_3$ and $KHCO_3$. $Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ were not tested due to their relative insolubility in water. All possible combinations of these ten salts, taken two at a time, were tested at the 0.03 normal level ($\sim$2.0 g/l as NaCl).

Another bioassay utilized Selenastrum, which had been acclimated for six months to a higher salinity environment by maintaining the stock culture in Lake Powell Synthetic medium instead of the usual AAM medium. The same ten salts as in Bioassay 3 were tested again as single salt additions at three levels: 0.3 N, 0.1 N, and 0.05 N.

Four additional bioassays were conducted using the diatom indigenous to Lake Powell, Synedra. Single salt additions of the same ten salts were tested at the 0.3 N, 0.2 N, 0.1 N, and 0.05 N concentrations. After this, all possible combinations at the 0.05 N level of the ten salts were tested. The medium used for all of these bioassays with Synedra was Lake Powell Synthetic; otherwise standard algal assay procedures were followed.

Another modified bioassay was conducted using three algae in mixed culture. These algae were: Synedra, Scenedesmus, and Anabaena. The standard test alga, Anabaena, was used with the two indigenous species because an appropriate cyanophyte was not

isolated in the water samples from Lake Powell. These algae were tested with single salt additions of the ten study salts at a concentration of 0.05 N. Because of the mixed algal cultures used as inoculum, the biomass monitoring techniques were adjusted. Fluorescence was monitored on the spectrofluorometer at three different settings to monitor three different algal pigments as summarized in Table 7. Direct cell counts of each alga and heterocyst counts of Anabaena were made. This was in addition to the total cell counts. These cell counts were made using a Sedwick-Rafter cell (APHA 1975). Each species was counted and the sum of the three species was the total cell count. The medium for this bioassay was Lake Powell Synthetic. Other than the above modifications, standard algal assay procedures were followed.

The oil shale used for the elutriation and leachate procedures are identified by an alphabetic code. The legend for this code (Appendix A-1) states that these are unhistoried samples from prototype processes and therefore may not be representative of a full-scale operation. Elutriates of shales CR, CP, DR, and DP, using elutriation procedure number one (Figure 5), were tested with acclimated Selenastrum as the test organism. Elutriates of shales AR, AP, BR, BP, using elutriation procedure number 2 (Figure 6), and leachate of shale AP were tested with Scenedesmus as the test organism.

Each of the elutriates and leachates were subjected to the special chemical analyses previously described. The salt composition of the extractions, as determined by analysis, was then used to prepare the salt additions used in the bioassay. These salt additions, composed of reagent grade salts and distilled water, were mixed to

Table 7. Fluorescence monitored for mixed algal cultures.

Excitation Wavelength (nm)	Emission Wavelength (nm)	Pigment Analyzed
400	500	Carotenoid
620	655	Phycocyanin
655	680	Chlorophyll A

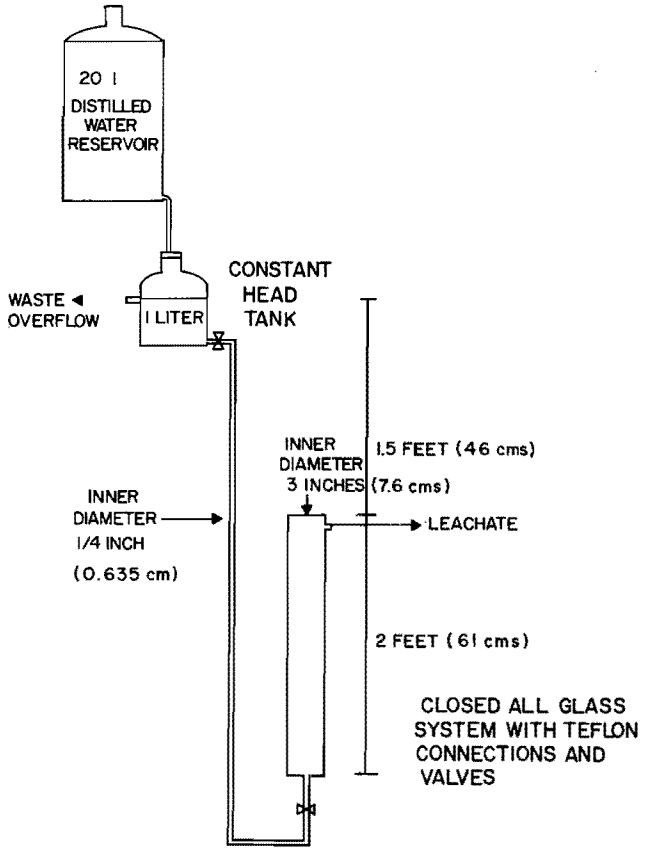


Figure 7. The up-flow column for leaching oil shale (Maase et al. 1975).

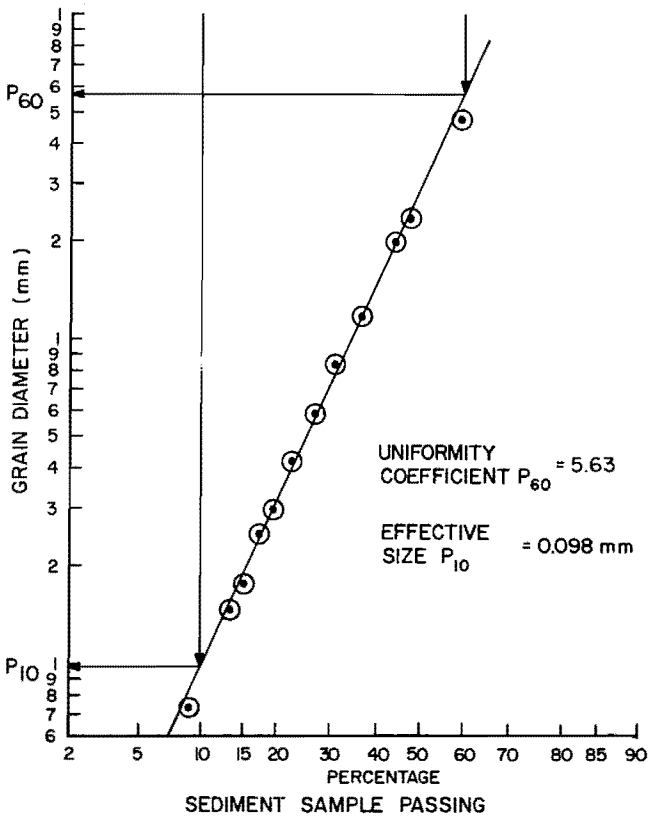


Figure 8. The sieve analysis of oil shale.

equal the salt composition of each elutriate and leachate. The elutriates, leachate, and salt additions were then tested at four different concentrations of additions.

Data and Statistical Analyses

The actual analytical measurements made during the bioassays were coded onto IBM cards and processed on a Burroughs 6700 computer. The algal biomass data were used to determine the maximum specific growth rate ($\hat{\mu}_b$) and the day it occurred. The value of the growth rate for each treatment was calculated by the formula (USEPA 1971):

$$\hat{\mu}_b = \frac{\ln (X_2/X_1)}{t_2 - t_1} \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

where

X_2 = biomass at time = t_2

X_1 = biomass at time = t_1

The maximum growth rate was then the highest growth rate determined for each treatment. The maximum standing crop ($\hat{X}$) of each treatment and the day on which it occurred was determined as the biomass achieved when the increase in biomass was less than 5 percent per day (USEPA 1971). The use of this definition of maximum standing crop was compared to the value of the maximum standing crop using the largest biomass reading as the definition. No significant difference occurred in the statistical conclusions when using either definition for the maximum standing crop determination. These parameters were used for statistical analyses. A summary of the data interpretations based on variations of $\hat{\mu}_b$ and $\hat{X}$ is presented in Table 8.

Statistical analyses were performed using STATPAC (Hurst 1972). All the treatments were tested against each other and the controls using the t-test (Middlebrooks 1976). Duncan's multiple range and multiple F tests were also conducted on the data (Duncan 1955). Both the t-tests and multiple range tests were paired by time of sampling to eliminate variation with time during the bioassay.

Table 8. Summary of probable responses for algal assay growth parameters.

Assay Protocol	$\hat{\mu}_b^{max}$	$\hat{X}$
Initial concentration of limiting nutrient	Defines rate limiting	Defines biomass limiting
Standard additions of limiting and other nutrients	Generally equal to maximum - no effect	Increased biomass
Toxic Materials	Decreases	Decreases
Growth rate stimulating chemicals	Increases	No effect

RESULTS

Effects of Increased Salt Concentrations on the Productivity of Selenastrum

Effects of Single Salt Additions

The concentrations of the salts under study were used at the same normality as NaCl. These salt concentrations, ranging from 3 g/l to 16 g/l, were selected based on current literature of estuarine salinity levels effects on the growth of the same species of Selenastrum, which would display no effect to algistatic on the growth of this green alga (Specht 1975). The levels and ion species were also within the potential ion load of leachate from spent oil shale and the soil overburden in the areas of oil shale development (Colorado State University 1971; Ward and Reinecke 1972).

A Duncan's multiple range analyses of the biomass data from this bioassay ranks the salt treatments from the least growth at the top of the listing to the greatest growth, at the bottom of the listing (Table 9). The growth depression of this alga was so great at all of the levels of salt additions, as compared to the control, that no differentiation could be made between the various treatments. Any group of treatments which are not significantly different from each other are connected by a line of stars to the right of the ranking list. All of the treatments produced biomass significantly lower than the controls.

Bioassay two was conducted at lower levels of salt additions (Table 10). The concentrations of salts under study were again equated to the normality of NaCl. The molarity, grams per liter, activity coefficients, and osmotic pressures of each of these solutions were also calculated. The activity coefficients were calculated using both the DeBye-Hückel equation and the Davies equation. The osmotic pressure calculations included both a van't Hoff factor (i) calculated from the literature and a van't Hoff factor calculated from the equivalent conductance of the solution.

A statistical analysis of the results was made using a split plot factorial analysis of variance (Table 11). Time was a known source of variance and was eliminated from the testing procedure by pairing the results. From this analysis it was established that significant differences at the 99 percent level, between the treatments, exist with differences in concentration and cation additions. However, a significant difference does not exist with differences in anion additions. The interactions of cations and anions produce significant differences at the 99 percent level between the different single salt additions. By grouping the biomass data by monovalent versus divalent cations and applying a completely randomized design analysis of variance, a significant difference was found at the 95 percent level of confidence for the cell count data (Table 12). Based on the optical density data, no significant difference was found. A significant difference in cell volumes was found when comparing the different salt treatments. Algal growth had no apparent effect on salinity because the electrical conductivity of the media did not change significantly from the first day of algal growth to the last day of algal growth.

Linear correlation coefficients with salt variables and growth explained very little of the variance (Table 13). However, the results show that correlations significantly different from zero occur between X versus the following concentration measurements in descending order of magnitude:

molarity > calculated osmotic pressure > normality > g/l

The activity coefficient did not correlate significantly with the $\hat{X}$ data. Correlations between $\hat{\mu}$ and the same concentration measurements occurred in the following descending order of magnitude:

calculated activity > normality > g/l > molarity > calculated osmotic pressure

The negative slopes and correlation coefficients show the inverse relationship between many of the salt concentration variables and the biomass data. Therefore, as the salt concentration increases, the algal growth response decreases. The cell volume data did not significantly correlate at the 95th percentile to any of these concentration measurements. The activity coefficient based on the Davies equation and the osmotic pressure based on the equivalent conductance calculation of the van't Hoff factor provided better correlations with the biomass data than the activity coefficient based on the DeBye-Huckel equation and the osmotic pressure based on the literature value of the van't Hoff factor. Because of the lack of sensitivity of the optical density measurements during the first few days of the

Table 9. Duncan's multiple range analyses of the biomass data from the initial bioassay with Selenastrum.

Test based on: Cell Counts					Optical Density				
Treatment Number	Concentration		Treatment		Treatment Number	Concentration		Treatment	
26	0.24	N	CASO4	*	26	0.24	N	CASO4	*
27	0.20	N	CASO4	*	27	0.20	N	CASO4	*
28	0.11	N	CASO4	*	28	0.11	N	CASO4	*
29	0.08	N	CASO4	*	29	0.08	N	CASO4	*
30	0.05	N	CASO4	*	30	0.05	N	CASO4	*
31	0.10	N	CASO4	*	31	0.10	N	CASO4	*
32	0.13	N	CASO4	*	32	0.13	N	CASO4	*
25	0.27	N	CASO4	*	25	0.27	N	CASO4	*
42	0.24	N	MGCL2	*	13	0.08	N	KCL	*
47	0.10	N	MGCL2	*	12	0.11	N	KCL	*
43	0.20	N	MGCL2	*	14	0.05	N	KCL	*
8	0.13	N	NACL	*	15	0.10	N	KCL	*
48	0.13	N	MGCL2	*	16	0.13	N	KCL	*
45	0.08	N	MGCL2	*	45	0.08	N	MGCL2	*
17	0.27	N	MGSO4	*	10	0.24	N	KCL	*
37	0.08	N	NA2SO4	*	11	0.20	N	KCL	*
38	0.05	N	NA2SO4	*	9	0.27	N	KCL	*
4	0.11	N	NACL	*	42	0.24	N	MGCL2	*
39	0.10	N	NA2SO4	*	43	0.20	N	MGCL2	*
40	0.13	N	NA2SO4	*	38	0.05	N	NA2SO4	*
3	0.20	N	NACL	*	1	0.27	N	NACL	*
1	0.27	N	NACL	*	47	0.10	N	MGCL2	*
36	0.11	N	NA2SO4	*	8	0.13	N	NACL	*
9	0.27	N	KCL	*	3	0.20	N	NACL	*
34	0.24	N	NA2SO4	*	17	0.27	N	MGSO4	*
10	0.24	N	KCL	*	2	0.24	N	NACL	*
24	0.13	N	MGSO4	*	4	0.11	N	NACL	*
35	0.20	N	NA2SO4	*	50	0.24	N	K2SO4	*
7	0.10	N	NACL	*	24	0.13	N	MGSO4	*
12	0.11	N	KCL	*	51	0.20	N	K2SO4	*
2	0.24	N	NACL	*	37	0.08	N	NA2SO4	*
44	0.11	N	MGCL2	*	49	0.27	N	K2SO4	*
23	0.10	N	MGSO4	*	39	0.10	N	NA2SO4	*
16	0.13	N	KCL	*	19	0.20	N	MGSO4	*
19	0.20	N	MGSO4	*	34	0.24	N	NA2SO4	*
22	0.05	N	MGSO4	*	18	0.24	N	MGSO4	*
21	0.08	N	MGSO4	*	33	0.27	N	NA2SO4	*
51	0.20	N	K2SO4	*	22	0.05	N	MGSO4	*
41	0.27	N	MGCL2	*	23	0.10	N	MGSO4	*
13	0.08	N	KCL	*	41	0.27	N	MGCL2	*
20	0.11	N	MGSO4	*	52	0.11	N	K2SO4	*
52	0.11	N	K2SO4	*	7	0.10	N	NACL	*
18	0.24	N	MGSO4	*	5	0.08	N	NACL	*
11	0.20	N	KCL	*	20	0.11	N	MGSO4	*
50	0.24	N	K2SO4	*	21	0.08	N	MGSO4	*
15	0.10	N	KCL	*	53	0.08	N	K2SO4	*
33	0.27	N	NA2SO4	*	48	0.13	N	MGCL2	*
49	0.27	N	K2SO4	*	35	0.20	N	NA2SO4	*
5	0.08	N	NACL	*	6	0.05	N	NACL	*
57	0.27	N	CACL2	*	44	0.11	N	MGCL2	*
53	0.08	N	K2SO4	*	40	0.13	N	NA2SO4	*
14	0.05	N	KCL	*	46	0.05	N	MGCL2	*
58	0.24	N	CACL2	*	54	0.05	N	K2SO4	*
6	0.05	N	NACL	*	36	0.11	N	NA2SO4	*
54	0.05	N	K2SO4	*	57	0.27	N	CACL2	*
56	0.13	N	K2SO4	*	58	0.24	N	CACL2	*
55	0.10	N	K2SO4	*	56	0.13	N	K2SO4	*
46	0.05	N	MGCL2	*	55	0.10	N	K2SO4	*
59	0.20	N	CACL2	*	64	0.13	N	CACL2	*
64	0.13	N	CACL2	*	59	0.20	N	CACL2	*
60	0.11	N	CACL2	*	63	0.10	N	CACL2	*
61	0.08	N	CACL2	*	62	0.05	N	CACL2	*
63	0.10	N	CACL2	*	61	0.08	N	CACL2	*
62	0.05	N	CACL2	*	60	0.11	N	CACL2	*
65	CONTROL			*	65	CONTROL			*

*Any group of treatments connected by a line of stars to the right of the ranking list are not significantly different from each other with 95 percent confidence.

Table 10. Single salt aadditions to AAM for bioassay 2 and the effects on Selenastrum.

Salt	Normality	Molarity	g/l	$\gamma_1^{\pm}$	$\gamma_2^{\pm}$	π_1(atm)	π_2(atm)
NaCl	0.05133	0.05133	3.000	0.80531	0.8111	2.37812	1.9926
	0.03422	0.03422	2.000	0.83277	0.8310	1.58541	1.3589
	0.01711	0.01711	1.000	0.87319	0.8639	0.80105	0.7021
	0.00856	0.00856	0.500	0.90550	0.8916	0.40493	0.3676
	0.00428	0.00428	0.250	0.93055	0.9106	0.20455	0.2041
KCl	0.05133	0.05133	3.8269	0.80531	0.7990	2.35309	2.1132
	0.03422	0.03422	2.5513	0.83277	0.8188	1.59376	1.4471
	0.01711	0.01711	1.2756	0.87319	0.8524	0.80105	0.7523
	0.00856	0.00856	0.6382	0.90550	0.8824	0.40493	0.3925
	0.00428	0.00428	0.3191	0.93055	0.8890	0.20455	0.2632
$MgCl_2$	0.05133	0.02567	2.4438	0.72342	0.6579	1.72134	1.3778
	0.03422	0.01711	1.6292	0.76247	0.6912	1.12231	0.9455
	0.01711	0.00856	0.8146	0.81992	0.7434	0.57400	0.5078
	0.00856	0.00428	0.4075	0.86592	0.7938	0.28909	0.2679
	0 00428	0.00214	0.2037	0.90153	0.8317	0.14559	0.1496
$CaCl_2$	0.05133	0.02567	2.8485	0.72342	0.6574	1.64623	1.3635
	0.03422	0.01711	1.8990	0.76247	0.6949	1.10562	0.9128
	0.01711	0.00856	0.9495	0.81992	0.7471	0.56357	0.4884
	0.00856	0.00428	0.4750	0.86592	0.7940	0.28700	0.2636
	0.00428	0.00214	0.2375	0.90153	0.8288	0.14507	0.1513
Na_2SO_4	0.05133	0.02567	3.6455	0.72342	0.6506	1.56486	1.3281
	0.03422	0.01711	2.4303	0.76247	0.6880	1.06390	0.8893
	0.01711	0.00856	1.2152	0.81992	0.7387	0.54687	0.4810
	0.00856	0.00428	0.6079	0.86592	0.7881	0.16176	0.2562
	0.00428	0.00214	0.3040	0.90153	0.8197	0.08662	0.1522
K_2SO_4	0.05133	0.02567	4.4725	0.72342	0.6299	1.53356	1.4219
	0.03422	0.01711	2.9817	0.76247	0.6701	1.16820	0.9344
	0.01711	0.00856	1.4908	0.81992	0.7242	0.45294	0.4986
	0.00856	0.00428	0.7459	0.86592	0.7727	0.28074	0.2706
	0.00428	0.00214	0.3729	0.90153	0.8191	0.14559	0.1436
$MgSO_4$	0.05133	0.02567	3.0894	0.52334	0.4851	0.81373	0.9001
	0.03422	0.01711	2.0596	0.58135	0.5210	0.69675	0.6257
	0.01711	0.00856	1.0298	0.67228	0.5924	0.31309	0.3297
	0.00856	0.00428	0.5152	0.74982	0.6557	0.16385	0.1772
	0.00428	0.00214	0.2576	0.81276	0.7045	0.08662	0.0999
$CaSO_4$	0.05133	0.02567	3.4941	0.52334	0.4966	0.99525	0.8491
	0.03422	0.01711	2.2394	0.58135	0.5260	0.66337	0.5957
	0.01711	0.00856	1.1647	0.67228	0.5905	0.33188	0.3178
	0.00856	0.00428	0.5827	0.74982	0.6472	0.17429	0.1738
	0.00428	0.00214	0.2913	0.81276	0.6939	0.08714	0.0988

$\gamma_1^{\pm}$ = Activity coefficient (DeBye-Hückel equation)

$\gamma_2^{\pm}$ = Activity coefficient (Davies equation)

π_1 = Osmotic Pressure

π_2 = Osmotic Pressure (equivalent conductance)

Table 11. Split plot factorial analysis of variance of single salt additions to Selenastrum (bioassay 2).

Source	DF	SS	MS	VAR	Var 1 (D.D.) Calc F	Var 1 (D.D.) dF	Var 1 (D.D.) F	Var 2 (C.C.) Calc F	Var 2 (C.C.) dF	Var 2 (C.C.) F
REP	1	.4932267E-02	.4932267E-02	1						
REP (Replicates)	1	2.137594	2.137594	2	2.92449727	1/47	N.S.	3.18	1/47	N.S.
A	5	1.884245	0.3768491	1						
A (Conc.)	5	688.4102	137.6820	2	223.45	5/47	S.01	204.89	5/47	S.01
B	3	1.396716	0.4655719	1						
B (Cations)	3	1090.982	363.6606	2	276.05	3/47	S.01	541.18	3/47	S.01
AB	15	0.4404872	.2936581E-01	1						
AB (Concentrations * Cations)	15	362.9585	24.19724	2	17.41	15/47	S.01	36.01	15/47	S.01
C	1	0.1632817	0.1632817	1						
C (Anions)	1	103.9508	103.9508	2	96.81	1/47	N.S.	154.69	1/47	N.S.
AC	5	0.1044255	.2088510E-01	1						
AC (Concentrations * Anions)	5	26.13784	5.227569	2	12.38	15/47	S.01	7.78	5/47	S.05
BC	3	0.2750743	.9169144E-01	1						
BC (Anions * Cations)	3	126.9280	42.30934	2	54.37	3/47	S.01	62.96	3/47	S.01
ABC (Anions * Cations *	15	0.1753275	.1168850E-01	1						
ABC Concentrations)	15	66.21303	4.414202	2	6.93	5/47	S.01	6.57	15/47	S.01
REP*ABC [(N-1) *	47	.7926713E-01	.1686535E-02	1						
REP*ABC (ABC-1)]	47	31.58291	0.6719767	2						
D	9	26.77909	2.975454	1						
D (Time)	9	8713.947	968.2163	2	8,467.23	9/432	S.01	4,482.84	9/432	S.01
AD	45	1.389440	.3087645E-01	1						
AD (Conccentration * Time)	45	708.1549	15.73678	2	87.86	45/432	S.01	72.86	45/432	S.01
BD	27	1.238859	.4588366E-01	1						
BD (Cations * Time)	27	937.2202	34.71186	2	130.57	27/432	S.01	160.72	27/432	S.01
ABD (Concentration *	135	0.8344270	.6180941E-02	1						
ABD Cations * Time)	135	339.9751	2.518334	2	17.59	135/432	S.01	11.66	135/432	S.01
CD	9	0.1700606	.1889562E-01	1						
CD (Anions * Time)	9	72.39926	8.044362	2	53.77	9/432	S.01	37.25	9/432	S.01
ACD (Concentration *	45	0.1208982	.2686627E-02	1						
ACD Anions * Time)	45	62.30643	1.384587	2	7.65	45/432	S.01	6.41	45/432	S.01
BCD (Cations * Anions	27	0.2495676	.9243244E-02	1						
BCD * Time)	27	438.6923	16.24786	2	26.30	27/432	S.01	75.23	27/432	S.01
ABCD (Concentration *	135	0.4209221	.3117941E-02	1						
ABCD Anions * Cations * Time)	135	178.3998	1.321480	2	8.87	135/432	S.01	6.12	135/432	S.01
ERROR [(N-1)*(d-1)*	432	0.1518086	.3514088E-03	1						
ERROR ABC]	432	93.30450	0.2159826	2	--	--	--	--	--	--
TOTAL	959	35.87883	.3741275E-01	1						
TOTAL	959	14043.70	14.64411	2						

Table 12. Summary of completely randomized design analyses of variance for Selenastrum single salt additions (bioassay 2).

F TESTS					
Alternate Hypotheses	Treatments	Variables Tested	Significance	Degrees of Freedom	F Value
Means of growth responses of cultures grown in the presence of divalent cations are different from those grown in the presence of monovalent cations	Na^+, K^+	Optical Density	N.S.	1/398	2.73
		Cell Counts	0.05	↓	6.58
	Mg^{++}, Ca^{++}				
Mean cell volumes of cultures grown in the presence of different salts are different	NaCl, KCl, $MgSO_4$, $CaSO_4$, Na_2SO4, $MgCl_2$, K_2SO4, $CaCl_2$, Control	Cell Volume (μ^3)	0.01	8/34 ↓	9.60
Means of electrical conductivity of the cultures changed from Day 0 to Day 15	Day 0, Day 15	Electrical Conductivity	N.S.	1/78	1.88

Table 13. Linear relations between salinity variables and different estimates of biomass for single salt additions to Selenastrum.

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
$\bar{X}$ (cells/ml)	Normality (N)	45	-0.4381**	y = -2.381E-9x + 2.868E15
	Concentration (g/l)	45	-0.3851**	y = -1.4314E-7x + 6.830E06
	Molarity (M)	45	-0.5083**	y = 1.914E-9x + 6.830E06
	π_2 (atm)	45	-0.4579**	y = 8.763E-8x + 6.830E06
	π_1 (atm)	45	-0.3817*	y = -1.052E-6x + 3.487E06
	Specific Conductivity (μmhos/cm)	45	-0.3782*	y = -332.163x + 3.6186E06
$\hat{\mu}$ (day^{-1}) (cell counts)	Normality (N)	45	-0.4753**	y = -0.0256x + 1.716
	Concentration (g/l)	45	-0.4008**	y = -1.462x + 3.677
	Molarity (M)	45	-0.3628*	y = -0.0138x + 1.716
	$\gamma^{\pm}_1$	45	0.4982**	y = +0.1152x + 1.627
	$\gamma^{\pm}_2$	45	0.5206**	y = +0.4180x + 2.0098
	π_2 (atm)	45	-0.3547*	y = -0.6763x + 2.1614

$\gamma_1^{\pm}$ = activity coefficient (DeBye-Hückel)

$\gamma_2^{\pm}$ = activity coefficient (Davies Equation)

π_1 = Osmotic pressure

π_2 = Osmotic pressure (equivalent conductance)

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at $P > 0.99$. If marked with (**), the value of the correlation coefficient is significantly different from zero at $P > 0.95$.

bioassay, the cell count data would appear to be more reliable.

Determination of which of the cations affected the Selenastrum biomass data the greatest was achieved by the use of the Duncan's Multiple Range Test (Table 14). This test uses the same output format as used in the previous Duncan's test output (Table 9). The treatments are ranked in the least to greatest values from the top to the bottom of the listing. All treatments connected by one line of vertical stars is not signficantly different from each other. Cell count data were used for this analysis because of the greater sensitivity of the measurement. Increasing concentrations of the individual salts caused decreasing productivity of Selenastrum when comparing groups of salts which are significantly different from each other. The cations depressed the $\hat{X}$ in the following order:

Mg, K > Na, Ca

For the Duncan's analysis of $\hat{\mu}$, the cation order was Mg, Ca > K, Na. Because depression occurred in both the $\hat{X}$ and $\hat{\mu}$ as compared to the controls, the toxic effect of these cations was established.

Effects of Single Salt Additions on Acclimated Selenastrum

Selenastrum, which was acclimated to higher salinity conditions by maintaining the culture in Lake Powell synthetic medium, was then tested in AAM with the concentrations of salt additions at the 0.3 N, 0.1 N, 0.05 N previously described first bioassay. A long lag time was noted as the acclimated Selenastrum adjusted to the AAM medium. The growth of the acclimated Selenastrum did not show a significant difference based on cations or anions (Table 15). Although the $\hat{X}$ for all of the salts was significantly lower than the controls, the $\hat{\mu}$ for the majority of the salts was not significantly different than the controls.

Effects of Two Salt Additions

The effects of the addition of two salts at one concentration did not maintain the cation dominance effect on algal growth depression. The Duncan's Multiple Range analysis (Table 16) of these data did not provide a clear relationship between monovalent and divalent toxicity.

Difficulties were encountered in calculation of activity coefficients and osmotic pressures with mixed salt solution. Therefore the electical conductivity of these salt solutions was linearly correlated with the Selenastrum biomass data from the two salt additions (Table 17). Although the maximum standing crop data do significantly correlate with the electrical conductivity, this correlation is slightly lower than the same correlation of the single salt addition data.

Growth measurements of $\hat{X}$ are plotted versus specific conductivity and identified by the cations (Figure 9) and anions (Figure 10) that were added to the medium. From the plots, regional effects of the salts added can be noted. The combination of monovalent and divalent cations encompasses a larger area than the monovalent or divalent cations alone and overlaps portions of each of these areas. This visually demonstrates the synergistic effects occurring between the monovalent and divalent cations present in the same salt solution. The areas of anion action overlapped more than the cation areas demonstrating less distinct regions of effect based on the anions.

Effects of Increased Salt Concentrations on the Productivity of Synedra

Effects of Single Salt Additions

The concentrations of the salts under study were normalized to the concentration of NaCl (Table 18). The Davies equation was used to calculate the activity coefficient and the osmotic pressure of the solutions because these calculations had provided better linear correlations with the biomass data in the single salt addition bioassay with Selenastrum.

A randomized block design was used to test if there was a significant difference in growth based on the different salts. There was a significant difference at the 99th percentile (Table 19). A significant difference in the growth of Synedra occurred based on differences of cations, which agreed with the results of the single salt addition bioassay with Selenastrum. A difference did occur in the result of anion difference, with Synedra there was a significant difference ($P \geq 99\%$) in growth between the different anions tested. There was no significant difference based on anion differences with Selenastrum. The bicarbonate anion was tested only with the Synedra and so there was a slight change in the experimental design between the two experiments. When HCO_3^- was eliminated from the experimental data, no significant difference in the biomass data could be found based on the anion differences. The electrical conductivity readings of the media did not change significantly over the time of the bioassay within each treatment flask.

Linear correlation coefficients with salt variables and growth explained less of the variability in general than they did for the Selenastrum correlations (Table 20). Normality and the concentration in g/l did not correlate significantly with either biomass variable. The only salinity concentration measurement which correlated significantly with both the $\hat{X}$ and $\hat{\mu}$ data was specific conductivity.

Table 14. Duncan's multiple range analysis for single salt additions to Selenastrum (bioassay 2).

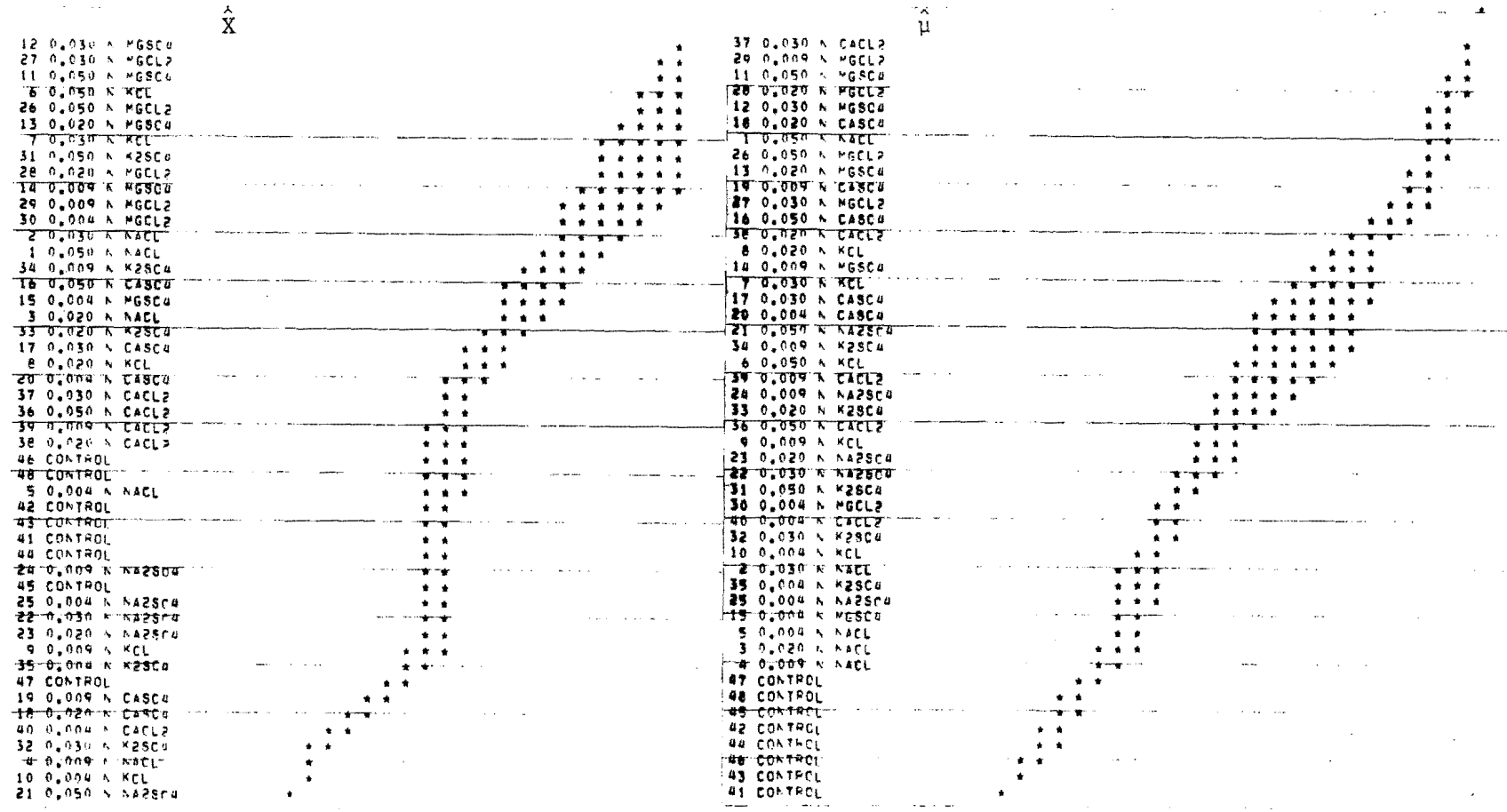


Table 15. Duncan's multiple range analysis for single salt additions to acclimated Selenastrum (bioassay 2).

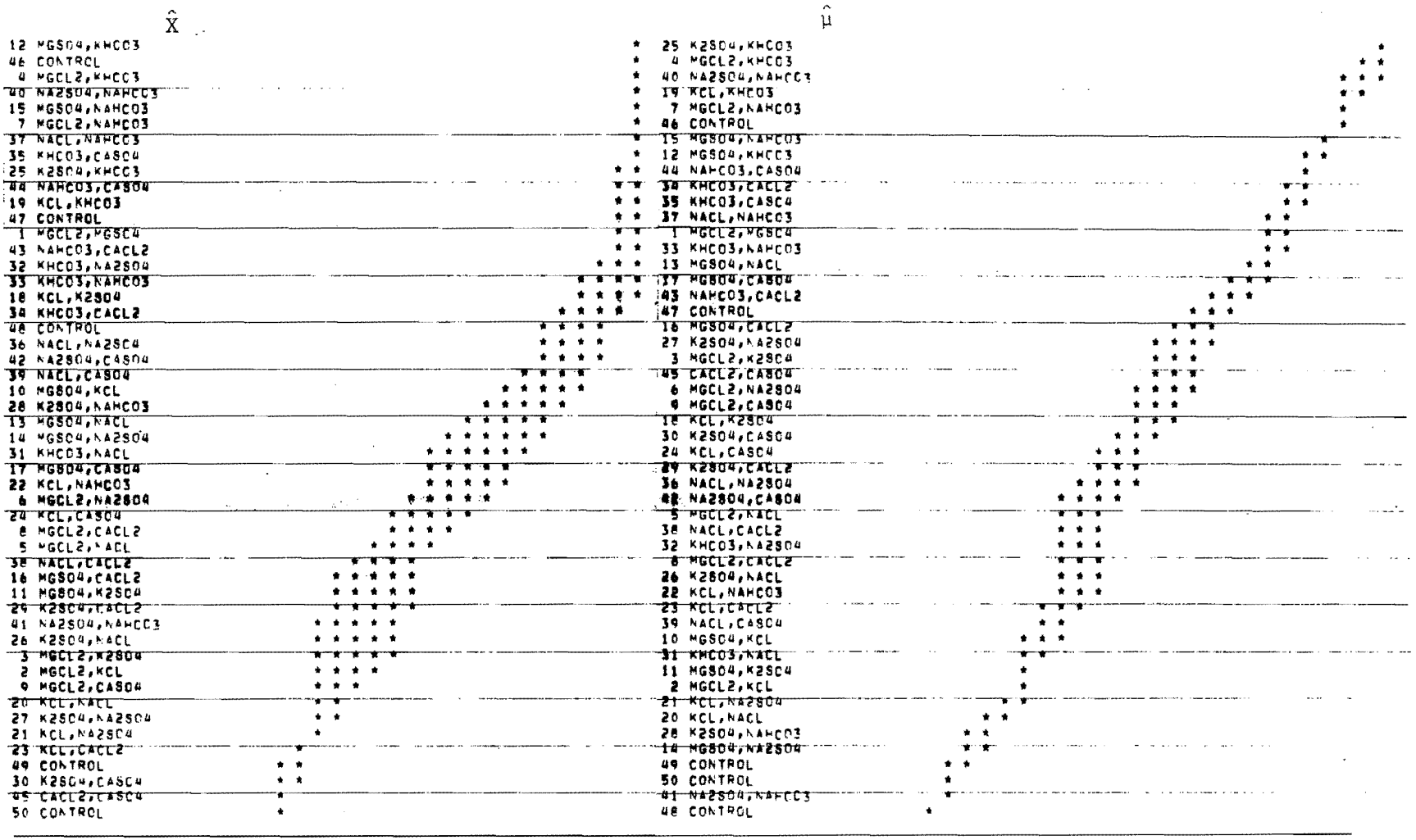
$\hat{X}$

Treatment Number	Concentration	Treatment	
27	0.30	N NAHCO3	*
28	0.05	N KHCO3	*
18	0.30	N K2SO4	*
29	0.10	N KHCO3	*
30	0.30	N KHCO3	*
17	0.10	N K2SO4	*
6	0.30	N KCL	*
35	10ML	CP ELUTRIATE	*
25	0.05	N NAHCO3	*
9	0.30	N MGCL2	*
14	0.10	N NA2SO4	*
16	0.05	N K2SO4	*
15	0.30	N NA2SO4	*
26	0.10	N NAHCO3	*
10	0.05	N CACL2	*
13	0.05	N NA2SO4	*
8	0.10	N MGCL2	*
12	0.30	N CACL2	*
5	0.10	N KCL	*
11	0.10	N CACL2	*
3	0.30	N NACL	*
21	0.30	N MGSO4	*
4	0.05	N KCL	*
19	0.05	N MGSO4	*
24	0.30	N CASO4	*
7	0.05	N MGCL2	*
20	0.10	N MGSO4	*
2	0.10	N NACL	*
23	0.10	N CASO4	*
22	0.05	N CASO4	*
1	0.05	N NACL	*
39	10ML	BP ELUTRIATE	*
37	10ML	DP ELUTRIATE	*
36	10ML	DP ELUTRIATE	*
38	10ML	BP ELUTRIATE	*
40	D.W.	BLANK	*
32	CONTROL		*
33	CONTROL		*
31	CONTROL		*
34	10ML	CP ELUTRIATE	*

$\hat{\mu}$

Treatment Number	Concentration	Treatment	
14	0.10	N NA2SO4	*
30	0.30	N KHCO3	*
28	0.05	N KHCO3	*
8	0.10	N MGCL2	*
13	0.05	N NA2SO4	*
29	0.10	N KHCO3	*
27	0.30	N NAHCO3	*
9	0.30	N MGCL2	*
15	0.30	N NA2SO4	*
26	0.10	N NAHCO3	*
12	0.30	N CACL2	*
16	0.05	N K2SO4	*
2	0.10	N NACL	*
7	0.05	N MGCL2	*
21	0.30	N MGSO4	*
11	0.10	N CACL2	*
4	0.05	N KCL	*
35	10ML	CP ELUTRIATE	*
25	0.05	N NAHCO3	*
10	0.05	N CACL2	*
5	0.10	N KCL	*
24	0.30	N CASO4	*
20	0.10	N MGSO4	*
3	0.30	N NACL	*
1	0.05	N NACL	*
23	0.10	N CASO4	*
22	0.05	N CASO4	*
40	D.W.	BLANK	*
32	CONTROL		*
33	CONTROL		*
38	10ML	BP ELUTRIATE	*
39	10ML	BP ELUTRIATE	*
36	10ML	DP ELUTRIATE	*
37	10ML	DP ELUTRIATE	*
34	10ML	CP ELUTRIATE	*
6	0.30	N KCL	*
18	0.30	N K2SO4	*
19	0.05	N MGSO4	*
31	CONTROL		*
17	0.10	N K2SO4	*

Table 16. Duncan's multiple range analysis for two salt additions to Selenastrum (bioassay 2).



Determination of salt effects on the biomass data of Synedra was done by the use of the Duncan's Multiple Range Test (Table 21). Fluorescence data were used for these analyses because of the greater sensitivity of this measurement as compared to the optical density measurement. Increasing concentrations of the individual salts decreased the growth of the Synedra when comparing salts which are significantly different from each other. The majority of the salts depressed growth below the levels of the controls. The cation orders were somewhat reversed from the first bioassay when comparing the cation and anion effects on $\hat{X}$ and $\hat{\mu}$.

Cations $\hat{X}$: K, Na > Mg, Ca

Anions $\hat{X}$: HCO_3, SO_4 > Cl

Cations $\hat{\mu}$: K, Na > Mg > Ca

Anions $\hat{\mu}$: HCO_3 > SO_4 > Cl

The relative inhibition of X is plotted versus specific conductivity and identified by valance of the cations (Figure 11) and anions (Figure 12) added to the medium. The large area of overlap between the monovalent and divalent cations displays the lack of differentiation in inhibition based on the valance of the cation. The total separation

Table 17. Linear relation between different estimates of biomass for two salt additions to Selenastrum (bioassay 3).

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
$\hat{X}$ (cells/ml)	Specific Conductivity (μmhos/cm)	50	-0.3414*	y = -1.223E-04x + 2.689E06

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at P > 0.99. If marked with (**), the value of the correlation coefficient is significantly different from zero at P > 0.95.

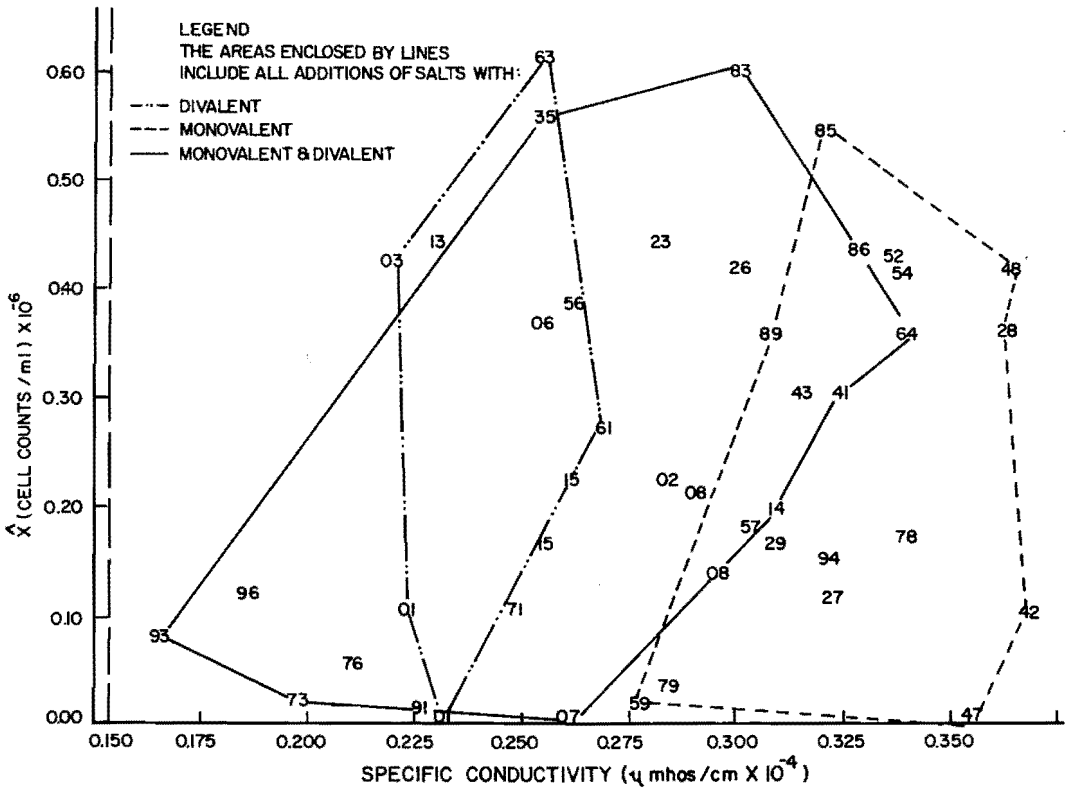


Figure 9. The regional effects of the cations added to the media (bioassay 2).

Table 18. Single salt additions to AAM and Synedra (bioassay 4).

Salt	Normality	Molarity	g/l	$\gamma_2^{\pm}$	π_2 (atm)
NaCl	0.300	0.300	17.5364	0.7347	11.9913
	0.200	0.200	11.6909	0.7471	8.0834
	0.100	0.100	5.8455	0.7747	4.2646
	0.050	0.050	2.9182	0.8057	2.2800
KCl	0.300	0.300	22.3636	0.7314	12.3804
	0.200	0.200	14.9091	0.7388	8.4527
	0.100	0.100	7.4545	0.7666	4.3222
	0.050	0.050	3.7273	0.7968	2.3157
$MgCl_2$	0.300	0.150	14.2818	0.5469	7.7318
	0.200	0.100	9.5182	0.5608	5.6234
	0.100	0.050	4.7636	0.6048	3.0101
	0.050	0.025	2.3818	0.6572	1.6169
$CaCl_2$	0.300	0.150	16.6455	0.5428	8.0083
	0.200	0.100	11.1000	0.5587	5.6429
	0.100	0.050	5.5455	0.5982	3.1022
	0.050	0.025	2.773	0.6542	1.6213
Na_2SO_4	0.300	0.150	21.3045	0.5346	15.8356
	0.200	0.100	14.2045	0.5368	11.3480
	0.400	0.050	7.1000	0.5656	6.0079
	0.050	0.025	3.5500	0.6089	3.2786
K_2SO_4	0.300	0.150	26.1409	0.5624	15.6339
	0.200	0.100	17.4273	0.5343	10.9261
	0.100	0.050	8.7136	0.5481	6.0593
	0.050	0.025	4.3545	0.5872	3.2320
$MgSO_4$	0.300	0.150	18.0545	0.3306	5.2435
	0.200	0.100	12.0364	0.3584	3.6142
	0.100	0.050	6.0182	0.4115	1.9475
	0.050	0.025	3.0091	0.4608	1.0990
$CaSO_4$	0.300	0.150	20.4182	0.5090	4.0259
	0.200	0.100	13.6182	0.5131	2.7954
	0.100	0.050	6.8091	0.5023	1.6066
	0.050	0.025	3.400	0.4885	1.0401
$NaHCO_3$	0.300	0.300	25.2000	0.7410	11.6096
	0.200	0.200	16.8000	0.7543	7.9759
	0.100	0.100	8.4000	0.7821	4.2591
	0.050	0.050	4.2000	0.8155	2.2387
$KHCO_3$	0.300	0.300	30.0364	0.7329	12.5914
	0.200	0.200	20.0273	0.7424	8.6224
	0.100	0.100	10.0091	0.7682	4.5669
	0.050	0.050	5.0091	0.8077	2.2770

$\gamma_1^{\pm}$ = activity coefficient (Davies Equation)

π_2 = Osmotic pressure (equivalent conductance)

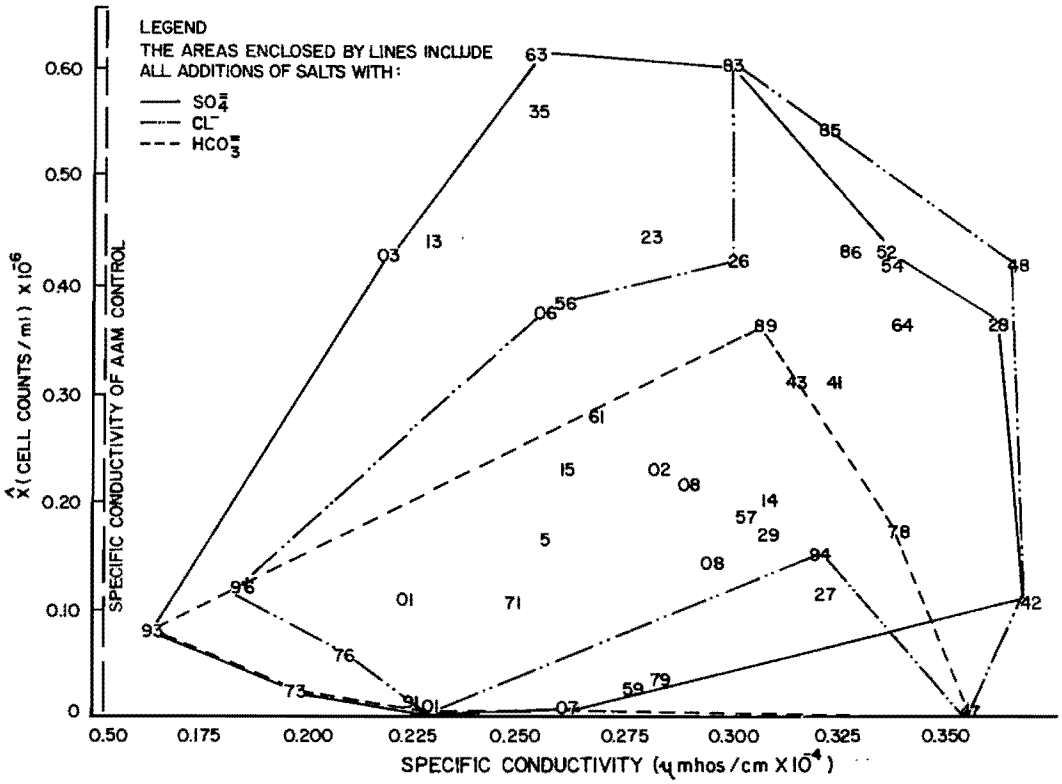


Figure 10. The regional effects of the anions added to the media (bioassay 3).

Table 19. Summary of analyses of variance for Synedra single salt additions (bioassay 4).

Alternate Hypotheses	Blocked By	Treatments	Variables Tested	Significance	Degrees of Freedom	F
Means of growth responses of cultures grown in the presence of different salts are different	Time	NaCl KCl $MgSO_4$ $CaSO_4$ Na_2SO_4 $MgCl_2$ K_2SO_4 $CaCl_2$ $NaHCO_3$ $KHCO_3$ Control	Optical Density	0.01	9/70	3.16
			Fluorescence	0.01	↓	5.00
Means of growth responses of cultures grown in the presence of different cations are different	-	Na K Mg Ca	Optical Density	0.01	3/316	24.68
			Fluorescence	0.01	↓	19.32
Means of growth responses of cultures grown in the presence of different anions are different	-	Cl SO_4 HCO_3	Optical Density	0.01	2/317	12.15
			Fluorescence	0.01		20.37

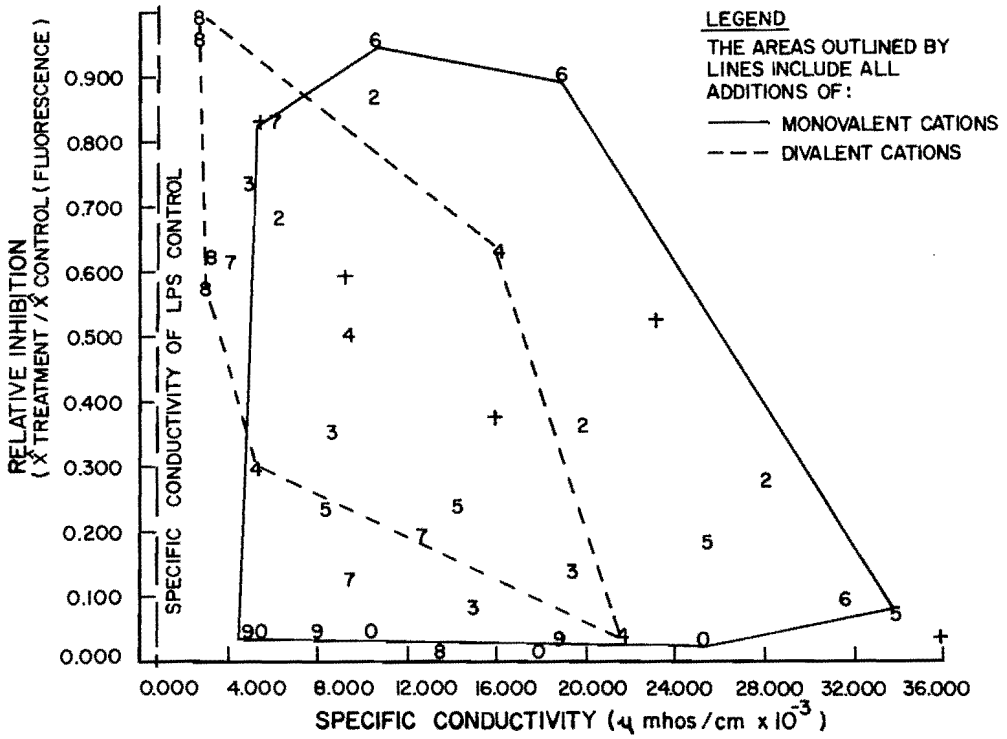


Figure 11. The relative inhibition of the $\hat{X}$ identified by the cation present during growth (bioassay 4).

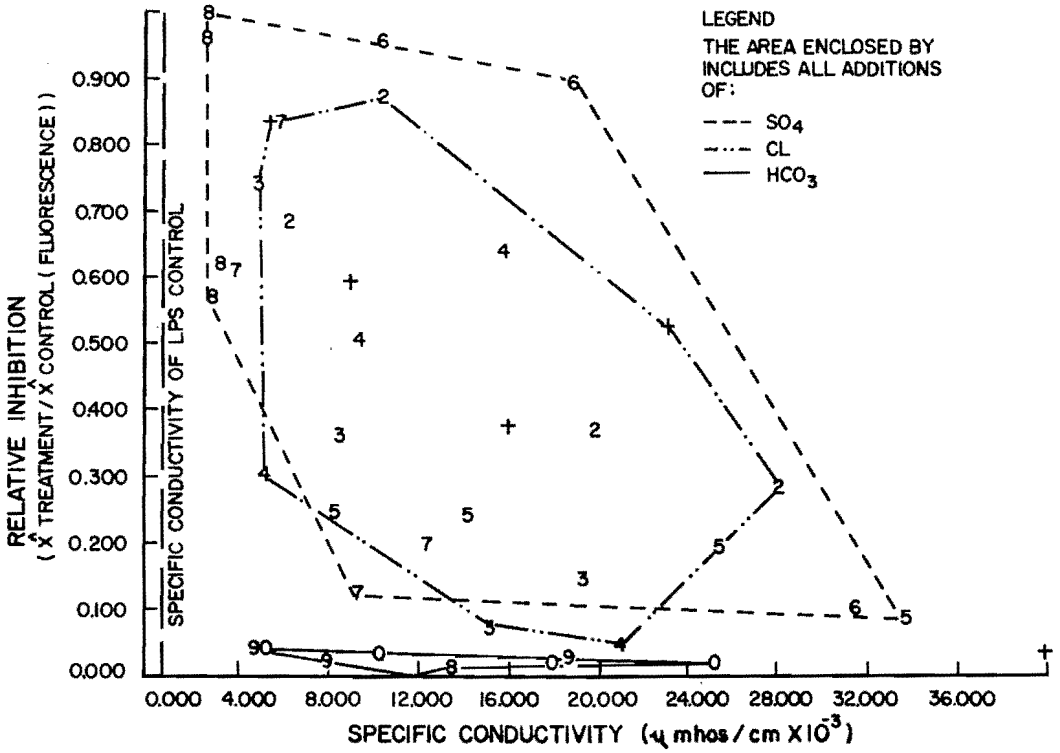


Figure 12. The relative inhibition of the $\hat{X}$ identified by the anion present during growth (bioassay 4).

Table 20. Linear relations between different estimates of biomass for single salt additions to Synedra (bioassay 4).

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
$\hat{X}$ (fluorescence)	$\gamma^{\pm}$	44	0.4400**	y = 1.14x + 6.77
	π	44	-0.4600**	y = -0.81x + 12.21
	Molarity (M)	44	-0.4100**	y = -33.12x + 11.64
	Specific Conductivity (μmhos/cm)	44	-0.5710**	y = -737.9x + 9.156E06
$\hat{\mu}$ (day^{-1}) (fluorescence)	Specific Conductivity (μmhos/cm)	44	-0.3900**	y = -3.54E04 x +4.394E08

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at P > 0.99. If marked with (**), the value of the correlation coefficient is significantly different from zero at P > 0.95.

of the HCO_3^- anion at a greater level of inhibition than the overlapping Cl^- and SO_4^- anion areas displays the significant difference in growth based on the anion present in the media when HCO_3^- is tested but not with the addition of Cl^- and SO_4^-.

In order to compare the bioassay data from two different algae based on fluorescence data, it was necessary to normalize them on the basis of the control data for that alga (Appendix B-1, B-2). This eliminated the variability due to different fluorescent characteristics of the different species of algae. After normalizing the data and comparing the bioassay results for single salt additions from acclimated Selenastrum to Synedra, 38 percent of the $\hat{X}$ and 18 percent of the $\hat{\mu}$ data for the acclimated Selenastrum was lower than the minimum Synedra results.

Effects of Two Salt Additions

The effects of the addition of two salts at one concentration were dominated by synergistic effects rather than the dominance of individual cations and anions (Table 22). There was also no clear relationship between monovalent and divalent toxicity. This same result occurred with the two salt additions to Selenastrum.

Linear relationships between biomass measurements and specific conductivity measurements and did not provide any significant correlations.

Effects of Multiple Salt Additions

Depression of algal growth was attained with the addition of multiple combinations of salts to Synedra (Tables 23 and 24). Again there was no relationship for monovalent to divalent toxicity.

Linear correlations of the fluorescence data with electrical conductivity measurements provided significant correlations with the $\hat{X}$ data for the multiple salt additions (Table 25). However, the $\hat{\mu}$ results (greater than four salt additions) did not produce a significant correlation. These correlation coefficients were comparable to the correlation coefficients obtained with the same relationship for the single salt additions to Synedra. When combining all of the Synedra biomass data and correlating that with specific conductance, this relationship did not hold. The slope of the line became positive but was not significant.

Effects of Increased Salt Concentrations on the Productivity of Three Algal Species

Effects of Single Salt Additions

The effect of single salt additions at one concentration (0.05 N) on Anabaena that was cultured with representatives of two other algal species was analyzed with Duncan's Multiple Range Tests (Table 26). Anabaena produces specialized cells, heterocysts, when nitrogen limitation in the growth medium is encountered. Counts of these cells were conducted separately from the total cell counts. The number of heterocysts were never greater than 0.001 percent of the total cell counts in any of the treatment flasks indicating that nitrogen fixation was insignificant and nitrogen was not limiting. The effects of these salts on the growth depression of Anabaena were as follows:

- $\hat{X}$ cell counts : all salts - $CaSO_4$, Na_2SO_4 > Anabaena control
- $\hat{\mu}$ cell counts : Anabaena control > KCl, NaCl, $CaCl_2$, $CaSO_4$, $MgCl_2$
- $\hat{X}$ heterocysts : all salts - $CaSO_4$ > Anabaena control

Table 21. Duncan's multiple range analysis of single salt additions to Synedra (bioassay 4).

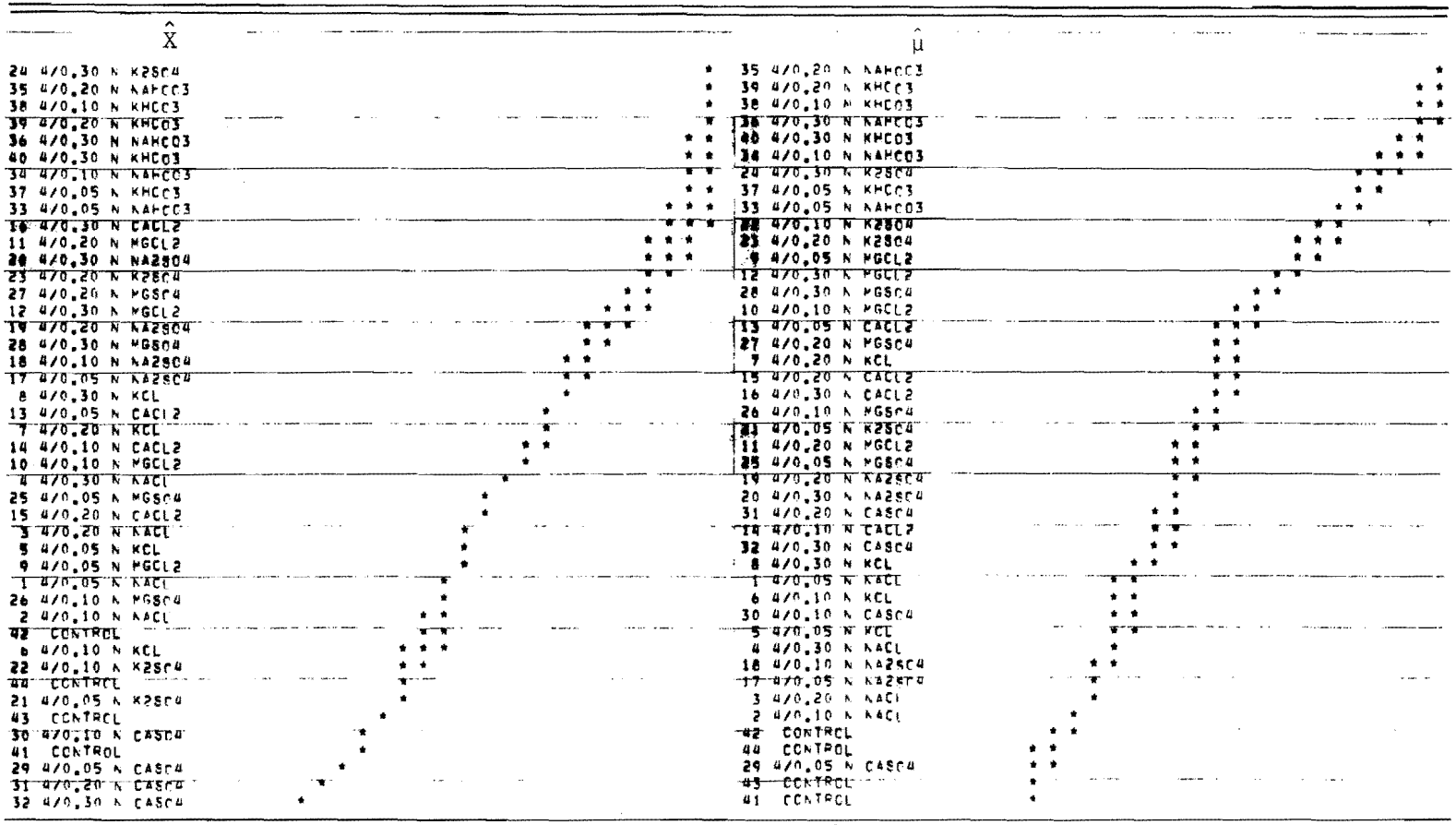


Table 22. Duncan's multiple range analysis for two salt additions to Synedra (bioassay 5).

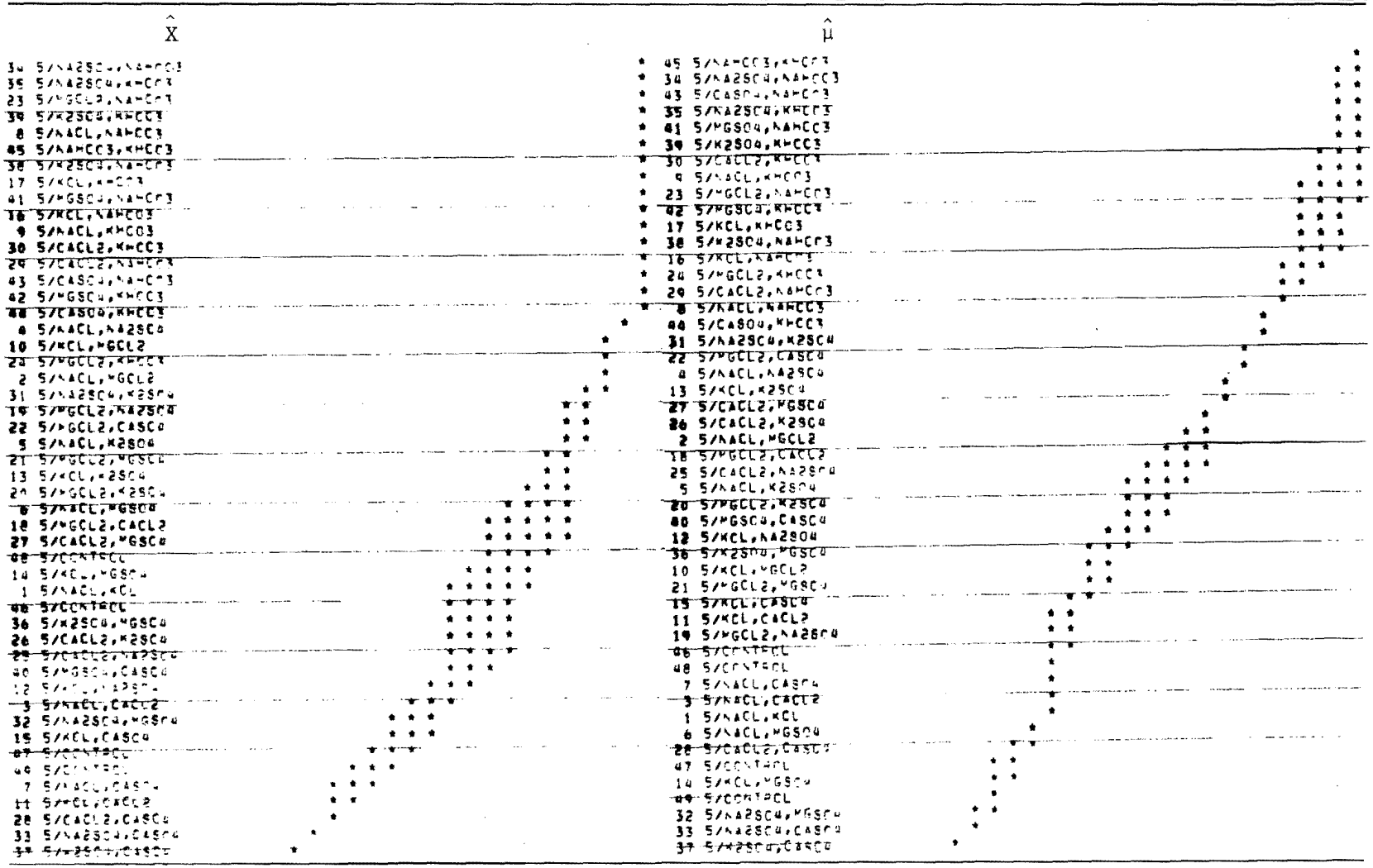
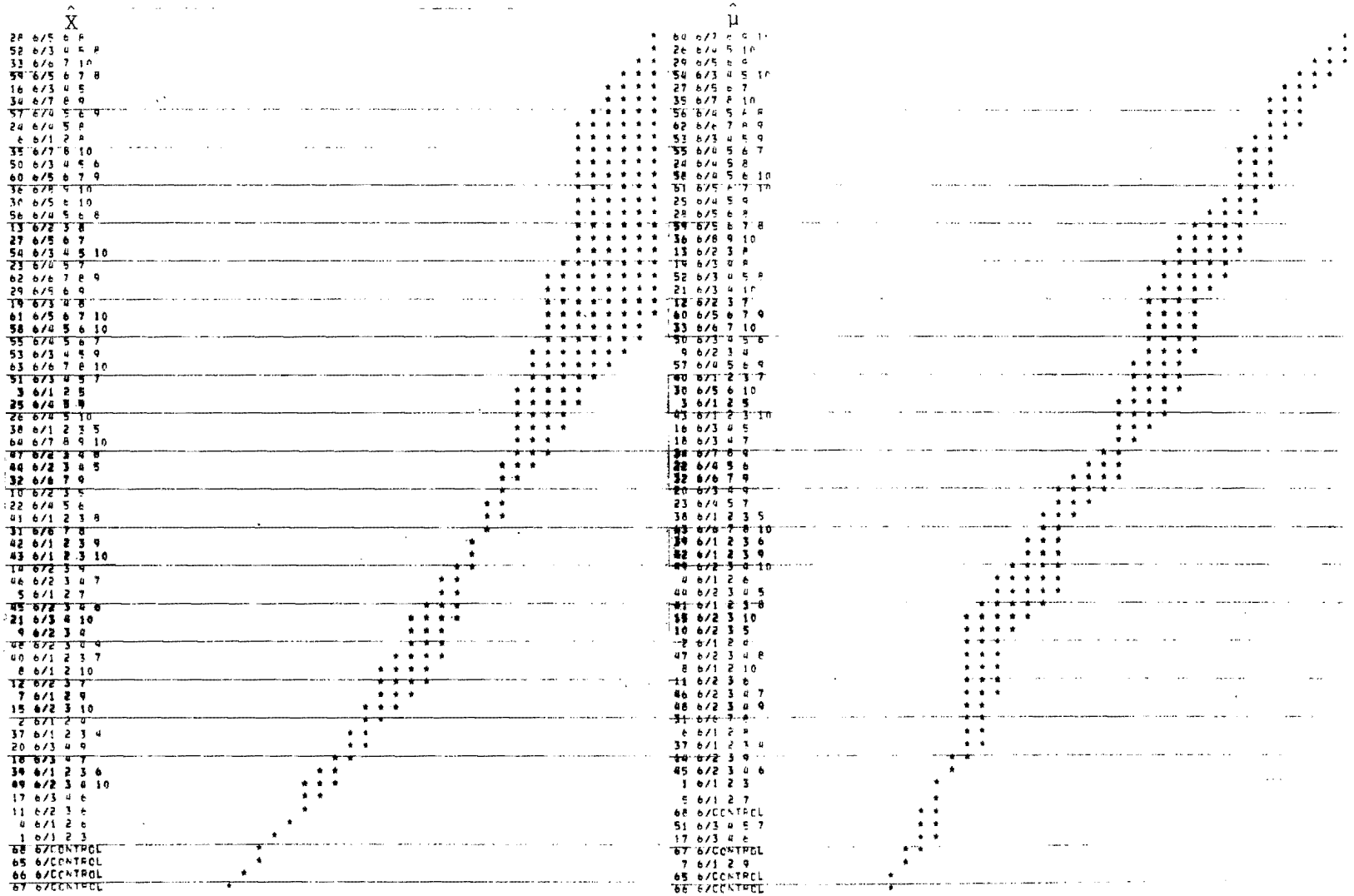


Table 23. Duncan's multiple range analysis of 3 and 4 salt additions to Synedra (bioassay 6).



Legend: 1 = mg SO_4, 2 = mg Cl_2, 3 = K_2SO_4, 4 = KCl, 5 = $KHCO_3$, 6 = Na_2SO_4, 7 = NaCl, 8 = $NaHCO_3$, 9 = $CaSO_4$, 10 = $CaCl_2$

Table 24. Duncan's multiple range analysis of multiple salt additions to Synedra (bioassay 7).

Table 25. Linear relations between different estimates of biomass for multiple salt additions to Synedra (bioassay 6 and 7).

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
$\hat{X}$ (fluorescence) (3 & 4 salt additions)	Specific Conductivity (µmhos/cm)	68	-0.5570**	y = -222.8x + 1.313E06
$\hat{X}$ (fluorescence) (> 4 salt additions)		60	-0.3227*	y = 1.200E03x + 1.906E07
$\hat{X}$ (fluorescence) (all Synedra data)		354	0.3750**	y = 1.526E04x + -3.571E08
$\hat{\mu}$ (fluorescence) (3 & 4 salt additions)		68	-0.4734**	y = -6.307E03x + 3.718E07

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at P > 0.99. If marked with (**), the value of the correlation coefficient is significantly different from zero at P > 0.95.

$\hat{\mu}$ heterocysts : $NaHCO_3$ > Anabaena control

$\hat{X}$ phycocyanin fluorescence : Anabaena control > all salts - $CaSO_4$

$\hat{\mu}$ phycocyanin fluorescence : $CaCl_2$, $KHCO_3$, $NaHCO_3$ > Anabaena control

The effects of these salts on the growth depression of Synedra (Table 27) were as follows:

$\hat{X}$ cell counts : all salts > Synedra control

$\hat{\mu}$ cell counts : Synedra control > NaCl, $CaCl_2$, $MgSO_4$, K_2SO_4, $CaSO_4$, $MgCl_2$

$\hat{X}$ carotenoid fluorescence : all salts - $NaHCO_3$, $CaSO_4$ > Synedra control

$\hat{\mu}$ carotenoid fluorescence : all salts - KCL > Synedra control

Scenedesmus data analyzed by the same method (Table 28) provided the following results:

$\hat{X}$ cell counts : All Salts - $MgSO_4$, Na_2SO_4 > Scenedesmus control

$\hat{\mu}$ cell counts : Scenedesmus control > $CaCl_2$, K_2SO_4, $MgCl_2$, $CaSO_4$, $MgSO_4$

$\hat{X}$ Chlorophyll *a* fluorescence : $CaCl_2$, KCL, $MgCl_2$, NaCl > Scendesmus control

$\hat{\mu}$ Chlorophyll *a* fluorescence : $CaCl_2$, NaCl, Na_2SO_4, $KHCO_3$, $NaHCO_3$ > Scendesmus control

Growth depression for the three algal general occurred in the following order:

$\hat{X}$ cell counts : All salts - $MgSO_4$ > 3 algae control

$\hat{\mu}$ cell counts : three algae control > NaCl, $CaCl_2$, $CaSO_4$, $MgCl_2$

Linear correlation coefficients were used to assess the cell count biomass measurements with the fluorescence biomass measurements (Table 29). Anabaena consistently provided the lowest correlation values with the fluorescence data and the total cell counts consistently provided the highest correlations with the fluorescence data. Although Scenedesmus correlated the best value with the chlorophyll *a* fluorescence measurements, Anabaena did not correlate the best with the phycocyanin fluorescence measurements and Synedra did not correlate the best with the carotenoid fluorescence measurements. Specific conductivity correlated better with the fluorescence measurements than with the cell count measurements.

Comparing the overall growth depression of the three algal genera bioassay to the growth depression produced in the comparable Synedra bioassay, growth depression occurred in the following order with the percentages quantifying the relationship:

$\hat{X}$ chlorophyll *a* fluorescence : Syndedra (38 percent) > three algae

$\hat{\mu}$ chlorophyll *a* fluorescence : Synedra (50 percent) > three algae

Table 26. Duncan's multiple range test for Anabaena (bioassay 9).

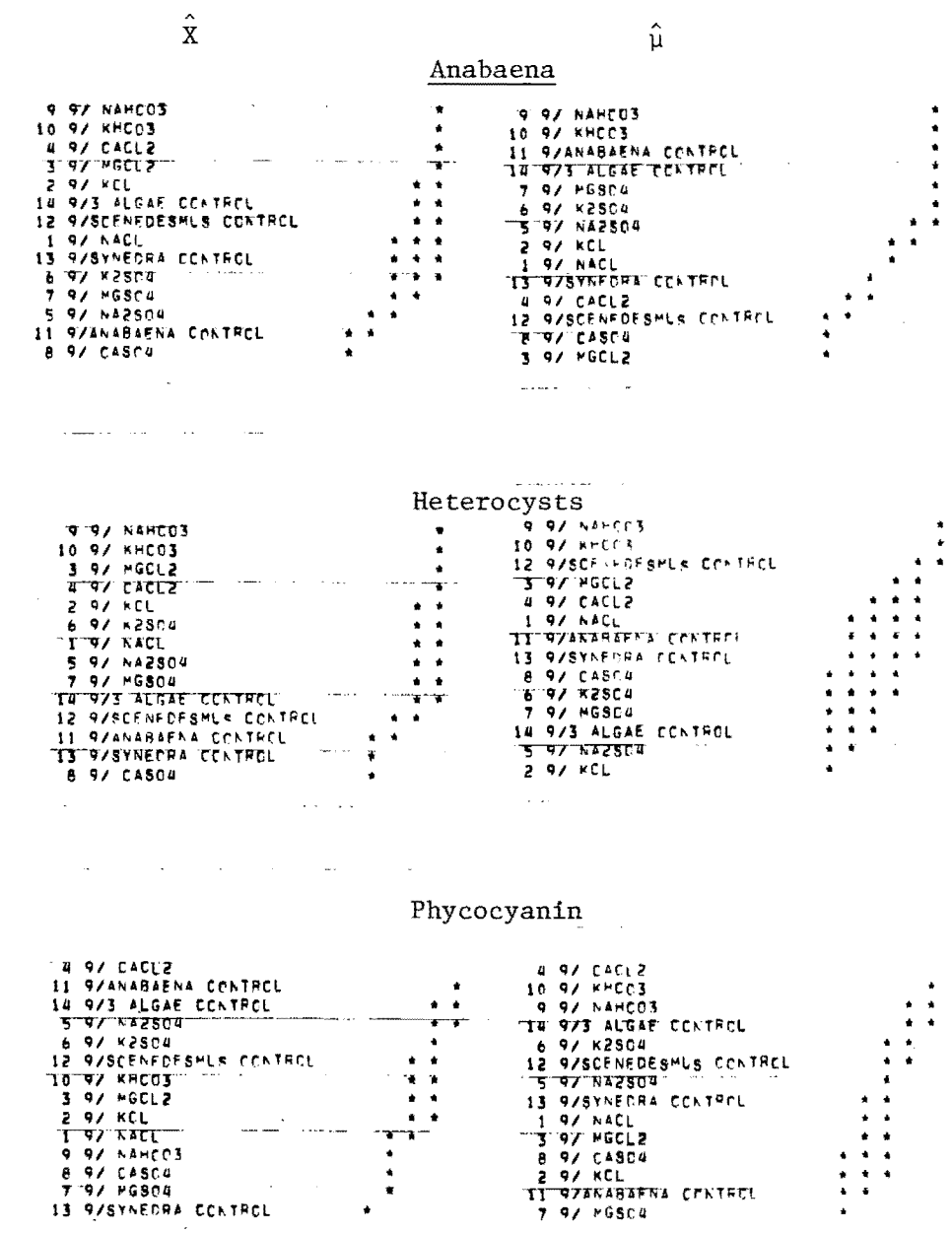


Table 27. Duncan's multiple range test for Synedra (bioassay 9).

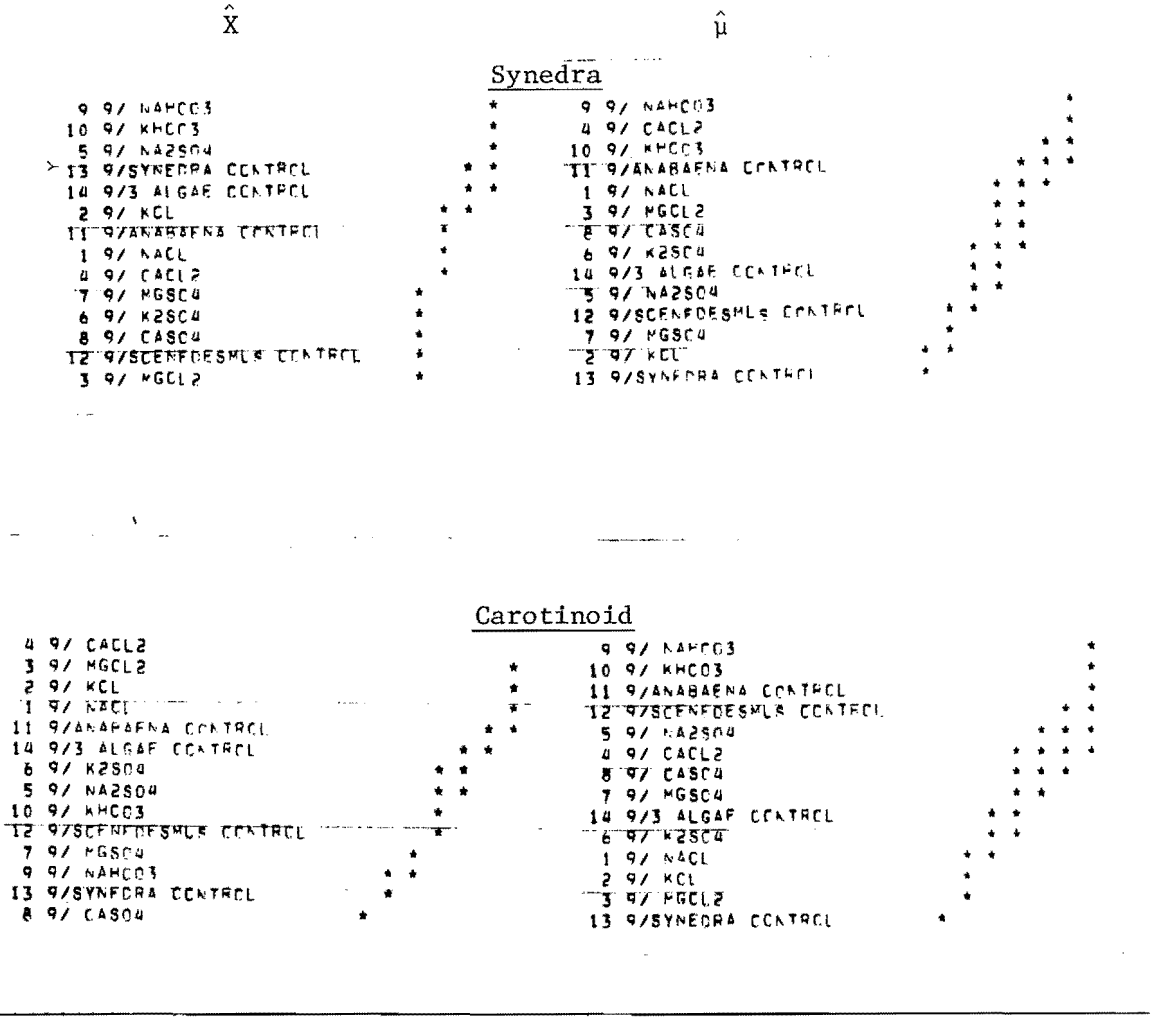


Table 28. Duncan's multiple range test for Scenedesmus (bioassay 9).

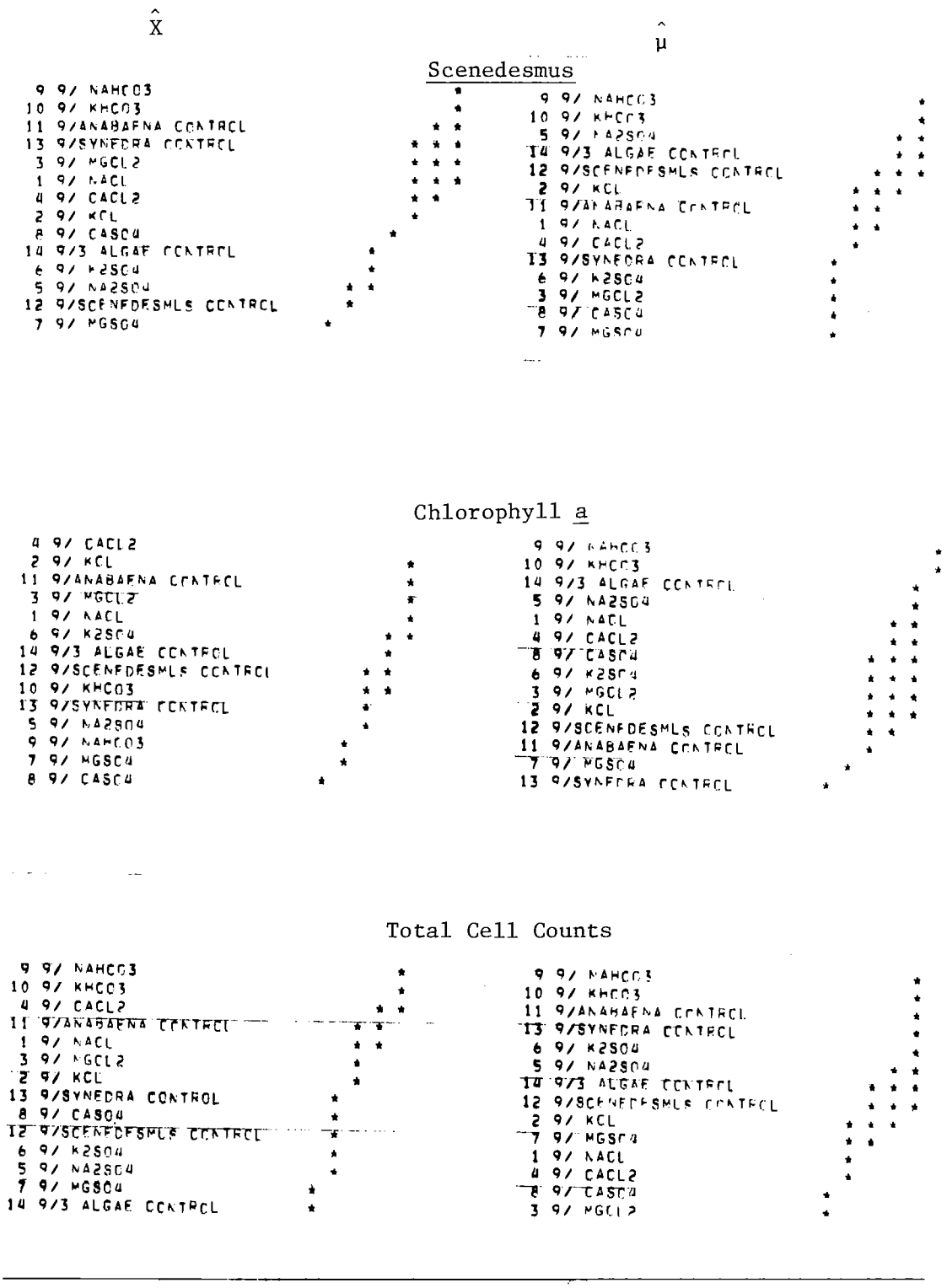


Table 29. Linear relations between different estimates of biomass for single salt additions to three algal genera.

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
Phycocyanin fluorescence	Number of Anabaena/ml	125	0.3052*	y = 2.105E11x - 2.604E21
Chrotenoid fluorescence	Number of Anabaena/ml	125	0.3679*	y = 9.038E10x - 1.118E21
Chlorophyll a fluorescence	Number of Anabaena/ml	125	0.3834*	y = 8.599E10x - 1.064E21
Phycocyanin fluorescence	Number of Scenedesmus/ml	125	0.5698*	y = 5.709E11x - 4.173E22
Carotenoid fluorescence	Number of Scenedesmus/ml	125	0.6917*	y = 2.436E11x - 1.701E22
Chlorophyll a fluorescence	Number of Scenedesmus/ml	125	0.7700*	y = 2.171E11x - 1.587E22
Phycocyanin fluorescence	Number of Synedra/ml	125	0.6568*	y = 2.823E11x - 1.118E22
Carotenoid fluorescence	Number of Synedra/ml	125	0.5550*	y = 1.731E11x - 6.852E21
Chlorophyll a fluorescence	Number of Synedra/ml	125	0.4408*	y = 2.162E11x - 8.558E21
Phycocyanin fluorescence	Total # algal cells/ml	125	0.7561*	y = 5.870E11x - 7.574E22
Carotenoid fluorescence	Total # algal cells/ml	125	0.8202*	y = 2.804E11x - 3.617E22
Chlorophyll a fluorescence	Total # algal cells/ml	125	0.8407*	y = 2.712E11x - 3.499E22
Phycocyanin fluorescence	Specific Conductivity (μmhos/cm)	125	-0.3061*	y = -1.907E04x + 5.700E07
Carotenoid fluorescence	Specific Conductivity (μmhos/cm)	125	-0.3019*	y = -1.002E04x + 2.995E07
Chlorophyll a fluorescence	Specific Conductivity (μmhos/cm)	125	-0.2627*	y = -1.142E04x + 3.412E07
Number of Anabaena/ml	Specific Conductivity (μmhos/cm)	125	N.S.	-
Number of Scenedesmus/ml	Specific Conductivity (μmhos/cm)	125	N.S.	-
Number of Synedra/ml	Specific Conductivity (μmhos/cm)	125	-0.3038*	y = -5.764E-08x + 3.957E10
Total # algal cells/ml	Specific Conductivity (μmhos/cm)	125	-0.2282*	y = -6.272E-01x + 1.290E11

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at $P > 0.99$. If marked with (**), the value of the correlation coefficient is significantly different from zero at $P > 0.95$.

Evaluation of Elutriates and Leachates of Oil Shales

Chemical Evaluations

The cations and anions prevalent in previous spent oil shale analyses in the literature were also prevalent in the analyses of AP shale (Appendix A-1) which was leached in the up-flow column (Appendix A-2). These analyses are grouped by the elapsed time at which the leachate was collected from the column. The analysis period extended over day 1 to day 12. The total concentration of the ions and the pH of the leachate decreased steadily. Throughout this time period the anion concentrations (meq/l) remained in the same order of dominance:

$$SO_4 > HCO_3 > Cl$$

The relative abundance of the cations with the exception of potassium and boron shifted during the analysis period as follows:

Day 1	:	Na > Mg > Ca > K > B
Day 2	:	Na > Ca > Mg > K > B
Day 3	:	Ca > Mg > Na > K > B
Day 5	:	Ca > Na > Mg > K > B
Day 9 & 12	:	Ca > Mg > Na > K > B

The cation and anion data were also normalized to the last analysis day to facilitate comparison of the relative abundance of these ions over the analysis period. Trace metals concentrations (μg/l) occurred in the following order of abundance:

$$Ba > Zn > Fe > Mn > Cu > Ag > Pb > Se$$

The chemical analyses of the Type I and Type II elutriates for all the shales studied are summarized in Appendix A-3. For each of the shale identification codes, the first letter refers to the process used to extract the oil and the second letter identifies whether it is processed (S) or unprocessed (R). The chemical analyses were checked by calculating the ion balance for the leachate

and type II elutriation procedures (Table 30). The chemical analyses data for the Type I elutriation procedure were balanced assuming an HCO_3 concentration. Alkalinity analyses were not conducted on the Type I elutriation samples. The Type I elutriate for the AP shale is comparable to the day 1 leachate composition, with similar electrical conductivities and the same dominance orders for cations and anions. The Type II elutriate for the same shale is comparable to the day 2 leachate with similar electrical conductivities and dominance order for the concentration of cations. The pH of the Type II elutriate was higher adding $CO_3^=$ to prevalent anions present in the solution. The concentration of $CO_3^=$ was less than the Cl and otherwise the dominance of the anions was the same as the day 2 leachate.

Effects of Oil Shale Elutriates on Acclimated Selenastrum

Type I elutriate (10 ml) was added to cultures of Selenastrum. Growth depression occurred in the following order:

$\hat{X}$: CP > BP = DP = DR = BR > Selenastrum = CR

$\hat{\mu}$: CP = BP = BR = DP = DR = CR > Selenastrum

Effects of Oil Shale Elutriates on Scenedesmus

The effect of varying concentrations of oil shale leachates and elutriates on the growth of Scenedesmus varied with the shale studied (Table 31). The AR elutriate and the AP leachate both showed variations in X with variations in concentrations, while the other elutriates did not show any effect. Figure 13 is a summary of the growth curves for the AP spent leachate comparing the growth curves at different concentrations of leachate addition to the growth curve of the Scenedesmus control.

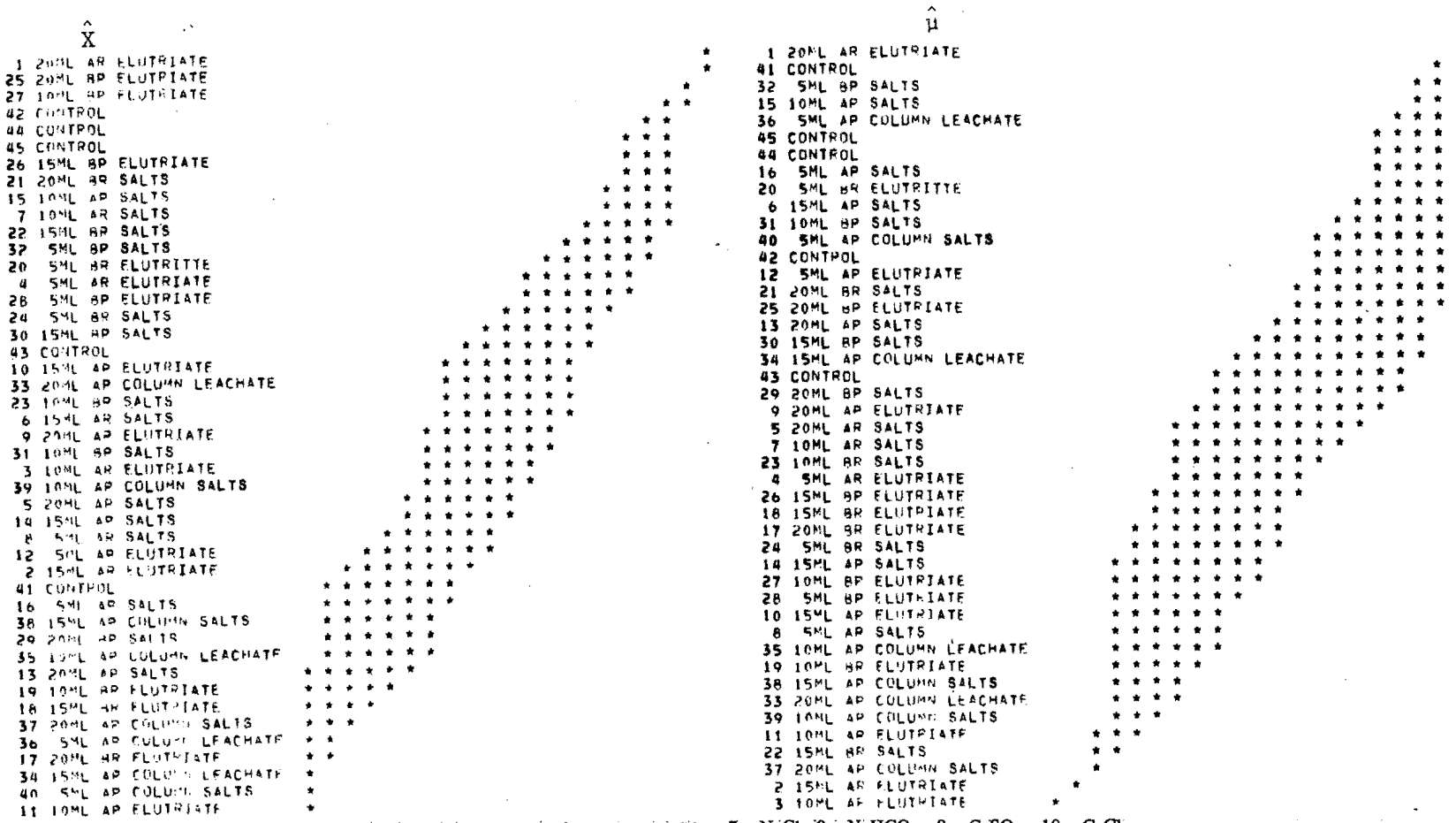
Raw and spent shales from A and B processes were tested. The raw shale elutriates exhibited approximately the same amount of growth depression of Scenedesmus. The spent shales depressed growth in the following order:

Table 30. Summations of cation and anion analyses of the oil shale leachates and elutriates.

Material Extracted	Extraction Procedure[a]	Cations	Anions
AP	Leaching	178.6	161.8
AP	Leaching	131.9	128.3
AP	Leaching	75.18	66.45
AP	Leaching	45.70	40.25
AP	Leaching	21.08	22.34
AP	Leaching	11.81	12.46
AP	Type I Elutriation	107.21	107.21
AP	Type II Elutriation	90.05	93.87
AR	Type I Elutriation	5.21	5.21
	Type II Elutriation	1.845	1.917
BP	Type I Elutriation	34.84	34.84
	Type II Elutriation	21.906	21.289
BR	Type I Elutriation	2.284	2.290
	Type II Elutriation	1.335	1.269
CP	Type I Elutriation	4.79	4.79
CR	Type I Elutriation	2.87	2.87
DP	Type I Elutriation	61.73	61.73
DR	Type I Elutriation	4.98	4.98

[a]Type I elutriation analyses balanced with assumed HCO_3^- concentration.

Table 31. Duncan's multiple range test of complex additions to Scenedesmus (bioassay 10).



Legend: 1 = mg SO_4, 2 = mg Cl_2, 3 = K_2SO_4, 4 = KCl, 5 = $KHCO_3$, 6 = Na_2SO_4, 7 = NaCl, 8 = $NaHCO_3$, 9 = $CaSO_4$, 10 = $CaCl_2$

AP elutriate > BP elutriate

The growth of Scenedesmus was less in the BP shale elutriate than the growth in the raw shale (Figure 14).

Significant linear correlation coefficients for the $\hat{X}$ and $\hat{\mu}$ data for Scenedesmus are summarized in Table 32. The electrical conductivity correlated at a low level with the $\hat{X}$ data and not at all with the $\hat{\mu}$ data. The heavy metals did not correlate with either $\hat{X}$ or $\hat{\mu}$.

Unlike the results with Selenastrum and Synedra, the electrical conductivity of the culture media did decrease significantly during the bioassay with Scenedesmus. An example of this is shown in Figure 15 by showing a linear regression on the electrical conductivity data versus time for the bioassay flask treated with AP leachate.

Comparison of the Salt Effects to the Oil Shale Elutriate Effects on the Productivity of Scenedesmus

The effects of oil shale elutriates were compared to the salt effects by comparing the growth of the controls consisting of AAM plus salts equivalent to the salinity of the extract (determined by analysis) to the growth of the extract additions The raw shales both showed better growth responses than their matching salt controls. The spent shales produced the opposite effect with the

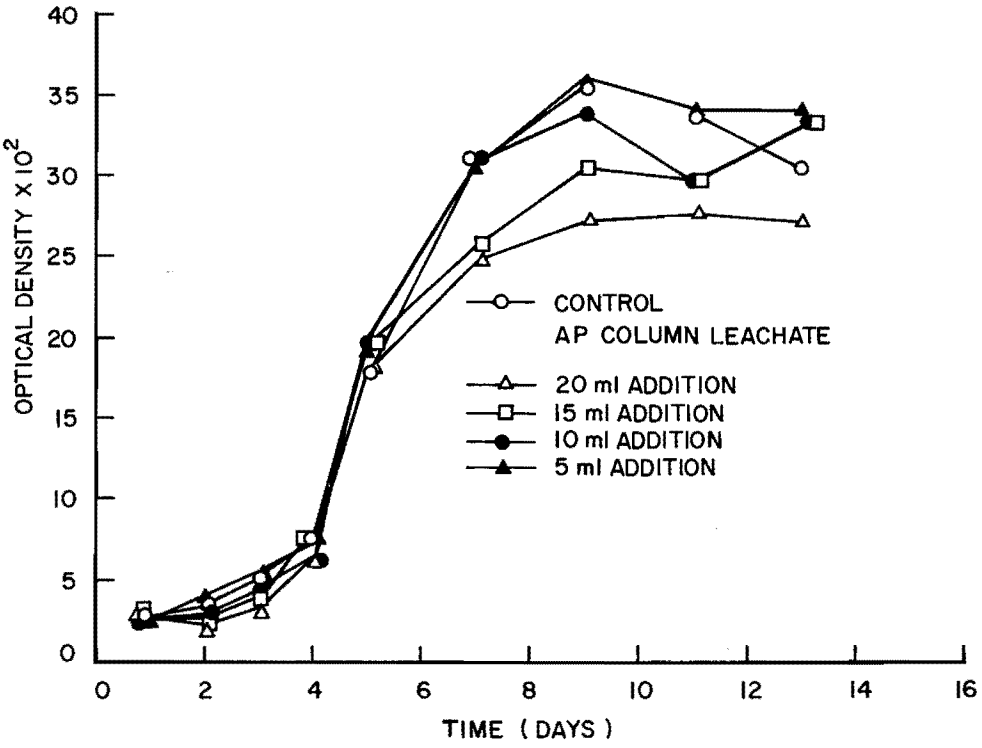


Figure 13. Concentration effects on the growth of Scenedesmus (bioassay 10).

Table 32. Linear relations between different estimates of biomass for complex additions to Scenedesmus (bioassay 10).

Dependent Variable (y)	Independent Variable (x)	Number of Data Points	Correlation Coefficient[a]	Equation y = mx + b
$\hat{X}$ fluorescence	Specific Conductivity (mhos/cm)	80	0.2407*	y = 7.619E04x - 7.480E07

[a]If marked with (*), the value of the correlation coefficient is significantly different from zero at P > 0.99. If marked with (**), the value of the correlation coefficient is significantly different from zero at P > 0.95.

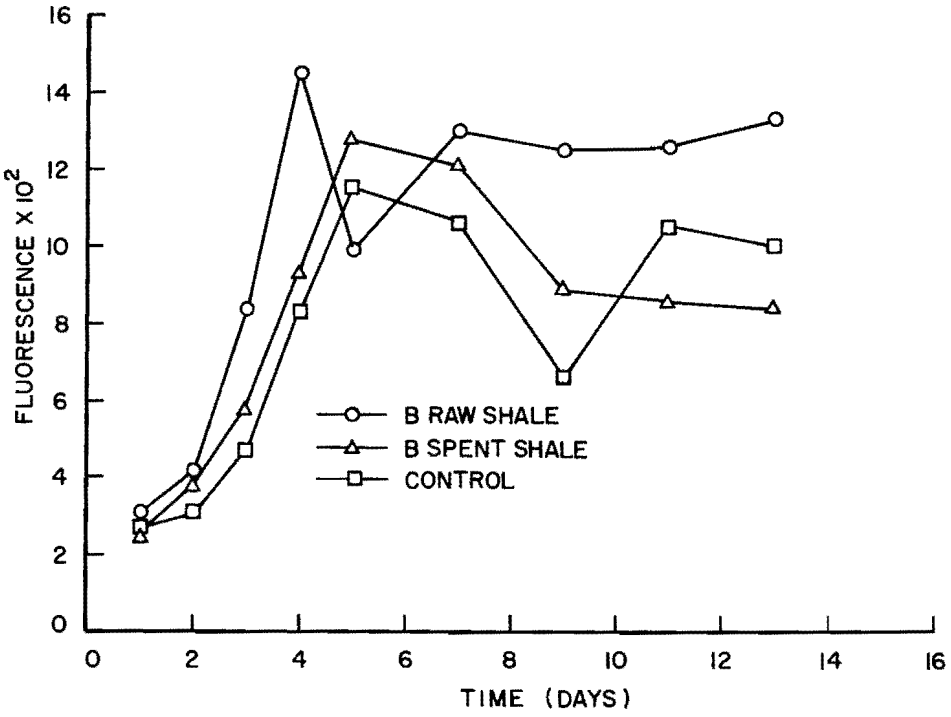


Figure 14. Comparison of the growth of Scenedesmus with the addition of BR and BP elutriates.

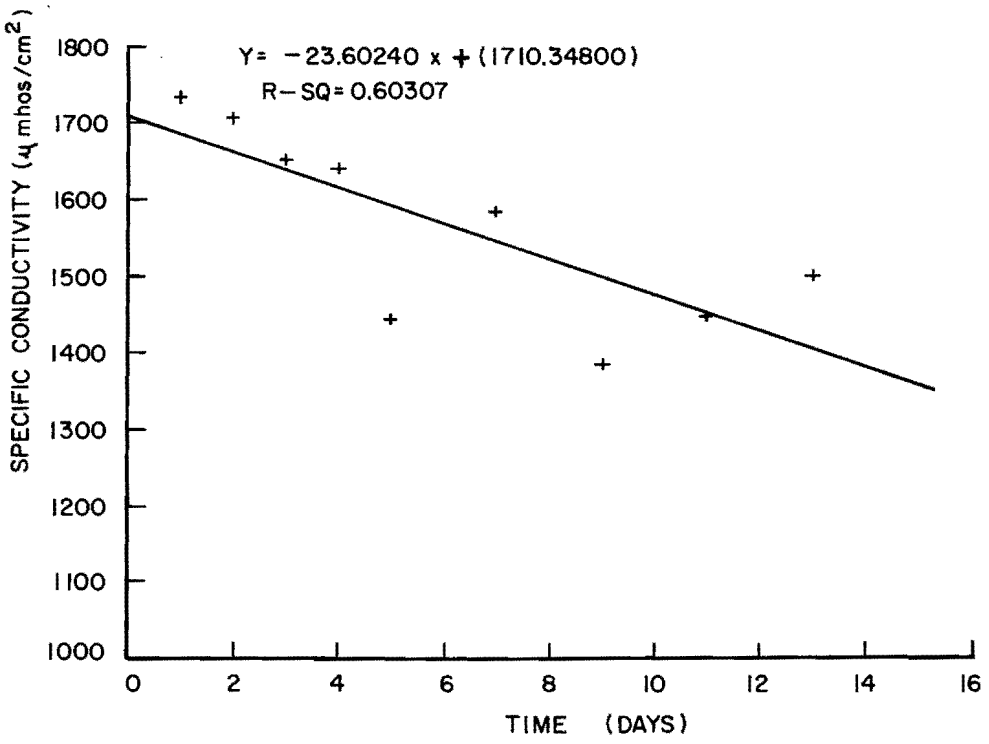


Figure 15. Decrease in the electrical conductivity of the culture medium plus AP leachate (20 ml) in the presence of Scenedesmus growth (bioassay 10).

spent shale showing less growth than the matching salt controls. This effect of the spent shale and matching salt control is shown in Figure 16. Significant differences ($P \leq 0.95$) in growth rate measured by fluorescence occurred although no significant difference in $\bar{X}$ was found.

Pearsall ion balances (Na + K/Mg + Ca in mg/l and meq/l) of the salt spikes and the oil shale elutriates were linearly correlated to the $\hat{X}$ and $\hat{\mu}$ for each concentration of additions. No correlation could be found between these variables.

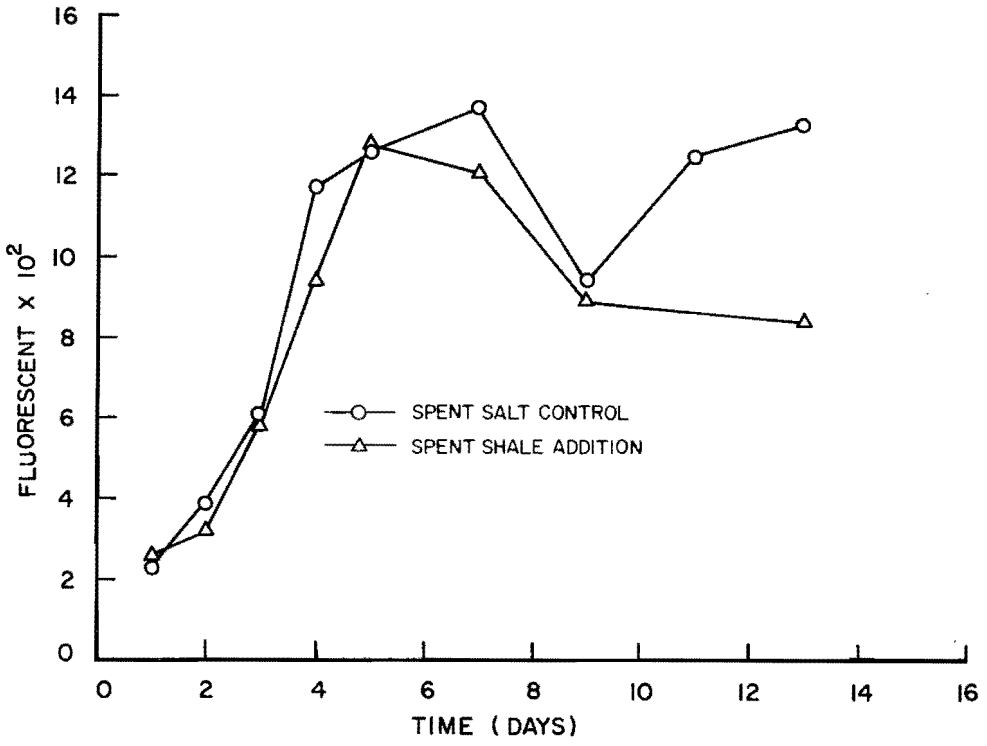


Figure 16. Comparison of the growth of Scenedesmus grown in the presence of BP oil shale elutriate and its matching salt control.

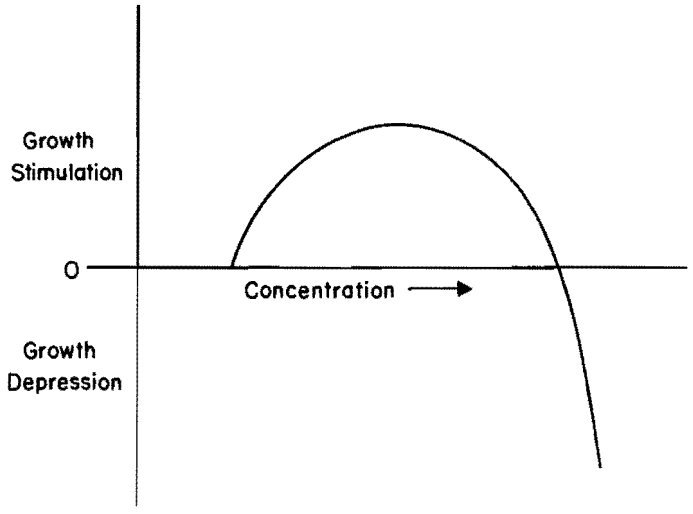


Figure 17. A beta toxicity curve (Luckey and Venugopal 1977).

DISCUSSION

The results with respect to each of the five study objectives stated at the beginning of the report are discussed below.

Utilization of Batch Bottle Bioassay for Toxicity Testing

Test Algae

Comparing the algal species tested for salt toxicity, these algal species displayed the following sensitivities to salt:

Selenastrum > acclimated Selenastrum > Synedra > 3 combined algal species

The indigenous diatom, Synedra, did tolerate higher salt concentrations than the test organism, Selenastrum. The acclimation of the Selenastrum did improve its ability to tolerate increased salt concentrations, but the acclimated Selenastrum did not display the same reactions to specific ions as did the Synedra. The addition of three algal species to each test flask displayed the least sensitivity to salt.

The greater tolerance of the indigenous algae to salt solutions illustrates the necessity of using indigenous organisms when testing for toxic responses. If indigenous algae are not available, then acclimation of the standard test algae to the receiving water is necessary. If possible, acclimated Selenastrum should also be used in order to establish a data base which is comparable to other algal bioassay data.

Variations in Biomass Monitoring Techniques

Variations occurred in the biomass measurements used to monitor the growth of the test algae. The optical density measurements were subject to interference from precipitates which occurred as the pH increased in test flasks of the less soluble salts such as $CaSO_4$. This measurement also displayed less sensitivity during the first days of the bioassay when the maximum specific growth rates ($\hat{\mu}_b$) occurred.

The fluorescence measurements were also subject to interference from precipitates, although this interference was not as great as the precipitate interference with optical density. The toxic response of algae to some compounds is chlorotic, which affects the algal fluorescence measurements. Chlorosis did not appear to be a problem with the toxic substances tested but should be considered as a possibility when considering biomass measurements with chlorophyll a fluorescence. The depression of algal photosynthesis after the transfer of algae to a higher salinity media has been stated in the literature (Stewart 1974).

Automated cell counts adjusted to the mean cell volume appeared to have the least variability of all the biomass measurements. Cell volume does significantly change in the presence of different toxicants, therefore the adjustment of the cell counts with the mean cell volume for biomass purposes is necessary and also more equivalent to biomass.

The results of the bioassays therefore were based on automated cell counts adjusted by the mean cell volume when these data were available. Otherwise chlorophyll a fluorescence was used. The optical density biomass measurements although collected, were not used in the analysis of results. For this reason, it is suggested that, if possible, an indigenous algal species be selected for toxicity testing that would be compatible with displacement cell counting techniques. This would necessitate an alga which is unicellular and has a morphologically simple shape.

The use of three different fluorescent characteristics to differently monitor the growth of three different algal species requires further research to develop a simple yet reliable method. The phycocyanin fluorescence, carotenoid fluorescence and chlorophyll a fluorescence all correlated better to the sum of the three algal direct cell counts than they did to the individual Anabaena, Synedra, and Scenedesmus cell counts respectively. The Anabaena cell counts correlated better with chlorophyll a fluorescence than the phycocyanin fluorescence suggesting that the chlorophyll a content of the Anabaena was easier to detect than the phycocyanin content. The Synedra cell counts correlated better with the phycocyanin fluorescence suggesting that the carotenoid peak of the mixed culture was probably closer to the phycocyanin wavelengths than the carotenoid wavelengths used for monitoring the bioassay. The proximity of these fluorescent peaks would be the cause of this interference at the 60 nanometer bandwidth used for this analysis.

Effects of Salinity on Freshwater Phytoplankton

Concentration Effects

The concentration of the compounds produced variable effects depending on the test alga and the compound. The ions being studied are hormetins, toxic agents at higher concentrations but stimulatory at lower concentrations (Luckey and Venugopal 1977). With the exception of Na and HCO_3, the ions are required ions for algal growth and so follow a beta toxicity curve (Figure 17).

AAM is a medium designed to provide all the required nutrients necessary for algal growth, with algal growth terminating from phosphorus limitation. Because of Liebig's Law of the minimum, an increase in biomass would have to include the addition of a form of phosphorus which the algae could utilize. Therefore, the growth stimulatory effects of the ions under study were eliminated, and the toxic concentration effects were expressed. This did occur using Selenastrum as the test organism, with growth depression occurring at the 0.004 N (250 mg/l/as NaCl) concentration. Growth depression increased as the concentration increased. Therefore, the full nutrient growth potential of the medium was not utilized by the Selenastrum because of the effects of salinity added to the AAM medium. Both the $\hat{X}$ and $\hat{\mu}_b$ were depressed.

LPS is an AAM based medium to which additional salinity has been added to equal the salinity of Lake Powell. No growth stimulatory effects were found using Synedra as the test organism. Growth depression began at the 0.05 N concentration and increased as the concentration of salts increased. However, Scenedesmus did exhibit growth stimulation with the addition of complex salt solutions. This would suggest that one of the salt ions under study and not phosphorus was limiting the growth of Scenedesmus. Vanadium (V) is a required trace element (Provasoli 1958) for the growth of Scenedesmus and LPS does not include V in the trace element addition. Provasoli (1958) also states that impurities in reagent salts used in nutrient media contain sufficient trace metals except Fe and Mn to support freshwater phytoplankton species. Therefore, not only the salt ions tested but also the trace metals, specifically V, may be limiting Scenedesmus growth. This apparent variability in the nutrient requirements for different algal species demonstrates the importance of identifying changes necessary in the media to maintain a known element of limitation when utilizing indigenous species for toxicity studies of complex wastes.

Effects of Different Ionic Species

Differences in ionic toxicity did exist with single salt additions to Selenastrum and Synedra. Mg was more toxic than the other cations for both of these algal genera. Mg is an essential ion for photosynthesis. It is the central chelated metal in chlorophyll a molecules. Mg is the ion with the smallest hydrated radius (8 Ångstrums) of the group II elements in the periodic table (Stumm and Morgan 1970). Na (4 Å) hydrated radius, which also exhibited the same toxicity as Mg with single salt additions to Synedra, has the smallest hydrated radius of the group I elements of the periodic table. This smaller hydrated radius may have allowed greater selective adsorption of these two cations compared to the other cations in solution. Synedra, unlike Selenastrum, did react selectively to anion toxicities.

The order of toxicity of the anions was reversed from their order of solubility and the size of their hydrated radius did not appear to be significant. SO_4 (4 Å) was the most toxic anion to Selenastrum. Most algae have the ability to reductively assimilate SO_4 to sulfide, which is essential for algal growth and cell division. Most algae also have the ability to reductively assimilate SO_3. The hydrated radii of HCO_3 and Cl are 4 Å and 3 Å respectively. HCO_3 was the most toxic anion to Synedra.

Anabaena is a cyanophyte which has been mentioned in the literature as a dominant genus in high salinity (TDS $\geq$ 1 g/l) environments. In the presence of all of the salts except $CaSO_4$, the maximum standing crop of the heterocysts decreased. Heterocysts are specialized cells of Cyanophyta which are present when nitrogen fixation by these algae occur. This would suggest that the medium may not be nitrogen limited for the Anabaena in the presence of these salts. The number of vegetative cells of Anabaena were low in the test flasks as compared to the Synedra and Scenedesmus. However, the short duration of the bottle test may have precluded Anabaena dominance. Blue-green algae are thought of as generally having slower growth rates than green algae (Stewart 1974). Also, this Anabaena was a standard test species rather than an indigenous alga as were the other two genera representatives tested. Also the bioassay light level may have been high enough to inhibit the growth of Anabaena because generally the standard test requires 200 f-c not 400 f-c for blue-greens (APHA, 1975).

A significant difference in the toxic response of Selenastrum because of in the synergistic interactions of cations and anions was shown. This effect appeared to control the amounts of depression of growth of all of the algal species tested when more than one salt was added to the medium. Therefore, when complex salt solutions are added to receiving waters, measurement of one dominant ion pair cannot be used to predict the effect of the salts on the productivity of the phytoplankton.

Evaluation of Concentration Measurements

Concentration measurements may provide a better means of assessing the effects of increased salinity on the productivity of freshwater phytoplankton. Osmotic pressure correlations from the bioassays with Selenastrum and Synedra were significant, but very low. This correlation was still less than a linear correlation of the same biomass data to electrical conductivity.

Although consistently low, the most consistently significant correlations with biomass measurements for all the algal species tested were obtained with specific conductivity. These correlations were better with $\hat{X}$ than with $\hat{\mu}$ results. This linear relationship did not hold when grouping data from all the Synedra bioassays. Therefore there were some inconsistencies between bioassays that were apparently a result of the different experimental conditions.

The other concentration measurements tested did not provide consistently significant correlations with any of the biomass measurements. The correlations of the activity coefficient with the biomass data may have been lowered due to the calculations used. The measured activities which were linearly correlated with the Scendesmus biomass data suffered from problems: 1) The activities of most of these solutions were at the lower end of the sensitivity range for the measurement technique utilized. 2) Calculations of activities and osmotic pressure based on limiting laws are not applicable to solutions of mixed electrolytic charges and the bioassay data support that conclusion (Stumm and Morgan 1970).

Evaluation of the Corps of Engineers Standard Elutriation Procedure

The chemical compositions of the two types of elutriation procedures and the leachate procedure were compared for the AP shale. The Type II elutriation procedure followed the Corps of Engineers standard elutriation protocol. The Type I elutriation procedure provided comparable data to the leachate produced from an up-flow column on the first day of operation. The Type II elutriation procedure produced comparable data to the day 2 leachate analyses, except the pH of the Type II elutriate was higher than the day 2 leachate. Otherwise the major ions present in the elutriate were comparable to the leachate of each day.

The leachate did provide the additional knowledge that the composition of the major cations changed in order of dominance over the extraction period and the pH increased steadily during the extraction period. Therefore, the ion composition and pH of the leachate from the spent oil shale disposal sites will change depending on the contact time of the disposal water. Both of these variables affects the biostimulatory or toxic responses of phytoplankton and so the contact time of the leachate with the disposal site shale could change the phytoplankton response to the leachate.

Problems in the utilization of the Corps of Engineers standard elutriation procedure could occur because of the difference demonstrated with the leachate procedure. The water passing through a spent shale disposal pile will be moving at all times. The contact times of the water and shale will vary but with the recycling of this water, a longer contact time, such as the 48 hour contact time of the Type I elutriate may provide an elutriate more characteristic of the leachate from spent shale disposal sites.

The Type II elutriate procedure did not totally wet the interior of the most hydrophobic shales. The standard elutriation procedure was obtained from standard soil analysis and designed for testing samples from dredged sites, but the hydrophobic nature of some of the shales did preclude complete extraction using this technique.

Effects of Oil Shale Leachates and Elutriates on Phytoplankton Productivity

The addition of many of the spent oil shale elutriates and leachates stimulated the growth of Scenedesmus. The concentration effects of these additions did not provide consistent conclusions. The extracts from the AP shale stimulated growth more than the extracts from the BP shale. Therefore, growth stimulation of Scenedesmus is dependent on the process applied to the shale.

In general the extracts from the spent shales stimulated growth more than did the extracts from the raw shales. The processing of the shale appears to make growth stimulating compounds more available to Scenedesmus. These compounds may be low molecular weight aromatic hydrocarbons, which were found to stimulate algal growth in other petroleum products (Dunstan et al. 1975). The spent shales did stimulate growth as compared to their matching salt controls. Therefore, this stimulation was not caused by the addition of any of the salt compounds.

This differed from the comparison of the growth of raw shale extracts to their matching salt controls. The growth of the raw shale extracts was less than the growth of the salt controls. This would suggest toxicity or a decrease in the limiting nutrient availability from a component of the oil shale extract other than the salt component. Linear correlations were made between the biomass and the trace metals present in the extracts but no consistent

correlations could be found between the Scenedesmus biomass data and the trace metal concentration in the extracts. Generally, the concentrations were lower than toxic level to algae and because of this the growth depression was probably not due to the trace metals present in the extracts.

Application of the Bioassay Results to the Colorado River System

Increased growth of the three algae grown competitively suggest that competition was occurring between the algae when grown in higher salt concentrations. The literature would suggest that this increase in salt concentrations would provide a competitive advantage for cyanophytes (Gupta 1972). The increased presence of cyanophytes would change the species composition of Lake Powell. At present no cyanophytes are common in Lake Powell. The literature also suggests that lower Pearsall ion ratio (< 1.5) also select for cyanophytes (Provasoli 1958). As the length of contact time with processed oil shale increases, the Pearsall ion balance decreases and therefore recycling of disposal water through the oil shale would decrease the Pearsall ion balance and may also favor cyanophytes.

An increase in the salinity of Lake Powell may inhibit the growth of Synedra. An increase to 0.05 N salinity will suppress the growth of Synedra in the laboratory, but an increase of 0.05 N salinity (1150 mg/l TDS as NaCl) would be a large increase in the salt content of this receiving water. The costs to agricultural water use of this salinity increase would probably prevent attaining such a level.

Leachates from oil shale sites may increase the productivity of Scenedesmus in Lake Powell. Leachates from the spent disposal sites would appear to stimulate Scenedesmus growth more than leachates from the raw shale. However, runoff leaching raw shale from ground disruption could also stimulate the growth of Scenedesmus in Lake Powell. The trace metals present in the oil shale leachates should not effect the growth of Scenedesmus.

Therefore, the increase in salinity because of water diversion will probably never reach a level high enough to affect the algal population in Lake Powell because of downstream agricultural interests. However, releases of leachates from the shale disposal sites may be biostimulatory to the algae of Lake Powell.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

In batch bioassay tests:

1. A single algal species indigenous to Lake Powell, Synedra, deli catissma var. angustissima, was more tolerant to salinity than the standard algal assay test alga, Selenastrum capricornutum, Printz.

2. Acclimation of the standard test alga, Selenastrum capricornutum in a higher salinity medium increased its tolerance to salinity but the acclimated Selenastrum capricornutum still was less tolerant to salinity than the indigenous alga, Synedra delicatissma.

3. A mixture of three algal species (Anabaena flosquae (culture), Synedra delicatissma (indigenous), and Scenedesmus capricornutum (indigenous)) were more tolerant to salinity than any of the other test algae.

4. Salinity toxicity in Selenastrum capricornutum occurs with the addition of salts at the 0.004 N concentration.

5. Salinity toxicity in Synedra delicatissma occurs with the addition of salts at the 0.05 N concentration.

6. With multiple salt additions, the interactions of cations and anions have more effect on the growth inhibition toxicity than any one cation and/or anion effect.

7. Specific conductivity correlates with algal productivity at a significant but a low level.

8. Calculated osmotic pressure and the activity coefficient do not correlate well with algal biomass variables.

9. Automated cell counts adjusted with mean cell volume measurements appear to be the best biomass monitoring technique when compared to chlorophyll a fluorescence and optical density.

10. The Corps of Engineers standard elutriation procedure does not extract ions from oil shales as completely as elutriation procedures with longer extraction periods or leachate procedures using an up-flow column.

11. The ion composition and pH of the oil shale leachate is dependent on the contact time of the water with the oil shale.

12. The addition of oil shale leachates to Lake Powell may be biostimulatory to the phytoplankton.

13. The increase in salinity in Lake Powell may not decrease algal productivity but higher salinity and/or a decrease in the ratio of monvalent to divalent ions of the salinity may increase the cyanophytes present in Lake Powell.

Recommendations For Further Research

1. Microcosm studies are needed to study the effects of sediment action on the cycling of these salts and leachates in the reservoir.

2. In situ studies in Lake Powell are needed to try to determine the possibility of algal population shifts in the presence of increased salinity or oil shale leachate concentrations.

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APPENDICES

Appendix A

Analytical Results of the Oil Shale

Leachate and Elutriate Analyses

Appendix A-1

Oil Shale Identification Listing

These samples of oil shale were provided by the companies for analysis. These are all unhistoried samples from prototype operations and as such may not be representative of samples from a full scale operation.

Table A-1. Oil shale identification listing.

AR	=	Raw Utah Shale
AP	=	Paraho Processed Utah Shale
BR	=	Raw Union Shale
BP	=	Union Processed Shale
CR	=	Raw Laramie Shale
CP	=	Laramie Processed Shale
DR	=	Raw Geokinetics Shale
DP	=	Geokinetics Processed Shale

Table A-2. Summary table for the characterization of oil shale leachates.

	AP											
	Leachate 1		Leachate 2*		Leachate 3		Leachate 4		Leachate 5		Leachate 6	
	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l
Elapsed Sampling Time (Hours)		30		42		78		127		223		295
(Days)		1.25		1.75		3.25		5.29		9.29		12.3
Cations												
z												
1 Na	2704.0	117.6	1990.8	86.60	438.6	19.08	183.2	7.971	35.57	1.550	21.87	0.95
2 Mg	317.1	26.08	176.6	14.53	226.8	21.95	88.47	7.280	45.09	3.709	24.10	1.98
1 K	454.98	11.64	159.7	4.083	357.1	9.134	72.0	1.84	12.62	0.323	8.28	0.212
2 Ca	465.5	23.23	534.9	26.69	501.5	25.02	573.3	28.61	309.71	15.46	172.06	8.586
		Σ 178.6		Σ 131.9		Σ 75.18		Σ 45.70		Σ 21.037		Σ 11.731
Anions												
z												
1 Cl	2.0	0.06	2.0	0.06	2.0	0.06	3.0	0.095	1.175	0.033	1.525	0.043
2 SO_4	6600.0	137.4	5250	109.3	2775	57.78	1800	37.48	966.8	20.13	482.24	10.04
1 HCO_3	1483	23.31	1156	18.94	525.4	8.610	163.4	2.680	133.0	2.180	145.09	2.378
2 CO_3	0	0	0	0	0	0	0	0	0	0	0	0
		Σ 161.8		Σ 128.3		Σ 66.45		Σ 40.25		Σ 22.34		Σ 12.461
Ion Balance		4.93%		2.77%		12.3%		12.6%		3.00%		3.02%
Trace Metals (μg/l)												
Se	2.2											
As	<1											
Fe	28.5											
Ba	206.6											
Pb	4.9											
Mn	16.4											
Cu	15.9											
Zn	55.6											
Cd	20.5											
Cr	14.6											
Ag	15.2											
B									139.0		299.0	

Table A-2. Continued.

	AP											
	Leachate 1		Leachate 2 *		Leachate 3		Leachate 4		Leachate 5		Leachate 6	
	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l	mg/l	meq/l
Total Organic Carbon (mg/l)									13		10	
pH		8.27		8.26		7.79		7.30	7.17		7.24	
Alkalinity (mg/l @ $CaCO_3$)		1215.6		947.4		430.7		140.0	109.02		118.93	
Total Dissolved Solids (mg/l)									1550		823	
Specific Conductivity (μmhos/cm)		10230		8258		4612		2956	1695		1007	
Pearsall Ion Balance		4.036		3.023		1.036		0.3856	0.1358		0.1100	
Leachates normalized to the 12.3 day leachate												
Cations												
Na		123.8		91.16		20.08		8.390	1.632		1	
Mg		13.17		7.338		11.09		3.977	1.873		1	
K		54.91		19.26		43.8		8.679	1.524		1	
Ca		2.706		3.109		2.914		3.332	1.801		1	
		Σ15.12		Σ11.164		Σ6.364		Σ3.868	Σ1.314		1	
Anions												
Cl		1.395		1.395		1.395		2.209	0.7674		1	
SO_4		13.69		10.89		5.755		3.733	2.005		1	
HCO_3		9.802		7.965		3.621		1.127	0.9167		1	
CO_3		0		0		0		0	0		0	
		Σ 12.99		Σ 10.30		Σ 5.33		Σ 3.23	Σ 1.79			

*Leachate 2 was used in the bioassay procedure.

Table A-3. Summary table for the characterization of oil shale elutriates.

	AP				AR			
	Type I* mg/l	Elutriate meq/l	Type II* mg/l	Elutriate meq/l	Type I* mg/l	Elutriate meq/l	Type II* mg/l	Elutriate meq/l
Cations								
Na	1045.8	45.49	821.75	35.74	58.0	2.523	13.62	0.592
Mg	475.3	39.10	245.73	20.215	12.1	0.995	2.42	0.199
K	110.4	2.823	54.60	1.396	2.0	0.051	11.1	0.284
Ca	396.8	19.80	653.8	32.63	32.87	1.640	12.15	0.606
		Σ 107.213		Σ 89.981		Σ 5.21		Σ 1.681
Anions								
Cl	92.51	2.609	30.175	0.851	9.16	0.258	3.025	0.085
SO_4	5013.1	104.37	4301.3	89.55	85.41	1.778	28.02	0.583
HCO_3	14.05	0.230	180.16	2.953	193.61	3.173	61.66	1.011
CO_3			15.46	0.515			7.14	0.238
		Σ 107.213		Σ 93.873		Σ 5.21		Σ 1.917
Ion Balance				2.12%				6.56%
Trace Metals			µg/l				µg/l	
Se			<1				<1	
As			10.5				2.5	
Fe			<25.0				66.5	
Ba			135.0				<78	
Pb			<1				1.7	
Mn			<7				<7	
Cu			<11				<11	
Zn			12.8				24.4	
Cd			<13				<13	
Cr			<11				<11	
Ag			<9				<9	
B			246				592	
Total Organic Carbon (mg/l)		--		1.985		--		7.38
Activity (Bars)		1.75		0.234		0.089		0.523
pH		--		8.84		--		9.04
Alkalinity (mg/l @ $CaCO_3$)		--		173.4		--		--
Total Dissolved Solids (mg/l)		--		7056		--		121
Specific Conductivity (µmhos/cm)		10060		6415		228		155
Pearsall Ion Balance		1.326		0.9742		1.334		1.697

*The Type I elutriation technique had a 48 hour extraction period. The Type II elutriation technique had a 30 minute extraction time. Further differences in these two elutriation procedures are shown in Figures 4 and 5 in the Materials and Methods section.

**Type I elutriation procedure balanced with assumed HCO_3 concentration.

Table A-3. Continued.

	BP				BR			
	Type I* mg/l	Elutriate meq/l	Type II* mg/l	Elutriate meq/l	Type I* mg/l	Elutriate meq/l	Type II* mg/l	Elutriate meq/l
Cations								
Na	625.6	27.21	109.5	4.545	5.7	0.248	2.68	0.117
Mg	41.5	3.41	58.13	4.782	7.0	0.576	4.49	0.369
K	8.1	0.207	7.43	0.190	0.8	0.020	1.60	0.041
Ca	80.16	4.000	242.91	12.121	28.86	1.440	16.19	0.808
		Σ 34.83		Σ 21.638		Σ 2.284		Σ 1.335
Anions								
Cl	24.59	0.6936	7.075	0.200	2.31	0.0652	1.425	0.040
SO_4	1510.5	31.448	877.77	18.275	58.15	1.211	37.14	0.773
HCO_3	164.24	2.6917	171.7	2.814	61.54	1.0085	25.391	0.416
CO_3			--	--			1.189	0.040
		Σ 34.83		Σ 21.289		Σ 2.284		Σ 1.269
Ion Balance				1.61%				5.07%
Trace Metals			µg/l				µg/l	
Se			<1				<1	
As			<1				<1	
Fe			<25.0				34.4	
Ba			<78				<78	
Pb			9.1				<1	
Mn			<7				<7	
Cu			<11				<11	
Zn			24.9				12.8	
Cd			15.9				<13	
Cr			<11				<11	
Ag			<9				<9	
B			966				<10	
Total Organic Carbon (mg/l)		--		11.3		--		0.153
Activity (Bars)		0.234		0.234		--		0.523
pH		--		8.33		--		8.85
Alkalinity (mg/l @ $CaCO_3$)				--				--
Total Dissolved Solids (mg/l)				1518				101
Specific Conductivity (µmhos/cm)		3210		1601		308		128
Pearsall Ion Balance		5.209		0.3718		0.1813		0.2070

*The Type I elutriation technique had a 48 hour extraction period. The Type II elutriation technique had a 30 minute extraction time. Further differences in these two elutriation procedures are shown in Figures 4 and 5 in the Materials and Methods section.

**Type I elutriation procedure balanced with assumed HCO_3 concentration.

Table A-3. Continued.

	CP		CR		DP		DR	
	Type I* mg/l	Elutriate meq/l	Type I* mg/l	Elutriate meq/l	Type I* mg/l	Elutriate meq/l	Type I* mg/l	Elutriate meq/l
Cations								
Na	94.9	4.13	6.9	0.30	541.5	23.55	106.7	4.64
Mg	0.57	0.047	6.7	0.55	0.20	0.016	1.50	0.123
K	19.4	0.496	0.70	0.018	34.4	0.879	0.80	0.0205
Ca	2.4	0.120	40.08	2.00	747.09	37.28	4.01	0.20
		Σ 4.79		Σ 2.87		Σ61.73		Σ 4.98
Anions								
Cl	57.74	1.629	5.20	0.147	29.19	0.823	4.19	0.118
SO_4	36.83	0.7668	65.52	1.364	2298.23	47.849	41.83	0.8709
HCO_3	145.83	2.39	82.98	1.360	796.89	13.06	243.46	3.99
CO_3								
		Σ 4.79		Σ2.87		Σ 61.73		Σ 4.98
Ion Balance								
Trace Metals (µg/l)								
Se								
As								
Fe								
Ba								
Pb								
Mn								
Cu								
Zn								
Cd								
Cr								
Ag								
Total Organic Carbon (mg/l)								
Activity (Bars)								
pH								
Alkalinity (mg/l @ $CaCO_3$)								
Total Dissolved Solids (mg/l)								
Specific Conductivity (µmhos/cm)		345		5100		4520		500
Pearsall Ion Balance		38.48		0.1625		0.7707		19.51

*The Type I elutriation technique had a 48 hour extraction period. The Type II elutriation technique had a 30 minute extraction time. Further differences in these two elutriation procedures are shown in Figures 4 and 5 in the Materials and Methods section.

**Type I elutriation procedure balanced with assumed HCO_3 concentration.

Appendix B

Listings of $\hat{X}$ and $\hat{\mu}$ Bioassay Results

All raw bioassay data are on file in the library at the Utah Water Research Laboratory

Selenastrum

Single Salt Additions

$\hat{X}$

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANK
26	0.24 N CASO4	0.	1
27	0.20 N CASO4	0.	2
28	0.11 N CASO4	0.	3
29	0.08 N CASO4	0.	4
30	0.05 N CASO4	0.	5
31	0.10 N CASO4	0.	6
32	0.13 N CASO4	0.	7
25	0.27 N CASO4	0.	8
3C	0.10 N NA2SO4	6444.0	9
R	0.13 N NACL	9068.5	10
43	0.20 N MGCL2	9331.0	11
3R	0.05 N NA2SO4	9785.0	12
47	0.10 N MGCL2	10898.	13
3	0.20 N NACL	11171.	14
42	0.24 N MGCL2	11503.	15
36	0.11 N NA2SO4	11729.	16
4R	0.13 N MGCL2	12480.	17
3C	0.24 N NA2SO4	12717.	18
4C	0.13 N NA2SO4	13391.	19
Q	0.27 N KCL	13419.	20
1	0.27 N NACL	13852.	21
4Q	0.27 N K2SO4	14075.	22
45	0.08 N MGCL2	14131.	23
4	0.11 N NACL	14328.	24
10	0.24 N KCL	14476.	25
2	0.24 N NACL	14731.	26
44	0.11 N MGCL2	15108.	27
17	0.27 N MGSO4	15110.	28
1Q	0.20 N MGSO4	15680.	29
7	0.10 N NACL	16299.	30
41	0.27 N MGCL2	16445.	31
3E	0.20 N NA2SO4	16685.	32
16	0.13 N KCL	17174.	33
37	0.08 N NA2SO4	17646.	34
12	0.11 N KCL	17738.	35
1R	0.24 N MGSO4	18113.	36
21	0.08 N MGSO4	19047.	37
24	0.13 N MGSO4	19464.	38
2I	0.10 N MGSO4	19871.	39
2C	0.11 N MGSO4	19973.	40
33	0.27 N NA2SO4	20326.	41
22	0.05 N MGSO4	20693.	42
13	0.08 N KCL	22540.	43
11	0.20 N KCL	23632.	44
51	0.20 N K2SO4	24220.	45
1E	0.10 N KCL	25765.	46
50	0.24 N K2SO4	25783.	47
52	0.11 N K2SO4	26052.	48
47	0.27 N CACL2	27103.	49
53	0.08 N K2SO4	27825.	50
5	0.08 N NACL	30555.	51
1C	0.05 N KCL	33852.	52
5R	0.24 N CACL2	33956.	53
6	0.05 N NACL	36367.	54
5E	0.10 N K2SO4	37143.	55
5A	0.13 N K2SO4	37386.	56
4A	0.05 N MGCL2	39148.	57
54	0.05 N K2SO4	40108.	58
59	0.20 N CACL2	49987.	59
64	0.13 N CACL2	80033.	60
60	0.11 N CACL2	83618.	61
6C	0.10 N CACL2	.10362E+06	62
61	0.08 N CACL2	.10715E+06	63
62	0.05 N CACL2	.11524E+06	64
65	CONTROL	.14268E+06	65

$\hat{\mu}$

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
26	0.24 N CASO4	0.	1
27	0.20 N CASO4	0.	2
28	0.11 N CASO4	0.	3
29	0.08 N CASO4	0.	4
30	0.05 N CASO4	0.	5
31	0.10 N CASO4	0.	6
32	0.13 N CASO4	0.	7
25	0.27 N CASO4	0.	8
49	0.27 N K2SO4	4.4215	9
39	0.10 N NA2SO4	4.4920	10
42	0.24 N MGCL2	6.4170	11
45	0.08 N MGCL2	6.6735	12
43	0.20 N MGCL2	6.7620	13
3	0.20 N NACL	8.7970	14
1	0.27 N NACL	8.8240	15
61	0.08 N CACL2	8.8340	16
62	0.05 N CACL2	8.8425	17
6	0.05 N NACL	8.8440	18
33	0.27 N NA2SO4	8.8540	19
24	0.13 N MGSO4	8.8565	20
64	0.13 N CACL2	8.8565	21
9	0.27 N KCL	8.8575	22
13	0.08 N KCL	8.8645	23
5	0.08 N NACL	8.8665	24
8	0.13 N NACL	8.8710	25
15	0.10 N KCL	8.8715	26
34	0.24 N NA2SO4	8.8755	27
2	0.24 N NACL	8.8775	28
10	0.24 N KCL	8.8865	29
14	0.05 N KCL	8.8905	30
63	0.10 N CACL2	8.8935	31
4	0.11 N NACL	8.8980	32
50	0.24 N K2SO4	8.9025	33
7	0.10 N NACL	8.9040	34
23	0.10 N MGSO4	8.9245	35
12	0.11 N KCL	8.9305	36
51	0.20 N K2SO4	8.9420	37
35	0.20 N NA2SO4	8.9460	38
48	0.13 N MGCL2	8.9490	39
44	0.11 N MGCL2	8.9605	40
20	0.11 N MGSO4	8.9730	41
47	0.10 N MGCL2	8.9740	42
40	0.13 N NA2SO4	8.9760	43
46	0.05 N MGCL2	8.9785	44
59	0.20 N CACL2	8.9800	45
60	0.11 N CACL2	8.9945	46
41	0.27 N MGCL2	8.9995	47
11	0.20 N KCL	9.0045	48
36	0.11 N NA2SO4	9.0120	49
52	0.11 N K2SO4	9.0130	50
57	0.27 N CACL2	9.0225	51
17	0.27 N MGSO4	9.0295	52
65	CONTROL	9.0350	53
21	0.08 N MGSO4	9.0470	54
55	0.10 N K2SO4	9.0480	55
22	0.05 N MGSO4	9.0550	56
56	0.13 N K2SO4	9.0580	57
37	0.08 N NA2SO4	9.0685	58
19	0.20 N MGSO4	9.0700	59
3A	0.05 N NA2SO4	9.0865	60
53	0.08 N K2SO4	9.0875	61
58	0.24 N CACL2	9.0960	62
16	0.13 N KCL	9.0985	63
54	0.05 N K2SO4	9.1150	64
1A	0.24 N MGSO4	9.1700	65

Single Salt Additions

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
12	0.030 N MGSO4	6392.0	1
27	0.030 N MGCL2	8120.5	2
11	0.050 N MGSO4	8360.5	3
5	0.050 N KCL	9075.0	4
26	0.050 N MGCL2	9620.0	5
13	0.020 N MGSO4	9990.0	6
7	0.030 N KCL	10504.	7
31	0.050 N K2SO4	13350.	8
28	0.020 N MGCL2	14100.	9
10	0.009 N MGSO4	17250.	10
29	0.009 N MGCL2	18750.	11
30	0.004 N MGCL2	19950.	12
2	0.030 N NACL	20850.	13
1	0.050 N NACL	21300.	14
30	0.009 N K2SO4	26950.	15
16	0.050 N CASO4	28700.	16
15	0.004 N MGSO4	28950.	17
3	0.020 N NACL	32350.	18
33	0.020 N K2SO4	34300.	19
17	0.030 N CASO4	38300.	20
8	0.020 N KCL	38550.	21
20	0.004 N CASO4	44850.	22
37	0.030 N CACL2	46250.	23
36	0.050 N CACL2	46800.	24
39	0.009 N CACL2	48250.	25
38	0.020 N CACL2	48500.	26
46	CONTROL	49350.	27
48	CONTROL	49350.	28
6	0.004 N NACL	49550.	29
42	CONTROL	50050.	30
43	CONTROL	50350.	31
41	CONTROL	50850.	32
44	CONTROL	51150.	33
24	0.009 N NA2SO4	51300.	34
45	CONTROL	51950.	35
25	0.004 N NA2SO4	52700.	36
22	0.030 N NA2SO4	52750.	37
23	0.020 N NA2SO4	53300.	38
9	0.009 N KCL	55950.	39
35	0.004 N K2SO4	58250.	40
47	CONTROL	65600.	41
14	0.009 N CASO4	72750.	42
18	0.020 N CASO4	83450.	43
40	0.004 N CACL2	90300.	44
32	0.030 N K2SO4	.10120E+06	45
4	0.009 N NACL	.10225E+06	46
10	0.004 N KCL	.10400E+06	47
21	0.050 N NA2SO4	.14085E+06	48

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
37	0.030 N CACL2	1.2115	1
29	0.009 N MGCL2	1.2146	2
11	0.050 N MGSO4	1.2780	3
28	0.020 N MGCL2	1.2965	4
12	0.030 N MGSO4	1.3345	5
18	0.020 N CASO4	1.3665	6
1	0.050 N NACL	1.3790	7
26	0.050 N MGCL2	1.3860	8
13	0.020 N MGSO4	1.4190	9
10	0.009 N CASO4	1.4340	10
27	0.030 N MGCL2	1.4395	11
16	0.050 N CASO4	1.5040	12
38	0.020 N CACL2	1.5510	13
8	0.020 N KCL	1.5735	14
14	0.009 N MGSO4	1.5785	15
7	0.030 N KCL	1.5930	16
17	0.030 N CASO4	1.6095	17
20	0.004 N CASO4	1.6205	18
21	0.050 N NA2SO4	1.6249	19
34	0.009 N K2SO4	1.6260	20
6	0.050 N KCL	1.6685	21
39	0.009 N CACL2	1.6925	22
24	0.009 N NA2SO4	1.7025	23
33	0.020 N K2SO4	1.7230	24
36	0.050 N CACL2	1.7345	25
9	0.009 N KCL	1.7480	26
23	0.020 N NA2SO4	1.7610	27
22	0.030 N NA2SO4	1.8155	28
31	0.050 N K2SO4	1.8425	29
30	0.004 N MGCL2	1.8915	30
40	0.004 N CACL2	1.8935	31
32	0.030 N K2SO4	1.8960	32
10	0.004 N KCL	1.9575	33
2	0.030 N NACL	1.9715	34
35	0.004 N K2SO4	1.9910	35
25	0.004 N NA2SO4	2.0010	36
15	0.004 N MGSO4	2.0255	37
5	0.004 N NACL	2.0455	38
3	0.020 N NACL	2.0655	39
4	0.009 N NACL	2.0840	40
47	CONTROL	2.1640	41
48	CONTROL	2.1990	42
45	CONTROL	2.2020	43
42	CONTROL	2.2985	44
44	CONTROL	2.3090	45
46	CONTROL	2.3300	46
43	CONTROL	2.4365	47
41	CONTROL	2.6400	48

Acclimated Selenastrum

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
27	0.30 N NAHCO3	0.13000	1
28	0.05 N KHCO3	0.15000	2
16	0.30 N K2SO4	0.16500	3
29	0.10 N KHCO3	0.17000	4
30	0.30 N KHCO3	0.21500	5
17	0.10 N K2SO4	0.32000	6
6	0.30 N KCL	0.38500	7
35	10ML CP ELUTRIATE	0.48000	8
25	0.05 N NAHCO3	0.55000	9
9	0.30 N MGCL2	0.98500	10
14	0.10 N NA2SO4	1.2500	11
16	0.05 N K2SO4	1.2500	12
15	0.30 N NA2SO4	1.3000	13
26	0.10 N NAHCO3	1.5650	14
10	0.05 N CACL2	1.6500	15
13	0.05 N NA2SO4	1.9350	16
8	0.10 N MGCL2	1.9500	17
12	0.30 N CACL2	2.5000	18
5	0.10 N KCL	2.8350	19
11	0.10 N CACL2	3.4000	20
3	0.30 N NACL	4.4650	21
21	0.30 N MGSO4	10.085	22
4	0.05 N KCL	22.000	23
19	0.05 N MGSO4	23.420	24
24	0.30 N CASO4	30.500	25
7	0.05 N MGCL2	52.885	26
20	0.10 N MGSO4	58.165	27
2	0.10 N NACL	60.000	28
23	0.10 N CASO4	75.340	29
22	0.05 N CASO4	103.34	30
1	0.05 N NACL	164.00	31
39	10ML BP ELUTRIATE	170.00	32
37	10ML DP ELUTRIATE	172.00	33
36	10ML DR ELUTRIATE	174.00	34
38	10ML BR ELUTRIATE	182.00	35
40	D.W.BLANK	188.00	36
32	CONTROL	200.00	37
33	CONTROL	203.00	38
31	CONTROL	204.00	39
34	10ML CR ELUTRIATE	208.00	40

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
14	0.10 N NA2SO4	0.39400	1
30	0.30 N KHCO3	0.39400	2
28	0.05 N KHCO3	0.45750	3
8	0.10 N MGCL2	0.46000	4
13	0.05 N NA2SO4	0.47800	5
29	0.10 N KHCO3	0.47800	6
27	0.30 N NAHCO3	0.50600	7
9	0.30 N MGCL2	0.52800	8
15	0.30 N NA2SO4	0.53250	9
26	0.10 N NAHCO3	0.55750	10
12	0.30 N CACL2	0.56800	11
16	0.05 N K2SO4	0.62000	12
2	0.10 N NACL	0.65550	13
7	0.05 N MGCL2	0.73100	14
21	0.30 N MGSO4	0.79300	15
11	0.10 N CACL2	0.81150	16
4	0.05 N KCL	0.83900	17
35	10ML CP ELUTRIATE	0.90450	18
25	0.05 N NAHCO3	0.93800	19
10	0.05 N CACL2	0.94700	20
5	0.10 N KCL	0.99250	21
24	0.30 N CASO4	1.1565	22
20	0.10 N MGSO4	1.2175	23
3	0.30 N NACL	1.2305	24
1	0.05 N NACL	1.3520	25
23	0.10 N CASO4	1.4105	26
22	0.05 N CASO4	1.4580	27
40	D.W.BLANK	1.5680	28
32	CONTROL	1.6135	29
33	CONTROL	1.6220	30
38	10ML BR ELUTRIATE	1.6665	31
39	10ML BP ELUTRIATE	1.6795	32
36	10ML DR ELUTRIATE	1.6825	33
37	10ML DP ELUTRIATE	1.7030	34
34	10ML CR ELUTRIATE	1.9245	35
6	0.30 N KCL	10.357	36
18	0.30 N K2SO4	10.887	37
19	0.05 N MGSO4	11.309	38
31	CONTROL	16.624	39
17	0.10 N K2SO4	20.646	40

Two Salt Additions

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CLACANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
12	MGSO4,KHCO3	255.00	1
46	CONTROL	515.00	2
4	MGCL2,KHCO3	570.00	3
40	NA2SO4,NAHCO3	1070.0	4
15	MGSO4,NAHCO3	1430.0	5
7	MGCL2,NAHCO3	1595.0	6
37	NACL,NAHCO3	1790.0	7
35	KHCO3,CASO4	1960.0	8
25	K2SO4,KHCO3	3405.0	9
44	NAHCO3,CASO4	4020.0	10
10	KCL,KHCO3	4155.0	11
47	CONTROL	4445.0	12
1	MGCL2,MGSO4	5560.0	13
43	NAHCO3,CACL2	5970.0	14
32	KHCO3,NA2SO4	9205.0	15
33	KHCO3,NAHCO3	15700.	16
18	KCL,K2SO4	18610.	17
34	KHCO3,CACL2	22200.	18
48	CONTROL	26150.	19
36	NACL,NA2SO4	27650.	20
42	NA2SO4,CASO4	27900.	21
39	NACL,CASO4	30400.	22
10	MGSO4,KCL	35275.	23
28	K2SO4,NAHCO3	40300.	24
13	MGSO4,NACL	43900.	25
14	MGSO4,NA2SO4	45950.	26
31	KHCO3,NACL	49150.	27
17	MGSO4,CASO4	50450.	28
22	KCL,NAHCO3	50450.	29
6	MGCL2,NA2SO4	58700.	30
20	KCL,CASO4	62850.	31
8	MGCL2,CACL2	64600.	32
9	MGCL2,NACL	66150.	33
38	NACL,CACL2	72200.	34
16	MGSO4,CACL2	74950.	35
11	MGSO4,K2SO4	74950.	36
29	K2SO4,CACL2	76500.	37
41	NA2SO4,NAHCO3	78900.	38
24	K2SO4,NACL	79450.	39
5	MGCL2,K2SO4	80550.	40
2	MGCL2,KCL	84550.	41
9	MGCL2,CASO4	86400.	42
20	KCL,NACL	92500.	43
27	K2SO4,NA2SO4	94650.	44
21	KCL,NA2SO4	97500.	45
23	KCL,CACL2	.13500E+06	46
49	CONTROL	.14450E+06	47
30	K2SO4,CASO4	.15105E+06	48
45	CACL2,CASO4	.15610E+06	49
50	CONTROL	.15940E+06	50

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CLACANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
25	K2SO4,KHCO3	0.29050	1
4	MGCL2,KHCO3	0.36000	2
40	NA2SO4,NAHCO3	0.36900	3
10	KCL,KHCO3	0.43350	4
7	MGCL2,NAHCO3	0.44400	5
46	CONTROL	0.44800	6
15	MGSO4,NAHCO3	0.54750	7
12	MGSO4,KHCO3	0.63300	8
44	NAHCO3,CASO4	0.64900	9
30	KHCO3,CACL2	0.70650	10
35	KHCO3,CASO4	0.71550	11
37	NACL,NAHCO3	0.76650	12
1	MGCL2,MGSO4	0.78000	13
33	KHCO3,NAHCO3	0.78450	14
13	MGSO4,NACL	0.81250	15
17	MGSO4,CASO4	0.85450	16
43	NAHCO3,CACL2	0.89500	17
47	CONTROL	0.93600	18
16	MGSO4,CACL2	0.95100	19
27	K2SO4,NA2SO4	0.97650	20
3	MGCL2,K2SO4	0.98650	21
45	CACL2,CASO4	0.99800	22
6	MGCL2,NA2SO4	1.0270	23
9	MGCL2,CASO4	1.0405	24
18	KCL,K2SO4	1.0410	25
30	K2SO4,CASO4	1.0665	26
20	KCL,CASO4	1.0900	27
28	K2SO4,CACL2	1.0955	28
36	NACL,NA2SO4	1.1025	29
42	NA2SO4,CASO4	1.1390	30
5	MGCL2,NACL	1.1600	31
38	NACL,CACL2	1.1645	32
32	KHCO3,NA2SO4	1.1675	33
8	MGCL2,CACL2	1.1710	34
26	K2SO4,NACL	1.1715	35
22	KCL,NAHCO3	1.1795	36
23	KCL,CACL2	1.1930	37
39	NACL,CASO4	1.1975	38
10	MGSO4,KCL	1.2295	39
31	KHCO3,NACL	1.2825	40
11	MGSO4,K2SO4	1.2900	41
2	MGCL2,KCL	1.2995	42
21	KCL,NA2SO4	1.3160	43
20	KCL,NACL	1.3980	44
28	K2SO4,NAHCO3	1.4325	45
14	MGSO4,NA2SO4	1.4485	46
49	CONTROL	1.5210	47
50	CONTROL	1.5805	48
41	NA2SO4,NAHCO3	1.5835	49
48	CONTROL	1.6755	50

Appendix B-2

Synedra

Single Salt Additions

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
24	4/0.30 N K2SO4	0.10000	1
35	4/0.20 N NAHCO3	0.11500	2
38	4/0.10 N KHCO3	0.13000	3
39	4/0.20 N KHCO3	0.18500	4
36	4/0.30 N NAHCO3	0.28500	5
40	4/0.30 N KHCO3	0.31500	6
34	4/0.10 N NAHCO3	0.31500	7
37	4/0.05 N KHCO3	0.33500	8
33	4/0.05 N NAHCO3	0.58500	9
16	4/0.30 N CACL2	0.68500	10
11	4/0.20 N MGCL2	1.2500	11
20	4/0.30 N NA2SO4	1.3200	12
23	4/0.20 N K2SO4	1.6350	13
27	4/0.20 N MGSO4	2.0150	14
12	4/0.30 N MGCL2	2.3150	15
19	4/0.20 N NA2SO4	3.1000	16
28	4/0.30 N MGSO4	3.2500	17
18	4/0.10 N NA2SO4	3.9500	18
17	4/0.05 N NA2SO4	4.1000	19
8	4/0.30 N KCL	4.6000	20
13	4/0.05 N CACL2	5.9150	21
7	4/0.20 N KCL	6.0500	22
14	4/0.10 N CACL2	6.4150	23
10	4/0.10 N MGCL2	7.5000	24
4	4/0.30 N NACL	8.6650	25
25	4/0.05 N MGSO4	10.170	26
15	4/0.20 N CACL2	10.500	27
3	4/0.20 N NACL	12.170	28
5	4/0.05 N KCL	12.330	29
9	4/0.05 N MGCL2	12.500	30
1	4/0.05 N NACL	13.830	31
26	4/0.10 N MGSO4	13.835	32
2	4/0.10 N NACL	14.165	33
42	CONTROL	14.165	34
6	4/0.10 N KCL	14.835	35
22	4/0.10 N K2SO4	15.000	36
44	CONTROL	15.330	37
21	4/0.05 N K2SO4	15.835	38
43	CONTROL	17.835	39
30	4/0.10 N CASO4	20.330	40
41	CONTROL	20.665	41
29	4/0.05 N CASO4	22.000	42
31	4/0.20 N CASO4	27.835	43
32	4/0.30 N CASO4	29.500	44

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
35	4/0.20 N NAHCO3	0.20800	1
39	4/0.20 N KHCO3	0.35250	2
38	4/0.10 N KHCO3	0.35700	3
36	4/0.30 N NAHCO3	0.35750	4
40	4/0.30 N KHCO3	0.45300	5
34	4/0.10 N NAHCO3	0.47200	6
24	4/0.30 N K2SO4	0.60200	7
37	4/0.05 N KHCO3	0.62850	8
33	4/0.05 N NAHCO3	0.75200	9
22	4/0.10 N K2SO4	0.81500	10
23	4/0.20 N K2SO4	0.86000	11
9	4/0.05 N MGCL2	0.93900	12
12	4/0.30 N MGCL2	1.0165	13
28	4/0.30 N MGSO4	1.1235	14
10	4/0.10 N MGCL2	1.2435	15
13	4/0.05 N CACL2	1.2820	16
27	4/0.20 N MGSO4	1.2960	17
7	4/0.20 N KCL	1.3590	18
15	4/0.20 N CACL2	1.3625	19
16	4/0.30 N CACL2	1.3665	20
26	4/0.10 N MGSO4	1.4430	21
21	4/0.05 N K2SO4	1.4520	22
11	4/0.20 N MGCL2	1.5595	23
25	4/0.05 N MGSO4	1.5715	24
19	4/0.20 N NA2SO4	1.6005	25
20	4/0.30 N NA2SO4	1.6295	26
31	4/0.20 N CASO4	1.7030	27
14	4/0.10 N CACL2	1.7060	28
32	4/0.30 N CASO4	1.7105	29
8	4/0.30 N KCL	1.8090	30
1	4/0.05 N NACL	1.8970	31
6	4/0.10 N KCL	1.9240	32
30	4/0.10 N CASO4	1.9340	33
5	4/0.05 N KCL	1.9550	34
4	4/0.30 N NACL	1.9915	35
18	4/0.10 N NA2SO4	2.0480	36
17	4/0.05 N NA2SO4	2.1900	37
3	4/0.20 N NACL	2.1915	38
2	4/0.10 N NACL	2.4510	39
42	CONTROL	2.5500	40
44	CONTROL	2.6445	41
29	4/0.05 N CASO4	2.6690	42
43	CONTROL	2.7845	43
41	CONTROL	2.8085	44

Two Salt Additions to Synedra

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
30	S/NA2SO4,NAHCO3	0,21500	1
35	S/NA2SO4,KHCO3	0,28500	2
23	S/MGCL2,NAHCO3	0,28500	3
39	S/K2SO4,KHCO3	0,31500	4
8	S/NACL,NAHCO3	0,47000	5
45	S/NAHCO3,KHCO3	0,50000	6
38	S/K2SO4,NAHCO3	0,55000	7
17	S/KCL,KHCO3	0,56500	8
41	S/MGSO4,NAHCO3	0,60000	9
16	S/KCL,NAHCO3	0,60000	10
9	S/NACL,KHCO3	0,96500	11
30	S/CACL2,KHCO3	1,1000	12
29	S/CACL2,NAHCO3	1,3350	13
43	S/CASO4,NAHCO3	1,6350	14
42	S/MGSO4,KHCO3	1,7000	15
44	S/CASO4,KHCO3	1,8850	16
4	S/NACL,NA2SO4	5,1850	17
10	S/KCL,MGCL2	8,3350	18
24	S/MGCL2,KHCO3	8,6850	19
2	S/NACL,MGCL2	8,8350	20
31	S/NA2SO4,K2SO4	9,8500	21
19	S/MGCL2,NA2SO4	11,500	22
22	S/MGCL2,CASO4	11,500	23
5	S/NACL,K2SO4	11,500	24
21	S/MGCL2,MGSO4	11,670	25
13	S/KCL,K2SO4	11,670	26
20	S/MGCL2,K2SO4	12,165	27
6	S/NACL,MGSO4	12,500	28
18	S/MGCL2,CACL2	12,835	29
27	S/CACL2,MGSO4	13,170	30
48	S/CONTROL	13,335	31
14	S/KCL,MGSO4	13,500	32
1	S/NACL,KCL	13,830	33
46	S/CONTROL	14,000	34
36	S/K2SO4,MGSO4	14,000	35
26	S/CACL2,K2SO4	14,000	36
25	S/CACL2,NA2SO4	14,000	37
40	S/MGSO4,CASO4	14,330	38
12	S/KCL,NA2SO4	15,170	39
3	S/NACL,CACL2	15,335	40
32	S/NA2SO4,MGSO4	16,170	41
15	S/KCL,CASO4	16,335	42
47	S/CONTROL	17,000	43
44	S/CONTROL	17,165	44
7	S/NACL,CASO4	18,335	45
11	S/KCL,CACL2	18,830	46
28	S/CACL2,CASO4	20,000	47
33	S/NA2SO4,CASO4	26,500	48
37	S/K2SO4,CASO4	30,835	49

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
45	S/NAHCO3,KHCO3	.90000E-02	1
34	S/NA2SO4,NAHCO3	.11000E-01	2
43	S/CASO4,NAHCO3	.13000E-01	3
35	S/NA2SO4,KHCO3	.13000E-01	4
41	S/MGSO4,NAHCO3	.13500E-01	5
39	S/K2SO4,KHCO3	.14500E-01	6
30	S/CACL2,NHCO3	.16500E-01	7
9	S/NACL,KHCO3	.16500E-01	8
23	S/MGCL2,NAHCO3	.19000E-01	9
42	S/MGSO4,KHCO3	.20500E-01	10
17	S/KCL,KHCO3	.21500E-01	11
38	S/K2SO4,NAHCO3	.21500E-01	12
16	S/KCL,NAHCO3	.28500E-01	13
24	S/MGCL2,KHCO3	.27000E-01	14
29	S/CACL2,NAHCO3	.30000E-01	15
8	S/NACL,NAHCO3	.36000E-01	16
44	S/CASO4,KHCO3	.65500E-01	17
31	S/NA2SO4,K2SO4	.71000E-01	18
22	S/MGCL2,CASO4	.84000E-01	19
4	S/NACL,NA2SO4	.94500E-01	20
13	S/KCL,K2SO4	0,10800	21
27	S/CACL2,MGSO4	0,11450	22
26	S/CACL2,K2SO4	0,13050	23
2	S/NACL,MGCL2	0,13150	24
18	S/MGCL2,CACL2	0,13550	25
25	S/CACL2,NA2SO4	0,14200	26
5	S/NACL,K2SO4	0,14300	27
20	S/MGCL2,K2SO4	0,14550	28
40	S/MGSO4,CASO4	0,14600	29
12	S/KCL,NA2SO4	0,15250	30
36	S/K2SO4,MGSO4	0,15500	31
10	S/KCL,MGCL2	0,15850	32
21	S/MGCL2,MGSO4	0,16200	33
15	S/KCL,CASO4	0,16550	34
11	S/KCL,CACL2	0,17450	35
19	S/MGCL2,NA2SO4	0,17500	36
46	S/CONTROL	0,17600	37
48	S/CONTROL	0,17900	38
7	S/NACL,CASO4	0,18100	39
3	S/NACL,CACL2	0,18250	40
1	S/NACL,KCL	0,18450	41
6	S/NACL,MGSO4	0,19750	42
28	S/CACL2,CASO4	0,20000	43
47	S/CONTROL	0,21100	44
14	S/KCL,MGSO4	0,21200	45
49	S/CONTROL	0,21800	46
32	S/NA2SO4,MGSO4	0,21850	47
33	S/NA2SO4,CASO4	0,23050	48
37	S/K2SO4,CASO4	0,34400	49

3 & 4 Salt Additions to Synedra

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
28	6/5 6 8	.70000E-01	1
52	6/3 4 5 8	0.14500	2
32	6/6 7 10	0.18000	3
56	6/5 6 7 8	0.20000	4
1A	6/3 4 5	0.21500	5
30	6/7 8 9	0.21500	6
57	6/4 5 6 9	0.25000	7
24	6/4 5 8	0.28500	8
6	6/1 2 8	0.31500	9
3E	6/7 8 10	0.35000	10
50	6/3 4 5 6	0.35000	11
60	6/5 6 7 9	0.35000	12
3A	6/8 9 10	0.35000	13
30	6/5 6 10	0.43000	14
56	6/4 5 6 8	0.43500	15
13	6/2 3 8	0.43500	16
27	6/5 6 7	0.45000	17
54	6/3 4 5 10	0.45000	18
23	6/4 5 7	0.48000	19
62	6/6 7 8 9	0.56500	20
29	6/5 6 9	0.56500	21
10	6/3 4 8	0.57000	22
61	6/5 6 7 10	0.60000	23
5A	6/4 5 6 10	0.76500	24
55	6/4 5 6 7	0.76500	25
53	6/3 4 5 9	0.78000	26
63	6/6 7 8 10	0.80000	27
51	6/3 4 5 7	0.81500	28
2	6/1 2 5	0.86500	29
2A	6/4 5 9	0.86500	30
2B	6/4 5 10	1.0500	31
3A	6/1 2 3 5	1.0500	32
60	6/7 8 9 10	1.0700	33
47	6/2 3 4 8	1.0850	34
40	6/2 3 4 5	1.3650	35
3D	6/6 7 9	1.4500	36
1C	6/2 3 5	1.6850	37
22	6/4 5 6	1.7000	38
41	6/1 2 3 8	1.8500	39
31	6/6 7 8	2.2750	40
42	6/1 2 3 9	3.1000	41
43	6/1 2 3 10	3.1000	42
1D	6/2 3 9	3.6500	43
4E	6/2 3 4 7	4.0000	44
E	6/1 2 7	4.0000	45
4C	6/2 3 4 6	4.1500	46
21	6/3 4 10	4.2000	47
9	6/2 3 4	4.2500	48
4B	6/2 3 4 9	4.3350	49
4A	6/1 2 3 7	4.5000	50
A	6/1 2 10	4.6500	51
12	6/2 3 7	4.6750	52
7	6/1 2 9	4.7500	53
15	6/2 3 10	5.0500	54
2	6/1 2 4	5.2000	55
37	6/1 2 3 4	5.3500	56
20	6/3 4 9	5.3750	57
1B	6/3 4 7	5.8500	58
39	6/1 2 3 6	6.0500	59
4D	6/2 3 4 10	6.3000	60
17	6/3 4 6	6.5000	61
11	6/2 3 8	6.6650	62
B	6/1 2 6	8.3000	63
1	6/1 2 3	13.500	64
4A	6/CONTROL	15.670	65
6E	6/CONTROL	16.000	66
6A	6/CONTROL	17.015	67
67	6/CONTROL	18.350	68

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
60	6/7 8 9 10	0.58350	1
26	6/4 5 10	0.61900	2
29	6/5 6 9	0.68350	3
54	6/3 4 5 10	0.73300	4
27	6/5 6 7	0.78750	5
35	6/7 8 10	0.86100	6
56	6/4 5 6 8	0.86700	7
62	6/6 7 8 9	0.87150	8
53	6/3 4 5 9	0.88900	9
55	6/4 5 6 7	0.93200	10
24	6/4 5 8	0.94850	11
5B	6/4 5 6 10	0.94850	12
61	6/5 6 7 10	0.96850	13
25	6/4 5 9	1.0170	14
28	6/5 6 8	1.0255	15
59	6/5 6 7 8	1.0500	16
3E	6/8 9 10	1.0725	17
13	6/2 3 8	1.0745	18
14	6/3 4 8	1.1200	19
52	6/3 4 5 8	1.1565	20
21	6/3 4 10	1.1725	21
12	6/2 3 7	1.1730	22
60	6/5 6 7 9	1.1755	23
33	6/6 7 10	1.1770	24
58	6/3 4 5 6	1.1790	25
0	6/2 3 4	1.1870	26
57	6/4 5 6 9	1.2000	27
40	6/1 2 3 7	1.2010	28
30	6/5 6 10	1.2040	29
3	6/1 2 5	1.2475	30
43	6/1 2 3 10	1.2515	31
16	6/3 4 5	1.3110	32
18	6/3 4 7	1.3415	33
50	6/7 8 9	1.3650	34
22	6/4 5 6	1.3755	35
32	6/6 7 9	1.3820	36
20	6/3 4 9	1.5015	37
23	6/4 5 7	1.5220	38
38	6/1 2 3 5	1.5270	39
63	6/6 7 8 10	1.5325	40
39	6/1 2 3 6	1.5395	41
42	6/1 2 3 9	1.5435	42
49	6/2 3 4 10	1.5510	43
8	6/1 2 8	1.6410	44
48	6/2 3 4 5	1.6470	45
41	6/1 2 3 8	1.6745	46
15	6/2 3 10	1.6815	47
10	6/2 3 5	1.6935	48
2	6/1 2 4	1.7005	49
47	6/2 3 4 8	1.7040	50
8	6/1 2 10	1.7290	51
11	6/2 3 6	1.7495	52
46	6/2 3 4 7	1.7745	53
4B	6/2 3 4 9	1.8045	54
31	6/6 7 8	1.8070	55
6	6/1 2 8	1.8120	56
37	6/1 2 3 4	1.8135	57
14	6/2 3 9	1.8225	58
45	6/2 3 4 6	1.9620	59
1	6/1 2 3	2.2455	60
5	6/1 2 7	2.2525	61
68	6/CONTROL	2.3225	62
51	6/3 4 5 7	2.3695	63
17	6/3 4 6	2.3795	64
67	6/CONTROL	2.4250	65
7	6/1 2 9	2.5585	66
65	6/CONTROL	2.7405	67
66	6/CONTROL	2.7755	68

Legend: 1 = mg SO_4, 2 = mg Cl_2, 3 = K_2SO_4, 4 = KCl, 5 = $KHCO_3$, 6 = Na_2SO_4, 7 = NaCl, 8 = $NaHCO_3$, 9 = $CaSO_4$, 10 = $CaCl_2$

Multiple Salt Additions to Synedra

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
29	7/1 2 3 4 8 8	0.23000	1
9	7/2 3 4 5 8	0.25000	2
31	7/3 4 5 6 7 8	0.25000	3
50	7/1 2 3 4 5 6 7 8 0	0.26500	4
14	7/4 5 6 7 8	0.28000	5
13	7/3 4 5 6 8	0.30000	6
35	7/4 5 6 7 8 0	0.32000	7
45	7/3 4 5 6 7 8 0	0.35000	8
41	7/2 3 4 5 6 7 8	0.40000	9
51	7/2 3 4 5 6 7 8 0	0.45000	10
10	7/4 5 6 7 0	0.45000	11
46	7/1 2 3 4 5 6 7 0	0.46500	12
38	7/1 2 3 4 5 6 8	0.48500	13
43	7/2 3 4 5 6 7 0	0.50000	14
28	7/2 3 4 5 6 8	0.50000	15
30	7/2 3 4 5 6 0	0.50000	16
20	7/5 6 7 8 0	0.51500	17
42	7/1 2 3 4 5 6 0	0.51500	18
11	7/2 3 4 5 0	0.55000	19
34	7/3 4 5 6 7 0	0.58500	20
18	7/3 4 5 6 0	0.63500	21
27	7/2 3 4 5 6 7	0.66500	22
44	7/3 4 5 6 7 8 9	0.68000	23
47	7/1 2 3 4 5 6 7 8	0.71500	24
12	7/3 4 5 6 7	0.73000	25
53	7/1 2 3 4 5 6 7 8 9	0.75000	26
23	7/1 2 3 4 5 7	0.76500	27
8	7/2 3 4 5 7	0.77000	28
36	7/1 2 3 4 5 6 9	0.80000	29
7	7/2 3 4 5 6	0.81500	30
40	7/4 5 6 7 8 9	0.81500	31
22	7/1 2 3 4 5 6	0.85000	32
16	7/5 6 7 8 9	0.90000	33
52	7/2 3 4 5 6 7 8 9 0	0.93000	34
37	7/1 2 3 4 5 6 7	0.93500	35
24	7/5 6 7 8 9 0	0.98000	36
21	7/6 7 8 9 0	1.0000	37
17	7/4 5 6 7 9	1.0150	38
54	7/1 2 3 4 5 6 7 8 9 0	1.0500	39
50	7/2 3 4 5 6 7 8 9	1.0650	40
10	7/2 3 4 5 9	1.1450	41
4	7/1 2 3 4 8	1.1500	42
58	7/CONTROL	1.2000	43
6	7/1 2 3 4 9	1.2500	44
50	7/CONTROL	1.4000	45
26	7/2 3 4 5 6 9	1.4000	46
42	7/2 3 4 5 6 7 9	1.4200	47
16	7/3 4 5 6 9	1.4200	48
48	7/1 2 3 4 5 6 7 9	1.5500	49
32	7/3 4 5 6 7 9	1.5500	50
6	7/1 2 3 4 0	1.5850	51
52	7/3 4 5 6 7 8 9 0	1.5850	52
57	7/CONTROL	1.7000	53
60	7/CONTROL	1.7650	54
48	7/4 5 6 7 8 9 0	1.9350	55
3	7/1 2 3 4 7	2.1850	56
1	7/1 2 3 4 5	2.2650	57
25	7/1 2 3 4 5 9	2.6500	58
2	7/1 2 3 4 6	3.3000	59
24	7/1 2 3 4 5 0	4.4850	60

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	7/2 3 4 5 8	0.35700	1
30	7/2 3 4 5 6 0	0.37200	2
46	7/4 5 6 7 8 9 0	0.45750	3
31	7/3 4 5 6 7 8	0.48800	4
55	7/2 3 4 5 6 7 8 9 0	0.62150	5
16	7/4 5 6 7 8	0.63150	6
44	7/3 4 5 6 7 8 9	0.64000	7
51	7/2 3 4 5 6 7 8 0	0.64700	8
54	7/1 2 3 4 5 6 7 8 0	0.65150	9
11	7/2 3 4 5 0	0.65600	10
45	7/3 4 5 6 7 8 0	0.68900	11
15	7/3 4 5 6 0	0.69700	12
41	7/2 3 4 5 6 7 8	0.69700	13
18	7/4 5 6 7 0	0.75300	14
13	7/3 4 5 6 8	0.75400	15
7	7/2 3 4 5 6	0.75650	16
24	7/1 2 3 4 5 8	0.79050	17
12	7/3 4 5 6 7	0.83850	18
56	7/2 3 4 5 6 7 8 9	0.84000	19
4	7/1 2 3 4 8	0.84650	20
3	7/1 2 3 4 7	0.87450	21
2	7/1 2 3 4 5	0.88150	22
17	7/4 5 6 7 9	0.91600	23
49	7/1 2 3 4 5 6 7 0	0.91950	24
34	7/4 5 6 7 8 9	0.96850	25
56	7/1 2 3 4 5 6 7 8 9 0	1.0010	26
52	7/3 4 5 6 7 8 9 0	1.0140	27
1	7/1 2 3 4 5	1.0205	28
14	7/3 4 5 6 9	1.0395	29
47	7/1 2 3 4 5 6 7 8	1.0550	30
8	7/1 2 3 4 0	1.0750	31
8	7/2 3 4 5 7	1.1120	32
10	7/2 3 4 5 9	1.1220	33
35	7/4 5 6 7 8 0	1.1300	34
53	7/1 2 3 4 5 6 7 8 9	1.1715	35
43	7/2 3 4 5 6 7 0	1.1765	36
40	7/1 2 3 4 5 6 0	1.1870	37
27	7/2 3 4 5 6 7	1.1900	38
28	7/2 3 4 5 6 8	1.2330	39
33	7/1 2 3 4 5 7	1.2950	40
39	7/1 2 3 4 5 6 7	1.3005	41
9	7/1 2 3 4 9	1.3360	42
22	7/1 2 3 4 5 6	1.3705	43
36	7/5 6 7 8 9 0	1.3705	44
33	7/3 4 5 6 7 0	1.4025	45
42	7/2 3 4 5 6 7 9	1.4390	46
60	7/CONTROL	1.4560	47
30	7/1 2 3 4 5 6 9	1.4625	48
29	7/2 3 4 5 6 9	1.4865	49
20	7/5 6 7 8 0	1.5435	50
32	7/3 4 5 6 7 9	1.6025	51
58	7/CONTROL	1.6365	52
19	7/5 6 7 8 9	1.6580	53
59	7/CONTROL	1.7400	54
57	7/CONTROL	1.8035	55
49	7/1 2 3 4 5 6 7 9	1.8315	56
38	7/1 2 3 4 5 6 8	1.8740	57
21	7/6 7 8 9 0	1.9050	58
26	7/1 2 3 4 5 0	2.0255	59
25	7/1 2 3 4 5 9	2.2975	60

Legend: 1 = mg SO_4, 2 = mg Cl_2, 3 = K_2SO_4, 4 = KCl, 5 = $KHCO_3$, 6 = Na_2SO_4, 7 = NaCl, 8 = $NaHCO_3$, 9 = $CaSO_4$, 10 = $CaCl_2$

Appendix B-3

Three Algal Genera

Single Salt Additions to 3 Algal Genera

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Anabaena

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
0	9/ CACL2	.88400E+10	3
5	9/ MGCL2	.50850E+10	4
2	9/ KCL	.11800E+11	5
14	9/3 ALGAE CONTROL	.13710E+11	6
12	9/SCENEDESMUS CONTROL	.14680E+11	7
1	9/ NACL	.24700E+11	8
13	9/SYNEDRA CONTROL	.25105E+11	9
6	9/ K2SO4	.28650E+11	10
7	9/ MGSO4	.64350E+11	11
5	9/ NA2SO4	.81550E+11	12
11	9/ANABAENA CONTROL	.13350E+12	13
8	9/ CASO4	.17590E+12	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
11	9/ANABAENA CONTROL	1.3635	3
14	9/3 ALGAE CONTROL	2.7920	4
7	9/ MGSO4	3.3230	5
6	9/ K2SO4	3.3250	6
5	9/ NA2SO4	3.4845	7
2	9/ KCL	7.8160	8
1	9/ NACL	10.572	9
13	9/SYNEDRA CONTROL	15.197	10
4	9/ CACL2	16.069	11
12	9/SCENEDESMUS CONTROL	20.336	12
8	9/ CASO4	20.717	13
3	9/ MGCL2	20.814	14

Heterocysts

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
0	9/ CACL2	392.00	3
4	9/ CACL2	2156.0	4
2	9/ KCL	15680.	5
6	9/ K2SO4	17640.	6
1	9/ NACL	23520.	7
5	9/ NA2SO4	26264.	8
7	9/ MGSO4	27440.	9
14	9/3 ALGAE CONTROL	27440.	10
12	9/SCENEDESMUS CONTROL	39200.	11
11	9/ANABAENA CONTROL	74480.	12
13	9/SYNEDRA CONTROL	82320.	13
8	9/ CASO4	94080.	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
12	9/SCENEDESMUS CONTROL	2.8175	3
3	9/ MGCL2	3.3320	4
4	9/ CACL2	3.8815	5
1	9/ NACL	5.0330	6
11	9/ANABAENA CONTROL	5.2080	7
13	9/SYNEDRA CONTROL	5.8380	8
8	9/ CASO4	6.3980	9
6	9/ K2SO4	6.5520	10
7	9/ MGSO4	7.0715	11
14	9/3 ALGAE CONTROL	7.0720	12
5	9/ NA2SO4	8.2635	13
2	9/ KCL	9.5960	14

Phycocyanin

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
0	9/ CACL2	0.23000	1
11	9/ANABAENA CONTROL	0.66500	2
14	9/3 ALGAE CONTROL	0.78000	3
5	9/ NA2SO4	0.78500	4
6	9/ K2SO4	0.82500	5
12	9/SCENEDESMUS CONTROL	0.83000	6
10	9/ KHCO3	0.85000	7
3	9/ MGCL2	0.86000	8
2	9/ KCL	0.86500	9
1	9/ NACL	0.95000	10
9	9/ NAHCO3	1.0250	11
8	9/ CASO4	1.0650	12
7	9/ MGSO4	1.0900	13
13	9/SYNEDRA CONTROL	1.3000	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
4	9/ CACL2	0.18300	1
10	9/ KHCO3	0.37150	2
9	9/ NAHCO3	0.47400	3
14	9/3 ALGAE CONTROL	0.49250	4
6	9/ K2SO4	0.58150	5
12	9/SCENEDESMUS CONTROL	0.59750	6
5	9/ NA2SO4	0.63250	7
13	9/SYNEDRA CONTROL	0.64950	8
1	9/ NACL	0.65250	9
3	9/ MGCL2	0.65350	10
8	9/ CASO4	0.68600	11
2	9/ KCL	0.69900	12
11	9/ANABAENA CONTROL	0.78400	13
7	9/ MGSO4	0.79550	14

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Synedra

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
5	9/ NA2SO4	1,9020	3
13	9/SYNEDRA CONTROL	2,0420	4
14	9/3 ALGAE CONTROL	2,7655	5
2	9/ KCL	8,1180	6
11	9/ANABAENA CONTROL	9,7495	7
1	9/ NACL	10,936	8
4	9/ CACL2	11,200	9
7	9/ MGSO4	18,811	10
6	9/ K2SO4	19,321	11
8	9/ CASO4	19,856	12
12	9/SCENEDESMUS CONTROL	20,480	13
3	9/ MGCL2	23,268	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
5	9/ NA2SO4	1,9020	3
13	9/SYNEDRA CONTROL	2,0420	4
14	9/3 ALGAE CONTROL	2,7655	5
2	9/ KCL	8,1180	6
11	9/ANABAENA CONTROL	9,7495	7
1	9/ NACL	10,936	8
4	9/ CACL2	11,200	9
7	9/ MGSO4	18,811	10
6	9/ K2SO4	19,321	11
8	9/ CASO4	19,856	12
12	9/SCENEDESMUS CONTROL	20,480	13
3	9/ MGCL2	23,268	14

Carotinoid

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
4	9/ CACL2	1,0000	1
3	9/ MGCL2	1,0150	2
2	9/ KCL	1,0250	3
1	9/ NACL	1,1000	4
11	9/ANABAENA CONTROL	1,1500	5
14	9/3 ALGAE CONTROL	1,3000	6
6	9/ K2SO4	1,4250	7
5	9/ NA2SO4	1,4750	8
10	9/ KHCO3	1,5600	9
12	9/SCENEDESMUS CONTROL	1,5750	10
7	9/ MGSO4	2,1250	11
9	9/ NAHCO3	2,3000	12
13	9/SYNEDRA CONTROL	2,3500	13
8	9/ CASO4	2,6500	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0,37750	1
4	9/ CACL2	0,41600	2
10	9/ KHCO3	0,48100	3
11	9/ANABAENA CONTROL	0,52350	4
1	9/ NACL	0,60800	5
3	9/ MGCL2	0,68050	6
8	9/ CASO4	0,69250	7
6	9/ K2SO4	0,70450	8
14	9/3 ALGAE CONTROL	0,71700	9
5	9/ NA2SO4	0,72000	10
12	9/SCENEDESMUS CONTROL	0,88850	11
7	9/ MGSO4	0,98750	12
2	9/ KCL	1,0480	13
13	9/SYNEDRA CONTROL	1,2205	14

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Scenedesmus

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
11	9/ANABAENA CONTROL	.78500E+09	3
13	9/SYNEDRA CONTROL	.21150E+11	4
3	9/ MGCL2	.21600E+11	5
1	9/ NACL	.30600E+11	6
4	9/ CACL2	.32850E+11	7
2	9/ KCL	.37600E+11	8
8	9/ CASO4	.13010E+12	9
14	9/3 ALGAE CONTROL	.25150E+12	10
6	9/ K2SO4	.25750E+12	11
5	9/ NA2SO4	.27850E+12	12
12	9/SCENEDESMUS CONTROL	.30100E+12	13
7	9/ MGSO4	.40300E+12	14

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE	RANKING
9/ NAHCO3	0.	1
9/ KHCO3	0.	2
9/ NA2SO4	2,3085	3
9/3 ALGAE CONTROL	2,6605	4
9/SCENEDESMUS CONTROL	5,0045	5
9/ KCL	7,0000	6
9/ANABAENA CONTROL	9,6780	7
9/ NACL	10,478	8
9/ CACL2	11,529	9
9/SYNEDRA CONTROL	20,630	10
9/ K2SO4	20,720	11
9/ MGCL2	20,790	12
9/ CASO4	21,299	13
9/ MGSO4	21,382	14

Chlorophyll A

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
4	9/ CACL2	0,87000	1
2	9/ KCL	1,0750	2
11	9/ANABAENA CONTROL	1,1000	3
3	9/ MGCL2	1,1850	4
1	9/ NACL	1,1500	5
6	9/ K2SO4	1,3300	6
14	9/3 ALGAE CONTROL	1,4750	7
12	9/SCENEDESMUS CONTROL	1,6250	8
10	9/ KHCO3	1,6500	9
13	9/SYNEDRA CONTROL	1,8250	10
5	9/ NA2SO4	1,8250	11
9	9/ NAHCO3	2,3250	12
7	9/ MGSO4	2,4100	13
8	9/ CASO4	2,7500	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0,27750	1
10	9/ KHCO3	0,36150	2
14	9/3 ALGAE CONTROL	0,55450	3
5	9/ NA2SO4	0,56650	4
1	9/ NACL	0,58450	5
4	9/ CACL2	0,58800	6
8	9/ CASO4	0,64700	7
6	9/ K2SO4	0,65100	8
3	9/ MGCL2	0,65150	9
2	9/ KCL	0,68450	10
12	9/SCENEDESMUS CONTROL	0,77200	11
11	9/ANABAENA CONTROL	0,81800	12
7	9/ MGSO4	1,0695	13
13	9/SYNEDRA CONTROL	1,3600	14

Total Cell Counts

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
4	9/ CACL2	.57981E+11	3
11	9/ANABAENA CONTROL	.13405E+12	4
1	9/ NACL	.15130E+12	5
3	9/ MGCL2	.17480E+12	6
2	9/ KCL	.17455E+12	7
13	9/SYNEDRA CONTROL	.29165E+12	8
8	9/ CASO4	.30185E+12	9
12	9/SCENEDESMUS CONTROL	.33560E+12	10
6	9/ K2SO4	.35125E+12	11
5	9/ NA2SO4	.37140E+12	12
7	9/ MGSO4	.50565E+12	13
14	9/3 ALGAE CONTROL	.56495E+12	14

DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
9	9/ NAHCO3	0.	1
10	9/ KHCO3	0.	2
11	9/ANABAENA CONTROL	1,3920	3
13	9/SYNEDRA CONTROL	2,0595	4
6	9/ K2SO4	2,3035	5
5	9/ NA2SO4	2,5645	6
14	9/3 ALGAE CONTROL	2,7925	7
12	9/SCENEDESMUS CONTROL	3,3200	8
2	9/ KCL	8,1930	9
7	9/ MGSO4	8,4065	10
1	9/ NACL	11,156	11
4	9/ CACL2	12,019	12
8	9/ CASO4	21,481	13
3	9/ MGCL2	23,395	14

Appendix B-4

Scenedesmus

Complex Additions to Scenedesmus

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
1	20ML AR ELUTRIATE	.89500E-01	1
25	20ML BP ELUTRIATE	.90500E-01	2
27	10ML BP ELUTRIATE	0.10100	3
42	CONTROL	0.10600	4
44	CONTROL	0.10900	5
45	CONTROL	0.11000	6
26	15ML BP ELUTRIATE	0.11050	7
21	20ML BR SALTS	0.11100	8
15	10ML AP SALTS	0.11150	9
7	10ML AR SALTS	0.11200	10
22	15ML BR SALTS	0.11350	11
32	5ML BP SALTS	0.11450	12
20	5ML BR ELUTRITTE	0.11650	13
4	5ML AR ELUTRIATE	0.11750	14
28	5ML BP ELUTRIATE	0.11750	15
24	5ML BR SALTS	0.11900	16
30	15ML BP SALTS	0.12000	17
43	CONTROL	0.12100	18
10	15ML AP ELUTRIATE	0.12250	19
33	20ML AP COLUMN LEACHATE	0.12250	20
23	10ML BR SALTS	0.12250	21
6	15ML AR SALTS	0.12250	22
9	20ML AP ELUTRIATE	0.12350	23
31	10ML BP SALTS	0.12400	24
3	10ML AR ELUTRIATE	0.12550	25
39	10ML AP COLUMN SALTS	0.12550	26
5	20ML AR SALTS	0.12700	27
14	15ML AP SALTS	0.12700	28
8	5ML AR SALTS	0.12750	29
12	5ML AP ELUTRIATE	0.12800	30
2	15ML AR ELUTRIATE	0.12850	31
41	CONTROL	0.13050	32
16	5ML AP SALTS	0.13050	33
38	15ML AP COLUMN SALTS	0.13100	34
29	20ML BP SALTS	0.13150	35
35	10ML AP COLUMN LEACHATE	0.13150	36
13	20ML AP SALTS	0.13400	37
19	10ML BR ELUTRIATE	0.13550	38
18	15ML BR ELUTRIATE	0.13600	39
37	20ML AP COLUMN SALTS	0.13650	40
36	5ML AP COLUMN LEACHATE	0.13750	41
17	20ML BR ELUTRIATE	0.13800	42
34	15ML AP COLUMN LEACHATE	0.14000	43
40	5ML AP COLUMN SALTS	0.14000	44
11	10ML AR ELUTRIATE	0.14000	45

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DUNCANS MULTIPLE RANGE TEST

	TREATMENT	AVERAGE	RANKING
1	20ML AR ELUTRIATE	0.47300	1
41	CONTROL	0.54700	2
32	5ML BP SALTS	0.56200	3
15	10ML AP SALTS	0.56300	4
36	5ML AP COLUMN LEACHATE	0.57000	5
45	CONTROL	0.57150	6
44	CONTROL	0.57150	7
16	5ML AP SALTS	0.58250	8
20	5ML BR ELUTRITTE	0.58800	9
6	15ML AR SALTS	0.59450	10
31	10ML BP SALTS	0.60250	11
40	5ML AP COLUMN SALTS	0.60350	12
42	CONTROL	0.60950	13
12	5ML AP ELUTRIATE	0.60950	14
21	20ML BR SALTS	0.61600	15
25	20ML BP ELUTRIATE	0.62050	16
13	20ML AP SALTS	0.63000	17
30	15ML BP SALTS	0.63750	18
34	15ML AP COLUMN LEACHATE	0.64200	19
43	CONTROL	0.64850	20
29	20ML BP SALTS	0.65000	21
9	20ML AP ELUTRIATE	0.65300	22
5	20ML AR SALTS	0.67050	23
7	10ML AR SALTS	0.68400	24
23	10ML BR SALTS	0.68550	25
4	5ML AR ELUTRIATE	0.69200	26
26	15ML BP ELUTRIATE	0.69550	27
18	15ML BR ELUTRIATE	0.70650	28
17	20ML BR ELUTRIATE	0.70850	29
28	5ML BP SALTS	0.70900	30
14	15ML AP SALTS	0.71350	31
27	10ML BP ELUTRIATE	0.71550	32
28	5ML BP ELUTRIATE	0.72300	33
10	15ML AP ELUTRIATE	0.72450	34
8	5ML AP SALTS	0.72500	35
35	10ML AP COLUMN LEACHATE	0.72900	36
19	10ML BR ELUTRIATE	0.73500	37
38	15ML AP COLUMN SALTS	0.73650	38
33	20ML AP COLUMN LEACHATE	0.74750	39
39	10ML AP COLUMN SALTS	0.77600	40
11	10ML AR ELUTRIATE	0.79050	41
22	15ML BR SALTS	0.79500	42
37	20ML AP COLUMN SALTS	0.85450	43
2	15ML AR ELUTRIATE	1.1860	44
3	10ML AR ELUTRIATE	1.3660	45