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TOXICITY OF HEAVY METALS AND SALTS TO EURASIAN WATERMILFOIL (*MYRIOPHYLLUM SPICATUM* L.)

RONALD A. STANLEY

Environmental Biology Branch
Division of Environmental Planning
Tennessee Valley Authority
Muscle Shoals, Alabama 35660

The toxicity of various heavy metals and salts to Eurasian watermilfoil (*Myriophyllum spicatum* L.) was determined under controlled growth conditions. Toxicants were added to water or to soil in systems with and without woods earth in the substrate.

Fifty precent inhibition of root weight occurred with concentrations of 0.25 ppm Cu^{+2}, 1.9 ppm $Cr_2O_7^{-2}$, 3.4 ppm Hg^{+2}, 2.9 ppm AsO_2^{-1}, 7.4 ppm Cd^{+2}, 2.5 ppm Al^{+3}, 9.9 ppm Cr^{+3}, 41.2 ppm Ba^{+2}, 21.6 ppm Zn^{+2}, 13.3 ppm NH_4^{+1}, 22.4 ppm CN^{-1}, 143 ppm $B_4O_7^{-2}$, 363 ppm Pb^{+2}, 10,228 ppm Na_2SO_4, and 8,183 ppm NaCl. Soil increased toxicity of Cr^{+2} and Ba^{+2} but decreased toxicity of $Cr_2O_7^{-2}$, Cu^{+2}, Cd^{+2}, Al^{+3}, and Hg^{+2}. In distilled water, $CaCl_2$ increased toxicity of Cr^{+3} but not $Cr_2O_7^{-2}$.

For most toxicants there was a consistent relationship between inhibition of length and inhibition of weight and between inhibition of roots and inhibition of shoots. However, $Cr_2O_7^{-2}$ disproportionately decreased dry weight, and Hg^{+2} and Na_2SO_4 disproportionately decreased stem length growth. With Cd^{+2} and Cu^{+2} stem length was greater relative to other measures of growth. Toxicity of Na_2SO_4 and NaCl was the same when concentrations were calculated as osmotic pressure but not when calculated as Na atoms or as total molecules/L.

Aquatic angiosperms are an important part of freshwater ecosystems. Some are considered beneficial for some water uses while others are considered generally detrimental. Despite the economic, ecological, and esthetic importance of this diverse group of aquatic plants, data on their response to toxic chemicals are fragmentary.

Most reported studies on toxicity have concerned copper and arsenic compounds because of their use as herbicides. Arsenite at ten ppm killed water hyssop and parrotfeather in Florida (Phillippy 1961) and at four ppm killed part of a population of *Potamogeton* and *Alisma* but did not kill *Sagittaria* (Cowell 1965). Arsenate at five ppm killed *Potamogeton, Najas,* and *Anacharis* but did not kill *Nymphaea* and *Scirpus* in hatchery ponds (Surber and Everhart 1950). At three ppm Cu^{+2} had no effect on *Potamogeton, Eriocaulon, Scirpus, Pontederia, Juncus,* or *Nymphaea* (Smith 1939) but at two ppm inhibited growth of parrotfeather (Sutton and Blackburn 1971).

9184

Several field observations and laboratory studies have been conducted on salinity. *Ruppia* was reported to tolerate 4.5% salinity in the field (Simmons 1957) and better growth was obtained in greenhouse experiments at 2.2% salinity than in fresh water (Bourn 1935). *Zostera* grows between 1.0 and 4.2% salinity (Biebl and McRoy 1971). Greenhouse results indicate inhibition of 50% of the growth at 3.1% salinity for *Potamogeton pectinatus*, at 3.05% for *P. perfoliatus*, at 1.15% for *Najas flexilis*, at 1.1% for *Vallisneria spiralis*, and at 0.38% for *Ceratophyllum demersum* (Bourn 1932). Tubers of *P. pectinatus*, on the other hand, were inhibited at 1.5% (Teeter 1965). Sodium chloride at 1.0% had no effect on *Lemna trisulca* (Vivier and Nisbet 1965). *Myriophyllum spicatum* is generally limited in the field to salinities below 1.5% (Anderson 1964). Results on photosynthesis and respiration in this species suggest that salinity may act by alteration of the photosynthesis-respiration ratio (McGahee and Davis 1971).

These reported results show that the threshold of toxicity of heavy metal salts is about two ppm, while the threshold for the relatively nontoxic salt, NaCl, is more than 3000 ppm. The purpose of this research was to determine more accurately the threshold of toxicity to *M. spicatum* L. for various salts, including salts of heavy metals and other less toxic salts.

Methods and materials

Eurasian watermilfoil (*M. spicatum* L.), from a clone collected in Friesland, Netherlands, in 1967, was grown under greenhouse conditions in seven-L earthen jars lined with plastic bags containing about 1500 cc of woods earth covered with about 1500 cc of ferric silicate and tap water. Tap water was replenished as needed to compensate for evaporation. Cultures were replanted periodically into fresh media, using apical cuttings. Replanted apices were treated as necessary with 50 ppm $CuSO_4$ for three to five min to reduce algae. Apices four cm long were cut to size in the greenhouse, transported to the laboratory in tap water, and planted the same day for experimental studies.

Apices were planted in 60 cc of ferric silicate or 30 cc of woods earth covered by 30 cc of ferric silicate in flat-bottomed tubes (made from glass tubing 50 mm in diameter) containing 200 ml of tap water. Samples of the tap water used contained 31 ppm of Ca, 4.5 ppm of Mg, 7.6 ppm of Na, 2.0 ppm of K, 1.2 ppm of NO_3, 0.11 ppm of N as organic compounds, 0.16 ppm of Zn, 0.1 ppm of Fe, and 0.034 ppm of P. Woods earth added to each tube weighed about 23 g and contained $>$ 34 mg of Ca, 11 mg of P, 2.3 mg of K, and 9 mg of Mg. Three types of treatment were routinely used: (1) the toxicant was added to soil in the substrate before ferric silicate and tap water were added, (2) the toxicant was added to water with soil in the substrate, and (3) the toxicant was added to water with only ferric silicate as substrate. In one experiment, a rinse procedure (Stanley, unpublished manuscript) was used to study the effect of calcium on uptake of $Cr_2O_7^{-2}$ and Cr^{+3}. Plants were grown for 32 days at 20°C with about 300 fc continuous light in all experiments.

At the end of the 32-day growth period, maximum lengths of roots and shoots were measured. After roots and shoots were oven-dried overnight at 70°C, dry weights were

determined separately on an analytical balance. There were ten replicates for all length determinations, and three replicates each composited from three plants for all weight determinations except for the comparison of NaCl and Na_2SO_4 in which 30 plants were composited into ten replicates. Data obtained were plotted on semilogarithmic paper, and the level of toxicant giving 50% inhibition (I_{50}) of each measured growth response was determined graphically. In addition, 10% and 90% inhibition levels were determined for root weight (I_{10}^{RW} and I_{90}^{RW}). Data obtained from treatments with the toxicant added to the soil were calculated as a "root zone effect" by the following formula:

$$R = \frac{r_{sx}/r_{so}}{r_x/r_o}$$

where R = root zone effect
r_{sx} = root weight with x amount of toxicant added to the soil
r_{so} = root weight control for r_{sx} (no toxicant added)
r_x = root weight with the same amount of toxicant added to water with soil in the substrate
r_o = root weight control for r_x (treatment identical to r_{so} but independently determined)

Similarly, a "soil synergism effect" was calculated from data obtained without woods earth in the substrate by the following formula:

$$S = \frac{r_{wx}/r_{wo}}{r_x/r_o}$$

where S = soil synergism effect
r_{wx} = root weight with x amount of toxicant and only sand substrate
r_{wo} = root weight control for r_{wx}

Results

The concentration giving 50% inhibition for root weight (I_{50}^{RW}) varied from 0.25 ppm for Cu^{+2} to 10,228 ppm for Na_2SO_4 (Table I). In general, roots were more sensitive to toxicants than were shoots, and weight growth was inhibited more than length growth.

The soil synergism effect was very dependent on concentration of the toxicant (Table II). The root zone effect apparently was largely independent of toxicant concentration (Table III). Different toxicants gave soil synergism effects ranging from 0.07 to 3.0 at concentrations selected near the I_{50} concentration for root weight (Table IV). Only Cr^{+3} and Ba^{+2} had soil synergism values >> 1: NH_4^{+1}, CN^{-1}, $B_4O_7^{-2}$, and NaCl gave unusually low ratios for this effect of the soil. For root zone effect (toxicant applied in the soil), Hg^{+2}, $Cr_2O_7^{-2}$, Cu^{+2}, Al^{+3}, Ba^{+2}, Cd^{+2}, and Pb^{+2} gave high ratios, and NaCl, NH_4^{+1}, $B_4O_7^{-2}$, and CN^{-1} gave low ratios (Table IV).

When plants were exposed for two days to 10.8 ppm of $Cr_2O_7^{-2}$, in distilled water and then grown in tap water, root weight was hardly affected by 40 ppm of Ca^{+2} in the

Table I. *Inhibition of growth of M. spicatum by various chemical compounds and comparison with surface water quality criteria for potable water*

Compound	Concentration (ppm) giving:[a]					Surface water criteria[b] (ppm)	Ratio (criteria: I_{50}^{RW})
	I_{50}^{RW}	I_{50}^{SW}	I_{50}^{RL}	I_{50}^{SL}	$I_{90}^{RW} : I_{10}^{RW}$		
Cu^{+2}	0.25	0.38	0.70	1.5	30	1.0	0.25
$Cr_2O_7^{-2}$	1.9	2.6	8.0	9.5	69	0.2	9.5
Hg^{+2}	3.4	4.4	12.0	1.2	37	0.1[c]	34.0
AsO_2^{-1}	2.9	2.6	3.6	4.1	15	0.07	41.4
Cd^{+2}	7.4	14.6	20.8	809	–	0.01	740.0
Al^{+3}	2.5	7.6	5.1	12.7	36	1.0[d]	2.5
Cr^{+3}	9.9	14.6	24.4	26.0	24	0.05	198.0
Ba^{+2}	41.2	103	113	83.8	34	1.0	41.2
Zn^{+2}	21.6	–	21.6	20.9	–	5.0	4.3
NH_4^{+1}	13.3	13.2	16.2	15.9	5	0.6	21.0
CN^{-1}	22.4	20.0	28.6	27.3	10	0.2	112
$B_4O_7^{-2}$	143	214	152	171	2	14	10.2
Pb^{+2}	363	808	767	725	63	0.05	7,260
Na_2SO_4	10,228	9,376	10,370	4,120	2	370	27.6
NaCl	8,183	5,962	8,008	7,423	2	410	20.0

[a] I_{50}^{RW} = 50% inhibition of root dry weight; I_{50}^{SW} = 50% inhibition of shoot dry weight; I_{50}^{RL} = 50% inhibition of root length; I_{50}^{SL} = 50% inhibition of shoot length.

[b] Adapted from potable water quality criteria (FWPCA 1968).

[c] Tennessee Water Quality Control Board, letter to Tennessee Valley Authority dated 7/19/72.

[d] Adapted from irrigation water quality criteria (FWPCA 1968).

distilled water. In a similar experiment with 46.8 ppm of Cr^{+3}, roots from plants exposed in the presence of 40 ppm of Ca^{+2} grew only 64% as much as those exposed in the absence of calcium.

Data from experiments with NaCl and Na_2SO_4 were plotted with sodium concentration as the abscissa, with osmotic pressure as the abscissa, and with total molecular concentration as the abscissa (Figure 1). In the sodium plot, data from NaCl all lay to the left of those for Na_2SO_4. When plotted on a molecular basis, the two curves were nearly coincidental at their upper ends but not at the lower ends. When plotted on an ionic basis, the data coincided at the lower end (higher concentrations). In this experiment, the lower concentrations of Na_2SO_4 stimulated growth.

Table II. *Relationship between concentration of Cr^{+3} and soil synergism effect*

Concentration (ppm)	Soil synergism effect[a]
5.2	3.00
10.4	1.20
26.0	0.04

[a]Percentage growth of roots with sand substrate divided by percentage growth of roots with soil substrate; numbers larger than 1 indicate synergism by soil and numbers smaller than 1 indicate blocking of inhibition by soil.

Table III. *Relationship between concentration of Pb^{+2} and NH_4^{+1} and root zone effect*

Chemical	Concentration (ppm)	Root zone effect[a]
Pb^{+2}	20.7	1.43
	207	1.88
NH_4^{+1}	36	0.38
	361	0.54

[a]Percentage growth of roots with toxicant added to the soil divided by percentage growth of roots with toxicant added to the water phase; numbers larger than 1 indicate soil adsorption of the toxicant and numbers smaller than 1 indicate preferential root uptake or preferential shoot exclusion of the toxicant.

Discussion

Compounds with low soil synergism effect ($S < 1$) and high root zone effect ($R > 1$) are presumed to be adsorbed by the woods earth used in these tests (Table IV). Only $Cr_2O_7^{-2}$, Cu^{+2}, Cd^{+2}, and Al^{+3} were in the category of strong soil adsorption as shown by both these tests. However, Hg^{+2} has an exceptionally high root zone effect and is probably also strongly adsorbed under these conditions. Other compounds in these tests that gave some evidence suggesting adsorption by soil include Ba^{+2} and Pb^{+2}. All chemicals may be adsorbed to some extent, and the degree of adsorption may be influenced by conditions such as soil type (Navrot and Gal 1971) and the presence of other chemicals (Feick *et al.* 1972).

Presumably, soil can influence toxicity in ways other than by removal of toxicants by adsorption. Soil may be synergistic with a toxicant by providing ions, such as calcium, that affect the membrane (Stanley, unpublished manuscript) or by providing compatible

Table IV. *Soil synergism and root zone effects at selected concentrations of various toxicants*

Toxic chemical	Concentration (ppm)	Soil synergism effect[a]	Root zone effect[b]
Cu^{+2}	1.3	0.23	4.30
$Cr_2O_7^{-2}$	10.8	0.16	4.70
Hg^{+2}	20.1	1.10	10.90
AsO_2^{-1}	1.1	1.10	1.10
Cd^{+2}	11.2	0.66	2.00
Al^{+3}	2.7	0.80	2.70
Cr^{+3}	5.2	3.00	0.97
Ba^{+2}	68.7	2.20	2.50
NH_4^{+1}	36.1	0.27	0.38
CN^{-1}	13.0	0.87	–
$B_4O_7^{-2}$	77.6	0.80	0.60
Pb^{+2}	103.6	0.97	1.40
NaCl	5,845	0.07	0.16

[a]Percentage growth of roots with sand substrate divided by percentage growth of roots with soil substrate; numbers larger than 1 indicate synergism by soil and numbers smaller than 1 indicate blocking of inhibition by soil.

[b]Percentage growth of roots with toxicant added to the soil divided by percentage growth of roots with toxicant added to the water; numbers larger than 1 indicate soil adsorption of the toxicant and numbers smaller than 1 indicate preferential root uptake or preferential shoot exclusion of the toxicant.

chemicals, such as chelators, that may couple to the toxicant and facilitate uptake (Wang and Newton 1971) or translocation (Wittwer *et al.* 1967). Synergism is suggested by a high value (much greater than 1) for soil synergism; only Cr^{+3} and Ba^{+2}, among the chemicals reported here (Table IV), were found to be in this category. Two chemicals were tested in a rinse experiment with $CaCl_2$: $Cr_2O_7^{-2}$, an ion antagonized rather than synergized by soil, and Cr^{+3}. In this experiment, the toxicity of dichromate was unaffected while that of the cationic chromium was increased by calcium as predicted from the results of the soil synergism effect. This is basically contradictory to the electronegative hypothesis of calcium effect, which predicts that cations will be repelled and uptake of anions will be facilitated by calcium in association with cell membranes.

Indirect evidence is also presented for preferential uptake by roots or preferential exclusion from shoots (these two possibilities cannot be distinguished by the techniques used). Low values for root zone effects suggested preferential root uptake for NaCl, NH_4^{+1}, $B_4O_7^{-2}$, and CN^{-1} (Table IV). These chemicals might be considered normal physiological compounds (in the broadest sense of the word *normal*) while others tested are not. Some authors suggest that preferential root uptake in aquatic plants is important (Bourn 1932; Stanley 1970) while others consider it of less importance in aquatic plants that are entirely submersed (Bristow and Whitcombe 1971; Demarte 1969; Seaman and Baldia 1967). The evidence here suggests preferential root uptake for essential nutrients (even when applied at toxic levels) but no preferential root uptake for non-essential chemicals.

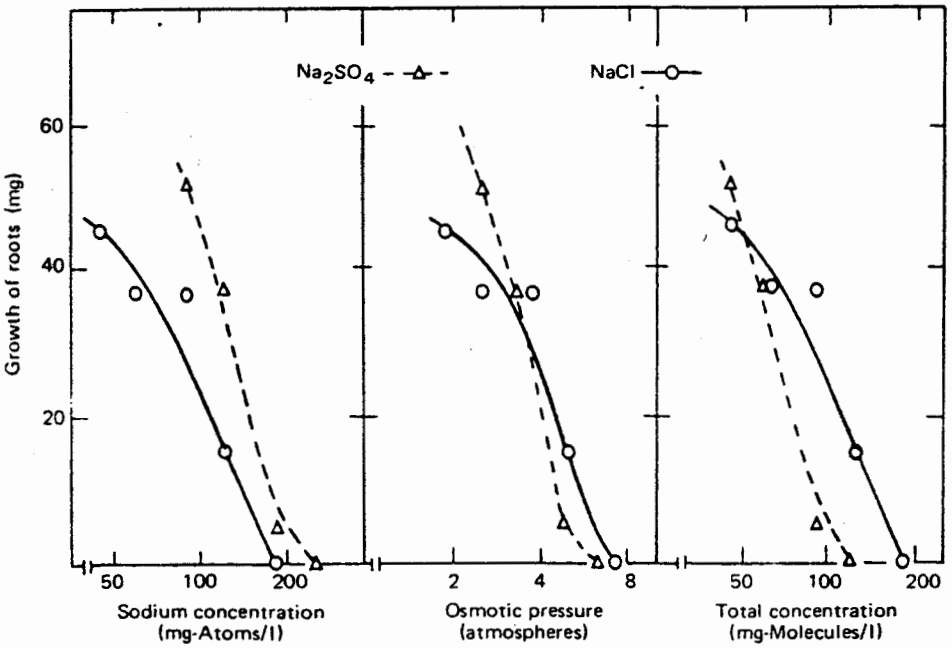

Fig. 1. Growth of roots of Eurasian watermilfoil in tap water in response to added NaCl or Na_2SO_4.

The pattern of inhibition of different measures of growth (Table I) provides evidence on the mechanism of action of each toxicant. Roots were about 10% more sensitive based on length and 20% more sensitive based on weight. Weight, in general, was 20% more sensitive than length based on shoots but 40% more sensitive based on roots. There were deviations from these general principles. When I_{50}^{SL} is divided into I_{50}^{SW} and into I_{50}^{RL}, the ratios for most toxicants fall into a rather narrow range, but there are the following six exceptions:

	$I_{50}^{RL}:I_{50}^{SL}$	$I_{50}^{SW}:I_{50}^{SL}$
Cd^{+2}	3	2
Cu^{+2}	48	26
Al^{+3}	40	60
$Cr_2O_7{}^{-2}$	84	27
Na_2SO_4	252	228
Hg^{+2}	1000	367
Mean and standard error for other 8 toxicants	103 ± 5	89 ± 10

These data show that the inhibition of growth in length of shoots by Hg^{+2} and Na_2SO_4 was much greater than expected from data on root lengths and on shoot weights. On the other hand, Cd^{+2} and Cu^{+2} inhibited the elongation of shoots much less than expected on the basis of other data. Root weights as well as root lengths were inhibited more by Al^{+3} than expected from growth of shoots, and $Cr_2O_7{}^{-2}$ resulted in more than expected inhibition of the increase in weight of roots in addition to the inhibition of shoots shown here.

This evidence suggests that dichromate specifically inactivates some protein(s) (enzyme?) concerned with bulk synthesis leading to dry weight increase; aluminum specifically affects root growth; and cadmium, copper, mercury, and Na_2SO_4 specifically alter some process of elongation. Sodium sulfate probably is acting osmotically on elongation (i.e., turgor pressure is reduced below the level necessary for driving extension of cell walls). Mercuric chloride apparently mimics this effect by specifically inhibiting some metabolic aspect of elongation. Cadmium and copper apparently stimulate shoot elongation to the detriment of other types of growth, thus upsetting the metabolic balance of the plant. Five of the six toxicants that seem to interfere with some specific metabolic process are among the ones to which this species is most sensitive.

It is inconsistent that Na_2SO_4 seems to inhibit specifically elongation and that NaCl does not (for NaCl $I_{50}^{SL}:I_{50}^{SW} = 1.2$), yet comparison of inhibition curves for these two compounds (Figure 1) indicates that the mechanism of action of both of them involves osmotic pressure. In fact, chloride may be taken up much more rapidly than sulfate; thus, cells may be able to maintain sufficient turgor pressure for partial elongation with NaCl added. Both may then be acting osmotically, Na_2SO_4 externally and NaCl internally.

Comparison of weight of roots with weight of shoots and with length of roots revealed two distinct groups of toxicants. The two comparisons produced identical groups except for $B_4O_7^{-2}$ and Zn^{+2}. The means of the ratios $I_{50}^{RW}:I_{50}^{RL}$ and $I_{50}^{RW}:I_{50}^{SW}$ were twice as great for the group of toxicants to which root weight increase was less sensitive than to the group of toxicants which was more inhibitory as shown below:

Ratio of I_{50}^{RW} to:	Toxicity Group	
	High	Low
I_{50}^{SW}	58 ± 5	114 ± 6
I_{50}^{RL}	37 ± 3	91 ± 4

The more toxic group included Al^{+3}, $Cr_2O_7^{-2}$, Ba^{+2}, Cd^{+2}, Cr^{+3}, Cu^{+2}, Hg^{+2}, and Pb^{+2}.

Only Cu^{+2} had I_{50} levels below usually accepted water quality criteria; however, Al^{+3}, $Cr_2O_7^{-2}$, and Zn^{+2} show a sufficiently wide range of partial inhibition that they will inhibit *M. spicatum* at "acceptable" concentrations. With strict adherence to quality criteria for potable water, only Cu^{+2} has promise as a herbicidal synergist for use near water intakes. However, treatment of areas of Eurasian watermilfoil where human consumption of water is not expected might be benefitted by treatment with herbicide in conjunction with some metal or salt.

Extrapolation of these results to natural waters requires careful comparison with field studies. Such comparison is possible in the case of NaCl assuming that estuarine water is only fresh water with certain amounts of NaCl added (an obviously false assumption). There is less than a twofold difference between laboratory determination of the toxicity of NaCl (I_{50}^{RW} = 8,183 ppm, Table I) and field observations on the maximum salinity tolerated (14,963 ppm) (Anderson 1964). This indicates that plants of Eurasian watermilfoil in the field can survive levels somewhat above the concentrations that give 50% inhibition in the laboratory.

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