CHROMIUM UPTAKE AND ITS EFFECTS ON THE GROWTH OF DUCKWEEDS

A Thesis

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Dedicated to my Mom and Dad

for their love, understanding, and friendship

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ABSTRACT

The uptake of chrome (Cr) and the effects of Cr on duckweed growth were examined using the radiotracer, ^{51}Cr. All examinations were conducted outdoors under natural conditions. In five separate experiments, duckweeds were subjected to Cr concentrations ranging from 4×10^{-5} to 40 ppm. Neither aeration nor stagnation of shallow tanks affected ($P > 0.05$) Cr uptake or growth of Spirodela punctata when exposed to a concentration of 1 ppm Cr. Data showed that Cr had increasingly negative effects on the growth of S. punctata, S. polyrrhiza, and Lemna gibba, at concentrations greater than 0.1 ppm. However, when growth was inhibited at increased levels of Cr, the absolute uptake by duckweeds was greater.

A comparison of responses of three duckweed species at 4 different Cr concentrations showed that for S. punctata, S. polyrrhiza, and L. gibba 10 ppm Cr was the concentration which effected greatest Cr uptake. At Cr levels greater than 1 ppm, the growth of S. polyrrhiza was greatly inhibited. S. punctata and L. gibba demonstrated greater tolerance to Cr as shown by larger biomass increases at all Cr levels above 0.1 ppm.

Because S. punctata, S. polyrrhiza, and L. gibba were able to show continued growth for 151 hr and maximum

Cr uptake when exposed to 10 ppm Cr some potential for using duckweeds for the removal of Cr from industrial and municipal wastewater was demonstrated. Future studies involving the long term exposure of duckweeds to Cr, differences in Cr removal caused by varying biomass, pH, harvest rate, etc. will help to illucidate the actual feasibility of biological removal of Cr using duckweeds.

INTRODUCTION AND LITERATURE REVIEW

The continued release of municipal, residential, industrial, and power generating plant wastewater containing heavy metals, in even low concentrations, has greatly increased the occurrence of these potentially dangerous substances in our streams, rivers (Mathis & Cummings, 1973), lakes (Ellis & Kanamori, 1977; Wentsel & Berry, 1975), swamps (Rodgers et al. 1978), and coastal areas (Eisler et al. 1977; Griggs & Johnson, 1978; Knauer, 1977; Matsumoto & Wong, 1977). The subsequent accumulation of these heavy metals within the biota and sediments in many regions decreases species diversity, inhibits productivity and has been linked to human mortality (Anderson, 1977; Cherry et al. 1979; DeMarte & Hartman, 1974; reviews by Leland et al. 1974, 1975, 1979; Mayes & McIntosh, 1977; Patrick & Loutit, 1976).

Because of its characteristic hardness, color, ability to be polished to a higher luster, and high melting point (1857 $\pm$ 20°C), chromium (Cr) has been used in industry for the production of stainless steels, tool steels, cast iron and steel, superalloys, refractories, pigments and paints, leather tanning chemicals, metal finishing chemicals, and an anti-corrosive and anti-algal agent in cooling towers (NAS, 1974).

Though this heavy metal has many benefits, its toxicity in aquatic systems has been demonstrated and the common occurrence of Cr in industrial effluents has been implicated as a primary source of Cr input to aquatic ecosystems.

Cr has a specific gravity of 7.18, an atomic weight of 51.99 g, and in aqueous solution exists complexed with water or other liquids, but never as free chromic ion (Mertz, 1969). Although Cr can exist in any oxidation state from -2 to +6, only the +3 and +6 forms are of biological interest. Cr^{+6} (soluble form) is a strong oxidizing agent which forms chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$) ions. It is often linked with oxygen and under acid conditions is readily reduced to the trivalent form (Cr^{+3}). The trivalent form is the most stable oxidation state. At neutral pH due to its coordination number of 6, Cr^{+3} forms octahedral compounds, complexes, and chelates which are often insoluble.

A debate continues regarding which form of Cr (+3 or +6) is the most toxic (Barth, 1965; Cheremisinoff, 1972; McKee & Wolf, 1963; Mertz, 1969; Mowat, 1976).

McKee & Wolf (1963) suggest that Cr^{+3} (1.2-40 ppm) is more toxic than Cr^{+6} (5-500 ppm) to fish, but that they are generally quite tolerant of Cr salts while lower trophic organisms are extremely sensitive (0.016-148 ppm).

Generally Cr^{+6} is considered more lethal than Cr^{+3} (Mertz, 1969). Because of this belief, Cr^{+6} in industrial cooling systems is reduced to Cr^{+3} prior to discharge (Mowat, 1976).

Due to chromium's toxicity, strict U. S. Environmental Protection Agency (EPA) guidelines for effluent limitations have been set for Cr (U. S. General Service Admin., 1979). See Appendix A for a list by industry of the limitations.

Cr found in water is either in the particulate or dissolved forms and is present in lower concentrations in seawater (usually less than 1 ppb), than in most rivers and ground water. The Cr content of river waters surveyed by the U. S. Geological Survey reported concentrations that range from less than 0.7 ppb to 84 ppb (NAS, 1974).

The adsorption of Cr^{+3} to particles and plankton was proved by Curl et al. (1965) to occur in seawater. Cutshall et al. (1966) suggested that this was one mechanism by which Cr is transferred from the water to the sediments. They demonstrated that Cr^{+6} dominated in the Columbia River below the Hanford Reactors and remained largely in the Cr^{+6} form 525 km from the Columbia River.

Under low oxygen concentrations and general reducing conditions, Canter & Gloyna (1969), found that 67-100% of the added $^{51}Cr^{+6}$ was reduced to $^{51}Cr^{+3}$ during

the 2 day radionuclide release period, whereas, under low organic load and high oxygen levels the tendency was for Cr to be in the +6 state regardless of its initial oxidation state.

Reported Cr levels in soils, marine algae, and several freshwater aquatic plants have varied widely, ranging from trace amounts (below the limits of detection) to 3900 ppm (Cowgill, 1974; Robinson et al. 1935; Saenko et al. 1976; Sivalingam, 1978). Cowgill (1974) reported general Cr levels within 6 common freshwater aquatic plant species collected from Linsley Pond, CT to be below 1 ppm. Cr levels in water hyacinths, (initially less than 0.1 ppm) increased to 286, 12, and 4 ppm in the roots, stems and leaves, respectively, when exposed to photographic and chemical wastewater (Wolverton & McDonald, 1976).

Studies regarding the toxicity and uptake of heavy metals to plants abound in the literature, however, Cr has been little studied. Some sensitive algal species were inhibited by Cr levels as low as 0.032 ppm. For most algal species, Cr concentrations greater than 5 ppm were very toxic, as measured by reduced growth and increased mortality, and at 10 ppm, most species could not survive beyond 120 hr of exposure. Oscillatoria limosa was an exception, surviving at 50 ppm Cr (Richards, 1936).

Duckweeds, members of the Family:Lemnaceae were chosen for this study based on their abilities to con-

centrate trace elements (Ernst & van Der Werff, 1978; Glandon & McNabb, 1978; Hutchinson & Czyrska, 1975; Lisiecki & McNabb, 1974; Rodgers et al. 1978), rapid vegetative growth, cold tolerance, hardiness, and importance within various aquatic ecosystems (Culley & Epps, 1973; Hillman & Culley, 1978).

Ernst & van Der Werff (1978) working with Lemna minor determined after three weeks a Cu LD50 of 6.35. The uptake of Cu, as well as, cadmium, lead, and zinc was described to be multiphasic. Factors such as pH, temperature, and biomass were shown to greatly affect the amount of uptake of Cu by macrophytes. Concentration factors for duckweeds subjected to various heavy metals (Cd, Cr, Cu, Ni, Zn) have been shown to vary depending on ambient metal concentrations (Ernst & van Der Werff, 1978; Glandon & McNabb, 1978; Hutchinson & Czyrska, 1975; Lisiecki & McNabb, 1974; Mangi et al. 1978; Rodgers et al. 1978).

Hutchinson & Czyrska (1975) reported maximum concentration factors for Lemna minor exposed to separate solutions of Cd (0.05); Cu (1.0); Ni (0.5); and Zn (0.05 ppm) of 9500, 54,500, 6100, and 9400 respectively while reported concentration factors for Cr uptake range between 60 (10 ppm), (Mangi et al. 1978) to 460 (0.16 ppm) (Rodgers et al. 1978).

Hutchinson & Czyrska (1975) also considered two other important factors: synergistic/antagonistic effects and changes in uptake when duckweeds are additionally stressed by competition. Zinc alone stimulated growth, however, Cd and Zn together inhibited growth. Likewise, Cu and Ni together killed plants, but both Cu and Ni uptake were increased by the presence of the other metal in solution. Competition with Salvinia natans produced levels of Ag in Lemna of 10.7 and 29.3 ppm at 0.05 and 0.5 ppm ambient levels. However, when grown separately no detectable Ag was found in the duckweed.

Brown & Rattigan (1979) reported the concentrations of heavy metals which caused 50% damage to Lemna minor, (Table 1). Mangi et al. (1978), demonstrated that at approximately 5 ppm Cr, there was a 50% reduction in the growth of L. minor. By placing the Cr results of Mangi et al. (1978) within the table of Brown & Rattigan (1979) it is evident that Cr lies in the intermediate range of toxicity for L. minor, when compared to other commonly studied heavy metals.

Mangi et al. 1978 showed that Lemna minor and Spirodela polyrrhiza grew at Cr^{+6} concentrations less than or equal to 10 ppm, but that growth was inhibited as Cr concentrations increased above 1.0 ppm.

Rodgers et al. (1978) showed that the duckweed, Lemna perpusilla was the predominant macrophyte in a swamp

Table 1. Heavy metal toxicity to duckweed: Lemna minor.

Metal	Chemical Compound	Concentration of Metal*
Thallium	Tl_2SO_4	0.008
Copper	$CuSO_4 \cdot 5H_2O$	0.13
Arsenic	$NaAsO_3$	0.15
Silver	$AgNO_3$	0.27
Nickel	$NiCl_2 \cdot 6H_2O$	0.34
Mercury	$HgCl_2$	1.0
Chromium**	K_2CrO_4	5.0
Cadmium	$Cd(NO_3)_2 \cdot 4H_2O$	14.8
Lead	$Pb(OAc)_2 \cdot 3H_2O$	16.3

Table modified from Brown & Rattigan (1979), p. 307, Table 2.

* Producing 50% damage after 28 days exposure.

** From Mangi et al. (1978) after 14 days exposure.

ecosystem contaminated with water from an ash settling basin had a mean annual Cr concentration of 65 ppm (dry wt basis).

In light of the tremendous cumulative abilities of aquatic plants for heavy metals, a wastewater treatment facility, though probably not capable of handling direct sewage loads, could prove very efficient in the removal of low-level heavy metal contamination. The added advantages of low technology, lower capital outlay and maintenance costs, likewise are added incentives.

Since not much is known of the uptake capabilities of duckweeds subjected to various concentrations of Cr or of differences in uptake between species or sampling periods, the following objectives were pursued: (1) determine ^{51}Cr uptake by duckweeds over time without the influence of added stable Cr; (2) estimate levels of Cr (as Na_2CrO_4) which would inhibit growth in 3 species of duckweed; (3) determine if different species of duckweed subjected to various Cr concentrations showed differences in Cr uptake.

MATERIALS AND METHODS

Plants

Three species of duckweeds, Family:Lemnaceae; Lemna gibba L., Spirodela punctata (Meyer) Thomp., and Spirodela polyrrhiza L. (Schleid), used in this study were collected from cultures maintained at the Louisiana State University (LSU) Dairy Science Teaching & Research Center's waste treatment facilities. These cultures have been in use at the waste treatment facility since 1975.

Monocultures of the plants were maintained outdoors in oblong metal tanks (8' x 2.5'). Duckweed cultures were thinned and a nutrient solution of 10-20 g fresh cow manure/L of water filtered through a screen, were added periodically in order to keep plants actively growing. This method is a variation of Said et al. (1979).

Chromium

Reagent grade Cr as sodium chromate crystal ($Na_2CrO_4 \cdot 4H_2O$), was purchased from Sargent Welch Corp. Chromium-51 (^{51}Cr), a soft gamma emitter (0.32 MeV) with a 27.7 day half life (Radiological Health Handbook, 1970), was obtained as Na_2CrO_4 in 0.9% saline solution (unsterile), from New England Nuclear for the first experiment and for subsequent tests from the Chemical and Radioisotope

Division of International Chemical and Nuclear (ICN). A ^{51}Cr standard (6.62 μCi), which was utilized to determine the detector efficiences of the two radioanalytical systems used, was obtained from Amersham/Searle Corp.

It was assumed that duckweeds would not discriminate between the radiotracer ^{51}Cr and stable Cr since their chemicals forms were the same and anomalies caused by isotopic effect would be insignificant (Mangi et al. 1978; Wang et al. 1975).

Instrumentation

Radioanalysis:

Two types of solid scintillation systems were used. The first experiment utilized a copper clad 3" x 3" (7.5 cm x 7.5 cm) NaI(Tl) well detector made by the Harshaw Chemical Company, Solon, Ohio, in combination with a Tracor Northern TN-1705 multichannel analyzer and a Canberra Inc., D.C. Power Supply. These apparatuses were located at the LSU Nuclear Science Center (NSC), and had no automatic sample changer. A detection efficiency of 52% was obtained for this system, during the study.

All other experimental samples were taken to the LSU School of Veterinary Medicine (SVM) where an automatic gamma counter equipped with a 2" x 2" (5 cm x 5 cm) NaI (Tl) well crystal was used for ^{51}Cr analysis. This instrument Model 1185 produced by Searle Analytic Inc.,

automatically sets the operating parameters for detection. A 43.7% detection efficiency for ^{51}Cr was determined for this apparatus.

In both analytical systems the complete photopeak was counted and background counts were subtracted. Both counters utilized the live-time counting mode and were operated at 900 volts.

Background Cr Concentration:

The determination of background stable Cr concentrations took place at the LSU Wilson Feed and Fertilizer Laboratory. A Perkin-Elmer (P-E) 5000 Atomic Absorption Spectrophotometer equipped with a P-E Intensitron Lamp, deuterium arc background corrector, P-E Printer Sequencer PRS-10, and a P-E HGA 500 Programmer Graphite Furnace was used.

Plant samples were dried at 65^{o}C to constant weight, ground and placed in 100 ml beakers with 20 ml of 12N nitric acid per dry wt of plant material. These beakers were covered with watch glasses and allowed to reflux until the plant matter was totally digested. Background Cr in three water samples (manure solution, water used to make up the manure solution, and distilled and deionized water, 300 ml each) were evaporated and then brought up to a total volume of 25 ml. All samples were

maintained at room temperature in the hood until they could be analyzed.

Sample Preparation for Radioanalysis

All samples: water, plants, and filters counted at the NSC were placed in 2-dram polyethylene vials (polyvials). The vials were then placed in the fingers of plastic gloves which were torn off. The samples, now surrounded by plastic, were placed in the well of the detector in order to prevent contamination of the detector crystal.

Samples counted at the VSM were placed in 15.6 mm x 125 mm gamma counting tubes purchased from Curtis Matheson Scientific Inc. An average difference of 0.0036 $\pm$ 0.0006 g was noted, by weighing 10 gamma tubes, placing them in the oven at 60^{o}C and reweighing them the next day. Therefore, it was decided that all gamma tubes to be used for plant samples would be first dried for at least 48 hours before determining their initial weight.

A preliminary study of the effects of changing sample geometry by increasing the sample volume for both radioanalytical systems demonstrated the need to correct for counts lost by self absorption utilizing correction factors. Only water samples were corrected since all other samples had a nearly uniform small sample size.

Geometry Correction Factors for Water

SVM		NSC	
1 ml	0.929	1 ml	0.963
2 ml	0.865	2 ml	0.945
3 ml	0.805	3 ml	0.918
5 ml	0.698	5 ml	0.886

Plant samples placed in gamma tubes were dried within the tubes and crushed into the lower 20 mm of the vials prior to counting.

Experimental Site and Radiation Safety Precautions

All experiments were performed outdoors (protected from the rain by clear 6 mil plastic) in a plastic sheltered artificial stream system described by Knaus (1978), or in plastic containers located within a greenhouse next to the stream system.

Proper precautions were made to insure that there was no leakage of ^{51}Cr into the surrounding area by utilizing plastic lined catch basins under all containers or plastic backed paper liners in trays.

Radiation warning signs and caution ropes were located around the study area. Samples were carried to and from the site in plastic-backed paper lined trays.

Plastic shoe boxes 12" x 6" x 3.5" (30 cm x 15 cm x 9 cm) used during greenhouse studies were lined by double bagged 1.02 mil white Hefty trash can liners, 17" x 18" (6.5 cm x 7 cm).

Any radioactively contaminated equipment was disposed of or was rinsed several times in tap water and acid washed in 6N nitric acid twice, with tap water rinses between acid washes. Subsequent to acid washing equipment was rinsed 3 times with tap water and 3 times with distilled and deionized water. Washed gamma tubes were recounted to insure that only normal background levels existed prior to reuse.

Plastic gloves were worn during all manipulations with ^{51}Cr and gloves were changed in between sample collections and counting in order to eliminate contamination of equipment.

At the termination of each experiment, radioactive wastes were placed in storage at the NSC's disposal area for a minimum of 270 days (10 half-lives).

Experimental Design and Laboratory Techniques

Five experiments were conducted to determine the influence of Cr on duckweed growth and Cr uptake.

Nutrient water for all experiments were the same as that water (10-20 g fresh cow manure/1 water filtered through a screen) used to maintain cultures.

Experiment 1: ^{51}Cr Uptake by Duckweeds

This experiment was performed to determine the rate of ^{51}Cr uptake over time by L. gibba and S. punctata within a static system without the influence of stable Cr. It was also important to determine if a total inventory of the ^{51}Cr was possible by examining the partitioning of ^{51}Cr within plants, water before filtering, filterable material, and water after filtering.

Forty-two Erlenmyer flasks (125 ml) were filled with 75 ml of nutrient medium to reduce the high suspended solids to volume ratio in the medium. The nutrient solution was filtered 18 hr prior to the beginning of the Experiment 1 test using #1 Whatman filter paper at 10 psi.

An average wt of 0.24 g L. gibba and 0.15 g S. punctata were added to each of 24 and 18 experimental flasks, respectively, 12 hr prior to the addition of ^{51}Cr. The plants had been grown in the laboratory under a 48" (122 cm) long bank of two General Electric Gro and Sho F40PL lights.

A radioactive stock solution was made by adding 424 μCi of ^{51}Cr to a volumetric flask. The solution was brought to a 5 ml volume by adding 4.5 ml. From the radioactive stock solution 60 μl were added to each flask. Therefore, each experimental flask contained 5.1 μCi of ^{51}Cr.

The purchased radioactive stock solution had a specific activity of 261 mCi/mg. The stable Cr, inadvertantly added as carrier of ^{51}Cr, resulted in a Cr concentration of 2.5×10^{-4} ppm (0.25 ppb) in each experimental flask. Consequently, the controls used throughout this work have stable Cr concentrations of less than a ppb and radiochrome concentrations in the 7×10^{-4} ppb range.

Water samples of one ml each were taken at the beginning of Experiment 1 to determine the initial radioactivity/ml in each experimental flask. All flasks were then placed outdoors at the stream. Each flask was covered with parafilm which had three holes. Four replicates of L. gibba and 3 replicates of S. punctata were set up to be terminated at the end of 6 different periods: 6, 18, 36, 52, 74, and 122 hr.

At the end of each of the 6 time periods, 4 replicates of L. gibba and 3 replicates of S. punctata (7 sample flasks) were returned to the laboratory and processed in the following manner: first, 5 ml water samples

were taken. The remaining contents of the flasks were then filtered with a #1 Whatman filter paper (15 cm) and the resulting filtrate was further filtered by a 0.45 μM millipore filter at 10 psi. The plants were removed from the #1 filter and placed in preweighed polyvials. The Whatman and Millipore filters were dried in petri dishes at 60°C for at least 24 hr. Five ml samples of the final filtrate were taken in order to determine the soluble ^{51}Cr content of the water. After wet weights of duckweed were determined, the vials were oven-dried at 60°C for 24 hr, plants removed, and dry weights obtained to determine their specific activity on a dry wt basis.

Because the same glassware was used to filter all samples, the following wash procedure was adopted to avoid cross contamination among samples.

- Rinse with tap water 5 times
- Rinse 3 times with 1N HCl
- Rinse 3 times with water followed by 3 rinses with distilled water and let drain.

Weights obtained for the #1 Whatman and Millipore filtered material were negligible. Therefore, it was decided that the best way to present all of the results, would be as a percent of the total ^{51}Cr activity initially added.

Experiment 2: Fall Growth of Duckweeds as Influenced by Cr

The purpose of this study was to show the effects of 5 Cr concentrations on growth and the Cr uptake of S. polyrrhiza and a mixed culture of L. gibba, S. polyrrhiza, and S. punctata.

Ten sections of the stream were lined with 6 mil clear plastic. Each section measuring 46" x 14.5" (114 cm x 36 cm) contained five plexiglass partitions 9" x 6" x 5" (23 cm x 15 cm x 13 cm). An aeration hose in each section caused the circulation of water between partitions. Holes in the lower portions of each partition allowed for the free flow of water between replicates.

Twenty liters of the nutrient solution (TKN 20 ppm) were added to each section.

Stable Cr solutions were all made from the same 2000 ppm Cr stock solution one day prior to the addition of ^{51}Cr. Mixtures of varying Cr concentrations: Control (7×10^{-5} ppm), 0.01, 1.0, 20, and 40 ppm of Cr, with a constant 188 μCi ^{51}Cr, were added to each section. The experiment consisted therefore, of 2 plant groups (S. polyrrhiza and a mixed culture of duckweeds) each exposed to 4 different Cr concentrations and controls. Subsequently a total of 50 experimental units were present, since each section (treatment level) contained 5 replicates.

The higher concentrations of Cr (20 and 40 ppm) were chosen since these Cr levels are within the range of

Cr concentrations found in the cooling tower blow-down water of a local industry in the Baton Rouge area.

Four parallel standards were made up directly from the radioactive stock used for each tank. These standards were used for decay correction and to determine the activity per μg stable Cr at each concentration.

Once the stable and radioactive Cr had been added to the system they were aerated overnight to provide thorough mixing of the Cr. An average 3 g wet wt of duckweeds were added to each partitioned area and 2 ml water samples taken, 18 hr later.

Additional water samples and observations of plant conditions, pH, and temperature readings were taken daily for 8 days. No make-up water was added during the study, but changes in the Cr concentrations were monitored.

On the last day of the study, additional water samples taken at the corners of each section showed no concentrated areas of Cr.

Plants were collected after 8 days using a dip net. Plants were washed from the net with distilled water into a Buchner funnel with a #1 Whatman filter under vacuum at 15 psi. The plants were then placed directly into preweighed gamma counting vials dried to constant weight at 60°C and were analyzed for radioactivity. Growth was determined on a dry wt basis (minus initial weight) and were related to the Cr concentration in the

plants and the water by the determination of concentration factors ($\frac{\mu g\ Cr}{g\ plants} / \frac{\mu g\ Cr}{ml\ water}$). Wet wt values were not considered for use in the results in this work. Wet wt biomass data in Experiment 2 showed that the mixed culture of duckweeds grew better than S. polyrrhiza. These findings are reversed when examined on a dry wt basis. Because differences in the water content of duckweeds could produce discrepancies in the results, when compared on both a dry and wet wt basis, only dry wt calculated data were utilized throughout this work.

Experiment 3: The Influence of Aeration vs Non-Aeration on Cr Uptake

The objectives of this study were to compare the uptake of Cr by S. punctata between aerated and non-aerated systems after 25, 88, 114, 120, and 147 hr of exposure to 1 ppm Cr.

Twenty plastic lined plastic tanks containing 2 liters of nutrient solution were used in Experiment 3. Only one Cr concentration (1 ppm) was utilized since in Experiment 2 it was found that at this level much of the algal growth had been inhibited while duckweed growth was evident. Ten experimental tanks were aerated by placing aeration stones under inverted short stem glass funnels. In this manner aeration was achieved with minimal disturbance of duckweed at the water surface. Ten experimental tanks were not aerated. A 200 ppm Cr stock

solution was used from which 10 ml were added in random order to each of the 20 tanks. To each tank 16.4 μCi of ^{51}Cr, and 3 g fresh wt of S. punctata were also distributed in a random sequence. After thorough mixing 3 ml water samples were taken.

During each of the 5 sampling periods 4 tanks (2 aerated: 2 non-aerated) were stripped of their plants. Water samples (3 ml) were taken from all tanks which still had plants at 26, 48, 89, 114, 120, and 147 hr.

No water was added to tanks and as a result the last 4 experimental tanks had lost an average of 757 ml over the 147 hr.

Plant samples were handled in the same manner as in Experiment 2, except that, due to the larger volume of plant material the plants were placed in paper towels. In this way all of the tanks for a particular time period could be harvested quickly and once back in the laboratory the plants could be placed in pre-weighed gamma tubes, reweighed, dried, and counted as before.

Experiment 4: Spring Growth of Duckweed as Influenced by Cr

The objective of this study was to determine the uptake by duckweeds and the effects of varying Cr concentrations on the growth of L. gibba, S. polyrrhiza, and S. punctata, at a different season of the year (compared with Experiment 2).

Since in Experiment 2, both 20 and 40 ppm Cr concentrations greatly inhibited growth and the lowest 0.01 ppm Cr level showed no negative effects on growth, the lowest concentration in Experiment 4 was raised to 0.1 ppm and the uppermost level was lowered to 20 ppm. The following Cr levels for Experiment 4 were utilized: Control (4×10^{-5} ppm), 0.1, 1.0, 10, and 20 ppm.

This experiment, carried out in the greenhouse, consisted of plastic tanks contained within a wooden frame lined with 6 mil clear plastic, topped with 4 mil black plastic. Water was placed within the wooden containment in an attempt to minimize fluctuations in water temperatures within the individual tanks.

The same nutrient solution of manure (TKN 35 ppm) was mixed as mentioned earlier. Due to the large number of samples involved, the starting time for each species was varied by a day so that harvesting on the last day could include only only the duckweed, but bottom sediments, filtered water, and a sample of the plastic liner as well. Nutrients were distributed to the tanks in a random order, the day before stable Cr and ^{51}Cr were to be added.

Seventy-five experimental units were arranged in fifteen rows of 5 tanks each within the wooden containment. Each species was represented at each Cr level by 5 replicates. Restricted randomization of the experimental

units took place. The Cr concentrations in each row were assigned randomly so that each Cr level was represented. The assignment of place species to tank locations were random, but without limitation to the number of tanks with the same species in a particular row.

Three g wet wt of plants were added per tank beginning on 3 March with S. punctata at 20:00. S. polyrrhiza and L. gibba were begun on the following 2 days at the same time.

Each species was removed 8 days from their respective starting times. Beginning with the controls and going to the higher concentrations, plants were removed with a dip net and placed between paper towels. Water samples (3 ml) were taken to monitor Cr concentration changes during the experiment. On the 3rd, 5th, and 8th days, approximately 250 ml of distilled/deionized water was added to the tanks to replace evaporated water.

Physical parameters such as temperature and pH were measured before water was added in order to monitor possible differences between the tanks of each Cr concentration. Photographs were taken daily to provide a record of general plant conditions.

Once plants were removed from the tanks, a scraper made of plexiglass was used to mix and suspend the bottom sediments and attached material. A representative aliquot was then quickly taken and filtered in a Buchner funnel

through #1 Whatman filter paper under vacuum at 15 psi until no water dripped from the funnel. The filter was folded and placed in a gamma counting tube. A 5 ml water sample of the filtrate was taken and the remaining contents were placed in a radioactive waste container.

The remaining tank contents and contaminated wash water was stored in a 55 gallon waste drum. A 150 cm^2 subsample of the plastic liner, rinsed with distilled water, rubbed with a paper towel and rinsed again, was analyzed for radioactivity to determine the amount of Cr sorbed to the plastic.

The effects of each Cr level on growth were measured on the basis of the dry wt of plants sampled at the end of the study. Estimated initial dry weights for each species added at time T_o were calculated on the basis of percent dry wt values obtained from plants at the end of the study. These initial weights were subtracted from end dry wt measurements, to resulting in biomass change values (used to quantitate growth).

Experiment 5: Uptake Over Time by Duckweeds Exposed to 10 ppm Cr

L. gibba, S. polyrrhiza, and S. punctata were used to determine the rate of uptake of Cr at a concentration typical of an industrial waste water (10 ppm).

The time at which the plants were harvested was varied so that the rate of uptake could be calculated.

As in Experiment 4, this study took place in the greenhouse in plastic containers. In addition to the 75 experimental tanks with Cr, 15 control tanks without Cr or ^{51}Cr (1 for each species per time period) were included. Restricted randomization of the 90 tanks took place. Tanks were arranged in 18 rows of 5 each and in no one row were two experimental tanks sampled during the same time period unless one of the two was a control. Five sampling periods, of 18 tanks/period (5 replicates/species and 1 control/species were collected at 20, 43, 77, 112, and 151 hr.)

Two liters of manure solution (TKN $\sim$ 29 ppm) were added to each tank and distilled/deionized water (200-300 ml) was added daily to replace evaporated water.

All tanks remained covered for a day before 12.5 ml of a stable Cr and ^{51}Cr solution, were added to each tank. The tank water was then mixed and water samples were taken immediately.

Sampling, weighing, and counting of plants, sediments, and water, were carried out as in Experiment 4.

Summary of Experimental Design and Statistical Analyses

Experiment 1: Total Number of Experimental Units = 42

24 units: *L. gibba*

18 units: *S. punctata*

Seven (7) experimental flasks (n = 4, *L. gibba*; n = 3, *S. punctata*) were collected during 6 sampling periods (6, 18, 36, 52, 74, and 122 hr).

The General Linear Models procedure of SAS79 was used for the Analysis of Variance using type III sum of squares.

Experiment 2: Total Number of Experimental Units = 49

24 units: *S. polyrrhiza*

25 units: Mixed culture of duckweeds

Each plant group was exposed to 4 different Cr concentrations (n = 5) 0.01, 1.0, 20, 40 ppm and control.

The General Linear Models procedure of SAS was used for the Analysis of Variance, and sum of squares type III was used.

The results were analyzed using orthogonal polynomials to examine the changes within species in growth (biomass change) on 2 different scales. Control Cr concentrations were considered equal to 0.0001 ppm. In this way the possibilities of orthogonal linear and/or quadratic relationships for biomass change of plants could

be examined on a log scale at 0.0001, 0.01, and 1.0 ppm Cr. Then the plant biomass change results at Control (0.0), 20 and 40 ppm Cr were tested for linear and/or quadratic relationships on an arithmetic scale (see Appendix C, Table for results).

Experiment 3: Total Number of Experimental Units = 19

10 units: aerated

9 units: non-aerated (one tank was omitted due to a mistake in the initial Cr dose)

The plants from 4 experimental tanks (n = 2, aerated; n = 2, non-aerated) were collected during 5 sampling periods (25, 88, 114, 120, and 147 hr).

The GLM procedure type III sum of squares was used. A 2 x 5 factorial was used for the analysis (see Appendix D, Table 4).

Experiment 4: Total Number of Experimental Units = 74

25 units: S. punctata

24 units: S. polyrrhiza

25 units: L. gibba

Each species was exposed to 5 different Cr levels, (n = 5) 0.1, 1.0, 10, 20 ppm and control.

GLM and sum of squares type III were used.

The results were first analyzed using orthogonal polynomials to examine the changes in growth within species

as Cr concentrations increased. The biomass change results at 0.1, 1.0, and 10 ppm Cr and also at 0, 10, and 20 ppm Cr were tested for linear and/or quadratic relationships (see Appendix C, Table 2).

A 3 x 5 two way ANOVA was completed for this experiment (see Appendix D, Table 5).

A special note should be made about the means obtained for each variable. Due to the large biomass of plants at the lower Cr concentrations it was necessary to split the replicates into subsamples and analyze each subsample individually for radiochrome. Once the results of the subsamples were obtained they were summed and the total weight and ^{51}Cr activity of complete replicates were calculated. One mean of the 5 replicates were then computed for each treatment. These results are tabulated in Tables 7 and 8.

Experiment 5: Total Number of Experimental Units = 74

25 units: S. punctata

24 units: S. polyrrhiza

25 units: L. gibba

The plants from 15 experimental tanks (5 replicates/species) were collected during 5 sampling periods after exposure to 10 ppm Cr for (20, 43, 77, 112, and 151 hr). Again because of the large mass of plants during the later sampling periods, subsamples were made of individual replicates.

The GLM procedure was used for the ANOVA using sum of squares type III. A 3 x 5 factorial ANOVA was used for the analysis (see Appendix D, Table 6).

All procedures used in the statistical analysis of data were taken from Barr et al. (1979 and Sokal & Rohlf (1969).

RESULTS AND DISCUSSION

Background Cr concentrations determined by atomic absorption spectrophotometry showed that Cr levels within the duckweed prior to experimental exposures were generally below 1 ppm: (S. punctata: 0.64±0.16, range of 0.20-1.28, n = 6; S. polyrrhiza: 0.27±0.04, range of 0.19-0.36, n = 4; L. gibba: 0.58±0.41 ppm Cr, range of 0.15-1.39, n = 3). The Cr present in one sample of the nutrient solution analyzed was 0.83 ppb. No detectable Cr was evident within the distilled and deionized water or the water used to make up the nutrient solutions.

Experiment 1: ^{51}Cr Uptake by Duckweeds

The objective of Experiment 1 was to demonstrate the removal of ^{51}Cr from water by duckweeds. By extension, then, it could be assumed that stable Cr would also be taken up by duckweeds in later studies.

Within any radioactive ^{51}Cr stock purchased, there is a small amount of stable Cr added by the supplier as a carrier. The computations shown below demonstrate how this stable Cr is calculated. The determination of the amount of Cr added as ^{51}Cr and the sensitivity of the radiotracer technique is also included. All calculations were made by using values obtained for flasks (75 ml) used in

Experiment 1. The values obtained from these flasks are the same as the controls used in later experiments.

1) Number of stable Cr atoms per microgram (μg):

$$\frac{6.023 \times 10^{23} \text{ atoms/mole}}{51.996 \text{ g/mole Cr}} \times \frac{10^{-6} \text{ g}}{\mu g} = \frac{1.16 \times 10^{16} \text{ atoms}}{\mu g \text{ Cr}}$$

2) Number of radioactive ^{51}Cr atoms/μCi, is found by using the formula:

$$\frac{dN}{dt} = \lambda N$$

where dN/dt represents the number of disintegrating ^{51}Cr atoms/min, λ is the decay constant, and N is the number of ^{51}Cr atoms.

$$\lambda = \frac{0.693}{\text{half-life}} \; ^{51}Cr \text{ in min} = 1.74 \times 10^{-5} \text{ min}^{-1}$$

Therefore, solving for N:

$$N = \frac{2.22 \times 10^{6} \text{ disintegrations/min}}{\mu Ci} \times \frac{1}{1.74 \times 10^{-5} \text{ min}^{-1}}$$

$N = 1.28 \times 10^{11}$ atoms/μCi of ^{51}Cr

Therefore, if 1 μCi of ^{51}Cr were added to a solution containing 1 μg Cr, there would be approximately 1 radioactive atom for every 10^5 stable Cr atoms.

3) The amount of stable carrier Cr in the radioactive stock is calculated as follows. Each flask contained 5.1 μCi of ^{51}Cr for a concentration of 0.068 μCi/ml. With a specific activity of 261 mCi/mg Cr, there were 1.95×10^{-5} mg Cr per 75 ml flask or 2.6×10^{-4} μg Cr/ml (0.26 ppb).

4) The concentration of elemental Cr added as ^{51}Cr:

$$^{51}Cr \text{ (ppb)} = \frac{5.1 \text{ μCi } ^{51}Cr}{75 \text{ ml}} \times \frac{1.28 \times 10^{11} \text{ at.}}{\text{μCi}} \times \frac{51 \text{ g}}{\text{mole}}$$

$$\times \frac{1 \text{ mole}}{6.023 \times 10^{23} \text{ at.}}$$

$$^{51}Cr \text{ (ppb)} = \frac{7.37 \times 10^{-13} \text{ g}}{\text{ml}} \text{ or } 7.37 \times 10^{-4} \text{ ppb.}$$

Consequently, in later experiments in which radioactive ^{51}Cr was added to the controls, the amount of stable Cr was less than 1 ppb and the concentration of ^{51}Cr was 3 orders of magnitude below the carrier stable Cr.

The values presented in Table 2 show that there is a significant change in the amount of activity per g dry wt of plant over time, but no particular patterns were evident. Significant differences between species ($P < 0.05$) with S. punctata taking up more ^{51}Cr than L. gibba were found after only 36 hr of exposure.

Table 2. Mean* ^{51}Cr activity per g dry wt and concentration factors for S. punctata (S.pu.) and L. gibba (L.g.) after 6, 18, 36, 52, 74, and 122 hr of exposure to tracer concentrations of Cr.

Time (hr)	cpm/g dry wt plant (values times 10^5)		Concentration factor[a]	
	S. pu.	L. g.	S. pu.	L. g.
6	24.0	24.1	252	240
18	50.2	45.6	555	526
36	87.1	51.1	1108	632
52	66.1	32.1	860	399
74	58.3	33.0	758	459
122	152.4	95.6	3127	1909

[a]Concentration factor - $\frac{\text{counts per min (cpm)}}{\text{g (dry wt plant)}}$ @ T_i / $\frac{\text{cpm}}{\text{ml filtrate}}$ @ T_i.

* S. pu. = 3
  L. g. = 4

Table 3. Mean* percentages of the total initial ^{51}Cr activity recovered within each partition of the system. Partitions which included duckweeds: S. punctata (S.pu.), and L. gibba (L.g.), large particulate matter (#1 Whatman filter), minute particulate matter (Millipore filter, 0.45μ), and final filtrate, were collected after 6, 18, 36, 52, 74, and 122 hr exposure to ^{51}Cr.

Partition Type	Time (hr) 6		18		36		52		74		122	
	S.pu.		L.g.		S.p.		S.pu.		L.g.		S.p.	
Plants	2.8	2.5	6.2	5.3	7.6	5.1	3.9	3.1	4.9	3.3	20.0	12.4
#1 Whatman filter	2.9	2.5	3.8	3.0	6.5	5.5	5.9	5.6	17.0	23.0	7.5	8.2
Millipore (0.45μ) filter	3.3	2.9	3.0	3.7	7.9	6.5	7.2	7.5	3.9	3.6	7.4	9.9
Filtrate[a]	86.6	91.2	82.2	79.3	71.6	73.3	70.7	75.4	70.5	65.8	44.6	45.4
Total percent ^{51}Cr for all partitions	95.6	99.1	95.2	91.3	92.2	91.8	87.7	91.6	96.3	95.7	79.4	75.9

[a] Filtrate - represents the percentages of the total ^{51}Cr activity added compared to the activity remaining in the water after filtering. (Total activity is equal to the activity in 74 ml (814,000 cpm/flask) since 1 ml was removed from each flask at the start of the experiment.)

* S. pu., n = 3
L. g., n = 4

A complete inventory of the ^{51}Cr activity initially added is presented in Table 3. There were a few scattered significant differences between species or between partitions from flasks containing the two duckweed species, at any one particular time (see Appendix B, Table 1). Though most of the ^{51}Cr remained in the filtrate there was a gradual decrease of ^{51}Cr over time. After 122 hr, 45% of the initial activity remained in the filtrate. The percent of the total activity retained by the #1 Whatman filter was essentially the same as that held by the Millipore filter.

By combining the results for the two filter types, the total percent ^{51}Cr removed from the water as the filterable material greater than 0.45 μ was shown to account for more ^{51}Cr uptake than that amount taken up by the plants. The only exception occurred for S. punctata which after 122 hr of exposure had stripped 20% of the total activity from the water, whereas the filterable material contained only 14%.

The ^{51}Cr within the organic portion (plants and total filterable material, Table 2) was shown to gradually increase with time. After 6 hr, 8.5% of the total ^{51}Cr pool was held within the organic material, and after 122 hr that amount had risen to 33%.

Generally, the total inventory was able to account for more than 90% of the ^{51}Cr initially added. The

discrepancy at 122 hr with only 79.4 and 75.9% of the ^{51}Cr accountable in the S. punctata and L. gibba flasks, respectively, could be explained by the presence of attached algae on the walls of the flasks, which were incompletely recovered. Samples taken from 3 control ^{51}Cr flasks containing the same medium and radioactive dose, but without plants, supports this explanation. The first 2 flasks collected at 6 and 74 hr showed radioactive losses of only 1.95 and 2.43%. The flask sampled at 122 hr showed extensive algal growth and a 23% loss in activity was noted.

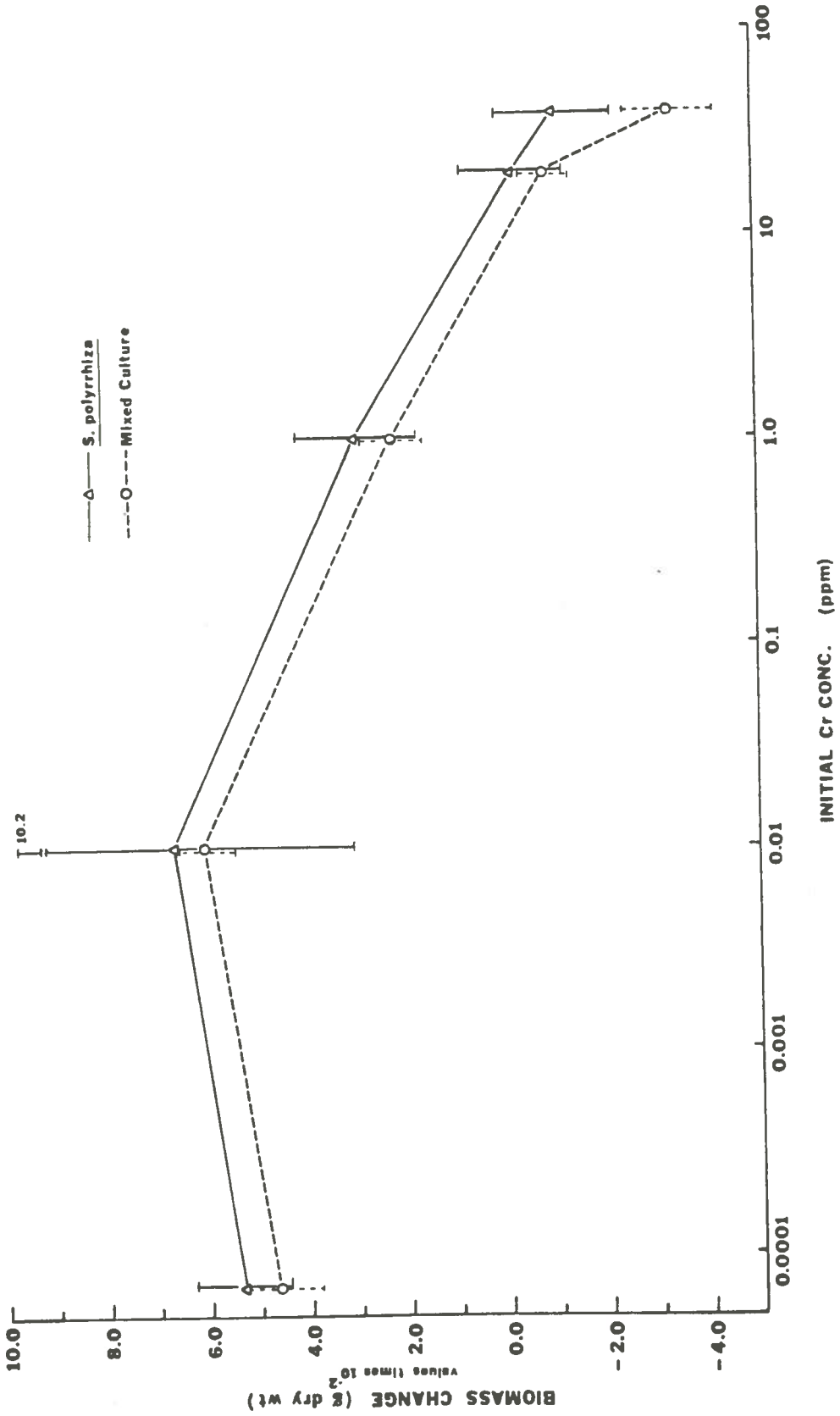
Experiment 2: Fall Growth of Duckweeds as Influenced by Cr

The purpose of Experiment 2 was to demonstrate the effects of 4 Cr concentrations on growth and Cr uptake of S. polyrrhiza and a mixed culture of L. gibba, S. polyrrhiza, and S. punctata (each in equal proportions).

Figure 1 illustrates the mean change in duckweed biomass (dry wt) after 8 days exposure to initial Cr concentrations of: Control (7×10^{-5} ppm), 0.01, 1.0, 20, and 40 ppm.

A significant difference in the dry wt change of S. polyrrhiza as a function of increasing Cr concentrations at the 0.05 level was demonstrated by analysis of variance (ANOVA). No significant difference could be found for S. polyrrhiza (Δ) wt change at the lower Cr levels, Control (0.01), 0.01, and 1.0 ppm. Orthogonal polynomials showed

Fig. 1. Mean (+ SE) biomass changes (dry wt) of S. polyrrhiza (Δ) and a mixed culture of duckweeds (O) after 8 days exposure to 4 Cr concentrations.


S. polyrrhiza
Mixed Culture
10.2
BIOMASS CHANGE (g dry wt)
values times 10^{-2}
10.0
8.0
6.0
4.0
2.0
0.0
- 2.0
- 4.0
0.0001
0.001
0.01
0.1
1.0
10
100
INITIAL Cr CONC. (ppm)

a highly significant ($P < 0.001$) linear decrease in biomass change at Control (0.0), 20 and 40 ppm Cr. No quadratic effect was found.

An analysis of variance for the mixed culture (o) showed a highly significant ($P < 0.001$) negative association between biomass wt change and increasing Cr concentration. At Cr levels of Control (0.0001), 0.01, and 1.0 ppm significant ($P < 0.05$) linear and quadratic orthogonal polynomials indicated that some form of curvi-linear function was present on a log scale. Examination of Figure 1 indicates a slight stimulation of growth at 0.01 ppm Cr with a decrease in dry wt change at concentrations greater than 0.01 ppm. A very significant negative linear decrease ($P < 0.001$), but not a quadratic relationship for the change in dry wt after 8 days exposure to initial Cr levels of Control (0.0), 20 and 40 ppm (arithmetic scale) was also shown.

By statistical inspection of the curve for S. polyrrhiza (Figure 1, Δ), it can be concluded that there exists a point between 1.0 and 20 ppm Cr beyond which only a negative effect on the growth of S. polyrrhiza would be evident. For the mixed culture of duckweed, the results suggest that at Cr concentrations beyond 0.01 ppm, only a decrease in dry wt change would be expected.

At each Cr level, no significant differences ($P > 0.05$) between the change in dry wt for the two plant groups existed.

Since no make-up water was added to the system over the 8 day period of the study, the final water concentrations of Cr to which plants were exposed had changed after 8 days to become: for S. polyrrhiza Control (3.2×10^{-5} ppm), 0.005, 0.91, 24 and 51 ppm and for the mixed culture Control (3.5×10^{-5} ppm), 0.006, 0.92, 25 and 54 ppm.

Air temperatures during the study fluctuated from -2^{o} to 26^{o}C (National Weather Service) with water temperatures approximately 5^{o}C higher than that of the air. Recorded pH valued ranged from 7.2 to 8.4 over the total period of study. The greatest pH ranges (7.2-8.4) were found in those sections with Cr concentrations less than 1 ppm. Algal populations which had established themselves over the 8 days at these levels could account for the large pH ranges noted. The amount of adsorption that could occur at these pH levels would be minimal, since Cr would be predominantly present in the anionic (CrO_4^{-2}) state (MacNaughton, 1977).

Because of near freezing weather conditions noted on days 1, 6, 7, and 8, duckweed growth was severely limited in all cultures. S. polyrrhiza cannot grow at temperatures less than 10 to 15^{o}C and begin to deteriorate at temperatures below 7^{o}C (Jacobs, 1947). L. gibba and

Table 4. Mean* µg Cr in plants and concentration factors on a dry wt basis, as well as, the total percentage Cr removed by plants per section, for S. polyrrhiza (S.p.) and a mixed culture[1] of duckweeds (Mix) after 8 days exposure to 4 different Cr concentrations.

Initial Cr Concentration (ppm)	µg Cr/g plant (dry wt)		Concentration[a] factor (dry wt)		Total percent Cr removed[b] by plants	
	S.p.	Mix	S.p.	Mix	S.p.	Mix
Control	0.0226_a	0.0142_a	709_a	396_a	2.40_a	1.05_a
0.01	2.30_a	3.22_a	461_b	533_b	1.80_b	1.75_b
1.0	125_b**	247_b	137_c**	268_c	0.85_c**	1.25_c
20	585_c	978_c	24_d	40_d	0.15_d	0.20_d
40	917_d	1178_d	18_d	22_d	0.10_d	0.10_d

[1]Mix culture: S. polyrrhiza, S. punctata, and L. gibba (in approximately equal proportions).

[a]Concentration factor: (µg Cr/g dry wt plant)/(µg Cr/ml water)

[b]Total percent Cr removed (total Cr in plants from all 5 replicates/total Cr in water of each treatment) * 100.

[a,b,c,d]Different subscripts for each species between different Cr concentrations indicate significant differences (Duncan's Multiple Range Test, $P < 0.05$).

* n = 5

** n = 4

S. oligrrhiza, however, have been shown to be able to grow under some conditions at temperatures as low as 4°C (Landolt, 1957). Observation of plant conditions within the control sections likewise suggested that S. polyrrhiza was the most affected by low temperatures, while L. gibba and S. punctata appeared healthier, having maintained much of their green pigmentation.

Even though all treatments were subject to the same environmental variations, the inhibition of duckweed growth mentioned earlier was shown. This effect could only have been caused by Cr or adverse seasonal conditions and Cr, but not by unfavorable weather conditions alone.

The values in Table 4 reflect the amount of Cr removed by S. polyrrhiza and the mixed culture of duckweeds at each of the Cr concentrations. No significance was found within plant groups at the control and 0.01 ppm Cr level on a μg Cr/g dry wt of plant basis. However, the μg Cr/g of plant did increase significantly as plants were exposed to Cr concentrations above 0.01 ppm (Duncan's Multiple Range Test, $P < 0.05$).

Significant differences ($P < 0.05$) in the uptake of Cr between species were noted with higher Cr concentrations (20 and 40 ppm) in the plants of the mixed culture at all Cr levels, except in controls.

Hutchinson and Czyrska, (1975) working with Salvinia natans and Lemna minor showed that when under the

Table 4. Mean* µg Cr in plants and concentration factors on a dry wt basis, as well as, the total percentage Cr removed by plants per section, for S. polyrrhiza (S.p.) and a mixed culture[1] of duckweeds (Mix) after 8 days exposure to 4 different Cr concentrations.

Initial Cr Concentration (ppm)	µg Cr/g plant (dry wt)		Concentration[a] factor (dry wt)		Total percent Cr removed[b] by plants	
	S.p.	Mix	S.p.	Mix	S.p.	Mix
Control	0.0226_a	0.0142_a	709_a	396_a	2.40_a	1.05_a
0.01	2.30_a	3.22_a	461_b	533_b	1.80_b	1.75_b
1.0	125_b**	247_b	137_c**	268_c	0.85_c**	1.25_c
20	585_c	978_c	24_d	40_d	0.15_d	0.20_d
40	917_d	1178_d	18_d	22_d	0.10_d	0.10_d

[1]Mix culture: S. polyrrhiza, S. punctata, and L. gibba (in approximately equal proportions).

[a]Concentration factor: (µg Cr/g dry wt plant)/(µg Cr/ml water)

[b]Total percent Cr removed (total Cr in plants from all 5 replicates/total Cr in water of each treatment) * 100.

a,b,c,d Different subscripts for each species between different Cr concentrations indicate significant differences (Duncan's Multiple Range Test, $P < 0.05$).

* n = 5

** n = 4

the stress of competition greater accumulation of Zn and Cd occurred in L. minor. The higher μg Cr/g concentrations within the mixed culture, composed of the 3 different duckweed species could possibly have been caused by this competition. A future study might examine this aspect.

Concentration factors, also shown in Table 4, decreased as the Cr levels increased. Yet, there was no significant differences ($P > 0.05$) in the concentration factors for either S. polyrrhiza or the mixed culture at 20 and 40 ppm Cr. From 0.01 to 20 ppm Cr, significant differences for each of the plant groups were noted. Although the concentration factors declined with the increasing Cr levels, the absolute amount of Cr taken up (μg Cr/g) by the plants increased, indicating that the concentration of Cr in the water does play a role in Cr uptake by duckweeds, actively or passively.

Experiment 3: The Influence of Aeration vs Non-Aeration on Cr Uptake

The uptake of Cr (1 ppm) by S. punctata under aerated or non-aerated conditions after 25, 88, 114, 120, and 147 hr of exposure was examined.

Air temperatures ranged from -1 to 27°C with averages of 17°C during the experiment.

Analysis of variance indicated that biomass changes of S. punctata (dry wt) for aerated or non-aerated treatments were significantly different ($P < 0.001$)

Table 5. Mean* biomass change (dry wt) of S. punctata exposed to 1 ppm Cr for 25, 88, 114, 120, and 147 hr under aerated (A) or non-aerated (N) conditions.

Time (hr)	Biomass change[a] A	N
25	-0.0159	0.0299
88	0.0897	0.0929
114	0.1121	0.1176
120	0.1342	0.1556**
147	0.2580	0.2672

[a]Biomass change: dry wt of plants (g) at T_i - dry wt of plants (g) at T_o.

* n = 2

** n = 1

over the 147 hr period of the study (see Appendix D, Table 4). There were, however, no significant differences ($P > 0.05$) between treatments for biomass change at any particular sampling time.

Differences within aerated or stagnant treatments analyzed over time were significant ($P < 0.001$) for each variable shown in Table 6 (µg Cr/g dry wt, concentration factors, total µg Cr in plants, and the percent of Cr removed by S. punctata, Appendix D, Table 4).

The ANOVA results shown in Appendix D, Table 4, indicate that there are no significant differences in µg Cr/g dry wt of plants or concentration factors, between aerated and non-aerated treatments ($P > 0.05$). However, ANOVA of the total µg Cr in plants and the percent Cr removed by plants suggests that there are significant differences between aerated vs non-aerated conditions. With the aid of LSmean t-test comparisons between treatments at each sampling time it was shown that the significant differences occurred only at 114 hr. Generally therefore, it can be concluded that in these shallow tanks aeration has no effect on S. punctata growth or Cr uptake.

Air temperatures, in this experiment, averaged over 8°C higher than in Experiment 2. Comparing the growth of S. polyrrhiza exposed to 1 ppm Cr, in Experiment 2, to the growth of S. punctata in Experiment 3, 8.5 times more growth was exhibited by S. punctata after only 6 days growth.

Table 6. Mean* µg Cr/g of plants, concentration factors, total µg Cr in plants, and percent Cr removed by S. punctata from the total Cr pool under aerated (A) or non-aerated (N) conditions.

Time (hr)	µg Cr/g dry wt in duckweed		Concentration factor[a]		Total µg Cr in plants[b]		Percentage of Cr removed by S. punctata[c]	
	A	N	A	N	A	N	A	N
25	100	92	105	99	22	22	1.12	1.09
88	372	393	384	425	107	118	5.35	5.89
114	536	605	569	657	155	178	7.76	8.89
120	459	434**	484	448**	158	174**	7.95	8.68**
147	518	506	520	554	262	266	13.11	13.30

[a] Concentration factors = (µg Cr/g dry wt plant)/(µg Cr/ml water).

[b] Total µg Cr in plants = (µg Cr/g dry wt plant) x Total dry wt of plants.

[c] Percent of Cr removed = (total µg Cr in plants/2000 µg Cr in total Cr pool initially) x 100.

* n = 2

** n = 1

By using individual tanks for each replicate with only 2000 ml of nutrient solution, the ratio of duckweed biomass to water volume was raised from 0.75 g/ℓ in Experiment 2 to 1.5 g/ℓ in this experiment. The higher biomass to volume ratio, in addition to more favorable seasonal conditions, allowed for greater plant exposure per unit volume and better plant growth. These factors could have then been responsible for the greater average percent of Cr (13% after 147 hr) removed from the total Cr pool by S. punctata. When compared to the maximum percent removal by the mixed culture of duckweeds (1.25%) after 8 days exposure to 1 ppm Cr during Experiment 2, it can be concluded that environmental conditions will have to be sufficient for duckweed growth. In addition a shallow recycling system with a high biomass to volume ratio or shallow ponds with a 5-10 cm maximum depth of wastewater will be a necessity. It is possible then under these conditions that duckweeds show potential as a remover of Cr from municipal waste effluent.

Experiment 4. Spring Growth of Duckweed as Influenced by Cr

Experiment 2 showed no negative effect on duckweed growth at 0.01 ppm Cr, and no significant difference in Cr removed by duckweeds at the 20 and 40 ppm Cr concentrations. Thus, a narrower range of Cr levels (Control (4×10^{-5} ppm), 0.1, 1.0, 10 and 20) was examined to disclose the effects of increasing Cr concentrations on 3 species of duckweeds and perhaps gain a more definitive measure of the toxic threshold.

Table 7 and Figure 2 show the biomass changes (dry wt) for S. punctata, S. polyrrhiza, and L. gibba after 8 days of exposure at 4 Cr levels. Significant weight changes in all 3 species as a function of increasing Cr concentrations were indicated by analysis of variance ($P < 0.001$).

The biomass change of each species at 0.1, 1.0, and 10 ppm showed a marked decrease in growth at levels above 0.1 ppm. An examination of this biomass decrease using orthogonal polynomials showed that a significant [and linear biomass decrease on a logarithmic scale] ($P < 0.001$) at 0.1, 1.0 and 10 ppm was evident for S. polyrrhiza and L. gibba. A linear negative relationship between increasing Cr concentration and growth was also shown for S. punctata, yet, unlike the other two species, a significant quadratic effect ($P < 0.01$) was also

Table 7. Mean* biomass changes (dry wt) of S. punctata (S. pu.), S. polyrrhiza (S. p.), and L. gibba (L. g.) after 8 days exposure to 4 concentrations of Cr.

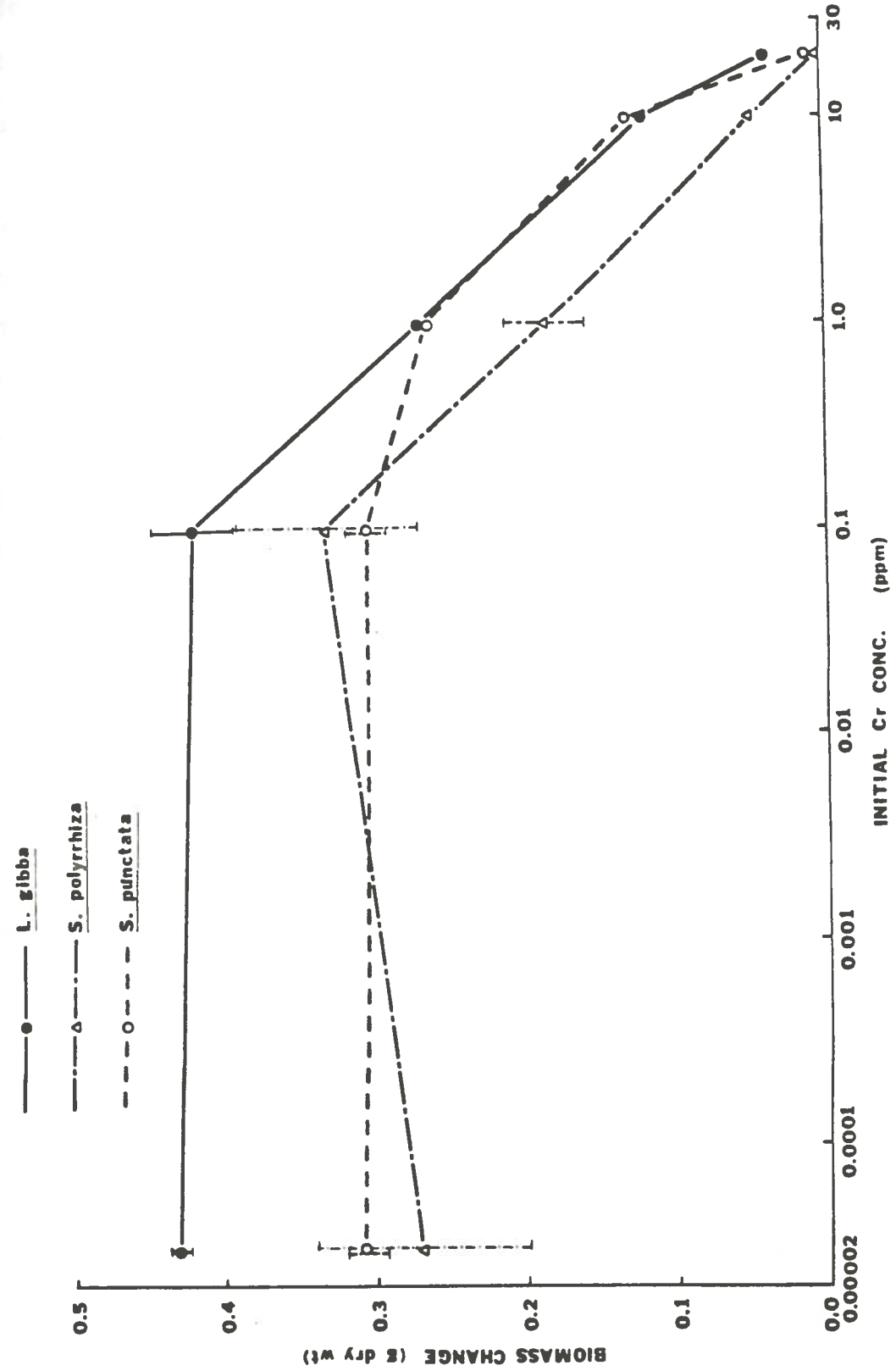
Initial Cr Concentration (ppm)	Biomass change[a]		
	S.pu.	S.p.	L.g.
Control	0.3087	0.2702	0.4315
0.1	0.3080	0.3333	0.4224
1.0	0.2653	0.1891**	0.2719
10	0.1304	0.0471	0.1194
20	0.0104	0.0044	0.0382

[a]Biomass change = dry wt of plants (g) at T_i - dry wt of plants (g) at T_o.

* n = 5

** n = 4

Fig. 2. Mean (± SE) biomass changes (dry wt) of L. gibba (●), S. polyrrhiza (Δ), and S. punctata (O) after 8 days exposure vs log concentrations of Cr.


L. gibba
S. polyrrhiza
S. punctata
BIOMASS CHANGE (g dry wt)
0.5
0.4
0.3
0.2
0.1
0.0
0.00002
0.0001
0.001
0.01
0.1
1.0
10
30
INITIAL Cr CONC. (ppm)

demonstrated. This implies that S. punctata is more tolerant of Cr at levels between 0.1 and 10 ppm than are L. gibba and S. polyrrhiza.

L. gibba appeared better able to tolerate 10 ppm Cr than was S. polyrrhiza or S. punctata. Orthogonal polynomials for the biomass changes of the 3 species at 0, 10, and 20 ppm (arithmetic scale) were made. Increasing Cr concentrations had significant decreasing linear effects ($P < 0.001$) on the growth of S. polyrrhiza and S. punctata. L. gibba, on the other hand, had both a significant linear and quadratic relationship.

The biomass changes at each Cr concentration among species were significant ($P < 0.001$). At the Control and 0.1 ppm Cr concentration biomass changes among species were significantly different. At 1.0 ppm, L. gibba and S. punctata grew significantly better than S. polyrrhiza. At 10 and 20 ppm no significant differences ($P > 0.05$) among species were evident.

Table 8 presents the data on the uptake by 3 species of duckweed of Cr (µg Cr/g dry wt) at 4 concentrations. Highly significant positive differences in uptake ($P < 0.001$) were found for all except L. gibba. The µg Cr/g for L. gibba exposed to 10 ppm Cr was significantly different demonstrating greater uptake on a per g basis than L. gibba maintained at all other Cr

Table 8. Mean* µg Cr in plants, concentration factors, and the percent Cr removed from the total Cr pool by S. punctata (S.pu.), S. polyrrhiza (S.p.), and L. gibba (L.g.) after 8 days exposure to 4 Cr concentrations.

Initial Cr Concentration (ppm)	µg Cr/g plant (dry wt)			Concentration factor (dry wt basis)			Percent Cr removed by plants		
	S.pu.	S.p.	L.g.	S.pu.	S.p.	L.g.	S.pu.	S.p.	L.g.
Control	0.0216	0.0178	0.0124	7223	2874	1415	18.6	14.5	11.1
0.1	76	60	64	5688	3207	2016	19.5	16.1	16.9
1.0	653	862**	696	2010	2200**	1248	15.2	15.5**	13.7
10	3778	3134	3714	738	565	521	5.8	3.2	4.7
20	5608	3718	2496	525	332	167	2.7	1.6	1.0

[a]Concentration factor = (µg Cr/g dry wt plant)/(µg Cr/ml filtrate)

[b]Percent Cr removed by plants = (total µg Cr in plants/µg Cr in total pool) x 100

* n = 5

** n = 4

concentrations. The decline at 20 ppm suggests that L. gibba may have exceeded its saturation or tolerance level. LSmean t-test comparisons (among species) indicated that no significant differences ($P > 0.05$) were present between the uptake in control plants or plants exposed to 0.1 ppm Cr.

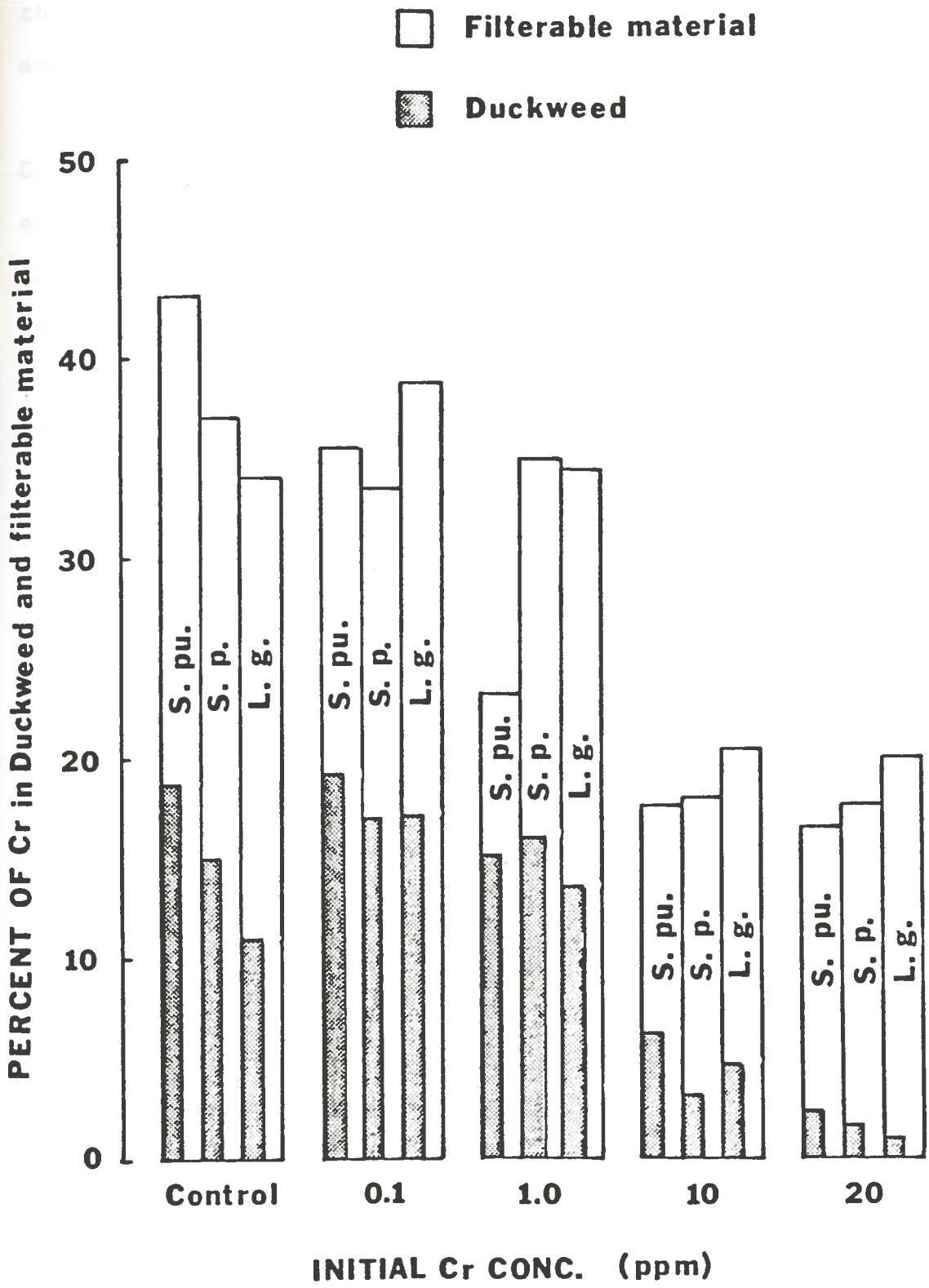
LSmean t-tests performed at each Cr level showed no significant differences ($P > 0.05$) among species exposed to 0.1 and 1.0 ppm Cr. Among species at 10 and 20 ppm Cr concentrations, there were significant differences ($P < 0.001$) in absolute Cr uptake. This suggests that at the lower Cr levels that L. gibba, S. polyrrhiza, and S. punctata are equally efficient in their removal of Cr. When stressed however, by higher Cr concentrations, differences in their ability to sorb Cr become evident. At 10 ppm, L. gibba and S. punctata were significantly different from S. polyrrhiza and at 20 ppm Cr all 3 species were significantly different from each other.

Both the concentration factors and the percent Cr removed (Table 8) resulted in highly significant decreases within species as Cr concentrations increased ($P < 0.001$). Exceptions were noted for L. gibba and S. polyrrhiza which showed their greatest concentrating efficiencies at 0.1 ppm (2016 and 3207, respectively). At 0.1 ppm, all species exhibited their greatest percent Cr removal.

Significant differences between species for concentration factors and the percent Cr removed by plants ($P < 0.001$) were indicated by ANOVA (see Appendix D. Table 5). The results might lead one to assume that Cr present in solution at the 0.1 ppm level would be the optimum Cr concentration from which to obtain maximum removal by duckweeds. This is indeed not the case. If duckweeds are to be used in the waste water removal of Cr, then the maximum weight of Cr removed is of greater importance. Twenty percent removal of Cr from a 0.1 ppm solution represents only 40 µg Cr in the plants. Yet, at the 10 ppm Cr level, removal as low as 5.5% would mean that the plants stripped 1100 µg Cr from the system.

The bar graph (Figure 3) was shown to demonstrate the importance of the filterable material in removing Cr relative to the Cr sorbed by plants. The percent of Cr found in the filterable material (clear bar) ranged from a high of 42.6% (Control, S. punctata), to a low of 16.5% at 20 ppm Cr (S. punctata). The percent Cr sorbed by the plants (shaded bar) ranged from a high of 19.5% for S. punctata at 0.1 ppm Cr to a low of 1% for L. gibba at 20 ppm. As is evident the Cr removed by duckweeds is not greatly affected except at levels greater than 1 ppm Cr. The percent of Cr retained by the filterable material,

Fig. 3. Bar graph of the percent Cr removed by S. punctata (S.pu.), S. polyrrhiza (S.p.), and L. gibba (L.g.) shaded bar, and the percent of Cr retained by the filterable material at each Cr concentration (clear bars).


Filterable material
Duckweed
PERCENT OF Cr in Duckweed and filterable material
50
40
30
20
10
0
S. pu.
S. p.
L. g.
Control
0.1
1.0
10
20
INITIAL Cr CONC. (ppm)

though reduced by approximately 50% from control levels, are similar at the 10 and 20 ppm Cr concentrations.

Mangi et al. (1978) suggested that adsorption of Cr by several algal species, and the duckweeds L. minor and S. polyrrhiza was largely responsible for the Cr uptake noted. L. gibba has the largest biomass at 20 ppm. If adsorption were the predominant mechanism of uptake, it should have removed the greatest percent of Cr from the system instead of the least. Hervey (1949) found when he subjected several algal species to sublethal Cr concentrations in the 0.0001-0.32 ppm range that algal growth was stimulated. No negative effects of Cr on uptake or growth of several duckweed species examined at 0.01 and 0.1 ppm Cr were found. The results in Table 8 for concentration factors and the percent Cr removed by plants, at 0.1 ppm Cr, imply that more active mechanisms of uptake are involved. At 0.1 ppm both L. gibba and S. polyrrhiza have their greatest concentration factors, and all three species exhibit their greatest percent Cr removal at this level.

The filterable material, consisting of suspended matter, algae, aufwuchs, and settleable particles, represents a major Cr removing segment which could be of great importance in the development of a waste water treatment facility for the removal of Cr and should not

be overlooked in studies involving the non-sterile examination of Cr uptake.

Sampling of the plastic tank liners after 8 days exposure to Cr at the 4 different concentrations, showed that adsorption to the liner represented less than 0.006% of the total Cr pool at all Cr levels. The Cr in the wash water was not sampled.

In Experiment 2 little Cr was removed by duckweeds. A comparison of the µg Cr/g dry wt of S. polyrrhiza in Experiment 2 and the absolute Cr uptake by S. polyrrhiza in this experiment indicates that Cr toxicity, as well as, seasonal changes are responsible for differences in the growth of duckweeds and the amount of Cr taken up. After 8 days exposure to Cr concentrations of 1 and 20 ppm during Experiment 2, S. polyrrhiza retained 125 and 585 µg Cr/g dry wt, respectively. In Experiment 4, Cr uptake by this same species was 903 (725%) and 3718 (635%) µg Cr/g at 1 and 20 ppm, respectively.

In Experiment 2, air temperatures ranged from -2° to 26°C (avg. 8.5°C). In Experiment 4 air temperatures ranged from 4° to 27°C (avg. 18°C). Although in Experiment 2 only negative duckweed growth was evident at 20 ppm Cr after 8 days exposure, in Experiment 4, an increase in duckweed biomass at the same Cr concentration was shown. At 1 ppm Cr in Experiment 4, biomass increases rose by

560% for S. polyrrhiza, and in the controls by 615% when compared to fall results (Experiment 2). These findings, therefore, suggest that favorable seasonal conditions helped the growth of plants and allowed duckweeds to grow at levels which would be toxic to plants under more adverse circumstances.

It is evident that many factors have to be taken into consideration when trying to decide at which Cr concentration duckweeds would be most efficient at removing Cr and which species would produce the best results.

The values of five variables (4 of which are given in Tables 7 and 8) characterize not only the amount of Cr in the plants (μg Cr/g dry wt, total μg Cr in plants), but also account for changes in growth (biomass changes), the Cr levels in the water relative to those in the plants (concentration factors), and the percent of Cr removed from the total Cr pool to which plants were originally exposed (percent Cr removed by plants). The values for each variable, listed above, taken individually by species, were made relative to one another at each Cr concentration by dividing the values for 20 ppm Cr into the values for that species at all other concentrations (see Tables 7 and 8). This made all values at each Cr level for a particular species relative to one another so that the experimental

Table 9. Examination of the most appropriate Cr concentration for optimum removal of Crs for L. gibba, S. polyrrhiza, and S. punctata at 4 different Cr concentrations. Values taken from Tables 7 and 8 were made relative to one another for easy comparison of the results for each species.

Species	Initial Cr Concentration	Comparative[a] biomass change	Comparative[b] conc. factors	Comparative µg Cr/g dry wt	Comparative[c] total µg Cr in plants	Comparative percent Cr removed by plants
	Control	11.3	8.5	--*	--*	11.1
	0.1	11.1	12.1	0.03	0.09	16.9
Lemna gibba	1.0	7.1	7.5	0.28	0.69	13.7
	10	3.1	3.1	1.49	2.38	4.7
	20	1	1	1	1	1
	Control	61.4	8.7	--*	--*	9.1
	0.1	75.8	9.7	0.02	0.05	10.1
Spirodela polyrrhiza	1.0	43.0	6.6	0.23	0.50	9.7
	10	10.7	1.7	0.84	1.03	2
	20	1	1	1	1	1
	Control	29.7	13.8	--*	--*	6.9
	0.1	29.6	10.8	0.01	0.04	7.2
Spirodela punctata	1.0	25.5	3.8	0.12	0.28	5.6
	10	12.5	1.4	0.67	1.07	2.1
	20	1	1	1	1	1

[a] Comparative biomass change - from Table 7 that value present for biomass change for each species at 20 ppm is divided into biomass change values for that particular species at the other Cr concentrations. (i.e., S.pu. - 0.0104 (value at 20 ppm) is divided into the values for S.pu. at each Cr level.).

[b] Comparative conc. factors - from Table 8 that value present for each species at 20 ppm is divided into the conc. factor value for that species at the other Cr concentrations.

[c] Comparative total µg Cr in plants - Total µg Cr in plant values were not given in Tables 7 or 8. These values were calculated by multiplying µg Cr/g dry wt of plants times the total dry wt of plants at a particular Cr concentration. After the total µg Cr in plant values were derived then the comparative values shown above were computed as previously described for the other variables.

* -- - the extremely small values of variables were obtained when the controls were considered. These values when divided by the large amounts of Cr in plants at 20 ppm produced comparative values which were meaningless.

results could be easily compared. In Table 9, the comparative total μg Cr in plants accounts for both duckweeds growth and absolute Cr uptake. Considering this variable a good estimator of overall Cr removal, it appears that the best Cr concentration to use for the maximum uptake of Cr is at the 10 ppm Cr level for the three species examined.

The same type of comparisons completed for Table 9 are given in Table 10, in order to determine which of the 3 species would be the best to use at each of the different Cr levels examined. Here, S. punctata was used as the arbitrary standard. In Tables 7 and 8, the S. punctata value was divided into the two values for the other two species. In this manner, results for each species became relative to each other. The comparative, total μg Cr in plant, values in Table 10, imply that S. punctata is the best species at the 0.1, 10 and 20 ppm Cr concentrations to use for the optimum removal of Cr. At the 1 ppm level, however, S. polyrrhiza and S. punctata had nearly equal comparative values.

Experiment 5. Uptake Over Time by Duckweeds Exposed to 10 ppm Cr

The relative comparisons shown in Table 9 of Experiment 4 suggested that conditions would be optimum for the removal of Cr at 10 ppm. Experiment 5 was designed

Table 10. Examination of the most appropriate species for the optimum removal of Cr at a particular Cr concentration. Values of individual variables found in Tables 7 and 8 were made of the results for L. gibba (L.g.), S. polyrrhiza (S.p.), and S. punctata (S.pu.), relative to one another for easy comparison at each Cr concentration.

Initial (Cr)	Species	Comparative[a] biomass change	Comparative[b] conc. factors	Comparative ug Cr/g dry wt	Comparative total ug Cr in plants	Comparative percent Cr removed by plants
Control	L.g.	1.40	0.20	0.57	0.60	0.60
	S.p.	0.88	0.40	0.82	0.78	0.81
	S.pu.	1	1	1	1	1
0.1	L.g.	1.37	0.35	0.84	0.87	0.87
	S.p.	1.08	0.56	0.79	0.83	0.83
	S.pu.	1	1	1	1	1
1.0	L.g.	1.02	0.62	1.07	0.90	0.90
	S.p.	0.71	1.09	1.32	1.02	1.02
	S.pu.	1	1	1	1	1
10	L.g.	0.92	0.71	0.98	0.82	0.81
	S.p.	0.36	0.77	0.83	0.55	0.55
	S.pu.	1	1	1	1	1
20	L.g.	3.67	0.32	0.44	0.37	0.37
	S.p.	0.42	0.63	0.66	0.58	0.59
	S.pu.	1	1	1	1	1

[a]Comparative biomass change - from Table 7 the value present for S.pu. is divided into the values for the other two species at a particular Cr concentration. (i.e., for S.pu. at control - 0.3082 is divided into the values for S.p. and L.g.).

[b]Comparative conc. factor - from Table 8 the value present for S.pu. is divided into the values for comparing each of the other two species at a particular Cr concentration.

to study the effects on duckweed growth and Cr uptake at this potential industrial waste treatment Cr concentration.

Canter & Gloyna (1969) reported that Cr^{+6} under oxidized conditions and low organic loads would remain in the Cr^{+6} state.

Though surface area to volume ratios within experimental tanks (Experiments 3 and 4) were fairly large (450 cm^2/2000 cm^3) and reducing conditions did not appear to be evident, it was considered important to monitor the dissolved oxygen (DO) levels as they changed during this experiment. The changes in DO levels are shown in Figure 4. The DO increased over the 151 hr test with a maximum DO concentration of 13.87 $\pm$ 0.22 ppm O_2 after 150 hr. The high DO values and basic pH indicate that ^{51}Cr and stable Cr added as chromate did remain in the Cr^{+6} anionic state (CrO_4^{-2}) within the experimental system over the 151 hr of the test.

In Experiments 2 and 4, plants, sampled once after 8 days exposure to Cr at 10 ppm were shown to have been inhibited. In this experiment, duckweeds exposed to an initial Cr concentration of 10 ppm showed substantial growth. Significant increasing biomass differences (Figure 5 and Table 11) were indicated for each species and were supported by ANOVA results ($P < 0.001$, Appendix D, Table 6).

Fig. 4. Mean ± SE dissolved oxygen (o) and pH (●) levels before plants or Cr were added (T_o) to tanks in which 10 ppm Cr was initially introduced.

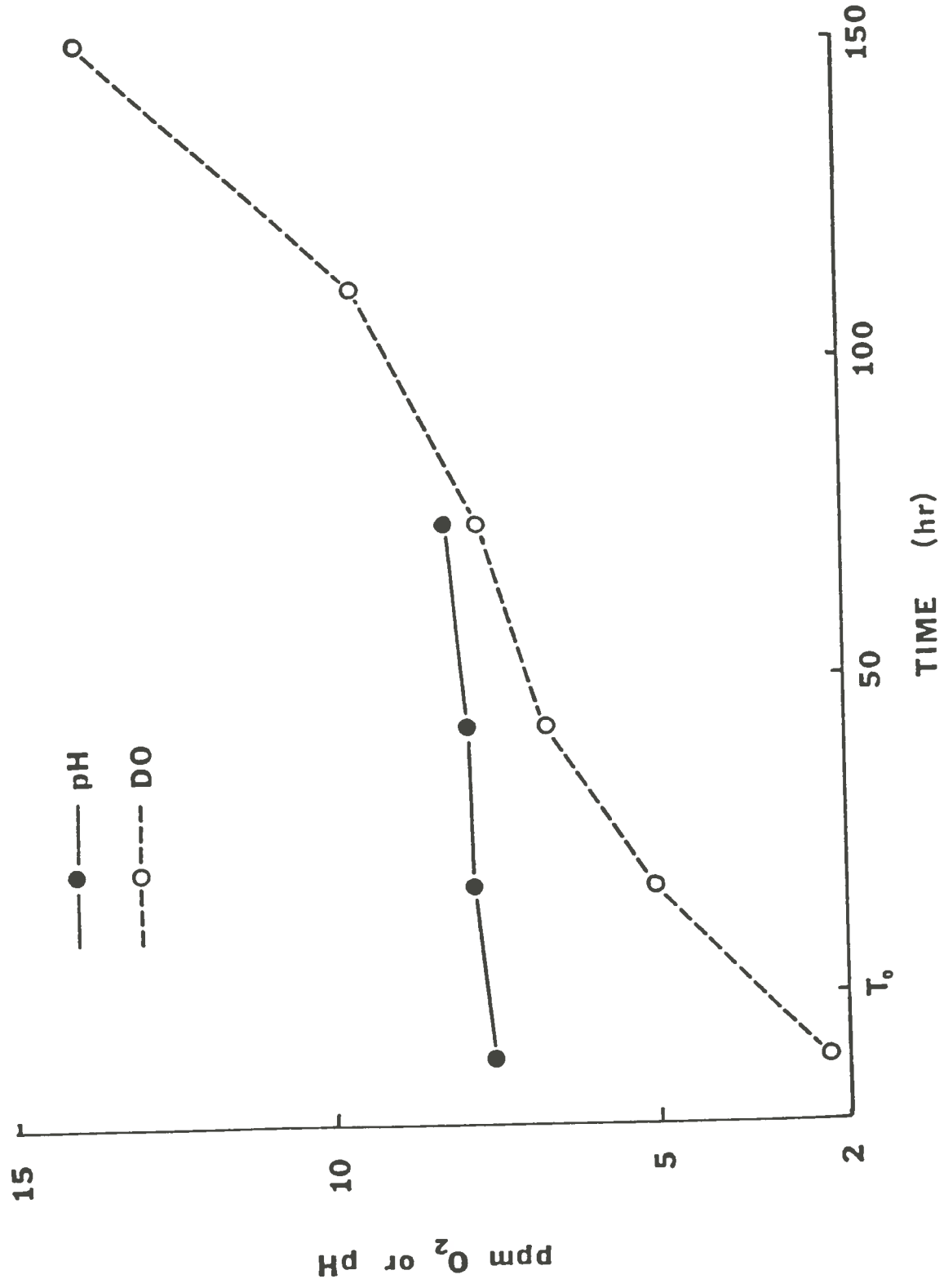

ppm O_2 or pH
15
10
5
2
pH
DO
T_o
50
100
150
TIME (hr)

Fig. 5. Mean biomass changes (dry wt) of S. punctata (o), L. gibba (●), and S. polyrrhiza (Δ) after 20, 43, 77, 112, and 151 hr of exposure to 10 ppm Cr.

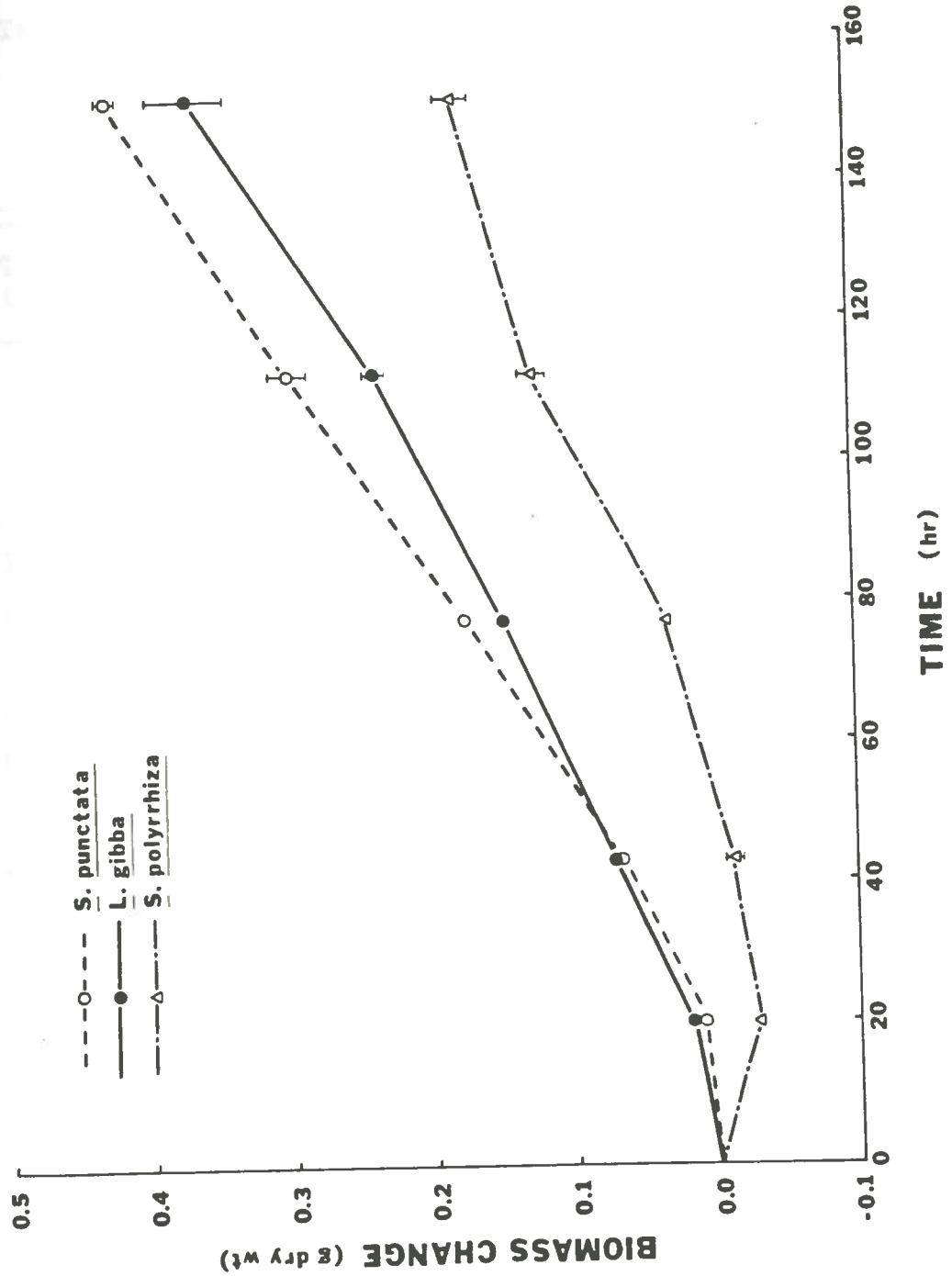

S. punctata
L. gibba
S. polyrrhiza
BIOMASS CHANGE (g dry wt)
TIME (hr)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
20
40
60
80
100
120
140
160

Table 11. Mean* biomass change (dry wt) of S. punctata (S.pu.), S. polyrrhiza (S.p.), and L. gibba (L.g.), after 20, 43, 77, 112, and 151 hr exposure to an initial Cr concentration of 10 ppm.

Time (hr)	Biomass change[a]		
	S.pu.	S.p.	L.g.
20	0.0111	-0.0283	0.0186
43	0.0660	-0.0125	0.0709
77	0.1787	0.0347**	0.1484
112	0.2986	0.1240	0.2372
151	0.4250	0.1806	0.3674

[a]Biomass change = dry wt of plants (g) at T_i - dry wt of plants (g) at T_o.

* n = 5 for all time periods.

** n = 4

Comparisons of growth between species at each time showed that all 3 species grew significantly different ($P < 0.001$) from each other. S. polyrrhiza appeared to be more sensitive to Cr at 10 ppm, since it grew significantly less than S. punctata and L. gibba in all sampling periods. In Experiment 4, S. polyrrhiza at this same Cr level after 8 days exposure, showed the least growth when the same 3 species of duckweed were compared. In Experiment 4, S. punctata had shown the greatest dry wt change when exposed to 10 ppm Cr. In Experiment 5, S. punctata also produced (from 77 to 151 hr) the largest biomass increase of the 3 species. The results in Experiments 4 and 5 therefore confirm the order of Cr tolerance between these 3 species of duckweed exposed to 10 ppm Cr. On the basis of growth, S. polyrrhiza is least tolerant, L. gibba is intermediate and S. punctata is the most tolerant of Cr at the 10 ppm level.

The µg Cr/g dry wt values in Table 12 show that the amount of Cr in the plants increased as longer periods of exposure to Cr are examined. An ANOVA indicated that within species, a highly significant ($P < 0.001$) difference in Cr uptake (µg Cr/g) by duckweeds over time was evident.

Between the species, L. gibba and S. punctata showed no significant ($P > 0.05$) differences in the µg Cr/g dry wt of plant, at each successive sampling period was

Table 12. Mean* µg Cr in plants, concentration factors, and the percent Cr removed by S. punctata (S.pu.), S. polyrrhiza (S.p.), and L. gibba (L.g.), exposed to an initial Cr concentration of 10 ppm for 20, 43, 77, 112, and 151 hr.

Time (hr)	µg Cr/g (dry wt)			Concentration[a] factor (dry wt)			Percent Cr removed by plants		
	S.pu.	S.p.	L.g.	S.pu.	S.p.	L.g.	S.pu.	S.p.	L.g.
20	1105	2412	752	170	393	117	1.28	1.99	0.59
43	1510	3434	903	228	593	160	2.25	3.17	0.96
77	1712	7227**	1399	275	1141**	214	3.67	8.08**	2.21
112	2417	8605	1510	430	1490	274	5.58	12.47	2.81
151	3137	6961	3553	533	1201	603	8.84	11.75	10.37

[a]Concentration factor = (µg Cr/g dry wt plant/(µg Cr/ml filtrate water)

* n = 5 at all time periods.

** n = 4

found. S. polyrrhiza (from 77 to 151 hr) had a significantly higher level of Cr uptake (µg Cr/g) than L. gibba or S. punctata. At 112 hr S. polyrrhiza had obtained an average Cr level which represented the highest Cr concentration (8605 µg Cr/g) obtained by any of the 3 species examined during the complete study.

For concentration factors and the percent Cr removed by plants, (Table 12), within species ANOVA results indicated significant differences over time ($P < 0.05$). Values for concentration factors and the percent Cr removed by plants both showed increasing trends as the periods of exposure to Cr became longer.

Between species, S. polyrrhiza had significantly greater concentration factors at all time periods ($P < 0.001$). There was no significant differences between S. punctata and L. gibba ($P > 0.05$).

For the percent Cr removed by plants, the only significant difference among species occurred at 112 hr for that sampling period. S. polyrrhiza was found to be different than L. gibba and S. punctata.

The bar graph shown in Figure 6 is a comparison of the µg Cr in the filterable material present in 50 ml of water sampled after thorough mixing of attached, settled, and suspended materials (shaded bars) to the µg Cr/g dry wt of duckweed (clear bars). The amount of

Fig. 6. Mean (µg Cr/g dry wt) removed by S. punctata (S.pu.), S. polyrrhiza (S.p.), and L. gibba (L.g.), (clear bars). The shaded bars represent the µg Cr retained by the filterable material (#1 Whatman Filter). This material, exposed to an initial Cr concentration of 10 ppm for 20, 43, 77, 112, and 151 hr was present in a 50 ml water sample collected after thorough mixing (see text).

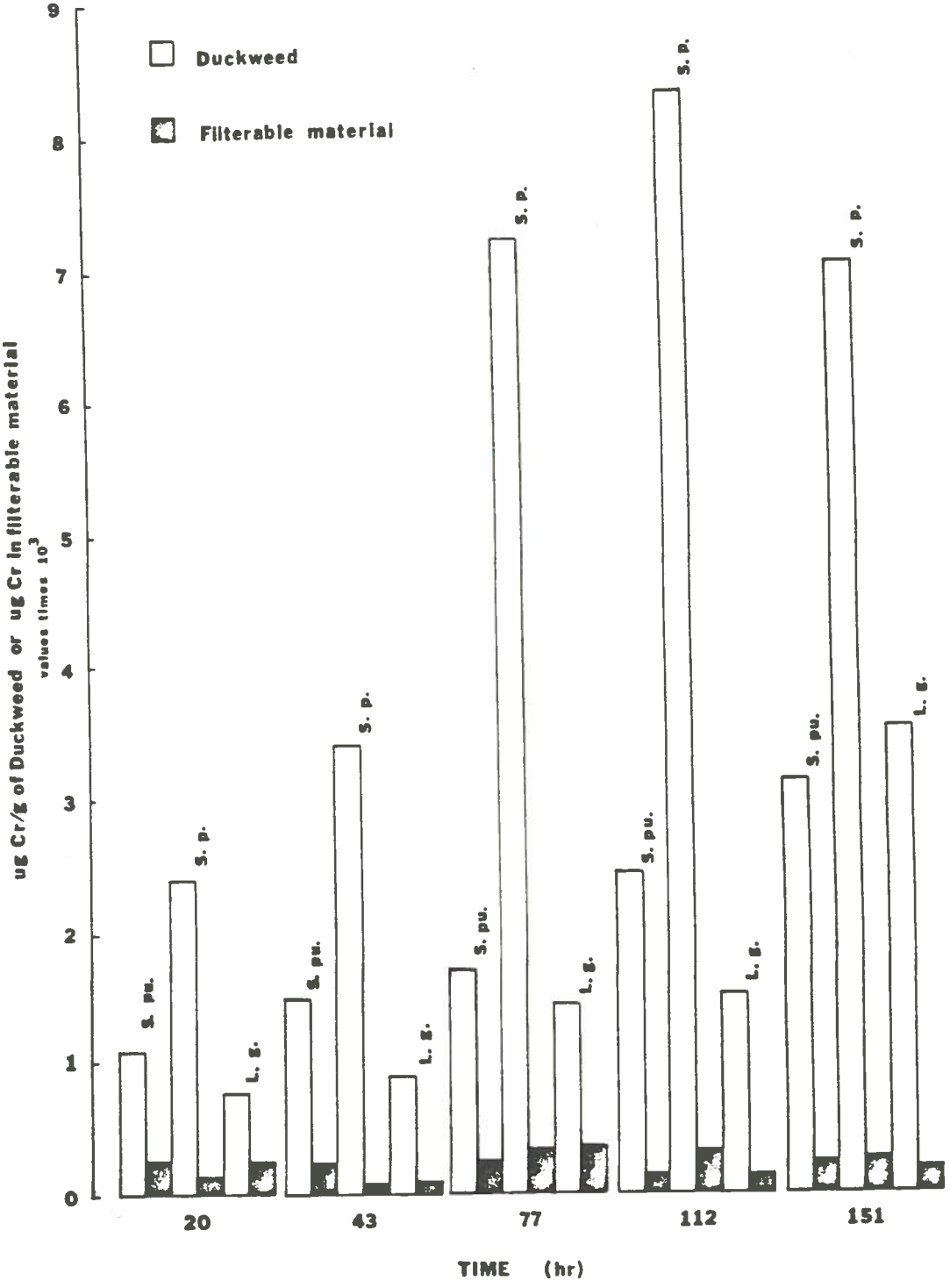

Duckweed
Filterable material
ug Cr/g of Duckweed or ug Cr in filterable material
values times 10^3
0
1
2
3
4
5
6
7
8
9
S. pu.
S. p.
L. g.
20
43
77
112
151
TIME (hr)

Table 13. Examination of the most appropriate species for the optimum removal of Cr at 10 ppm for 5 sampling times. Values of data found in Tables 11 and 12 were made of the results for L. gibba (L.g.), S. polyrrhiza (S.p.), and S. punctata (S.pu.) relative to one another for easy comparison at each time.

Time (hr)	Species	Comparative[a] biomass change	Comparative[b] conc. factors	Comparative ug Cr/g dry wt	Comparative[c] total ug Cr in plants	Comparative percent Cr removed by plants
20	L.g.	1.68	0.69	0.68	0.46	0.46
	S.p.	--*	2.31	2.18	1.55	1.55
	S.pu.	1	1	1	1	1
43	L.g.	1.07	0.70	0.60	0.43	0.43
	S.p.	--*	2.60	2.27	1.41	1.41
	S.pu.	1	1	1	1	1
77	L.g.	0.83	0.78	0.82	0.60	0.60
	S.p.	0.19	4.16	4.23	2.20	2.20
	S.pu.	1	1	1	1	1
112	L.g.	0.79	0.64	0.62	0.50	0.50
	S.p.	0.42	3.47	3.56	2.23	2.23
	S.pu.	1	1	1	1	1
151	L.g.	0.86	1.13	1.13	1.17	1.17
	S.p.	0.43	2.25	2.22	1.33	1.33
	S.pu.	1	1	1	1	1

[a]Comparative biomass change - from Table 11 that value present for S.pu. was divided into the values for the other two species at a particular sampling time. (i.e., for S.pu. at 151 hr - 0.4249 is divided into the values for S.p. and L.g. The values obtained by this comparison are presented in this table.

[b]Comparative conc. factors - from Table 12 that value present for S.pu. is divided into the values for the other two species at a particular sampling time.

[c]Comparative total ug Cr in plants - total ug Cr in plant values were not presented in Tables 11 or 12. These values were calculated as follows: (ug Cr/g dry wt plant) x total dry wt of plants at a particular sample time. After the total ug Cr in plant values were derived, the comparative values shown above were computed as described for the other variables.

*Since these values could not be made relative to S.pu. and still remain positive they were omitted.

Cr in the filterable material did not change appreciably. The uptake of Cr by the plants, on the other hand, increased. Thus, the ratio of the μg Cr/g of duckweed to the μg Cr retained by the filtered matter rose during the study for S. punctata and L. gibba, from a low of 20 hr of 4:1 to a high of 18:1 for L. gibba after 151 hr of exposure. The ratio of Cr in plants and the Cr in the filterable material for S. polyrrhiza did not increase as the time of exposure increased. A peak of 25:1 was obtained at 112 hr but this remained constant at 151 hr. These results suggest that the filterable material, when exposed to 10 ppm Cr, is not actively involved in Cr uptake but that it is a passive process.

The same type of comparative values developed for Table 9 in Experiment 4 are shown in Table 13 for Experiment 5. The values for 4 of the 5 variables used for the comparison are shown in Tables 11 and 12. The S. punctata value, for a particular variable and time (Table 11 and 12) was divided into the two values for the other two species. In this manner, the results for each species became relative to each other. The data could then be easily compared.

Of the 3 duckweed species exposed to 10 ppm Cr for 151 hr, S. polyrrhiza showed optimum Cr removal based on the comparative total μg Cr removed by plants.

The results from Experiment 4 had shown that the removal of Cr by S. polyrrhiza would probably be best at 1 ppm, based on the comparative total µg Cr removed by plant values presented in Table 10. Of the 3 species examined at 10 ppm Cr level, S. polyrrhiza showed the least Cr removing potential having a comparative value of only 0.55.

In Experiment 5 findings suggested that some other parameter within the system had to be considered in order to account for this unexpectedly high Cr removal by S. polyrrhiza. Comparison of the µg Cr/g dry wt of S. punctata and L. gibba from Experiment 4 after 8 days and Experiment 5, after 151 hr, demonstrates that values are relatively the same (3000-3600) for plants exposed to an initial Cr concentration of 10 ppm. The 7000 and 8000 µg Cr/g found within S. polyrrhiza from Experiment 5, are 2-3 times higher (though the plants were exposed for a shorter period of time) than levels for S. polyrrhiza shown in the previous study.

The presence of a diverse algal population within the S. polyrrhiza stock cultures could well be the source of the unique uptake experienced in Experiment 5. Algae are known for their ability to concentrate trace metals (Filip et al. 1979; Saenko et al. 1976; Sivalingam, 1978).

This discrepancy in the expected results for S. polyrrhiza could be of great significance in a Cr removing waste treatment system. Possible algal species that can grow among the duckweed roots and also take up Cr could be selectively cultured with the plants. The efficiency of Cr waste water treatment using duckweeds might thereby be enhanced.

Since Klein et al. (1974) reports Cr concentration common in residential wastewater to be generally less than 1 ppm, it would seem that duckweeds have some potential for use in waste water removal of Cr.

The use of duckweeds for industrial waste water treatment is unlikely since at the concentrations of Cr commonly found in waste water the retention times required would make it unfeasible.

Future studies should include longer term examination of duckweeds to various Cr levels so that the uptake of Cr obtained at equilibrium is determined. It is of importance also to know how long it takes for equilibrium to be reached. Factors such as changing pH, biomass, harvest rate and DO would also affect Cr removal and should be considered. Chrome resistant duckweed ecotypes might also be found which could actively grow and remove Cr at higher Cr concentrations. The results of these future studies could present a bright future for the use

of duckweeds in Cr waste water management. In any case the foundation from which to proceed has been laid.

CONCLUSIONS

1. Radiochrome (^{51}Cr) is a very sensitive tracer for monitoring stable Cr concentrations in duckweed.
2. Adverse seasonal conditions, in combination with Cr concentrations greater than 1 ppm, have a synergistic effect on the growth of duckweed and uptake of Cr.
3. Neither aeration nor non-aeration of shallow tanks had any effects ($P > 0.05$) on Cr uptake or growth of S. punctata when exposed to a concentration of 1 ppm Cr.
4. At Cr concentrations of 0.01 and 0.1 ppm, no negative effects on duckweed growth were shown.
5. At Cr concentrations above 0.1 ppm, increasingly negative effects on the growth of S. punctata, S. polyrrhiza, and L. gibba were demonstrated.
6. Lemna gibba grew best at 0.1, 1.0, and 20 ppm Cr, S. punctata grew best at 10 ppm Cr, and S. polyrrhiza exhibited the least Cr tolerance of the 3 species examined.
7. A direct relationship exists between Cr concentration and Cr uptake by duckweeds. As Cr levels increase the absolute Cr uptake by duckweeds increases.

8. The results indicated indicated that for comparative total µg Cr in plant the species studied, 10 ppm Cr was the optimum level at which the greatest quantity of Cr was removed.
9. Comparison of the results for L. gibba, S. punctata, and S. polyrrhiza, exposed to 4 different Cr concentrations, indicated that S. punctata at 0.1, 10, and 20 ppm was the most efficient species for Cr removal. S. polyrrhiza was the most efficient species for Cr removal at 1 ppm.
10. The continued growth of S. punctata, S. polyrrhiza, and L. gibba at 10 ppm for 151 hr suggests that these species can tolerate the lower range of Cr concentrations common in industrial waste water for short periods of time. Their use in residential wastewater where Cr concentrations are lower however has greater potential.

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Appendix A

Cr Effluent Limitation Guidelines

The following table presents the ranges representing the degree of effluent reduction attainable by the application of the best practicable control technology currently available. Guidelines are listed by industry.

Industry	Maximum for any 1 day:	Average daily Cr values for 30 consecutive days shall not exceed:
Textile	0.04-0.14 $\frac{kg}{kkg\ wool}$ or product	0.02-0.07 $\frac{kg}{kkg\ wool}$ or product
Electroplating	Total Cr 80-160 mg/sq m of operation	40-80 mg/sq m of operation
	Cr^{+6} 8-16 mg/sq m of operation	4-8 mg/sq m of operation
Inorganic Chemicals	Total Cr 0.0088 ppm	0.0044 ppm
	Cr^{+6} 0.009 ppm	0.0005 ppm
Iron & Steel Manufacturing	Total Cr 0.0225 kg/kkg of product	0.0075 kg/kkg of product
	Cr^{+6} 0.00015-0.0009	0.00005-0.0003
	Dissolved Cr 0.0030-0.0075	0.0010-0.0025

Industry	Maximum for any 1 day:	Average daily Cr values for 30 consecutive days shall not exceed:
Ferroalloy Manufacturing	Total Cr 0.0006-0.008 kg/MWh Cr^{+6} 0.0006-0.0008 Total Cr 0.053-0.106 kg/kkg processed or product	0.0003-0.004 kg/MWh 0.0003-0.0004 0.026-0.0532 kg/kkg processed or product
Leather Tanning & Finishing	0.10-0.24 kg/kkg raw product	0.06-0.12 kg/kkg raw product

Appendix B

Table 1

^{51}Cr Uptake by Duckweeds

Means* ± SE for all variables used in Experiment 1.

Time (hr)	cpm/g dry wt (values times 10^5) including SE				Concentration factor				Percent of total activity in plants			
	S. punctata	DF	P(T)	L. gibba	S. punctata	DF	P(T)	L. gibba	S. punctata	DF	P(T)	L. gibba
6	24.0 ± 1.7	5.0	0.964	24.1 ± 1.4	252.0 ± 18.2	5.0	0.623	240.0 ± 13.7	2.8 ± 0.32	2.2	0.507	2.5 ± 0.07
18	50.2 ± 1.6	5.0	0.309	45.6 ± 3.2	555.0 ± 18.7	5.0	0.596	526.0 ± 41.8	6.2 ± 0.50	5.0	0.282	5.3 ± 0.54
36	87.1 ± 9.8	5.0	0.019	51.1 ± 5.6	1108.0 ± 136.0	5.0	0.017	632.0 ± 61.0	7.6 ± 0.74	5.0	0.046	5.1 ± 0.58
52	66.1 ± 8.6	5.0	0.048	32.1 ± 9.2	860.0 ± 141.0	5.0	0.060	399.0 ± 126.0	3.9 ± 0.64	5.0	0.635	3.1 ± 1.19
74	58.3 ± 2.5	5.0	0.001	33.0 ± 2.1	758 0 ± 65.3	5.0	0.008	459.0 ± 36.8	4.9 ± 0.04	3.1	0.009	3.3 ± 0.26
122	152.4 ± 7.1	5.0	0.005	95.6 ± 8.5	3127.0 ± 251.0	5.0	0.008	1909.0 ± 148.0	20.0 ± 4.72	5.0	0.176	12.4 ± 2.23

Time (hr)	Percent of total activity in large filtered material (#1 Whatman)				Percent of total activity in minute filterable material (millipore filter)				Percent of the total activity in final filtrate water			
	S. punctata	DF	P(T)	L. gibba	S. punctata	DF	P(T)	L. gibba	S. punctata	DF	P(T)	L. gibba
6	2.9 ± 0.004	5.0	0.030	2.5 ± 0.08	3.3 ± 0.52	5.0	0.689	2.9 ± 0.32	86.6 ± 0.97	5.0	0.011	91.2 ± 0.68
18	3.8 ± 0.31	5.0	0.020	3.0 ± 0.08	3.0 ± 0.02	3.0	0.139	3.7 ± 0.30	82.2 ± 0.25	3.1	0.306	79.3 ± 2.42
36	6.5 ± 0.98	2.1	0.403	5.5 ± 0.11	7.9 ± 0.54	5.0	0.240	6.5 ± 0.75	71.6 ± 0.75	5.0	0.318	73.3 ± 1.22
52	5.9 ± 0.70	5.0	0.727	5.6 ± 0.36	7.2 ± 0.45	5.0	0.853	7.5 ± 1.14	70.7 ± 2.74	5.0	0.411	75.4 ± 3.97
74	17.0 ± 1.35	5.0	0.078	23.0 ± 2.02	3.9 ± 0.33	5.0	0.370	3.6 ± 0.38	70.5 ± 2.94	5.0	0.220	65.8 ± 1.94
122	7.5 ± 1.69	2.1	0.749	8.2 ± 0.27	7.4 ± 1.49	2.2	0.252	9.9 ± 0.31	44.6 ± 2.24	5.0	0.714	45.4 ± 0.73

t - tests shown for variables shown in Tables 2 and 3.

* L. gibba: n = 4
S. punctata: n = 3

Appendix B (cont'd)

Table 2

Fall Growth of Duckweeds as Influenced by Cr

Means* ± SE for all variables used in Experiment 2.

Initial Cr concentration (ppm)	Biomass change		ug Cr/g plant (dry wt)	
	S. polyrrhiza	Mix	S. polyrrhiza	Mix
Control	0.0538 ± 0.0091	0.0463 ± 0.0082	0.0226 ± 0.0008	0.0142 ± 0.0004
0.01	0.0670 ∓ 0.0353	0.0607 ∓ 0.0064	2.30 ∓ 0.213	3.2 ∓ 0.0329
1.0	0.0303 ∓ 0.0125	0.0228 ∓ 0.0056	125.0 ∓ 1.86	247.0 ∓ 9.84
20	-0.0018 ∓ 0.0103	-0.0082 ∓ 0.0054	585.0 ∓ 11.05	978.0 ∓ 27.64
40	-0.0102 ∓ 0.0115	-0.0334 ∓ 0.0088	917.0 ∓ 60.68	1178.0 ∓ 25.54

Initial Cr concentration (ppm)	Concentration factor	
	S. polyrrhiza	Mix
Control	709.0 + 25.86	396.0 + 13.81
0.01	461.0 ∓ 44.96	533.0 ∓ 7.45
1.0	137.0 ∓ 2.67	268.0 ∓ 10.36
20	24.0 ∓ 0.52	40.0 ∓ 1.25
40	18.0 ∓ 1.76	22.0 ∓ 0.47

* n = 5

Appendix B (cont'd)

Table 3

The Influence of Aeration (A) vs Non-Aeration (N) on Cr Uptake

Means* ± SE for all variables used in Experiment 3.

Time (hr)	Biomass change		µg Cr/g plant (dry wt)	
	A	N	A	N
25	- 0.0159 ± 0.0011	0.0299 ± 0.0013	100.0 ± 5.32	92.0 ± 8.93
88	0.0897 ∓ 0.0181	0.0929 ∓ 0.0133	372.0 ∓ 26.92	393.0 ∓ 5.46
114	0.1121 ∓ 0.0089	0.1176 ∓ 0.0223	536.0 ∓ 39.82	605.0 ∓ 2.42
120	0.1342 ∓ 0.0252	0.1556**	459.0 ∓ 32.19	434.0**
147	0.2580 ∓ 0.0017	0.2672 ± 0.0067	518.0 ∓ 13.85	506.0 ± 7.89

Time (hr)	Concentration factor		Total g Cr in plants		Percent of total Cr removed by plants	
	A	N	A	N	A	N
25	105.0 ± 6.47	99.0 ± 9.23	22.0 ± 0.77	22.0 ± 2.67	1.12 ± 0.04	1.09 ± 0.13
88	384.0 ∓ 22.82	425.0 ∓ 10.66	107.0 ∓ 6.30	118.0 ∓ 3.86	5.35 ∓ 0.32	5.89 ∓ 0.19
114	569.0 ∓ 36.98	657.0 ∓ 10.83	155.0 ∓ 6.48	178.0 ∓ 2.83	7.76 ∓ 0.32	8.89 ∓ 0.14
120	484.0 ∓ 25.81	448.0**	158.0 ∓ 5.41	174.0**	7.95 ∓ 0.27	8.68**
147	520.0 ∓ 10.51	554.0 ± 7.07	262.0 ∓ 6.96	266.0 ± 2.62	13.11 ∓ 0.35	13.30 ± 0.13

* n = 2
**n = 1

Appendix B (cont'd)

Table 4

Spring Growth of Duckweed as Influenced by Cr

Means* ± SE for all variables used in Experiment 4

Initial Cr concentration	Biomass change			µg Cr/g plant (dry wt)		
	S. punctata	S. polyrrhiza	L. gibba	S. punctata	S. polyrrhiza	L. gibba
Control	0.3087 ± 0.0120	0.2702 ± 0.0645	0.4315 ± 0.0132	0.0216 ± 0.0004	0.0178 ± 0.0013	0.0124 ± 0.0006
0.1	0.3080 ∓ 0.0116	0.3333 ∓ 0.0608	0.4224 ∓ 0.0292	76 ∓ 2.81	60 ∓ 5.34	64 ∓ 1.22
1.0	0.2653 ∓ 0.0073	0.1891 ∓ 0.0269**	0.2719 ∓ 0.0027	653 ∓ 18.3	862 ∓ 124**	696 ∓ 13.0
10	0.1304 ∓ 0.0129	0.0471 ∓ 0.0025	0.1194 ∓ 0.0025	3778 ∓ 212	3134 ∓ 288	3714 ∓ 170
20	0.0104 ∓ 0.0038	0.0044 ∓ 0.0037	0.0382 ∓ 0.0058	5608 ∓ 237	3718 ∓ 185	2496 ∓ 75.1

Initial Cr concentration	Concentration factor			Percent of total Cr removed by plants		
	S. punctata	S. polyrrhiza	L. gibba	S. punctata	S. polyrrhiza	L. gibba
Control	7223 ± 1123	2874 ± 628	1415 ± 61.3	18.6 ± 0.66	14.5 ± 0.50	11.1 ± 0.66
0.1	5688 ∓ 767	3207 ∓ 497	2016 ∓ 54.2	19.5 ∓ 0.72	16.1 ∓ 0.60	16.9 ∓ 1.22
1.0	2010 ∓ 122	2200 ∓ 281**	1248 ∓ 34.9	15.2 ∓ 0.62	15.5 ∓ 0.92**	13.7 ∓ 0.31
10	738 ∓ 75.6	565 ∓ 61.3	521 ∓ 29.0	5.8 ∓ 0.44	3.2 ∓ 0.17	4.7 ∓ 0.21
20	525 ∓ 29.1	332 ∓ 13.1	167 ∓ 5.2	2.7 ∓ 0.14	1.6 ∓ 0.07	1.0 ∓ 0.05

* n = 5
**n = 4

Appendix 8 (cont'd)

Table 5

Over Time by Duckweeds Exposed to 10 ppm Cr

Means* ± SE for all variables used in Experiment 5.

Time (hr)	Biomass change			ug Cr/g plant (dry wt)		
	S. punctata	S. polyrrhiza	L. gibba	S. punctata	S. polyrrhiza	L. gibba
20	0.0111 ± 0.0033	- 0.0283 ± 0.0036	0.0186 ± 0.0020	1105.0 ± 156.0	2412.0 ± 230.0	752.0 ± 45.6
43	0.0660 ± 0.0036	- 0.0125 ± 0.0036	0.0709 ± 0.0022	1510.0 ± 347.0	3434.0 ± 261.0	903.0 ± 42.8
77	0.1787 ± 0.0052	0.0347 ± 0.0027**	0.1484 ± 0.0068	1712.0 ± 329.0	7227.0 ± 1575.0**	1399.0 ± 252.0
112	0.2086 ± 0.0192	0.1240 ± 0.0085	0.2372 ± 0.0108	2417.0 ± 475.0	8605.0 ± 2255.0	1510.0 ± 195.0
151	0.4250 ± 0.0100	0.1806 ± 0.0121	0.3674 ± 0.0476	3137.0 ± 575.0	6961.0 ± 1643.0	3553.0 ± 1644.0

Time (hr)	Concentration factor			Percent of total Cr removed by plants		
	S. punctata	S. polyrrhiza	L. gibba	S. punctata	S. polyrrhiza	L. gibba
20	170.0 ± 17.9	393.0 ± 34.8	117.0 ± 6.09	1.28 ± 0.19	1.99 ± 0.19	0.59 ± 0.03
43	228.0 ± 30.3	593.0 ± 33.6	160.0 ± 7.51	2.25 ± 0.51	3.17 ± 0.24	0.96 ± 0.04
77	275.0 ± 38.6	1141.0 ± 222.0**	214.0 ± 29.0	3.67 ± 0.71	8.08 ± 1.75**	2.21 ± 0.37
112	430.0 ± 80.2	1490.0 ± 373.0	274.0 ± 33.0	5.58 ± 1.07	12.47 ± 3.45	2.81 ± 0.34
151	533.0 ± 83.5	1201.0 ± 245.0	603.0 ± 262.0	8.84 ± 1.52	11.75 ± 2.74	10.37 ± 6.15

* n = 5
**n = 4

Appendix C

Table 1

Experiment 2

Orthogonal Comparisons of Biomass Change

0.0001, 0.01, 1 (log scale)

Source	DF	Mix: Biomass change MS	P(F)	DF	S. polyrrhiza MS	P(F)
Treatment levels	2	0.00183	0.007	2	0.00152	0.572
linear 1		0.00139	0.031		0.00123	0.504
quadratic 1		0.00228	0.009		0.00200	0.397
Error	12	0.000232		11	0.00258	

0, 20, 40 (Arithmetic scale)

Source	DF	Mix: Biomass change MS	P(F)	DF	S. polyrrhiza: Biomass change MS	P(F)
Treatment levels	2	0.00831	0.001	2	0.00605	0.002
linear 1		0.0159	0.001		0.01023	0.001
quadratic 1		0.00072	0.141		0.00186	0.087
Error	12	0.000288		12	0.00053	

Appendix C (continued)

Table 2

Experiment 4

Orthogonal Comparisons of Biomass Change

Source	DF	*L. gibba* MS	P(F)	*S. polyrrhiza* MS	P(F)	*S. punctata* MS	P(F)
		0.1, 1.0, 10 (Log scale)					
Treatment levels	2	0.1147	0.001	0.1024	0.001	0.0430	0.001
linear 1		0.229	0.001	0.205	0.001	0.0788	0.001
quadratic 1		0.0000039	0.960	0.0000034	0.984	0.00710	0.005
Error	12	0.00145		0.00753		0.00059	
		0, 10, 20 (Arithmetic Scale)					
Treatment levels	2	0.2156	0.001	0.1018	0.001	0.1176	0.001
linear 1		0.3867	0.001	0.1765	0.001	0.2225	0.001
quadratic 1		0.0445	0.001	0.0271	0.072	0.0128	0.066
Error	12	0.00036		0.000696		0.000313	

Appendix D

Table 1

Experiment 1: ^{51}Cr Uptake by Duckweeds

Source	DF	Differences within species: *L. gibba* MS	P(F)	DF	*S. punctata* MS	P(F)
Concentration Factors						
Time	5	1486000	0.001	5	3181000	0.001
Error	18	30000		12	53100	
cpm/g dry wt						
Time	5	2.66×10^{13}	0.001	5	5.81×10^{13}	0.001
Error	18	1.36×10^{12}		12	1.16×10^{12}	
Percent of Total ^{51}Cr Activity in Duckweeds						
Time	5	53.9	0.001	5	119.0	0.001
Error	18	4.74		12	11.8	
#1 Whatman Filter						
Time	5	223.0	0.001	5	73.4	0.001
Error	18	2.88		12	3.11	
Millipore Filters						
Time	5	30.8	0.001	5	11.1	0.003
Error	18	1.54		12	1.55	
Filtrate						
Time	5	942.0	0.001	5	640.0	0.001
Error	18	18.6		12	11.4	

Appendix D (cont'd)

Table 2

Experiment 2: Fall Growth of Duckweeds as Influenced by Cr

Source	Differences within plant groups: Mix: Biomass changes DF	MS	P(F)	S. polyrrhiza: biomass changes DF	MS	P(F)
Biomass Changes						
µg Cr/ml solution	4	0.00748	0.001	4	0.00567	0.034
Error	20	0.000246		19	0.00175	
µg Cr/g dry wt						
µg Cr/ml solution	4	1558700	0.001	4	825100	0.001
Error	20	1513		19	4007	
Concentration Factors						
µg Cr/ml solution	4	274900	0.001	4	459200	0.001
Error	20	355		19	7838	
Percent Cr Removed by Plants						
µg Cr/ml solution	4	0.1023	0.001	4	0.20190	0.001
Error	20	0.00019		19	0.00080	

Appendix D (cont'd)

Table 3

Experiment 2: Fall Growth of Duckweeds as Influenced by Cr

Source	DF	Control MS	P(F)	0.01 MS	P(F)	1.0 MS	P(F)	20 MS	P(F)	40 MS	P(F)
					Differences among species						
					Biomass Changes						
Species	1	0.000139	0.559	0.0000991	0.865	0.000123	0.577	0.000102	0.597	0.00135	0.147
Error	8	0.000373		0.00321		0.000359		0.000337		0.000524	
					ug Cr/g dry wt						
Species	1	0.000177	0.001	2.142	0.003	33045	0.001	384200	0.001	170058	0.004
Error	8	0.00000209		0.116		283		2215		10836	
					Concentration Factors						
Species	1	70030	0.223	4750	0.521	23320	0.024	594	0.001	26.92	0.056
Error	8	40200		10520		3030		4.57		5.12	
					Percent Cr Removed by Plants						
Species	1	0.174	0.001	0.00016	0.591	0.01226	0.001	0.00002	0.050	0.00004	0.076
Error	8	0.00165		0.00052		0.00022		0.000003		0.00001	

Appendix D (cont'd)

Table 4

Experiment 3: The Influence of Aeration (A) vs Non-Aeration (N) on Cr Uptake

		Variable: Biomass change		Variable: ug Cr/g dry wt		Variable: Concentration factor	
Source	DF	MS	P(F)	MS	P(F)	MS	P(F)
Model	9	0.0155	0.001	61244	0.001	69852	0.001
A vs N	1	0.00132	0.100	165	0.663	2713	0.079
Time	4	0.0343	0.001	136451	0.001	13970	0.001
A vs N * Time	4	0.000307	0.564	1305	0.264	1952	0.090
Error	9	0.000396		832		689	

		Variable: Total ug Cr in plants		Variable: Percent Cr removed by plants	
Source	DF	MS	P(F)	MS	P(F)
Model	9	125057	0.001	34.7	0.001
A vs N	1	476	0.009	1.190	0.009
Time	4	31104	0.001	77.76	0.001
A vs N * Time	4	80.7	0.204	0.202	0.204
Error	9	393		0.109	

Appendix D (cont'd)

Table 5

Experiment 4: Spring Growth of Duckweeds as Influenced by Cr

Variable: Biomass change

Source	DF	MS	P(F)
Model	14	0.1044	0.001
Species	2	0.04778	0.001
µg Cr/ml	4	0.327	0.001
Species * µg Cr/ml	8	0.00678	0.059
Error	59	0.00336	

Variable: µg Cr/g dry wt

MS	P(F)
1.776×10^7	0.001
2.665×10^6	0.001
5.559×10^7	0.001
2.573×10^6	0.001
8.886×10^4	

Variable: Concentration factor

Source	DF	MS	P(F)
Model	14	2.078×10^7	0.001
Species	2	3.009×10^7	0.001
µg Cr/ml	4	4.035×10^7	0.001
Species * µg Cr/ml	8	8.598×10^6	0.001
Error	59	8.720×10^5	

Variable: Percent Cr removed by plants

MS	P(F)
223.6	0.001
56.21	0.001
730.20	0.001
11.32	0.001
1.633	

VITA

Roger Staves was born in Rockville, Connecticut on August 27, 1955, the son of Gerard O. and Theresa L. Staves. Following his secondary education at Tolland High School, Tolland, CT., in 1973, he proceeded to Southeastern Massachusetts University (SMU), North Dartmouth, MA. He recieved the degree of Bachelor of Science from SMU in May 1978. In that same year he began at Louisiana State University (LSU), in the Fisheries Program within the School of Forestry and Wildlife Management. In conjuction with the Nuclear Science Center he has been trained for two years. He is currently a candidate for the Master of Science degree in Fisheries. He will venture from LSU to the University of Kiel, West Germany. At the Marine Science Institute he will begin a research program involving municipal waste water and larval fish. His work will be supported by a DAAD Fellowhip from the Federal Republic of Germany.