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Growth responses of selected freshwater algae to trace elements and scrubber ash slurry generated by coal-fired power plants

by

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ABSTRACT[1]

The freshwater algae Ankistrodesmus falcatus, Scenedesmus obliquus, Selenastrum capricornutum, and Microcoleus vaginatus were exposed to potential pollutants from coal-fired power plants and their growth responses were evaluated. The algae were exposed to As(V) as $Na_2HAsO_4 \cdot 7H_2O$, Cd(II) as $CdSO_4$, Hg(II) as $HgSO_4$, Se(VI) as Na_2SeO_4, in solution, and scrubber ash slurry generated by the Colstrip, Montana coal-fired power plant complex, using a modification of the EPA Algal Assay Procedure Bottle Test.

Water quality criteria for the potential coal-fired power plant pollutants are proposed. These criteria are based on the American Public Health Association concept "maximum allowable toxicant concentration" (MATC) values and were estimated using median effective concentration (EC50) values. The EC50 values for the potential pollutants ranged from 0.048-30.761 mg L^{-1} As(V), 0.005-0.019 mg L^{-1} Cd(II), 0.033-0.253 mg L^{-1} Hg(II), 0.033-8.511 mg L^{-1} Se(VI), and 3.048-15.417% scrubber ash slurry extract (SASE). The MATC values ranged from 0.016-10.000 mg L^{-1} As(V), 0.001-0.005 mg L^{-1} Cd(II), 0.004-0.032 mg L^{-1} Hg(II), 0.006-1.447 mg L^{-1} Se(VI), and 0.344-1.742% SASE.

The data presented in this dissertation have been generated as part of a U.S. Department of Energy Project which was designed to assess the impact of the Colstrip coal-fired power complex on the surrounding range ecosystem in southeastern Montana.

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INTRODUCTION

Increasing energy demands have resulted in the development of vast coal deposits in the western United States. In addition to off-site shipment of coal, mine-mouth coal-fired generating complexes have been and are being constructed. The Colstrip power plant, located at Colstrip in southeastern Montana, is such a complex.

Abernethy et al. (1969), Berry and Wallace (1974), Joensuu (1971), and Tsao and Wicks (1974) reported coals contain a variety of trace elements. Among these trace elements, arsenic, cadmium, mercury, and selenium are of prime concern because they are volatilized during combustion of coal (Andren et al., 1975; Billings and Matson, 1972; Klein and Russell, 1973; Klein et al., 1975a,b; Tsao and Wicks, 1974). Pollution control equipment for coal-fired power plants has not been designed for removal of trace elements in combustion gases. However, wet venturi scrubber systems, such as those installed at the Colstrip power plant, are believed to be among the most effective control systems for reducing stack emissions of trace elements. Even so, considerable quantities of volatile trace elements are expected to escape. Wet venturi scrubber systems used in coal-fired generating plants produce an ash slurry that is a potential pollutant. This slurry contains toxic trace elements and high concentrations of suspended and dissolved solids (Tsao and Wicks, 1974). Dreesen et al. (1977) indicated that molybdate, borate, fluoride, selenate, and arsenate were soluble contaminants from coal ash disposed in alkaline environments. Additionally, selenium has been reported in fly ash (Gutenmann et al., 1975).

The potential deposition of trace elements from coal-fired generating plants has received increasing attention in recent years due to their adverse effects on the environment (Cannon and Swanson, 1975; Crockett and Kinnison, 1977; Horton and Dorsett, 1976; Lewis and Lefohn, 1976; Lewis et al., 1976; Lindberg et al., 1975; Van Hook and Shultz, 1977; Vaughan et al., 1975). Because of their potentially deleterious effects (Ferens, 1974; Fishbein, 1974; Harriss, 1971; Kania et al., 1976; Kolb et al., 1973; Overnell, 1975; Peakall and Lovett, 1972; Reiniger, 1977; Tyler, 1972), trace elements should not exceed concentration ranges which are compatible with naturally functioning ecosystems. Moreover, the compatible level for each trace element depends on the physicochemical and biological components of the ecosystem, which directly or indirectly determine the chemical state of the element and its availability (Lee, 1973).

Toxicity tests provide one approach to determining compatible levels of trace elements, although care must be taken to create a test situation which is representative of the natural situation being examined. A chronic exposure toxicity test is required when investigating the potential toxicity of pollutants from coal-fired power plants because they typically enter aquatic ecosystems in small quantities over long periods (Van Hook and Shultz, 1977). Toxicity tests measure the biological impact of pollutants by monitoring, in a given test system, the physiological reaction of living organisms to the concentration of a test material made available. The available concentration in many cases is not the same as the total quantity of the test material initially introduced to the test

system. A difficulty encountered in the interpretation and application of toxicity tests is that different procedures are employed by various investigators which, in turn, complicate the comparison and reconciliation of data and conclusions (Black et al., 1975; Brown, 1975; Lee, 1973).

When one is dealing with the aqueous phase of aquatic ecosystems, selected species of planktonic algae make ideal test organisms. They are easily cultured, require minimal laboratory space and facilities, and can be easily subjected to a range of environmental conditions. Algal toxicity tests are significant because algae are primary producers in aquatic systems and permit utilization of population growth as a criterion of response.

The objectives of this study were to develop an algal toxicity test system based on the EPA Algal Assay Procedure Bottle Test (U.S. EPA, 1971) and, by using it, to develop water quality criteria for potential pollutants from coal-fired power plants. The criteria which have been developed for As(V), Cd(II), Hg(II), and Se(VI), in solution, and for scrubber ash slurry from the Colstrip coal-fired generating plant complex are based on dose-response relationships for algae indigenous to southeastern Montana and for Selenastrum capricornutum an EPA test alga.

The data have been generated as part of a U.S. Department of Energy Project (Potential of energy extraction processes in the Northern Great Plains for heavy metal contamination and consequent uptake and turnover in a range ecosystem model; Ames Laboratory, Department of Energy, Iowa State University, Ames, Iowa) designed to assess the impact of the Colstrip power plant complex on the surrounding range ecosystem.

LITERATURE REVIEW

Arsenic

Arsenic occurs ubiquitously in nature and can be both acutely and chronically toxic to man. No known form of arsenic has been shown to be essential (National Academy of Sciences, 1972). The toxic properties of arsenic are due partially to its actions in biochemical cycles. A major action of arsenate is to block the production of ATP and consequently, to block all syntheses dependent on a supply of energy-rich phosphate. Arsenate replaces one of the phosphate radicals in the oxidation of diphosphoglyceraldehyde, thus leading to the formation of 1-arseno-3-phosphate instead of diphosphoglycerate (Doby, 1965). Arsenate is also a powerful inhibitor of the oxidation of both pyruvate and α-ketoglutarate (Bonner and Varner, 1965). By blocking α-keto acid oxidation, which inhibits the Krebs cycle at the substrate level, arsenate can inhibit respiration in most plant tissues (Krogmann, 1973).

Arsenic can exist in trivalent and pentavalent inorganic and organic states. Physicochemical conditions which favor chemical and biological oxidation promote the shift to the pentavalent species; and conversely, those that favor reduction will shift the equilibrium to the trivalent state (National Academy of Sciences, 1972). Crecelius (1975) reported that arsenate was the dominant chemical form present in the waters of Lake Washington near Seattle, Washington. Ridley et al. (1977a,b), Wood (1974), and Woolson (1977) discussed the chemical and biochemical transformations of arsenic in different environmental substrates.

Few investigators have published work concerning the growth response of algae exposed to arsenic. den Dooren de Jong (1965) reported that the highest concentration of As(V) as $Na_2HAsO_4 \cdot 7H_2O$ which was tolerated without effect by Chlorella vulgaris was 0.03 mg L^{-1}. The lowest concentration preventing growth was 0.06 mg L^{-1}. Irgolic et al. (1977) reported that Tetraselmis chuii, a marine alga, initially grew well in a medium containing up to 50 mg L^{-1} As(V) as $Na_2HAsO_4 \cdot 7H_2O$, and with additional increments of As(V), the alga was eventually able to adapt and thrive at 1000 mg L^{-1}. A direct transfer back to arsenic-free medium resulted in death. Conway (1978) reported no detrimental effects to Asterionella formosa when the freshwater diatom was exposed to 0.16 mg L^{-1} As(V).

Cadmium

Cadmium is a biologically nonessential, nonbeneficial element and is an extremely dangerous cumulative poison (National Academy of Sciences, 1972). The toxic effects of cadmium have been reviewed by Cook (1977), Fishbein (1974), Flick et al. (1971), and Friberg et al. (1971).

Cadmium can cause enzyme inactivation by binding to sulfhydryl groups that have key positions at or near the active site of the enzyme (Katagiri, 1975). Additionally, cadmium competes with other biochemically important cations (e.g., zinc, iron, calcium, and magnesium). Eichhorn et al. (1970) indicated cadmium could bind with nucleic acid molecules at the phosphate groups of the ribose phosphate backbone. This binding can stabilize the ordered conformation of multiple stranded helical structures such as DNA by neutralizing the repelling negative charges of the phosphate groups. However, Cd^{+2} binding with heterocyclic bases destabilizes

the conformation of the ordered structure of DNA by displacing hydrogen bonds. Thus, it appears there are many sites where cadmium, by binding, may be detrimental to the metabolic processes of organisms.

The chemistry of cadmium in natural waters was discussed by Gardiner (1974a,b). Shephard (1976) and Shephard and McIntosh (1976) reported that the Cd(II) ion was the dominant chemical species in the freshwater lakes which they investigated.

The responses reported for algal species exposed to different levels of cadmium compounds varies significantly. Bartlett et al. (1974) reported that growth of Selenastrum capricornutum was initially inhibited at 0.05 mg L^{-1} and completely inhibited with 0.08 mg L^{-1} Cd(II) as $CdCl_2 \cdot 2\ 1/2\ H_2O$, using the Algal Assay Procedure Bottle Test (U.S. EPA, 1971). Conway (1978) and Conway and Williams (1978) indicated that populations of Asterionella formosa exhibited a linear decrease in the percent reduction of growth rate with increasing ambient $CdCl_2$ concentrations from approximately 0.002 to 0.009 mg L^{-1} Cd(II). Growth ceased with concentrations greater than 0.01 mg L^{-1}. The growth rate of Fragilaria crotonensis was not affected over this range (Conway and Williams, 1978). The growth of natural phytoplankton populations was not altered by 0.124 mg L^{-1} Cd(II) as $Cd(CH_3CO_2)_2 \cdot 2H_2O$, and the upper range of tolerance appeared to be below 11.24 mg L^{-1} (Cook, 1975). den Dooren de Jong (1965) found that the highest concentration of Cd(II) as $CdCl_2 \cdot 2\ 1/2\ H_2O$ tolerated without effect by Chlorella vulgaris was 0.09 mg L^{-1}, with the lowest concentration preventing growth being 0.14 mg L^{-1}. Hart (1975) reported that a Cd(II) concentration of 0.25 mg L^{-1} as $Cd(CH_3CO_2)_2 \cdot 2H_2O$ inhibited the growth rate of Chlorella pyrenoidosa cultures in the logarithmic growth phase.

Hutchinson and Stokes (1975) found Chlorella vulgaris to be more sensitive and reported an abrupt inhibition of growth by concentrations of cadmium above 0.05 mg L^{-1}. When Chlorella vulgaris and Haematococcus capensis were treated with selenium and cadmium in combination, an antagonistic response was noted. Katagiri (1975) indicated that the oxygen evolution rate of Anacystis nidulans in the presence of 0.1 mg L^{-1} Cd(II) as $Cd(CH_3CO_2)_2 \cdot 2H_2O$ was 10% of the control. No oxygen evolution occurred at 0.5 mg L^{-1} Cd(II). Klass et al. (1974) observed that Cd(II) concentrations as low as 0.0061 mg L^{-1} $CdCl_2$ had a significant inhibitory effect on Scenedesmus quadracauda, and 0.061 mg L^{-1} severely inhibited its growth. Alterations in the fine structure of Ankistrodesmus falcatus, Chlorella pyrenoidosa, and Scenedesmus quadracauda occurred with exposure to 0.03 to 0.10 mg L^{-1} Cd(II) as $CdCl_2$ (Silverberg, 1976). Miller et al. (1976) reported Cd(II) as $CdCl_2$ to be toxic to Selenastrum capricornutum between 0.1 and 0.2 percent of the major cations and anions of synthetic algal nutrient medium (calculated on the basis of $meqL^{-1}$). Rosko and Rachlin (1977) reported that cell division in Chlorella vulgaris was inhibited 50% by 0.06 ± 0.001 mg L^{-1} Cd(II) as $CdCl_2$.

Investigators have reported that certain physicochemical parameters partially mask the toxic effects of cadmium. Interactions occurred when organic chelators such as EDTA were used while conducting toxicity investigations with cadmium (Hart, 1975; Hart and Scaife, 1977; Katagiri, 1975). Gardiner (1974a,b) reported that EDTA displays a strong tendency to complex with cadmium. Kinkade and Erdman (1975) reported the accumulation of cadmium to be less in hard water than in soft water. Gardiner (1974b) speculated that cadmium was occluded when calcium carbonate precipitated.

Hart (1975) and Hart and Scaife (1977) reported that cadmium accumulation was regulated by pH as well as the concentration of manganese and possibly of iron in the culture medium. Additionally, research has indicated that adsorption of cadmium on the sides of Pyrex test containers could be a problem in toxicity studies (Gardiner, 1974b).

Mercury

Mercury is an extremely toxic element which is distributed throughout the environment (National Academy of Sciences, 1972). The toxic effects of mercury have been reviewed by Cook (1977), Fishbein (1974) and Friberg and Vostal (1972). Mercury's very toxic nature to biological organisms may, in part, be due to its affinity for sulfhydryl and disulfide groups of proteins (Vallee and Ulmer, 1972). Kuramitsu (1968) suggested that the effect of mercury on enzymatic activity was the result of various structural changes produced following mercaptide formation with different -SH groups of the enzyme. Mercury, like cadmium, displays the ability to compete with other biochemically important cations and can bind with the phosphate groups of structures such as DNA (Eichhorn et al., 1970).

The chemical species of mercury present at any given time is dependent upon the biota present and the physicochemical conditions within the freshwater ecosystem (National Academy of Sciences, 1972). Mercury can exist in the natural environment in contact with water in three oxidation states: elemental, mercurous, and mercuric (Gavis and Ferguson, 1972). These investigators reported that Hg(II) as $Hg(OH)_2$ and $HgCl_2$ were the dominant inorganic forms of mercury in surface waters because

surface waters exhibit Eh potentials near 0.5 V unless the oxygen concentration becomes very low. Investigations have shown that both CH_3Hg^+ and $(CH_3)_2Hg$ are synthesized from inorganic mercury in solution by bacteria living in the sediments. The environmental chemistry of mercury has been discussed extensively (D'Itri, 1975; D'Itri et al., 1971; Fagerström and Jernelöv, 1972; Gavis and Ferguson, 1972; Gilmour, 1971; Greesen, 1970; Hem, 1970; Holm and Cox, 1974; Jenne, 1970; Jernelöv, 1973; Land et al., 1973; Langley, 1973; Lockwood and Chen, 1974; Zepp et al., 1974).

Investigators have reported the inhibition of a variety of biological responses on exposure of numerous algal species to a wide range of mercury concentrations. Ben-Bassat et al. (1972) reported that the growth of Chlamydomonas reinhardi y^{-1} was completely retarded by 2 mg L^{-1} Hg(II) as $HgCl_2$, with concentrations from 0.0 to 1.0 mg L^{-1} causing an increased lag period. den Dooren de Jong (1965) found that the highest concentration of Hg(II) as $HgCl_2$ tolerated without effect by Chlorella vulgaris was 0.018 mg L^{-1}, with the lowest concentration preventing growth being 0.037 mg L^{-1}. Utilizing 6000-liter experimental chambers in Lake Powell, Arizona, Blinn et al. (1977) reported that a treatment with more than 1.0 mg L^{-1} Hg(II) as $HgCl_2$ resulted in less than 15% of the photosynthetic activity of control populations. At least a 40% reduction in photosynthetic activity occurred at Hg(II) concentrations as low as 0.06 mg L^{-1}. Hannan and Patouillet (1972) reported 0.1 and 1.0 mg L^{-1} Hg(II) as $HgCl_2$ to be partially and totally inhibitory, respectively, to the marine alga, Phaeodactylum tricornutum. Cyclotella nana and Chaetoceros galvestonensis, marine algae, and the freshwater alga Chorella pyrenoidosa were totally

inhibited by 0.1 mg L^{-1} Hg(II) as $HgCl_2$. They indicated $(CH_3)_2Hg$ was less toxic than $HgCl_2$. The gross photosynthetic rate of Coelastrum microporum was slightly increased by 0.0008 mg L^{-1} CH_3HgCl and drastically reduced at levels above 0.006 mg L^{-1} (Holderness et al., 1975). Photosynthesis and growth of the marine diatom Nitzschia delicatissima and several natural phytoplankton communities from Florida lakes were significantly reduced by organomercurial fungicides at 0.001 mg L^{-1} (Harriss et al., 1970). Hutchinson and Stokes (1975) reported that total inhibition of Chlorella vulgaris growth occurred at 0.2 mg L^{-1} inorganic Hg(II). Matson et al. (1972) reported that 1.0 mg L^{-1} Hg(II) as $HgCl_2$ resulted in significant inhibition of chlorophyll and galactolipid biosynthesis in Ankistrodesmus braunii and, to a slightly lesser degree, in Euglena gracilis. A more toxic response was noted for CH_3HgCl. Nuzzi (1972) reported Hg(II) as phenylmercuric acetate ($C_6H_5HgO_2C_2H_3$) to be inhibitory to Phaeodactylum tricornutum, Chlorella sp., and Chlamydomonas sp. as low as 0.06 µg L^{-1}. He found complete inhibition occurred with less than 25 µg L^{-1} Hg(II) as $HgCl_2$, which was less inhibitory than phenylmercuric acetate. Richardson et al. (1975) noticed a gradual delay in the release of Pediastrum boryanum zoospores and a reduction in the number of colonies produced when the Hg(II) concentration as $HgCl_2$ was increased from 0.2 to 1.0 mg L^{-1}. Rosko and Rachlin (1977) reported that cell division of Chlorella vulgaris was inhibited 50% by 1.03 ± 0.03 mg L^{-1} Hg(II) as $HgCl_2$. Tompkins and Blinn (1976) reported that total inhibition of Fragilaria crotonensis and Asterionella formosa occurred at 0.1 and 0.5 mg L^{-1} Hg(II) as $HgCl_2$, respectively. Ethyl mercuric phosphate ($C_2H_5HgPO_4$) was very toxic to marine

phytoplankton, causing 100% inhibition in Protococcus sp., Chlorella sp., and Monochrysis lutheri at 0.006 mg L^{-1} Hg(II) (Ukeles, 1962). Dunaliella euchlora and Phaeodactylum tricornutum were inhibited 100% at 0.06 mg L^{-1} Hg(II). Zingmark and Miller (1975) reported that 0.001 mg L^{-1} Hg(II) as $HgCl_2$ inhibited growth of Amphidinium carterae, a marine alga, by 50%.

De Filippis and Pallaghy (1976a,b,c) reported the sublethal effects of mercury as $HgCl_2$ or phenylmercuric acetate on Chlorella. Barber et al. (1973) reported on aspects of uptake of Hg(II) as $HgCl_2$ by Chlorella pyrenoidosa, and Burkett (1975) investigated the uptake and release of methylmercury by Cladophora glomerata. Sheih and Barber (1973) reported on uptake of mercury by Chlorella and its effect on potassium regulation. Fujita et al. (1977) reported on the intracellular distribution of mercury in Synedra, a freshwater diatom.

Several complicating factors have been encountered in determining the effect of mercury on algal species which make the interpretation and comparison of results difficult. Harriss et al. (1970), Kamp-Nielsen (1971), and Ben-Bassat and Mayer (1975) noted that the initial inoculum level is of importance when assessing the toxic effect of mercury compounds. Also, the toxicity of mercury compounds has been reported to vary inversely with the ionic strength or nutrient concentration of the growth medium (Fujita and Hashizume, 1972; Hannan and Patouillet, 1972). Experimental evidence has indicated that the reduction of the initial treatment level through volatilization is a serious problem when studying the effect of mercury compounds on algae (Ben-Bassat et al., 1972; Ben-Bassat and Mayer, 1975,

1977; Knowles and Zingmark, 1975; Zingmark and Miller, 1975). Filip and Lynn (1972), Fujita and Hashizume (1975), and Glooschenko (1969) reported that adsorption of mercury compounds on the walls of glassware was a complicating factor in toxicity studies. Polycarbonate flasks have been used to reduce adsorption of mercury compounds in toxicity tests (Tompkins and Blinn, 1976). Additionally, Zingmark (1975) found that mercury complexes accumulated in the particulate fraction of his culture medium when he investigated the toxicity of $HgCl_2$.

Selenium

A detailed review of selenium in the environment and its toxic effects has been provided by Whanger (1974). It has been hypothesized that selenium is toxic because it is assimilated into proteins, replaces sulfur at critical places in the polypeptide chain, and gives rise to altered, malfunctioning enzymes (Shrift, 1972). Selenium can replace sulfur in critical sulfur amino acids producing selenocystathionine, selenomethionine, selenocysteine, and methylselenocysteine (Hewitt and Smith, 1975). Selenate can be activated by the same ATP sulfurylase which is used to reduce SO_4^{-2} to S^{-2} (Krogmann, 1973).

Elemental selenium is highly insoluble and requires oxidation to transform it to the selenite or selenate state before appreciable quantities appear in solution. The relative abundances of selenite or selenate are pH dependent. Alkaline environments favor the formation of selenate. Chau et al. (1976) reported methylation of selenium in aquatic environments.

Several investigators have reported the toxic effects of selenium on various species of algae under a variety of environmental conditions. den Dooren de Jong (1965) indicated that the highest concentration of Se(IV) as Na_2SeO_3 tolerated without effect by Chlorella vulgaris was 5.8 mg L^{-1}, while the lowest concentration preventing growth was 12 mg L^{-1}. A Se(VI) concentration of 20 mg L^{-1} as Na_2SeO_4 was lethal to a strain of Anacystis nidulans (Kumar, 1964). Kumar and Prakash (1971) reported the highest growth-permitting concentration of Se(VI) as Na_2SeO_4 was 70 mg L^{-1} for Anacystis nidulans, with Anabaena variabilis being two to three times more sensitive. They found the highest growth-permitting concentrations of Se(IV) as $Na_2SeO_3 \cdot 5H_2O$ were 175 and 65 mg L^{-1}, respectively, for Anacystis nidulans and Anabaena variabilis. Sielicki and Burnham (1973) reported that 0.79 mg L^{-1} Se (IV) as $Na_2SeO_3 \cdot 5H_2O$ inhibited chlorophyll production in Phormidium luridum. Chlorophyll production was not inhibited by 0.079 mg L^{-1}. Sandholm et al. (1973) found that Scenedesmus dimorphus actively concentrated ^{75}Se-selenomethionine but not Se(IV) from $Na_2SeO_3 \cdot 5H_2O$. Kumar and Prakash (1971) reported the highest growth-permitting concentrations of Se(IV) as $Na_2SeO_3 \cdot 5H_2O$ in sulfur-containing and sulfur-free media were 175 and 50 mg L^{-1}, respectively, for Anacystis nidulans and 65 and 50 mg L^{-1}, respectively, for Anabaena variabilis. Shrift (1954a) found the growth response remained the same with any particular ratio of sulfate to selenate from 10 to 360 mg L^{-1} Se(VI) as K_2SeO_4. Shrift (1954b) reported on the sulfur-selenium antagonism with the antimetabolite action of selenomethionine on the growth of Chlorella vulgaris.

Algal species have displayed the ability to adapt to selenium. Kumar (1964) reported that 20 mg L^{-1} Se(VI) as $NaSeO_4$ was initially completely toxic to Anacystis nidulans, but after repeated exposure the alga tolerated 150 mg L^{-1}. Stability and reversibility of mass adaptation to selenomethionine in populations of Chlorella vulgaris was indicated by Shrift et al. (1961a,b).

MATERIALS AND METHODS

The Algal Assay Procedure Bottle Test (AAPBT) (U.S. EPA, 1971) was adapted to evaluate the effect of As(V), Cd(II), Hg(II), and Se(VI), in solution, and of scrubber ash slurry from the Colstrip coal-fired power generating plant on selected algal species. The algae used in these investigations were the freshwater chlorophytes Ankistrodesmus falcatus (Corda) Ralfs, Scenedemus obliquus (Turp.) Kütz., and Selenastrum capricornutum Printz, and the freshwater cyanophyte Microcoleus vaginatus (Vauch.) Gom. (per Drouet, 1968). These species will be referred to after this only by generic name.

Ankistrodesmus, Scenedesmus, and Microcoleus were isolated from phytoplankton collections from stock ponds in Rosebud County, southeastern Montana (Sec20, T3N, R42E; Sec8, T1S, R43E; and Sec22, T3N, R39E, respectively). The unialgal, bacteria-free cultures of Ankistrodesmus and Scenedesmus were obtained by repeated streaking of isolated colonies on petri plates containing 1.5% Bacto-Agar (Difco) in Algal Assay Medium (AAM) (Table 1) (U.S. EPA, 1971). Stock cultures of Ankistrodesmus and Scenedesmus were maintained in AAM. Microcoleus was isolated using similar streaking techniques but substituting Gorham's nutrient medium (Table 2) (Hughes et al., 1958) for AAM. Stock cultures of Microcoleus were grown in Gorham's medium because Microcoleus could not be maintained in AAM. The unialgal Microcoleus was not bacteria-free. An axenic culture of Selenastrum was obtained from the U.S. EPA and maintained in AAM. The axenic cultures were checked for bacteria with each transfer using Difco Tryptone Glucose Extract Agar (TGEA).

Table 1. Chemical composition of algal assay medium (AAM) (U.S. EPA, 1971)

Macronutrients

Compound	Concentration (mg L^{-1})	Nutrient	Concentration (mg L^{-1})
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
		K	0.469
$MgCl_2$	5.700	Mg	2.904
$MgSO_4 \cdot 7H_2O$	14.700	S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	Ca	1.202
$NaHCO_3$	15.000		

Micronutrients

Compound	Concentration (µg L^{-1})	Nutrient	Concentration (µg L^{-1})
H_3BO_3	185.520	B	32.460
$MnCl_2$	264.264	Mn	115.374
$ZnCl_2$	32.709	Zn	15.691
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$FeCl_3$	96.000	Fe	33.051
$Na_2EDTA \cdot 2H_2O$	300.000		

Table 2. Chemical composition of Gorham's nutrient medium (Hughes et al., 1958)

Compound	Concentration (mg L^{-1})	Nutrient	Concentration (mg L^{-1})
$NaNO_3$	496	N	82
K_2HPO_4	39	P	7
		K	17
$MgSO_4 \cdot 7H_2O$	75	Mg	7
$CaCl_2 \cdot 7H_2O$	36	Ca	10
Fe citrate	6	Fe	1
$Na_2SiO_3 \cdot 9H_2O$	58	S	10
Na_2CO_3	20		
Citric acid	6		
EDTA	1		

Laboratory toxicity testing procedures included cleaning labware as outlined by the American Public Health Association (1975). Polycarbonate flasks and all glassware in contact with trace element solutions or scrubber ash slurry extract were rinsed with 10% HNO_3 solutions. All other glassware was rinsed with recommended 10% HCl solutions. Toxic waste solutions were filtered and passed through a water purification system before disposal. The toxic solid wastes were disposed of by the Department of Environmental Health and Safety, Iowa State University.

Similar test conditions were imposed for all toxicity tests. The culture media used for toxicity tests were the same as those used for stock cultures. The chelator, EDTA, was omitted from both culture media,

although small quantities were introduced with the algal inoculums, and the micronutrients were omitted from AAM. Chemical precipitates remaining in the culture media after autoclaving were dissolved by the addition of 1N HCl.

The test elements were introduced as analytical reagent grade salts of $Na_2HAsO_4 \cdot 7H_2O$, $CdSO_4$, $HgSO_4$, and Na_2SeO_4. Stock solutions containing 1 g L^{-1} element were prepared using deionized water. The stock solutions were acidified with HCl to pH 1.5. The appropriate range of test concentrations was obtained by diluting the stock solutions aseptically with sterile culture media. Element levels of 0 (control), 0.01, 0.1, 1, 10, and 100 mg L^{-1} were used in the initial toxicity screenings. Treatment levels of subsequent experiments were dependent on initial toxicity screening results. The pH of each test culture solution was adjusted to 7.0 ± 0.3 with NaOH or HCl solutions prior to inoculation with the test alga.

The scrubber ash slurry toxicity tests were conducted using an extract of the scrubber ash slurry obtained from the settling pond for Units 1 and 2 of the coal-fired generating plant located at Colstrip, Montana. The extract was prepared by filtering the slurry (10% solids) through 0.45 µm Millipore filters, mixing the filtrate with the appropriate algal medium, and filter-sterilizing through 0.22 µm Millipore filters. The toxicity tests were conducted using percentages of algal medium/slurry extract stock solutions in the appropriate algal medium. The pH of the test solution was allowed to vary as a function of the extract concentration.

The scrubber ash slurry extract (SASE), without nutrients, was analyzed for pH, specific conductance, alkalinity, and sulfate (American Public Health Association, 1975). Total phosphate, ammonia nitrogen, nitrate-nitrite nitrogen, total organic carbon, and silica analyses were performed by the Analytical Services Laboratory, Engineering Research Institute, Iowa State University using techniques outlined by the American Public Health Association (1975). Analyses for selected elements were made using inductively coupled plasma-atomic emission spectroscopy by Ames Laboratory, Department of Energy, Iowa State University.

All treatment levels of the trace elements and SASE were run in triplicate for each experiment. Test flasks were inoculated from 10- to 14-day axenic cultures of Ankistrodesmus, Scenedesmus, or Selenastrum to give a final cell concentration of 1×10^4 cells ml^{-1} ± 10%. The Microcoleus inoculum, of the same age, was 1 ml of a stock culture reading 30% transmission at 450 nm on a Hach DR-EL2 spectrophotometer. All test cultures were incubated in 500-ml polycarbonate flasks containing 100 ml of test culture solution. The cultures were incubated at 24 ± 2°C under 400 ft-c ± 10% mixed illumination ("cool white" fluorescent and incandescent), measured adjacent to the flask at the liquid level, on a 16:8 hour light: dark regime.

After two weeks the algae were harvested and analyzed for chlorophyll a (Trichromatic Method) as outlined by the American Public Health Association (1975). The extracted chlorophyll was read on a modified Gilford spectrophotometer, with a 1-cm light path. Percent of control (response) values were calculated using chlorophyll a values. The chlorophyll a

detection limit of the algal assay method was approximately the same as the chlorophyll a inoculation level. Therefore, either an algistatic or algicidal response could be indicated by a chlorophyll a value of zero.

A standard two-tailed t-test

$$t = \frac{(y - 100\%)}{\sqrt{\frac{S^2}{n}}}$$

was used to test for significant differences in response, where y, S^2, and n are equal to the % of control mean, variance of the % control values, and number of observations, respectively.

Concentrations of the toxic substances which produced 50% of the control responses, median effective concentrations (EC50 values), were predicted from linear regression models. The regression models were estimated using the least squares criterion. For each experimental combination, those treatment levels were selected for regression analysis whose percent of control values approached a straight line when transformed using $\log_{10}$. An average standard error was estimated for each EC50 value using

$$\text{average standard error} = \frac{\left[\text{standard error of estimate of intercept}\right]^2}{(\text{slope})^2} + \frac{\left[\text{standard error of estimate of slope}\right]^2}{(\text{slope})^4}$$

The statistical analysis of all data was completed using the Statistical Analysis System (Barr et al., 1976).

The test algae were exposed to their respective median effective concentrations of Cd(II) using the test procedures previously outlined to check the validity of this methodology.

Water quality criteria based on maximum allowable toxicant concentrations (MATC) of As(V), Cd(II), Hg(II), Se(VI), and SASE for the algal species indigenous to southeastern Montana and Selenastrum were calculated, as outlined by the American Public Health Association (1975).

According to the American Public Health Association (1975), the water quality criterion for a given region is based on the safe concentration (SC) for the important local species, fauna or flora, that are most sensitive to the material or waste under consideration, and is calculated as follows: SC = application factor (AF) × incipient median lethal concentration (LC50). The SC is the maximum concentration of a toxicant that has no observable harmful effects after long-term exposure over one or more generations in the waters of the region. When water other than the actual receiving water must be used in the toxicity tests and where the most sensitive important species in the biota for the toxicant under consideration is not known definitely, as in these investigations, the maximum allowable toxicant concentration (MATC) can be substituted for the SC according to the American Public Health Association. Also, in these investigations EC50 values were substituted for LC50 values. Thus, the MATC was calculated as follows: MATC = AF × EC50. An AF was derived for each test substance from the toxicant-alga combination providing the best MATC from the dose-response relationship developed for each pollutant by substituting the MATC value and the EC50 value into the equation and solving for AF. The AF value for each test substance was then used with the appropriate EC50 values for the other algal dose-response relationships to calculate MATC values for each test substance.

RESULTS

The growth responses (% of control values) of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus on exposure to As(V), Cd(II), Hg(II), Se(VI), and SASE are presented in Tables 3-7, respectively. Selenastrum is increasingly inhibited with an increasing As(V) concentration between 10 and 75 mg L^{-1}. A similar response was found for Ankistrodesmus and Scenedesmus, from 0.01 to 5 and 0.01 to 50 mg L^{-1}, respectively. The first treatment level to cause a statistically significant inhibitory response to Selenastrum was 25 mg L^{-1}. The first significant growth inhibition of Ankistrodesmus and Scenedesmus occurred at 0.1 and 0.01 mg L^{-1} As(V), respectively. Microcoleus was not significantly inhibited at any treatment level. In fact, a stimulatory response was noted at 75 mg L^{-1}. Algistatic-algicidal responses were noted for Ankistrodesmus and Scenedesmus at 5 and 50 mg L^{-1} As(V), respectively.

Cadmium(II) was extremely inhibitory, causing significant inhibition of Ankistrodesmus, Scenedesmus, and Microcoleus at 0.01 mg L^{-1} and Selenastrum at 0.05 mg L^{-1} (Table 4). All the algae responded algistatically-algicidally at or below 0.3 mg L^{-1}. A significant stimulatory response was noted for Ankistrodesmus with 0.001 mg L^{-1}.

The sensitivity of the algal species to Hg(II) was variable (Table 5). The first significant inhibition of Ankistrodesmus, Scenedesmus, Selenastrum and Microcoleus occurred at 0.05, 0.1, 0.01, and 0.4 mg L^{-1} Hg(II), respectively. Algistatic-algicidal responses for Ankistrodesmus, Scenedesmus, and Selenastrum were first noted at 0.4 mg L^{-1}. Microcoleus approached an algistatic response at 1.0 mg L^{-1}.

Table 3. Growth responses of Ankistrodesmus, Scenedesmus, Selenastrum and Microcoleus to As (V)

As(V)	Ankistrodesmus[a]		Scenedesmus[a]		Selenastrum[a]		Microcoleus[b]	
mg L^{-1}	n[c]	y±sd[d]	n	y±sd	n	y±sd	n	y±sd
0.01	3	61±18	5	58±20*	6	78±26	6	106±23
0.10	3	72±4*	6	48±15*	6	87±30	6	97±13
1.0	6	52±22*	6	26±10*	6	76±24	6	99±17
5.0	3	0	-[e]	-	-	-	-	-
10	6	0	5	4±5*	5	104±26	6	106±13
25	3	0	3	1±2*	3	50±18*	3	90±17
50	6	0	3	0	3	9±2*	3	78±26
75	6	0	3	0	3	4±10*	3	115±4*
100	6	0	3	0	6	14±15*	6	97±17

[a]Grown in AAM.

[b]Grown in Gorham's medium.

[c]Number of observations.

[d]% of control ± standard deviation.

[e]No data.

*Statistically significant (P = 0.05).

The sensitivity of the algal species to Se(VI) varied (Table 6). The first significant inhibition of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus occurred at 0.01, 0.1, 0.3, and 10 mg L^{-1} Se(VI), respectively. Microcoleus was stimulated by treatment levels of 1.0 mg L^{-1} and below. Algistatic-algicidal responses by Ankistrodesmus, Scenedesmus, and

Table 4. Growth responses of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus to Cd(II)

Cd(II) mg L^{-1}	Ankistrodesmus[a] n[c]	Ankistrodesmus[a] y±sd[d]	Scenedesmus[a] n	Scenedesmus[a] y±sd	Selenastrum[a] n	Selenastrum[a] y±sd	Microcoleus[b] n	Microcoleus[b] y±sd
0.001	3	156±13*	2	118±11	2	89±73	3	118±23
0.005	3	99±34	3	78±18	3	106±58	3	89±21
0.01	7	69±29*	8	8±12*	6	61±28	6	69±12*
0.05	6	8±5*	6	0	6	17±7*	6	3±3*
0.10	9	1±1*	9	0	8	7±5*	6	1±1*
0.20	3	0	3	0	3	2±1*	-[e]	-
0.30	6	0	6	0	6	0	3	0

[a]Grown in AAM.

[b]Grown in Gorham's medium.

[c]Number of observations.

[d]% of control ± standard deviation.

[e]No data.

*Statistically significant (P = 0.05).

Selenastrum appeared at 10, 4, and 1.7 mg L^{-1}, respectively. Microcoleus approached an algistatic response at 50 mg L^{-1}.

The first significant inhibition of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus occurred at 1, 5, 10, and 5% SASE, respectively (Table 7). Algistatic-algicidal responses for Ankistrodesmus, Scenedesmus, and Selenastrum were indicated at 50, 100, and 75% SASE. Microcoleus approached an algistatic response at 100% SASE.

Table 5. Growth responses of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus to Hg(II)

Hg(II) mg L^{-1}	Ankistrodesmus[a] n[c]	Ankistrodesmus[a] y±sd[d]	Scenedesmus[a] n	Scenedesmus[a] y±sd	Selenastrum[a] n	Selenastrum[a] y±sd	Microcoleus[b] n	Microcoleus[b] y±sd
0.001	3	91±28	6	150±78	3	99±12	-[e]	-
0.01	5	92±14	9	86±19	6	88±11*	6	99±10
0.05	3	70±11*	5	86±39	3	52±21	5	79±40
0.1	8	48±32*	8	55±46*	6	6±10*	6	102±12
0.4	3	0	6	0	3	0	5	42±22*
0.7	3	0	6	0	3	0	5	12±16*
1.0	9	0	9	0	9	0	5	1±2*

[a]Grown in AAM.

[b]Grown in Gorham's medium.

[c]Number of observations.

[d]% of control ± standard deviation.

[e]No data.

*Statistically significant (P = 0.05).

The chemical analysis of the SASE is presented in Table 8. The extract was high in sulfate, nitrogen (nitrate-nitrite), total phosphate, silica, calcium, magnesium, manganese, specific conductance, and alkalinity. Several potentially toxic trace elements were found to be present at low levels, however, interferences prevented analysis for the presence of mercury, molybdenum, chromium, lead, selenium, strontium, and vanadium.

The regression lines used for predicting EC50 values of As(V), Cd(II), Hg(II), Se(VI) and SASE are presented in Figures 1-5, respectively. No

Table 6. Growth responses of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus to Se(VI)

Se(VI)	Ankistrodemus[a]		Scenedesmus[a]		Selenastrum[a]		Microcoleus[b]	
mg L^{-1}	n[c]	y±sd[d]	n	y±sd	n	y±sd	n	y±sd
0.01	7	70±31*	3	236±129	3	89±8	3	118±1*
0.05	3	46±1*	6	152±70	-[e]	-	3	105±12
0.10	9	30±14*	15	48±32*	8	84±19	3	116±9
0.20	3	19±2*	3	58±15*	3	81±19	-	-
0.30	3	18±3*	3	21±5*	3	48±17*	-	-
0.40	3	10±4*	8	19±10*	6	20±14*	3	128±6*
0.50	3	13±3*	-	-	3	32±11*	-	-
0.60	3	10±2*	-	-	2	33±5*	-	-
0.70	3	9±2*	7	18±8*	6	16±8*	3	117±7*
0.80	2	12±2*	-	-	3	15±1*	-	-
0.90	3	11±3*	-	-	3	18±5*	-	-
1.0	8	10±10*	12	12±8*	9	5±6*	6	110±3*
1.4	3	12±0*	2	11±4*	3	2±3*	-	-
1.7	3	7±1*	3	8±3*	3	0	-	-
2.0	3	8±3*	2	8±8*	3	0	-	-
4.0	-	-	6	0	-	-	-	-
5.0	-	-	-	-	-	-	3	73±11
10	3	0	-	-	3	0	3	50±6*
20	-	-	-	-	-	-	2	16±4*
30	-	-	-	-	-	-	3	6±2*
40	-	-	-	-	-	-	3	4±3*
50	-	-	-	-	-	-	3	5±2*

[a] Grown in AAM.

[b] Grown in Gorham's medium.

[c] Number of observations.

[d] % of control ± standard deviation.

[e] No data.

* Statistically significant (P = 0.05).

Table 7. Growth responses of Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus to SASE

	Ankistrodesmus[a]		Scenedesmus[a]		Selenastrum[a]		Microcoleus[b]	
% SASE	n[c]	y±sd[d]	n	y±sd	n	y±sd	n	y±sd
0.01	6	98±32	5	103±26	4	98±42	5	106±15
0.1	6	72±42	5	72±26	6	88±31	5	103±6
1.0	6	72±18*	5	115±61	4	135±66	6	98±15
5	6	57±17*	5	65±9*	6	98±42	5	19±4*
10	6	61±15*	5	62±17*	6	61±20*	6	8±3*
25	6	4±3*	6	5±3*	6	40±10*	6	7±1*
50	5	0	6	1±1*	6	2±3*	6	4±4*
75	6	0	6	2±2*	6	0	6	1±2*
100	6	0	6	0	6	0	6	1±1*

[a]Grown in AAM.

[b]Grown in Gorham's medium.

[c]Number of observations.

[d]% of control ± standard deviation.

*Statistically significant (P = 0.05).

regression line or model is presented for the As(V)-Microcoleus combination because Microcoleus was not significantly inhibited at the highesc treatment level. Log transformations were required because the absolute increase in concentration of a substance is not nearly as proportional to its effect on the algal population as is the percentage increase. The number of experimental runs ranged from 2 to 5 for each experimental combination and was dictated by success in selecting treatment levels and

Table 8. Chemical analysis of SASE

Parameter	Concentration[a]	Parameter	Concentration
pH	7.3	Ba	0.044
Specific conductance	9,750	Be	0.002
Alkalinity[b]	248	Ca[c]	360
$SO_4^{=}$	10,162	Cd	0.042
C-total organic	1	Co	0.321
NH_3-N	0.40	Cu	0.002
NO_2^-+NO_3^--N	21.4	Fe	0.067
$PO_4^{\equiv}$-total	1.6	Mg	>100
Si	91.0	Mn[d]	47
Al	0.557	Ni	0.302
As	0.251	Zn	0.121

[a] µmhos/cm for specific conductance and mg L^{-1} for all other parameters except pH.

[b] pH 4.5 as $CaCO_3$.

[c] Estimated.

[d] Approximation.

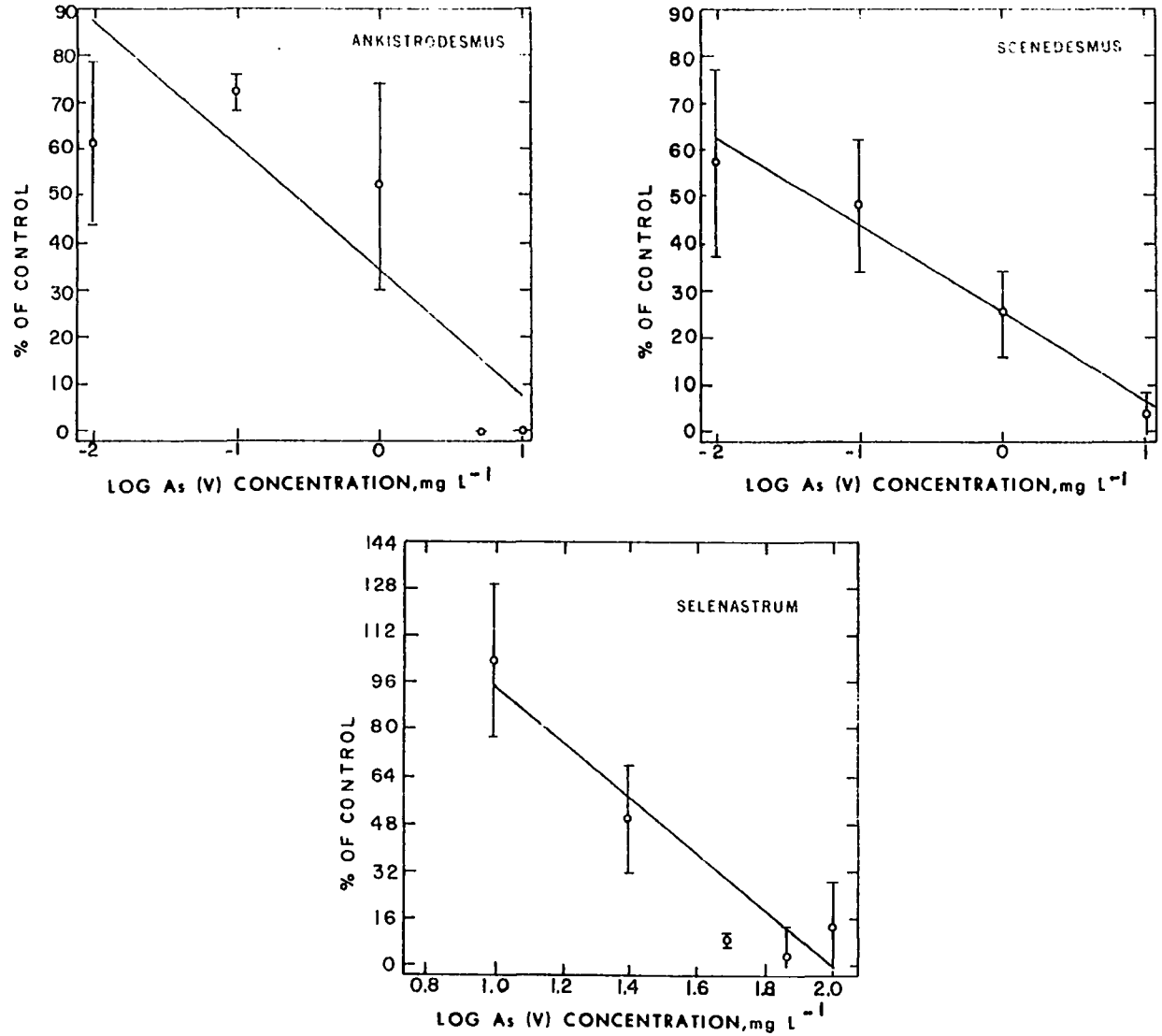

Figure 1. Regression lines of algae exposed to As(V), showing means with standard deviations.

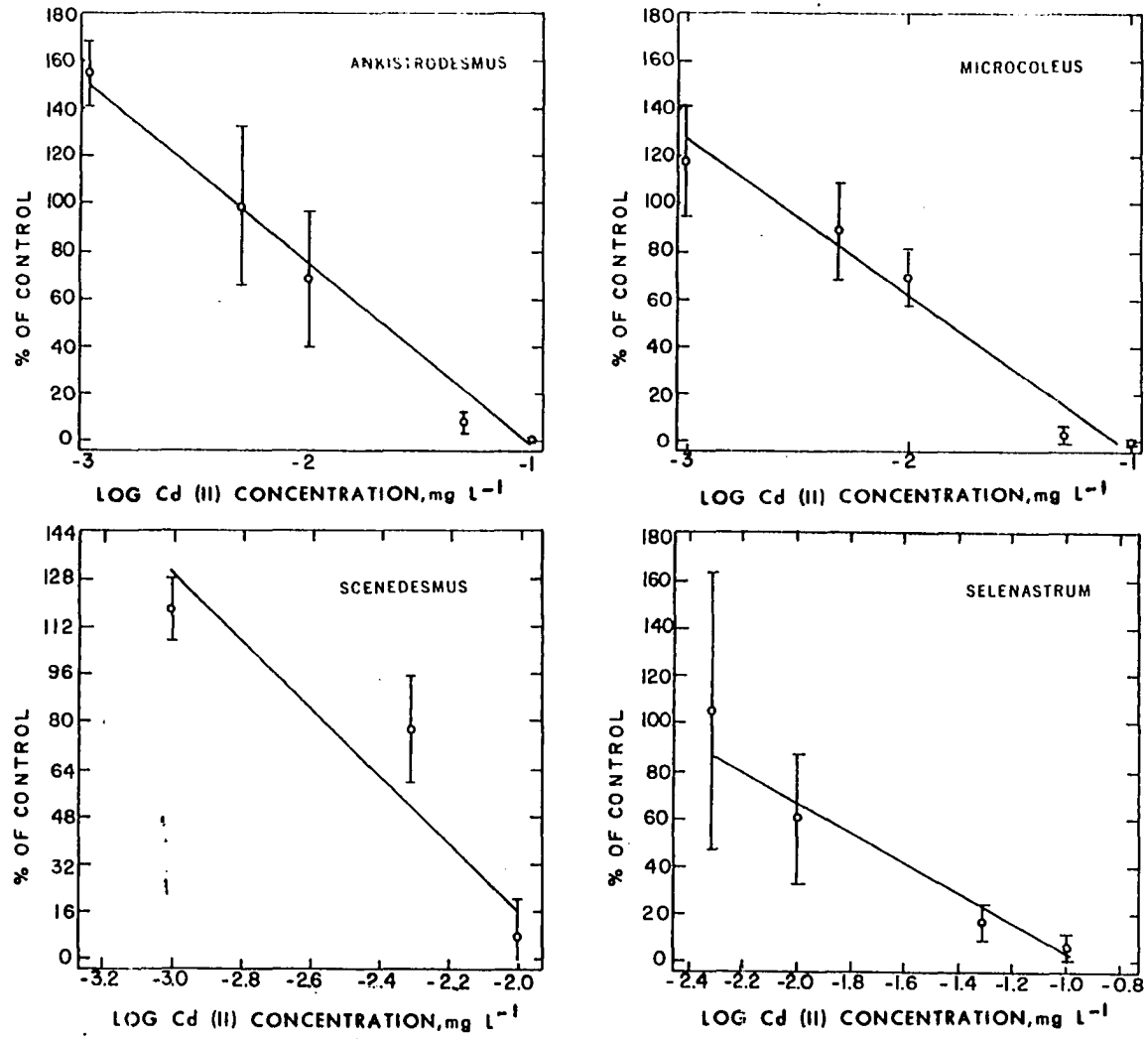

Figure 2. Regression lines of algae exposed to Cd(II), showing means with standard deviations.

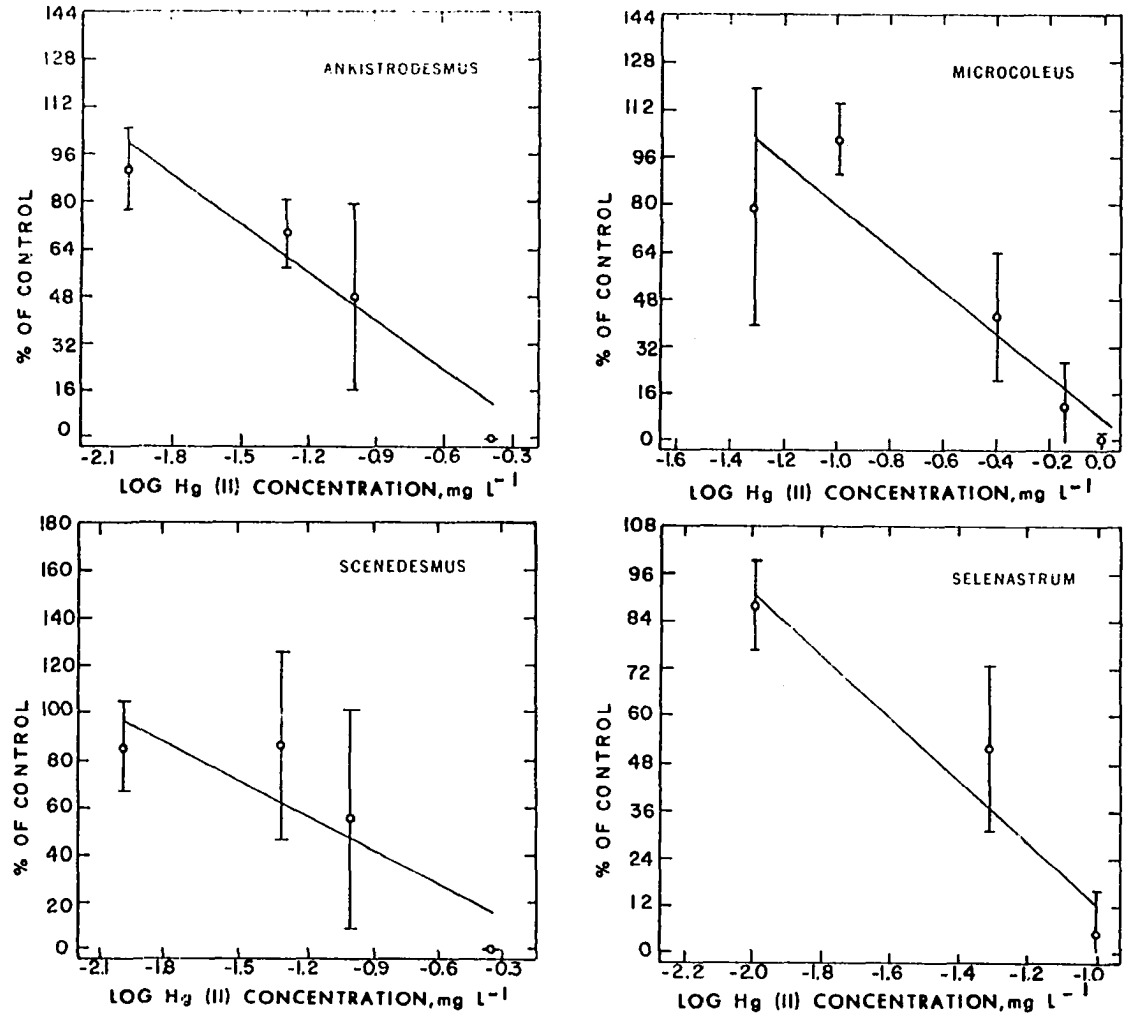

Figure 3. Regression lines of algae exposed to Hg(II), showing means with standard deviations.

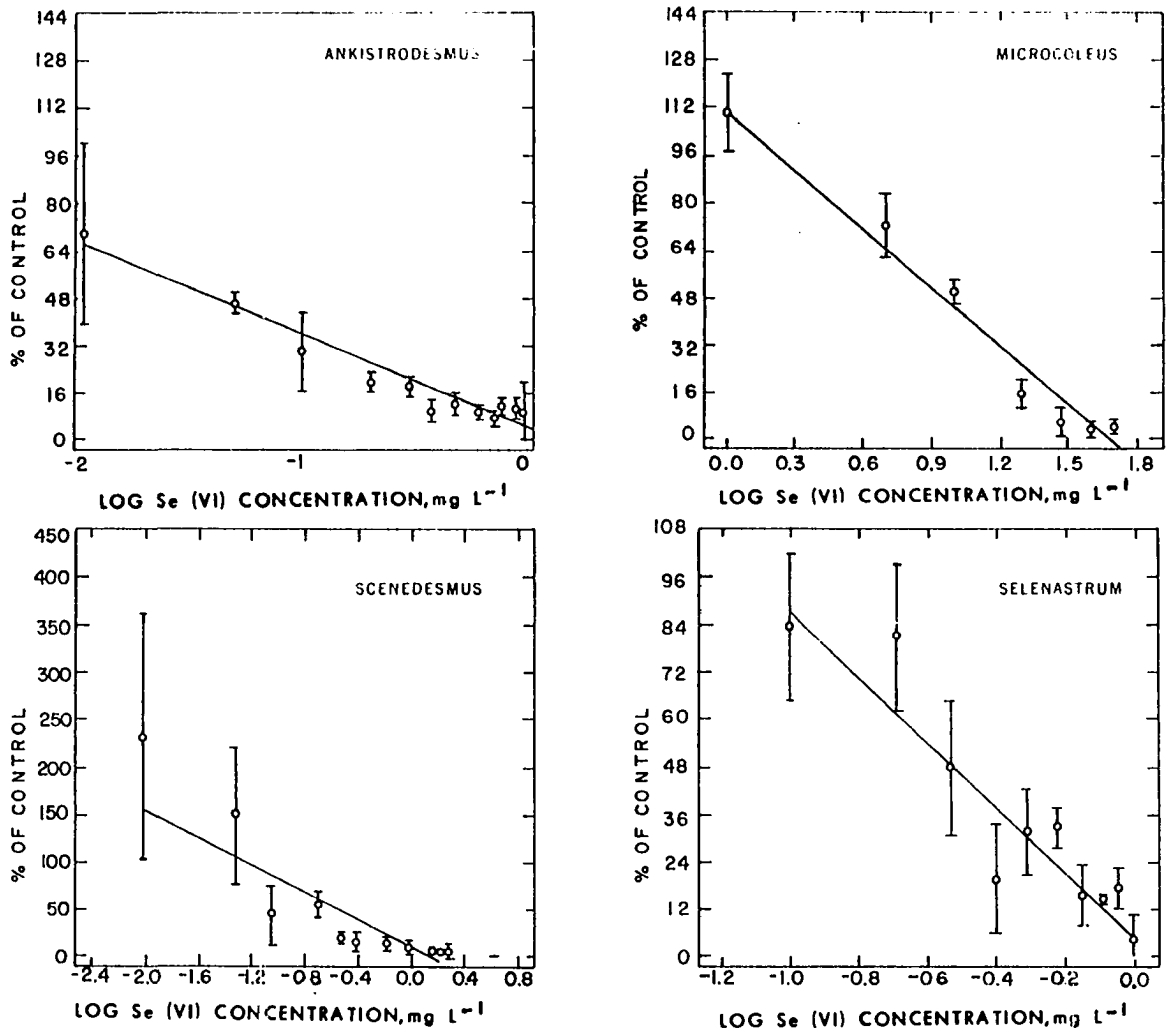

Figure 4. Regression lines of algae exposed to Se(VI), showing means with standard deviations.

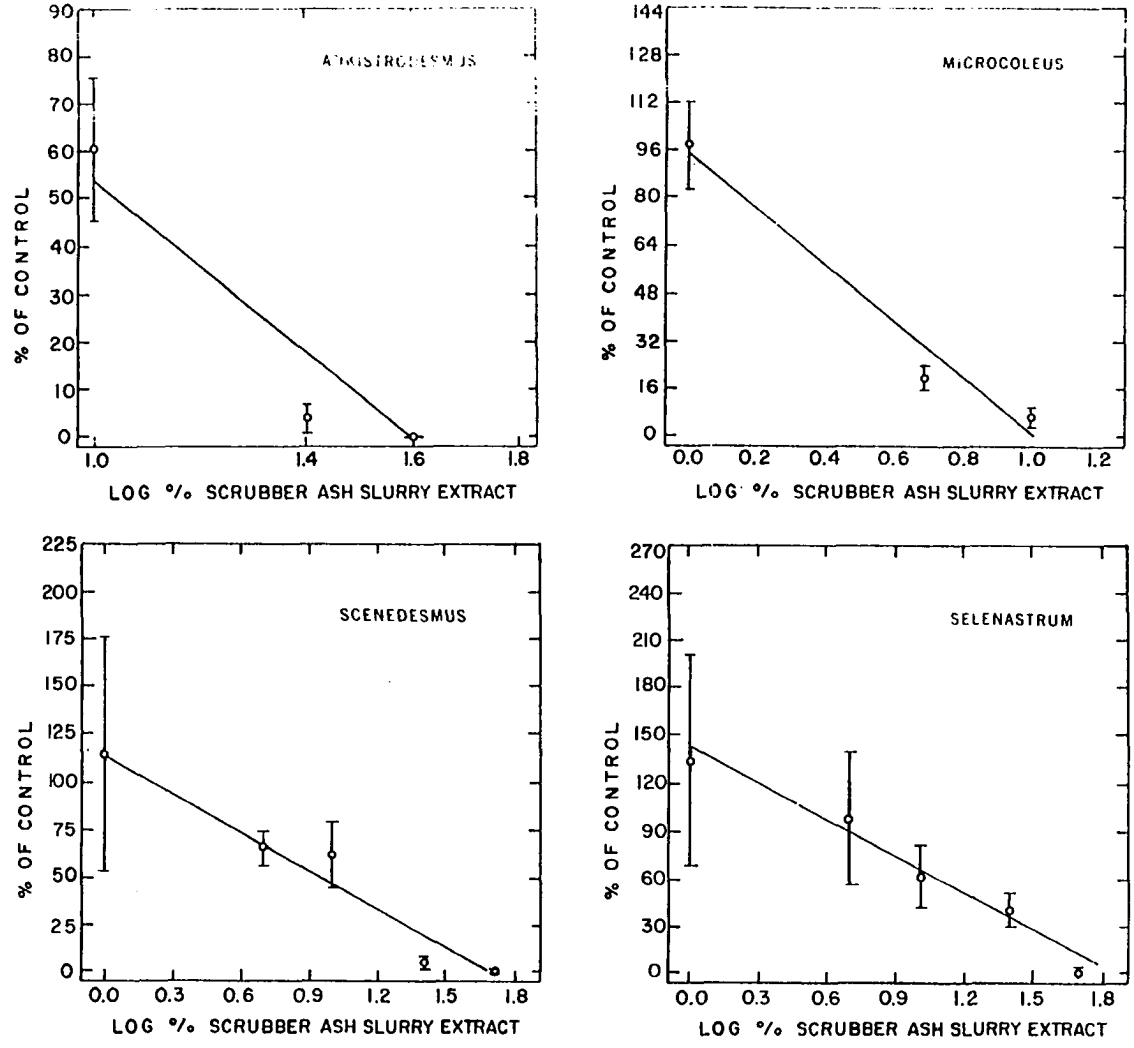

Figure 5. Regression lines of algae exposed to scrubber ash slurry extract, showing means with standard deviations.

agreement of results between experimental runs. All the final regression models have coefficient of determination (R^2) values above 0.60 except Hg(II)-Scenedesmus and Se(VI)-Scenedesmus (Table 9). R^2 equals the percentage of the corrected sum of squares which is explained by the fitting of the simple linear regression model.

The EC50 values for the test algae with average standard errors of the trace elements and SASE are presented in Table 10. The EC50 values ranged from 0.048-30.761 mg L^{-1} As(V), 0.005-0.019 mg L^{-1} Cd(II), 0.033-0.253 mg L^{-1} Hg(II), 0.033-8.511 mg L^{-1} Se(VI), and 3.048-15.417% SASE. The test algae with the lowest EC50 values were Scenedesmus for As(V) and Cd(II), Selenastrum for Hg(II), Ankistrodesmus for Se(VI), and Microcoleus for SASE.

The following % of control values were recorded when Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus were exposed to the median effective concentrations of Cd(II) in triplicate, 59 ± 32, 32 ± 11, 36 ± 30, and 46 ± 25, respectively. A mean value for all the test algae of 43 ± 25 was recorded.

Figures 6-10 display graphically the dose-response relationships of the algae exposed to As(V), Cd(II), Hg(II), Se(VI), and SASE, respectively. A greater interspecies variation is indicated for the algae when exposed to Se(VI) and As(V) than when exposed to Hg(II), Cd(II), and SASE. Additionally, the extremely toxic nature of Cd(II) and Hg(II) can be seen by the toxicant-alga dose-response relationships.

The MATC values for the test algae exposed to the trace elements and SASE are presented in Table 11. Also presented is the AF for each potential toxicant which provided the best MATC estimate direction from the dose-

Table 9. Regression equations of algal responses to selected trace element or SASE treatment levels

Test substance	Alga	Regression equation	R^2
As	Ankistrodesmus	$Y = -26.963(\log_{10}x) + 34.053$	0.64
	Scenedesmus	$Y = -18.700(\log_{10}x) + 25.251$	0.70
	Selenastrum	$Y = -96.029(\log_{10}x) + 192.868$	0.79
Cd	Ankistrodesmus	$Y = -78.401(\log_{10}x) - 84.306$	0.89
	Scenedesmus	$Y = -115.248(\log_{10}x) - 215.247$	0.82
	Selenastrum	$Y = -65.792(\log_{10}x) - 63.788$	0.64
	Microcoleus	$Y = -65.747(\log_{10}x) - 70.207$	0.91
Hg	Ankistrodesmus	$Y = -54.274(\log_{10}x) - 10.201$	0.63
	Scenedesmus	$Y = -50.592(\log_{10}x) - 4.257$	0.46
	Selenastrum	$Y = -78.388(\log_{10}x) - 66.496$	0.86
	Microcoleus	$Y = -72.355(\log_{10}x) + 6.822$	0.67
Se	Ankistrodesmus	$Y = -30.462(\log_{10}x) + 4.754$	0.69
	Scenedesmus	$Y = -73.596(\log_{10}x) + 10.811$	0.53
	Selenastrum	$Y = -79.893(\log_{10}x) + 5.465$	0.80
	Microcoleus	$Y = -66.669(\log_{10}x) + 112.036$	0.96
SASE	Ankistrodesmus	$Y = -92.339(\log_{10}x) + 147.314$	0.79
	Scenedesmus	$Y = -70.753(\log_{10}x) + 116.941$	0.71
	Selenastrum	$Y = -78.803(\log_{10}x) + 143.654$	0.67
	Microcoleus	$Y = -93.000(\log_{10}x) + 94.993$	0.93

Table 10. EC50 values[a] with average standard errors of the trace elements and SASE for the test algae

Test substance	Alga	Log EC50	Log average standard error	EC50
As	Ankistrodesmus	-0.592	0.167	0.256
	Scenedesmus	-1.323	0.173	0.048
	Selenastrum	1.488	0.200	30.761
Cd	Ankistrodesmus	-1.713	0.122	0.019
	Scenedesmus	-2.302	0.318	0.005
	Selenastrum	-1.729	0.253	0.019
	Microcoleus	-1.828	0.126	0.015
Hg	Ankistrodesmus	-1.109	0.245	0.078
	Scenedesmus	-1.072	0.239	0.085
	Selenastrum	-1.486	0.170	0.033
	Microcoleus	-0.597	0.110	0.253
Se	Ankistrodesmus	-1.485	0.095	0.033
	Scenedesmus	-0.532	0.089	0.294
	Selenastrum	-0.557	0.032	0.277
	Microcoleus	0.930	0.055	8.511
SASE	Ankistrodesmus	1.054	0.184	11.324
	Scenedesmus	0.946	0.148	8.831
	Selenastrum	1.188	0.161	15.417
	Microcoleus	0.484	0.045	3.048

[a] $mg\ L^{-1}$ trace elements and % SASE.

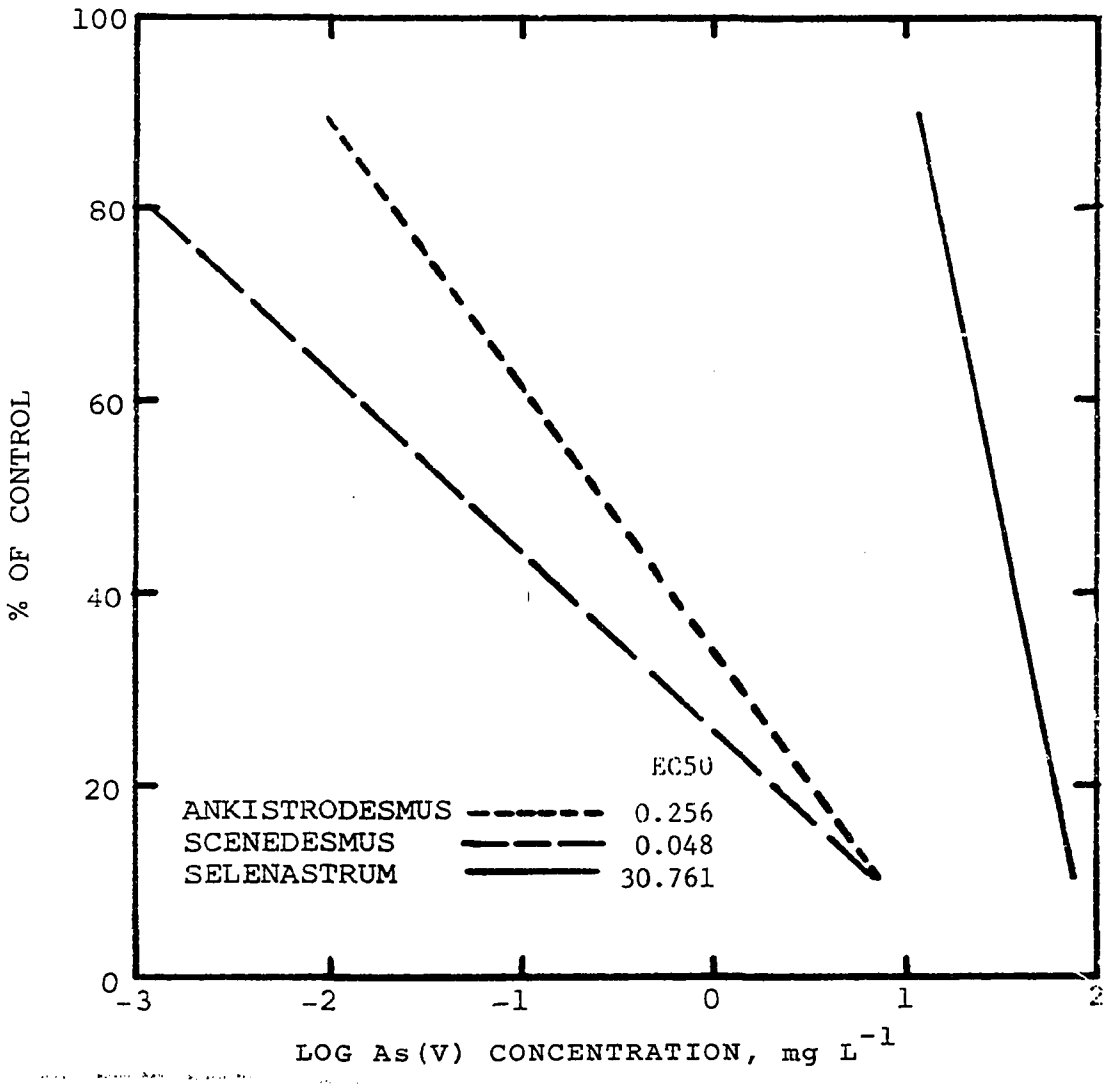

Figure 6. Regression lines of algae exposed to As(V), with EC50 values (mg L^{-1}).

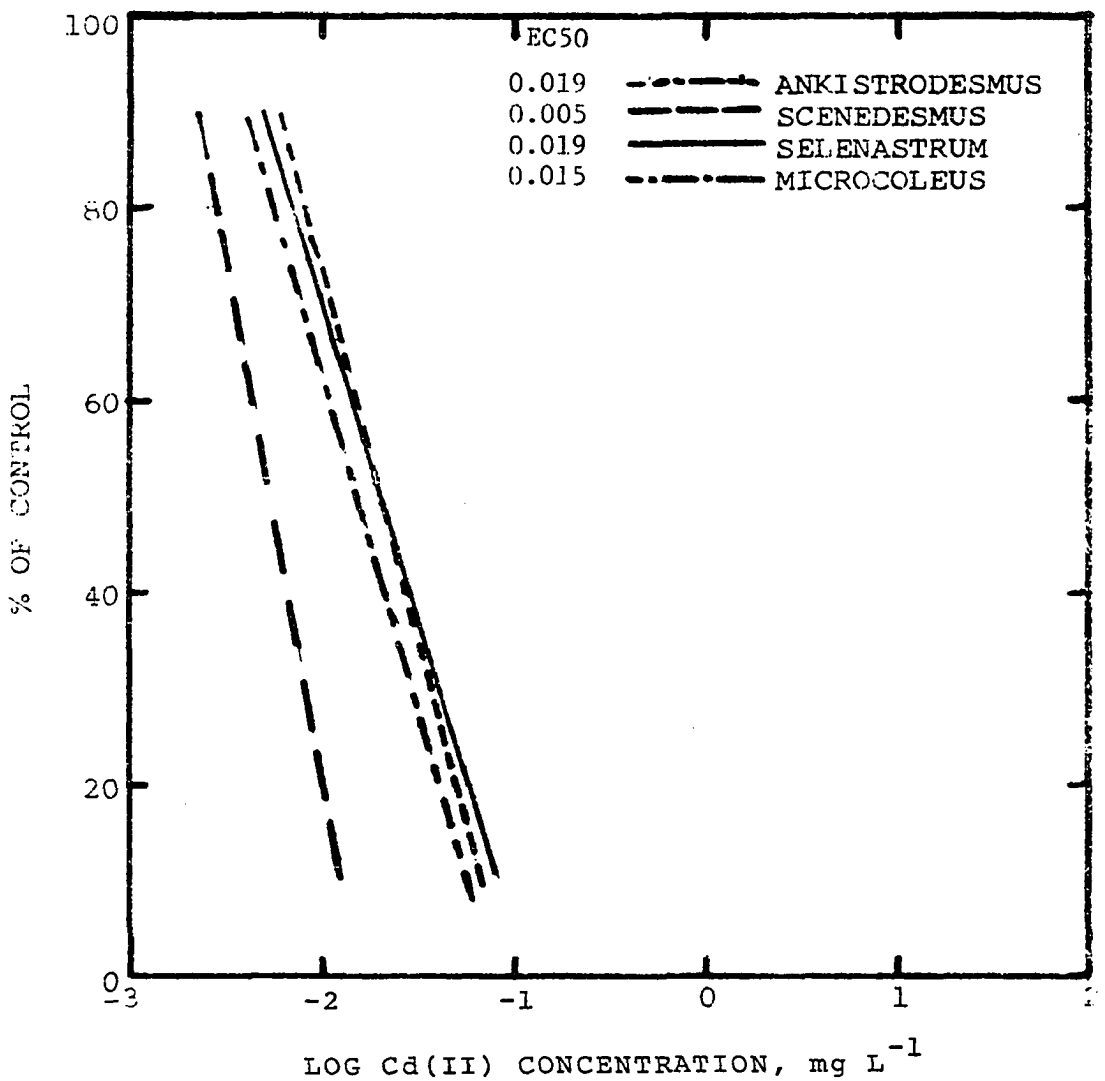

Figure 7. Regression lines of algae exposed to Cd(II), with EC50 values (mg L^{-1}).

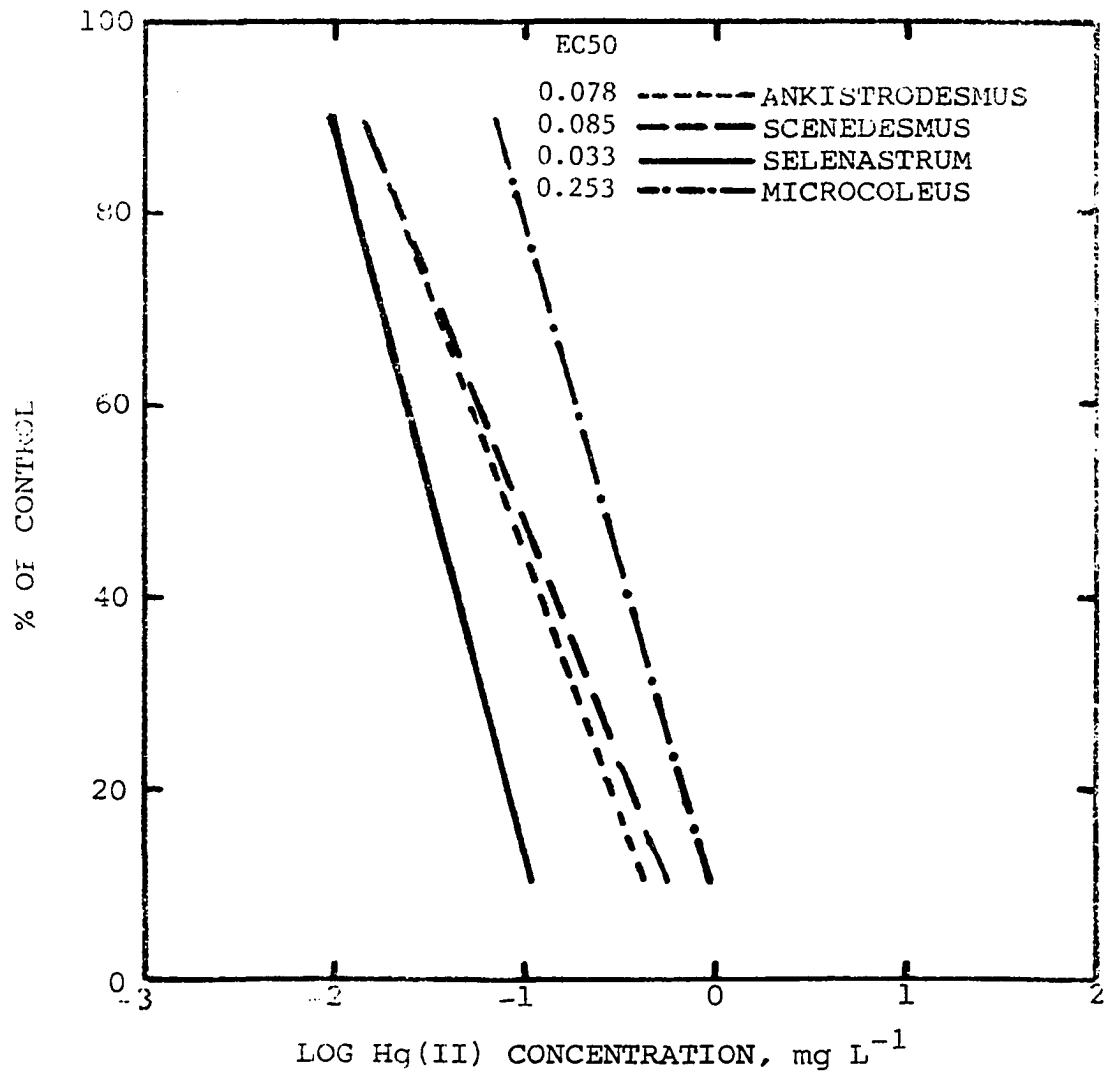

Figure 8. Regression lines of algae exposed to Hg(II), with EC50 values (mg L^{-1}).

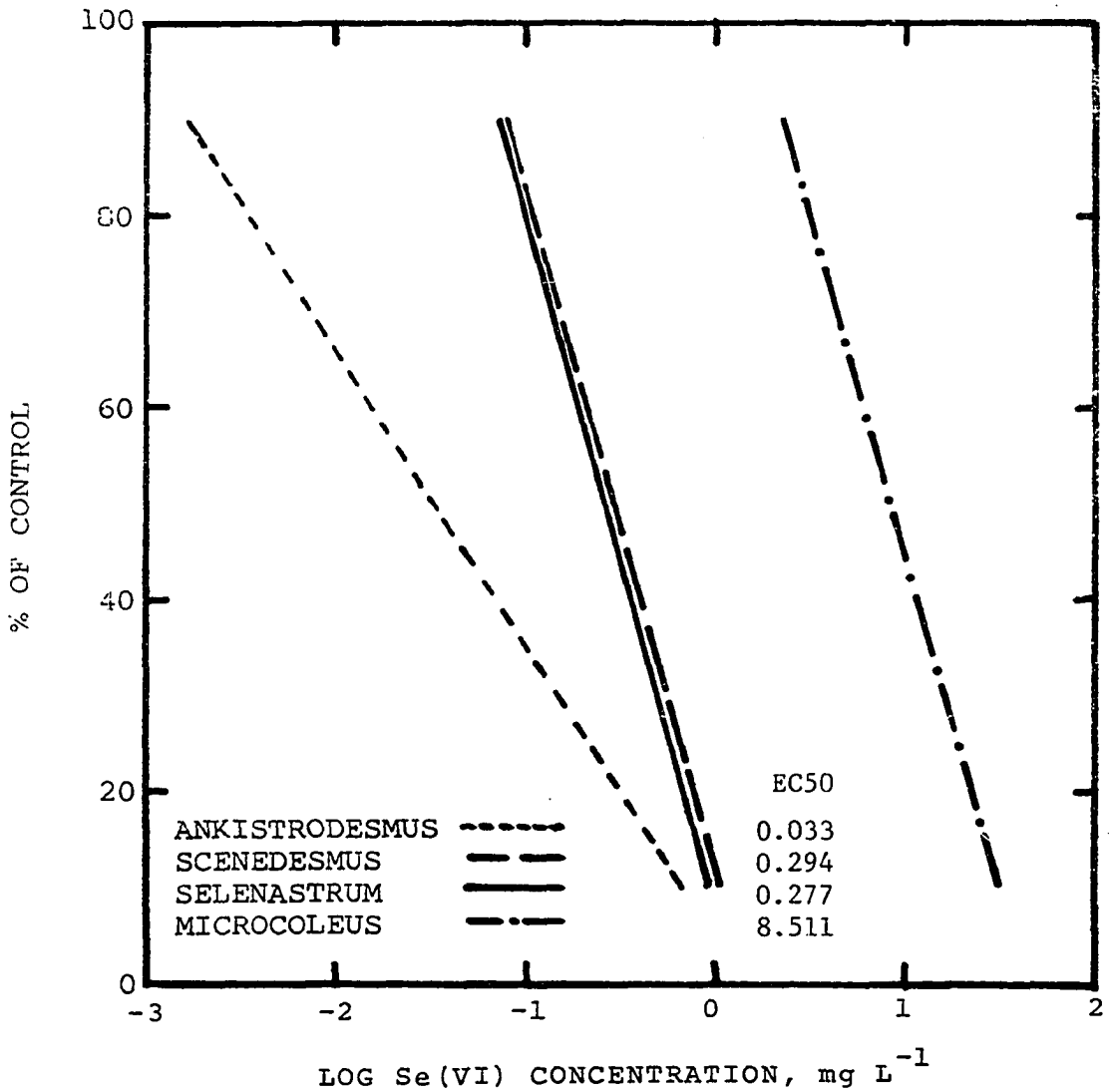

Figure 9. Regression lines of algae exposed to Se(VI), with EC50 values (mg L^{-1}).

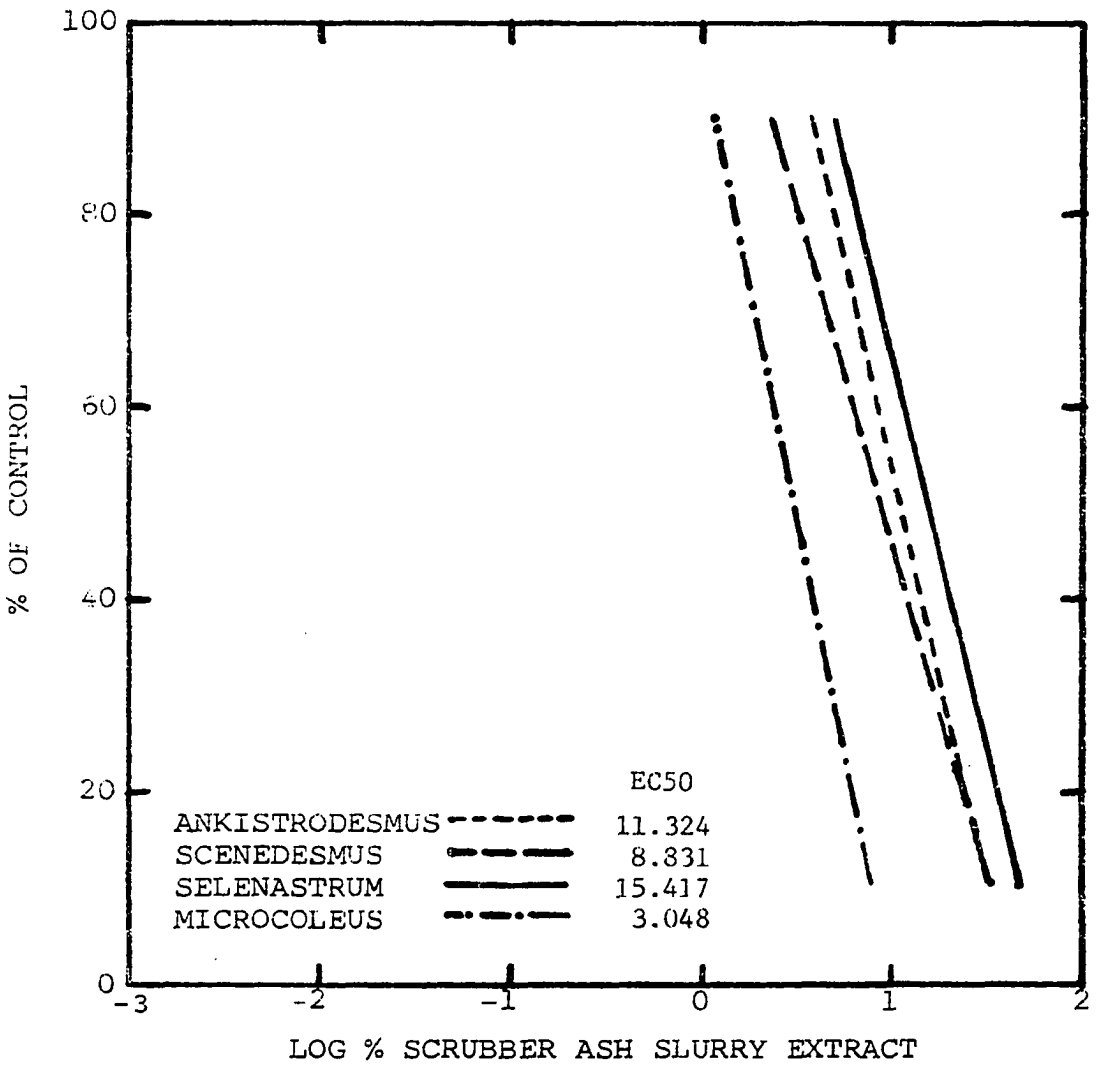

Figure 10. Regression lines of algae exposed to SASE, with EC50 values (% SASE).

Table 11. Safe concentrations[a] of the test substances for the test algae

Test substance	Alga	AF	MATC
As	Ankistrodesmus		0.083
	Scenedesmus		0.016
	Selenastrum	0.325	10.000
Cd	Ankistrodesmus	0.263	0.005
	Scenedesmus		0.001
	Selenastrum		0.005
	Microcoleus		0.004
Hg	Ankistrodesmus	0.128	0.010
	Scenedesmus		0.011
	Selenastrum		0.004
	Microcoleus		0.032
Se	Ankistrodesmus		0.006
	Scenedesmus	0.170	0.050
	Selenastrum		0.047
	Microcoleus		1.447
SASE	Ankistrodesmus		1.280
	Scenedesmus	0.113	1.000
	Selenastrum		1.742
	Microcoleus		0.344

[a] mg L^{-1} trace elements and % SASE.

response relationship. The AF was calculated as follows: AF = MATC/EC50. Microcoleus vaginatus was not used to estimate AF values because the alga was cultured in Gorham's medium and was not bacteria-free. The MATC values for Cd(II), Hg(II), Se(VI), SASE are in good agreement with the highest treatment levels that did not cause a significant inhibition (Tables 4-7). The MATC values for As(V) fall within an acceptable range although the fit is not as good as for the other test substances (Table 3). This lack of agreement is probably due to the large interspecies variation in response to As(V). The water quality criteria for the potential coal-fired power plant pollutants based on the lowest MATC values are 0.016 mg L^{-1} As(V), 0.001 mg L^{-1} Cd(II), 0.004 mg L^{-1} Hg(II), 0.006 mg L^{-1} Se(VI), and 0.344% SASE.

DISCUSSION

Toxicity tests provide a direct method of assessing the biological availability of elements in solution. The adapted AAPBT-AAM was used because AAM provides a good culture medium for evaluating the effect of substances on algae and for extrapolating the results to the natural environment. The adapted AAM has a defined chemical composition comparable to natural waters (Table 1). The medium is buffered to maintain pH near neutrality, has a total dissolved solids concentration of less than 70 mg L^{-1}, and, when formulated without EDTA, is completely inorganic.

The importance of using a medium similar in ionic strength to the lower ionic strength natural waters to provide a sensitive toxicity test is reported in the literature. Kinkade and Erdman (1975) found the accumulation of cadmium (0.1 mg L^{-1} cadmium initial concentration) by Nitella flexilis and by Elodea canadensis was less in hard water [total Ca(II) and mg(II) 150 mg L^{-1}] than in soft water [total Ca(II) and Mg(II) 0 mg L^{-1}]. The concentrations of cadmium found in Nitella flexilis and Elodea canadensis grown in hard water were 40.6% and 9.5% of the soft water plants, respectively, after 21 days. The toxicity of mercury has been reported to vary inversely with the ionic strength or nutrient concentration of the growth medium (Fujita and Hashizume, 1972; Hannan and Patouillet, 1972). Greene et al. (1975) reported that the sensitivity of Selenastrum capricornutum to zinc was inversely proportional to the ionic strength of the test substances. Foster (1976) reported that in highly polluted environments the concentration of minor elements (cadmium, lead, and chromium) in Fucus vesiculosus and Ascophyllum nodosum did not reflect the concentra-

tions dissolved in the water to which they had been exposed. He speculated that the algae may possess a finite number of nonspecific binding sites. The extent to which these sites are occupied by a particular metal depends upon the concentration of other competing elements.

Chelating agents were omitted from the culture solutions because they have been shown to counteract growth inhibition in toxicity studies (Hart, 1975; Hart and Scaife, 1977; Katagiri, 1975). Hutchinson and Stokes (1975) omitted organic chelators to minimize chemical complications when investigating heavy metal toxicity to algae. Additionally, Gardiner (1974a,b) reported EDTA displays a strong tendency to complex with cadmium, providing an environment that is not representative of natural, unpolluted waters.

The adjustment of the algal assay solution to pH 7.0 ± 0.3 with NaOH or HCl reduced the possibility of pH affecting the algal responses to the trace elements. Laboratory investigations indicated that small additions of NaOH and HCl did not affect growth of the algal species. This methodology was also used by Hutchinson and Stokes (1975). Kamp-Nielsen (1971) speculated that a decrease in the hydrogen ion concentration resulted in a lowered sensitivity of Chlorella pyrenoidosa to Hg(II) as $HgCl_2$ due to a competition between the hydrogen and mercury ions. Katagiri (1975) and Hart and Scaife (1977) reported that cadmium accumulation was pH dependent. Glooschenko (1969) reported that the adsorption of mercury on siliconed glass was in part a function of pH.

The use of HCl to dissolve particulates formed during autoclaving of the medium reduced the possibility of occlusion, adsorption, or precipitation of the trace element being studied. Zingmark (1975) found that

mercury accumulated in the particulate fraction of the culture medium. Gardiner (1974b) speculated that cadmium was occluded when calcium carbonate precipitated in his test solutions.

Micronutrients were omitted to reduce the possibility of synergistic or antagonistic effects. Hart (1975) reported that the amount of cadmium accumulated in Chlorella pyrenoidosa was regulated by the concentration of manganese in the medium. Virtually no cadmium accumulation occurred in cells grown in medium containing 0.2 mg L^{-1} manganese. Hutchinson and Stokes (1975) also omitted heavy metal micronutrients from the medium when investigating heavy metal toxicity with algal bioassays.

It is important to use only a small quantity of sulfur, as in AAM, because sulfur and selenium compounds have been shown to display antagonistic effects (Hurd-Karrer, 1938; Kumar and Prakash, 1971).

The use of an inoculum of uniformly low density in these investigations reduced the possibility of an algal overload. Kamp-Nielsen (1971) and Ben-Bassat and Mayer (1975) indicated that cell concentration affected the toxicity of Hg(II) to Chlorella pyrenoidosa. Harriss et al. (1970) reported that the toxicity of mercurial compounds decreased with increasing cell concentrations.

Variations in the growth phase and metabolic activity of the inoculum were reduced by inoculating the test solutions from 10- to 14-day stock cultures. Laboratory investigation indicated that cultures of this age were healthy and actively growing. Additionally, the AAPBT recommends the use of 10- to 14-day cultures of Selenastrum capricornutum (U.S. EPA, 1971).

A standard temperature of 24 ± 2°C was used because temperature-trace metal interactions have been shown to affect algal responses (Knowles and Zingmark, 1975; Zingmark, 1975). This temperature is also recommended by the AAPBT.

A two-week test period for the algal toxicity studies was chosen over a shorter duration in order to simulate chronic exposure and to give a better indication of the long-term impact to be expected on release of the substance into the environment. Zingmark and Miller (1975) reported that increased exposure time of mercury to Amphidinium carterae increased its toxic effects.

Polycarbonate flasks rather than glass were used for toxicity test containers in the trace element toxicity studies as recommended by the U.S. EPA (1971). Other investigators also have used polycarbonate flasks (Tompkins and Blinn, 1976; Miller et al., 1976). Glooschenko (1969) reported that mercury adsorption on the walls of siliconed glass was a serious problem. Gardiner (1974b) found adsorption of cadmium on the walls of Pyrex glass also a cause for concern. Project investigations with $^{76}As(III)$, $^{115m}Cd(II)$, and $^{75}Se(IV)$ in our laboratories indicated that less than 5% of the elements were adsorbed on the walls of polycarbonate flasks at 0.01, 0.001, and 0.01 mg L^{-1} of the respective elements.

Axenic unialgal cultures were used, when possible, for these static algal toxicity tests. Baier et al. (1975) reported that the apparent mercury loss in nonacidified media was by bacterial conversion to the organic and/or elemental form and subsequent volatilization. Micro-

organisms vary from species to species in their sensitivity to toxic substances, because they have different dose-response relationships and display different bioconcentration abilities. Thus, if a mixed inoculum were used, experimental results might be misinterpreted. Greater experiment-to-experiment variation also could be expected to occur with a mixed inoculum because the relative species composition of the inocula would not be consistent. Unless all components of mixed inocula are monitored from the beginning to the end of a toxicity study, in terms of identification, quantification, and bioconcentration by species, unknown interactions could occur making interexperimental comparisons of dose-response relationships difficult.

To provide a viable extrapolation from the laboratory to the natural environment, those oxidation states of the trace elements were selected which are the dominant states occurring in natural, unpolluted, slightly alkaline freshwaters (Crecelius, 1975; Gavis and Ferguson, 1972; Shephard, 1976; Shephard and McIntosh, 1976; Whanger, 1974).

The static toxicity tests employed in this investigation tended to underestimate the toxicity of low-level treatments. Underestimation occurred because the initial treatment concentrations of the potential pollutants were reduced during the tests through bioaccumulation and possibly volatilization in the case of Hg(II) (Ben-Bassat et al., 1972; Ben-Bassat and Mayer, 1975, 1977; Knowles and Zingmark, 1975; Zingmark and Miller, 1975). The depletion was most critical for substances at low treatment levels where the percentage reduction was the greatest. If the tests had been conducted in a continuous-flow algal system, then the first

significant inhibition levels of As(V), Cd(II), Hg(II), and Se(VI) and SASE would have been reduced. The EC50 values, however, are much less affected by this depletion. The validity of using EC50 values was confirmed through the cadmium EC50 experiments. Although it must be pointed out that EC50 values are, in fact, means, and the data here indicate a substantial degree of variation about these means.

Selected growth responses reported in the literature for freshwater algae exposed to arsenic, cadmium, mercury, and selenium are presented in Tables 12-15. Little information is available concerning the toxicity of arsenic to freshwater algae. The algistatic-algicidal value reported for Chlorella vulgaris of 0.06 mg L^{-1} As(V) by den Dooren de Jong (1965) is well below the algistatic-algicidal value of 5.0 mg L^{-1} As(V) for Ankistrodesmus, the alga with the lowest algistatic-algicidal value. Additionally, the EC50 values (0.048 to >100 mg L^{-1}) and the algistatic-algicidal values (5 to >100 mg L^{-1}) displayed a large degree of variation from alga to alga. The blue-green alga Microcoleus was much more tolerant of As(V) than the green algae investigated. This could be due in part to differences in the assay media, the fact that the Microcoleus was not axenic, or possibly because Microcoleus is a procaryotic organism differing in some metabolic pathways from the other eucaryotic algae.

This investigation confirms reports that cadmium is extremely toxic to freshwater algae (Table 13). Cadmium concentrations of 0.13 and 0.08 mg L^{-1} for Selenastrum capricornutum and of 0.14 mg L^{-1} for Chlorella vulgaris were reported as algistatic-algicidal. In this investigation, algistatic-algicidal values between 0.2-0.2, 0.01-0.05, 0.2-0.3, and 0.1-

Table 12. Selected growth responses reported for freshwater algae exposed to arsenic

Reference	Element	Compound	Exposure level mg L^{-1}	Alga	Growth response[a]
den Dooren de Jong (1965)	As(V)	$Na_2HAsO_4 \cdot 7H_2O$	0.03	Chlorella vulgaris	No I[b]
	As(V)	$Na_2HAsO_4 \cdot 7H_2O$	0.06	Chlorella vulgaris	100% I
Conway (1978)	As(V)	Arsenate	0.16	Asterionella formosa	No I

[a]Responses have been transformed to simplify comparisons.

[b]Inhibition.

Table 13. Selected growth responses reported for freshwater algae exposed to cadmium

Reference	Element	Compound	Exposure level mg L^{-1}	Alga	Growth response[a]
Conway (1978)	Cd(II)	$CdCl_2$	>0.010	Asterionella formosa	100% I[b]
Klass et al. (1974)	Cd(II)	$CdCl_2$	0.0061	Scenedesmus quadracauda	Significant I
Miller et al. (1976)	Cd(II)	$CdCl_2$	0.13	Selenastrum capricornutum	100% I
Rosko and Rachlin (1977)	Cd(II)	$CdCl_2$	0.06	Chlorella vulgaris	50% I
Bartlett et al. (1974)	Cd(II)	$CdCl_2 \cdot 2\ 1/2\ H_2O$	0.08	Selenastrum capricornutum	100% I
den Dooren de Jong (1965)	Cd(II)	$CdCl_2 \cdot 2\ 1/2\ H_2O$	0.09	Chlorella vulgaris	No I
	Cd(II)	$CdCl_2 \cdot 2\ 1/2\ H_2O$	0.14	Chlorella vulgaris	100% I
Hart (1975)	Cd(II)	$Cd(CH_3CO_2)_2 \cdot 2H_2O$	0.25	Chlorella pyrenoidosa	Partial I

[a]Responses have been transformed to simplify comparisons.

[b]Inhibition.

Table 14. Selected growth responses reported for freshwater algae exposed to mercury

Reference	Element	Compound	Exposure level mg L^{-1}	Alga	Growth response[a]
Ben-Bassat et al. (1972)	Hg(II)	$HgCl_2$	2.0	Chlamydomonas reinhardi y^{-1}	100% I[b]
den Dooren de Jong (1965)	Hg(II)	$HgCl_2$	0.018	Chlorella vulgaris	No I
	Hg(II)	$HgCl_2$	0.037	Chlorella vulgaris	100% I
Hannan and Patouillet (1972)	Hg(II)	$HgCl_2$	0.1	Chlorella pyrenoidosa	Significant I
	Hg(II)	$HgCl_2$	1.0	Chlorella pyrenoidosa	100% I
Rosko and Rachlin (1977)	Hg(II)	$HgCl_2$	1.03	Chlorella vulgaris	50% I
Tompkins and Blinn (1976)	Hg(II)	$HgCl_2$	0.1	Fragilaria crotonensis	100% I
	Hg(II)	$HgCl_2$	0.5	Asterionella formosa	100% I
Holderness et al. (1975)	Hg(II)	CH_3HgCl	0.005	Coelastrum microporum	Extreme I

[a] Responses have been transformed to simplify comparisons.

[b] Inhibition.

Table 15. Selected growth responses reported for freshwater algae exposed to selenium

Reference	Element	Compound	Exposure level mg L^{-1}	Alga	Growth response[a]
den Dooren de Jong (1965)	Se(IV)	Na_2SeO_3	5.8	Chlorella vulgaris	No I[b]
	Se(IV)	Na_2SeO_3	12	Chlorella vulgaris	100% I
Kumar and Prakash (1971)	Se(IV)	Na_2SeO_3	13	Anabaena variabilis	LD50[c]
	Se(IV)	Na_2SeO_3	31	Anacystis variabilis	LD50
	Se(VI)	Na_2SeO_4	18	Anabaena variabilis	LD50
	Se(VI)	Na_2SeO_4	42	Anacystis nidulans	LD50
Kumar (1964)	Se(VI)	Na_2SeO_4	20	Anacystis nidulans	100% I

[a]Responses have been transformed to simplify comparisons.

[b]Inhibition.

[c]Lethal dose to 50% of the organisms.

0.3 mg L^{-1} Cd(II) were indicated for Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus, respectively. The EC50 value reported for Chlorella vulgaris of 0.06 mg L^{-1} is slightly higher than the EC50 values of 0.019, 0.005, 0.019, and 0.015 mg L^{-1} Cd(II) found in this investigation for Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus, respectively. A difference in sensitivity between blue-green and green algae to Cd(II) was not noticed.

The algistatic-algicidal values of 0.1-0.4 mg L^{-1} Hg(II) for the green algae and approximately 1.0 mg L^{-1} Hg(II) for Microcoleus compare favorably with values reported in the literature (Table 14). The EC50 values of 0.078, 0.085, 0.033, and 0.253 mg L^{-1} Hg(II) for Ankistrodesmus, Scenedesmus, Selenastrum, and Microcoleus, respectively, are smaller than the 1.03 mg L^{-1} reported by Rosko and Rachlin (1977) for Chlorella vulgaris. The blue-green alga Microcoleus appears to be somewhat less sensitive to Hg(II), but this could be due to differences in test media or to the fact that Microcoleus was not axenic.

The response values obtained for Microcoleus exposed to Se(VI) are similar to those reported for other blue-green algae exposed to Se(VI) (Table 15). Response values for green algae exposed to Se(VI) were not found in the literature. Although it does appear that green algae are more sensitive to Se(VI) than blue-green algae, the antagonistic response between sulfur and selenium in the different media complicates this relationship. The response values obtained from the literature for Se(IV) are not directly comparable to Se(VI) response values.

The MATC water quality criteria values obtained in this investigation for algae should supplement the U.S. EPA quality criteria for water which are periodically updated. The 1976 quality criteria do not give any arsenic recommendations for the protection of aquatic life (U.S. EPA, 1976). Cadmium concentrations of 0.4 μg L^{-1} and 1.2 μg L^{-1} in waters with hardness values of less than and greater than 150 mg L^{-1} $CaCO_3$, respectively, are recommended for the protection of the most sensitive aquatic species. A mercury level of 0.05 μg L^{-1} is recommended for the protection of aquatic life. Safe selenium levels are to be determined using toxicity tests for a specific geographical area.

A problem arises when one extrapolates from single element toxicity studies to energy-generated pollutants such as stack effluents and scrubber ash slurries. These complex mixtures of elements have the potential to cause complex synergistic or antagonistic effects on exposed biota. When many toxicants are present, the National Technical Advisory Committee recommends use of the following relationship:

$$\frac{C_a}{L_a} + \frac{C_b}{L_b} \ldots + \frac{C_n}{L_n} \leq 1$$

where C_a, C_b, and C_n are the measured concentrations of the toxic substances in a receiving water, and L_a, L_b, and L_n are the concentrations permissible for each substance individually. This relationship is valid when the toxic effects of the materials are simply additive (Warren, 1971). Antagonistic effects would allow greater concentrations and synergistic effects lesser concentrations of the toxic substances than indicated by the National Technical Advisory Committee relationship.

CONCLUSION

The development and implementation of standard toxicity tests is a necessity if consistent and reliable data are to be obtained for water quality criteria. The adapted EPA AAPBT is an ideal static algal toxicity test system. The algal test medium has a chemical composition similar to natural unpolluted waters of low ionic strength. The medium is buffered to maintain pH near neutrality, has a dissolved solids concentration of less than 70 mg L^{-1}, and when used without EDTA, is completely inorganic. The use of polycarbonate flasks and low level inoculums of axenic cultures increases the sensitivity of the toxicity tests. The use of standard algal species such as Selenastrum capricornutum for interlaboratory comparisons is recommended for purposes of quality control. The static toxicity tests employed in this investigation probably tended to underestimate the toxicity of low-level treatments because of depletion of the test substances in the test system.

It is appropriate to use MATC water quality criteria when assessing the potential impact of pollutants generated by coal-fired power stations because these energy-generated pollutants typically enter aquatic systems in small quantities over long periods.

The MATC water quality criteria are estimates of trace element and SASE levels, based on the most sensitive alga investigated, that will not cause significant changes in naturally-functioning algal populations. These levels are 0.016 mg L^{-1} As(V), 0.001 mg L^{-1} Cd(II), 0.004 mg L^{-1} Hg(II), 0.006 mg L^{-1} Se(VI), and 0.344% SASE. To provide viable working water quality criteria, an extrapolation from the laboratory to the

natural environment must be made. Therefore, those oxidation states of the trace elements were selected which are the dominant states occurring in natural, unpolluted, slightly alkaline freshwaters.

It must be pointed out that these MATC values are based on algal responses to single toxicants and no allowance is made for synergistic, additive, or antagonistic relationships which could occur in natural aquatic systems. Additionally, natural chelation may influence toxicity.

The highly toxic nature of potential pollutants from coal-fired generating plants emphasizes the need for minimizing stack effluent pollutants and detaining scrubber ash slurry for proper disposal in an effort to maintain trace elements in concentration ranges which are compatible with naturally-functioning ecosystems.

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APPENDIX: KEY TO ACRONYMS

AAM	Algal Assay Medium
AAPBT	Algal Assay Procedure Bottle Test
AF	Application Factor
EC50	Median Effective Concentration
EDTA	Ethylenediaminetetraacetic Acid
EPA	Environmental Protection Agency
LC50	Median Lethal Concentration
MATC	Maximum Allowable Toxicant Concentrations
SASE	Scrubber Ash Slurry Extract
SC	Safe Concentration
TGEA	Tryptone Glucose Extract Agar