Bull. Environ. Contam. Toxicol. (1994) 53:442–449


Environmental Contamination and Toxicology

# Effects of Zinc and Copper on Growth and Metal Accumulation in Duckweed, *Lemna minor*

N. Dirilgen, Y. İnel

Department of Chemistry, Boğaziçi University, Bebek, P.K.2., 80815, Istanbul, Turkey

Received: 9 August 1993/Accepted: 15 March 1994

Heavy metal pollutants are known to be quite toxic to a wide variety of aquatic plants. *Lemna* (duckweed), due to its special features, is sought as a test organism for aquatic pollutant studies and for wastewater treatment (Culley et al. 1981; O' Brien 1981; Nasu and Kugimoto 1981; Taraldsen et al. 1990). *Lemna* grows rapidly and reproduces vegetatively; its biomass is measured easily. It is adaptable to various aquatic conditions; it extacts and also accumulates metals in its frond bodies. Among the metals, Cu is classified as extremley toxic (Nasu et al. 1983) and Zn is classified as moderately toxic to *Lemna* (Wang 1986). It is reported that both Cu and Zn concentrations in the medium have a great impact on the growth responses and the physiological processes in *Lemna*. Deficiences in Cu and Zn resulted in chlorosis of *L.minor* fronds and low concentrations of Cu interfered with the floral induction in *L.minor* and *L.gibba* (Hillman 1961). Excess Cu inhibited both frond growth and frond multiplication of *L.paucicostata* (Nasu et al. 1984) and it decreased the content of chloropyll *a* and photosynthetic $CO_2$ uptake in *L.minor* (Filbin and Hough 1979).

In water bodies, metals always are present in combination. Consequently, metal pair interaction is a factor to be considered. However, there are few studies on the effects of metal pair interactions on duckweed growth and metal accumulation (Hutchinson and Czyrska 1975; Nasu and Kugimoto 1984; Mo et al. 1988; Jain et al. 1989).

The purpose of this study was to investigate the effects of increased concentrations of Zn and Cu in combination on growth and metal accumulation by *Lemna minor* L. under controlled laboratory conditions. Zn and Cu were chosen since they are known as essential trace elements for duckweed up to a certain concentration; above that growth inhibition might occur.

## MATERIALS AND METHODS

*L.minor* plants were collected from Çekmece Nuclear Research Center laboratory in İstanbul where they were maintained since 1978. A full-strength Jacob culture medium (McLay 1976) containing all the essential microelements was used to maintain stock and experimental cultures. Iron was used as $Fe^{+3}$-EDTA (ethlenediaminetetraacetic acid). Jars of about 300-ml capacity were used as test vessels. They were covered with aluminum foil to exclude side lighting and with watch glasses to prevent loss due to evaporation.The chemicals tested were all

*Correspondence to:* Y. İnel

analytical grade. Cu and Zn were used as $CuCl_2$ and $ZnCl_2$. Studies were carried out at concentrations of 0.10, 0.20, 0.50, 1.00 and 2.00 ppm of Zn+Cu. The purpose was to keep the concentrations close to the toxicity level of Cu whose inhibition concentration ($IC_{50}$) was reported as 1.1 ppm (Wang 1986). The media were not renewed during the experiment. The stock and test cultures were kept under continuous illumination with preheat day-light fluorescent tubes (Philips TLD 36W/54) giving an intensity of 3190-3320 lux at plant level. Temperature was kept constant at 25-28 $^{0}$C.

Experimental cultures of *L.minor*, each inoculated with twenty-six healthy looking fronds of about 32.0 to 50.0 mg total fresh weight, were grown in 200-ml of the nutrient medium. The pH of the medium was adjusted to 6.30-6.40 with 0.1 M KOH. On the 7$^{th}$ day of frond incubation, plants were washed three times with distilled water and weighed. Subsequently, they were dried at 80 $^{0}$C to constant weight. The plants, whose dry weights had been determined, were digested in 3 ml of 16N $HNO_3$ on a hot plate set at a low temperature, 80 $^{0}$C, for a period of about 5 days and their Zn-Cu content were then measured by a Varian Techtron Atomic Absorption Spectrometry (AAS) (Kunkel and Manahan 1973). The results were expressed on a dry weight basis.

Each experimental set was composed of control samples and five to six replicates for each treatment. Plant growth was defined in terms of the relative growth rate value (RGR) according to the formula : $RGR=100\ (\ln W_2-\ln W_1)/t$ where $W_1$ and $W_2$ are the fresh weights at the beginning and at the end of the time interval (t) (Ericsson et al. 1982).

Dry to fresh weight ratios (DFR) as percentage of control sets were given to indicate the metal toxicity to the plant. An increase in this ratio occurred when the frond cells became loaded with starch grains under stress conditions (Hillman 1961). Final pH values of the test and the control samples also were measured at the end of each experiment. Each experiment was repeated two to four times.

A preliminary test was performed to check whether any probable metal adsorption occurred on the surface of the test vessels or not. At each concentration, control samples were prepared under identical experimental conditions as the test samples, except that they were not inoculated with *L.minor* fronds. Initial and final metal concentrations on the 7$^{th}$ day of the test period were checked with AAS. For the metals tested a perfect linear correlation with R= 0.9998 was observed between the initial and the final ambient metal levels.

Statistical parameters of the experimental results were evaluated by using a statistical package program, Systat, (Willkonson 1987).

## RESULTS AND DISCUSSION

Test solutions at 0.10 and 0.20 ppm were contaminated slightly with algae and their final pH values were raised to a mean value of $6.78 \pm 0.08$. The rest of the test solutions maintained their initial pH range.Fronds looked green and healthy at the 0.10-1.00 ppm levels. Only at 2.00 ppm were the fronds covered with yellow spots and even smaller in size than the ones incubated at lower ambient metal levels.

The effects of increased concentrations of Zn and Cu in combination were correlated with the corresponding relative growth rates (RGR), dry to fresh weight ratios (DFR) and the concentrations of metal in *L.minor*.

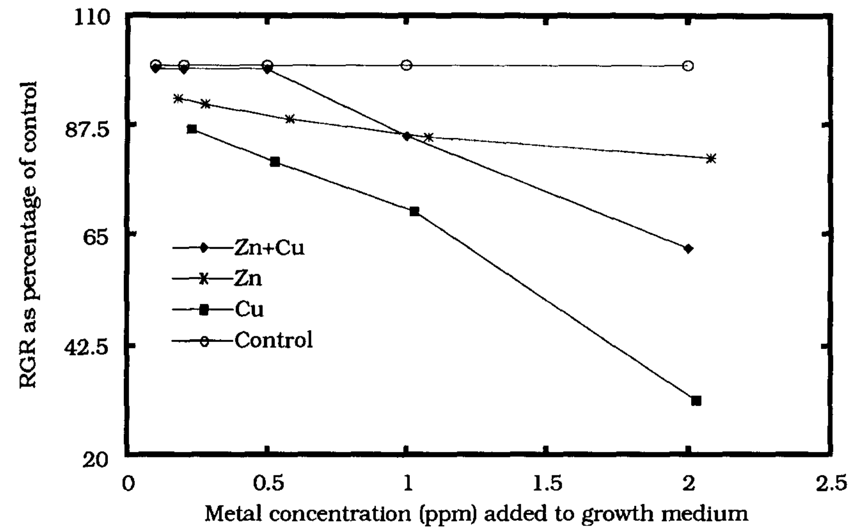


Fig. 1. Effects of Zn and Cu on the relative growth rates of *L. minor*. Each value is the mean of 4-11 independent measurements.

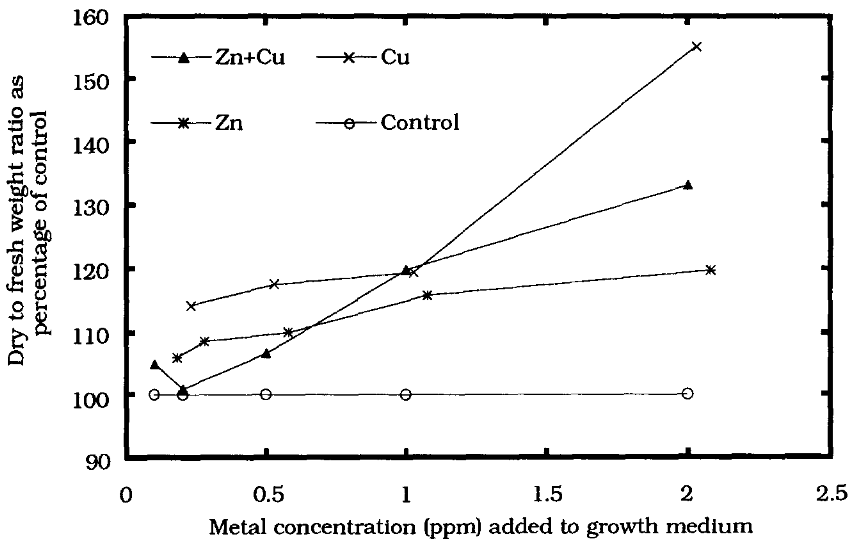


Fig. 2. Effects of Zn and Cu on the dry to fresh weight ratios of *L. minor*. Each value is the mean of 4-9 independent measurements.

According to Fig.1, which represents the relative growth rate (RGR) values of *L.minor* as a percentage of control sets, a monotonic decrease of the growth existed when the corresponding metal concentrations in the medium were increased. Growth yields were reduced from 86.5% (at 0.23 ppm) to 31.2% (at 2.03 ppm) for Cu sets and from 93.1% (at 0.18 ppm) to 80.6% (at 2.08 ppm) for Zn sets. Copper being more toxic than Zn showed a greater effect in decreasing the RGR. When the two metals co-existed at concentrations of 0.10-2.00 ppm, Zn suppressed the inhibitory effect of Cu and, yet, at the 0.10-0.50 ppm levels the co-existence of the two had a stimulating growth effect on *L.minor*. The corresponding RGR values of the Zn-Cu pair changed from 99.3% at 0.10 ppm to 62.1% at 2.00 ppm.

Dry to fresh weight ratio (DFR) values of *L.minor* (Fig.2) showed the expected trend. At 0.10-0.50 ppm of Zn+Cu, the corresponding DFR values were slightly greater than that of Zn alone and lower than that of Cu alone in the growth medium.

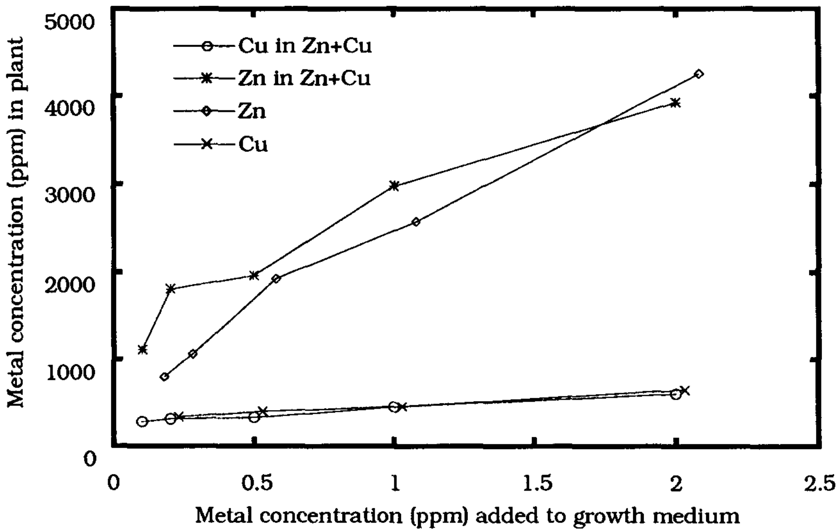


Fig.3. Effects of Zn and Cu on metal accumulation by *L.minor* .Values are mean numbers ±SE. Since SE values are small with respect to axis options, they do not appear on the figure.

Fig.3 represents the results for the metal accumulation studies by *L.minor*. The increased concentrations of Zn and Cu in the medium caused an increase in the corresponding metal concentrations in the fronds. The Zn concentration in the plant was higher than that of the Cu concentration at each concentration level. The presence of Cu in the growth medium increased the accumulation of Zn at the 0.10-1.00 ppm levels; e.g., in a solution containing Zn alone at 1.08 ppm, the accumulation was 2571 ppm and it was increased to 2979 ppm in the presence of 1.00 ppm each of Zn and Cu. Only at 2.00 ppm was the accumulation of Zn decreased by the presence of Cu; most probably because of the fact that the concentration of Zn was insufficient to reduce the toxicity of Cu. On the other hand, the accumulation of Cu was not affected by the presence of Zn; e.g., in a solution containing Cu alone at 1.03 ppm, the accumulation was 458 ppm and in the presence of 1.00 ppm each of Zn and Cu, it was 449 ppm.

Table 1. Relative growth rate (RGR) and the metal accumulation by *L.minor.*

| Conc. (ppm) added to medium | RGR | Conc. (ppm) in fronds | |
|---|---|---|---|
| Cu | | | |
| Control[a] .03 | 314±14.6 (4) | 120±14.6 (5) | |
| 0.20 | 272±11.4 (4) | 331±22.5 (4) | |
| 0.50 | 251±9.04 (4) | 400±49.5 (4) | |
| 1.00 | 219±11.2 (6) | 458±45.6 (4) | |
| 2.00 | 98.2±7.89 (6) | 647±55.0 (4) | |
| Zn | | | |
| Control[a] .08 | 199±15.0 (7) | 199±15.0 (7) | |
| 0.10 | 185±11.4 (7) | 797±80.9 (5) | |
| 0.20 | 183±13.5 (7) | 1064±53.2 (6) | |
| 0.50 | 177±10.6 (7) | 1926±62.4 (7) | |
| 1.00 | 169±60.2 (7) | 2571±88.7 (5) | |
| 2.00 | 161±8.80 (11) | 4254±99.7 (4) | |
| Zn-Cu | | Zn | Cu |
| Control[a] | 207±5.75 (9) | 889±96.2 (5) | 153±16.1 (7) |
| 0.10 | 205±8.28 (9) | 1114±60.7 (6) | 279±13.3 (8) |
| 0.20 | 205±11.8 (8) | 1813±71.0 (5) | 316±25.7 (7) |
| 0.50 | 205±11.4 (8) | 1962±78.4 (8) | 335±12.9 (8) |
| 1.00 | 177±8.39 (9) | 2979±211 (7) | 449±34.4 (7) |
| 2.00 | 128±9.57 (7) | 3923±73.8 (6) | 605±45.6 (7) |

Values are means ± SE. Values in parentheses are the number of replicates for each set. [a]Control samples contain 0.03 ppm of Cu and 0.08 ppm of Zn, respectively; higher concentrations are amounts added to control levels.

Experimental data for the results of growth and metal accumulation studies are presented in Table 1. It can be seen that the growth responses and metal accumulation by *L.minor* were both affected by the metal type and corresponding concentration levels.

Statistical parameters of the experimental results were evaluated by using a factorial model at $\alpha=0.05$ significance level (Willkonson 1987). In this factorial design we tested the significance of metal type and concentration on the RGR and on the concentration of metal accumulated by *L.minor*. These individual effects were called tests of main effects. However, the main concern was whether the metal type and concentration were operating independently of each other or a differential effect occurred when the two were combined. Thus, the significance of joint effect (metal*concentration) which was defined as interaction effect was tested.

For this design we established two different models : model 1 tested the significance of main effects, i.e., metal type and concentration; model 2 tested the significance of main and interaction effects together. Dependent variables were the RGR and concentration of metals accumulated by *L.minor* fronds. Categorical variables and levels of each variable are presented in Table 2.

Table 2. Categorical variables and levels of each variable.

| | Metal type | | |
|---|---|---|---|
| | Zn | Cu | Zn-Cu |
| Conc (ppm) | | | |
| 1 | 0.10 | 0.10 | 0.10 |
| 2 | 0.20 | 0.20 | 0.20 |
| 3 | 0.50 | 0.50 | 0.50 |
| 4 | 1.00 | 1.00 | 1.00 |
| 5 | 2.00 | 2.00 | 2.00 |

The results of the statistical parameters are represented by two-way analysis of variance (ANOVA) Tables 3-6.

Table 3. Two-way ANOVA summary for growth experiment of Zn-Cu pair
Model 2: Growth=constant+metal+concentration.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 2 | 8840.023 | 4420.01 | 120.320 | 0.000 |
| Error | 61 | 13268.426 | 217.515 | | |

According to the first model which tested the significance of the main effects, growth was affected significantly both by the type and concentration of metals added to growth medium (Table 3).

Table 4. Two-way ANOVA summary for growth experiment of Zn-Cu pair
Model 2: Growth=constant+metal+concentration+metal*concentration.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 10528.214 | 3509.405 | 18.813 | 0.000 |
| Metal*Conc | 1 | 1688.192 | 1688.192 | 8.747 | 0.004 |
| Metal | 1 | 1213.329 | 1213.329 | 6.287 | 0.015 |
| Conc | 1 | 0.489 | 0.489 | 0.003 | 0.960 |
| Error | 60 | 11580.234 | 193.004 | | |

The F-values of the second model, which tested the significance of the main and interaction effects together, indicated that the main effect due to metal type was significant, but the main effect due to concentration level was insignificant on the growth of *L.minor* (Table 4).

Table 5. Two-way ANOVA summary for metal accumulation experiment
Model 1: Accumulation=constant+metal+concentration.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Zn, Zn-Cu | | | | | |
| Regression | 2 | 0.4759E+08 | 0.2379E+08 | 247.827 | 0.000 |
| Error | 46 | 4416609.694 | 96013.254 | | |
| Cu, Zn-Cu | | | | | |
| Regression | 2 | 535585.336 | 267792.668 | 123.511 | 0.000 |
| Error | 40 | 86726.973 | 2168.174 | | |

Table 6. Two-way ANOVA summary for metal accumulation experiment
Model 2:Accumulation=constant+metal+concentration+metal*concentration.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Zn, Zn-Cu | | | | | |
| Regression | 3 | 0.4922E+08 | 0.1634E+08 | 246.443 | 0.000 |
| Metal*Conc | 1 | 1432819.963 | 1432819.963 | 21.609 | 0.000 |
| Metal | 1 | 1974326.774 | 1974326.774 | 29.776 | 0.000 |
| Conc | 1 | 0.1059E+08 | 0.1059E+08 | 159.785 | 0.000 |
| Error | 45 | 2983789.730 | 66306.438 | | |
| Cu, Zn-Cu | | | | | |
| Regression | 3 | 535585.754 | 178528.585 | 80.282 | 0.000 |
| Metal*Conc | 1 | 0.417 | 0.417 | 0.000 | 0.989 |

The F-values of Table 5 indicated that the metal accumulation by *L.minor* was affected by the main effects, i.e., metal type and concentration. The F-values of Table 6 showed that the interaction effect of Cu and concentration was also significant on metal accumulation. Since there was no interaction effect of Zn and concentration, the influence of the main efffects due to metal type and concentration were not analyzed (Table 6).

The experimental results tested by the factorial model for Zn-Cu pair at 0.10-2.00 ppm levels with a constant pH value showed that the interaction effect of metal type and concentration was not as significant as the individual main effects on growth and metal accumulation by *L.minor*. Thus, the metal type and concentration operated independently of each other.

Acknowledgements. This research was supported by the Boğaziçi University Research Fund ( Project no: 90B0522 ). The authors thank Dr. Nilsun Ince for her help with the statistical calculations.

**REFERENCES**

Culley DD, Rejmankova E, Kvet J, Frye JB (1981) Chemical quality and use of duckweeds (Lemnaceae) in aquaculture, waste management, and animal feeds. J World Maricul Soc 12 : 27-49

Ericsson T, Larsson CM, Tillberg E (1982) Growth responses of *Lemna* to different levels of nitrogen limitation. Z Pflanzenphysiol 105: 331-340

Filbin GJ, Hough RA (1979) The effects of excess copper sulfate on the metabolism of the duckweed *Lemna minor.* Aqua Bot 7:79-86

Hillman WS 1961) The Lemnacea, or duckweeds, a review of the descriptive and experimental literature. Bot Rev 27: 221-287

Hutchinson TC, Czyrska H (1975) Heavy metal toxicity and synergism to floating aquatic weeds. Verh Internat Verein Limnol 19: 2102-2111

Jain SK, Vasudevan P, Jha NK (1989) Removal of some heavy metals from polluted water by aquatic plants : studies on duckweed and water velvet. Biol Wastes 28 : 115-126

Kunkel R, Manahan SE (1973) Atomic absorption analysis of strong heavy metal chelating agents in water and wastewater. Anal Chem 45 : 1465-1468

McLay CL (1976) The effect of pH on the population growth of three duckweeds: *Spirodela oligorrhiza, Lemna minor and Wolffia arrhiza* Freshwat Biol 6 : 125-136

Mo SC, Choi DS, Robinson JW (1988) A study of the uptake by duckweed of aluminum, copper and lead from aqueous solution. J Environ Sci Health (A) 23 : 139-156

Nasu Y, Kugimoto M (1981) *Lemna* (duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna paucicostata* to heavy metals. Bull Environ Contam Toxicol 10 : 159-169

Nasu Y, Kugimoto M, Tanaka O, Takimoto A (1983) Comparative studies on the absorption of cadmium and copper in *Lemna paucicostata* . Environ Pollut Series A 33 : 201-209

Nasu Y, Kugimoto, M, Tanaka O, Yanase D, Takimoto A (1984) Effects of cadmium and copper co-existing in the medium on the growth and flowering of *Lemna paucicostata* in relation to their absorption. Environ Pollut Series A 33 : 267-274

O'Brien WJ (1981) Use of aquatic macrophytes for wastewater treatment. ASCE 107(EE4) : 681-698

Taraldsen JA, Norberg-King TJ (1990) New method for determining effluent toxicity using duckweed ( *Lemna minor* ). Environ Toxicol Chem 9 : 761-767

Wang W (1986) Toxicity tests of aquatic pollutants by using common duckweed. Environ Pollut Series B 11 : 1-14

Willkonson L (1987) SYSTAT : The system for statistics. Evanston, IL: SYSTAT, Inc.