Bull. Environ. Contam. Toxicol. (1991) 46:466–472

Archives of Environmental Contamination and Toxicology

Chemical Speciation and Bioavailability of Cu(II). Study of the Ionic Copper(II) and Bis(Glycinate)–Copper(II) Accumulation by *Lemna* Species

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The mechanism of accumulation of d-metals by aquatic plants, including the mechanism of toxic effects of the metals, is a topic of current interest (Stauber and Florence 1987). In the case of copper(II), these properties are to a great extent related to the ability of the metal to form various complex species. In water, these metallic species comprise particularly coordination compounds with the organic ligands present and these are involved in equilibrium systems containing also various species of hydrated Cu^{2+} ions, depending on the acid-base conditions. Uptake of these substances by algae and some macrophytes such as duckweed (Lemna species) is primarly controlled by the thermodynamic and kinetic parameters of the bonding interactions of the metal ions and complex species with the cell wall (Florence et al. 1983, Gavis 1983). This implies that uptake is also dependent on the thermodynamic properties of these "intermediates" and, hence, on the concentration of the "free" metal ions in the medium. The kinetics of their formation will be controlled by the nucleophilic properties of the ligands in the cell wall with respect to the "free" metal ions in the medium. The direct interaction of the complex species of metal elements present in the surrounding aquatic medium then will include substitution reactions of the ligands (functional groups) of the cell wall with the ligands of the free complex species. This process is governed by the

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thermodynamic stability of the initial and formed complex species and by the kinetics of the processes, which will also be highly dependent on their stereochemistry. Furthermore, direct diffusion of some of the complex species also takes place. If these species are toxic, the manifestation of their toxicity is related to their degradation inside the cell; the ligand can exert toxic effects as well. All this suggests that the resulting effect characterized as accumulation of metal ions by floating aquatic plants such as duckweed, or more generally, the toxic effect, cannot be treated in terms of simplified concepts such as that claiming that the so-called ionic species are more toxic than species referred to as complex (Magnuson et al.1979). In the case studied, we examined the accumulation of copper(II) in, and its toxic effect on, duckweed, a plant which exhibits extremely high concentration factors (as high as 28 500) (Hutchinson and Cryrska 1974). The effect of copper(II) was investigated by adding it to the "minimal" medium in two forms : $CuSO_4$ and $[Cu(Gly)_2]$.

The neutral (2:1) tetracoordinated bis(glycinate)-copper(II) complex is constituted by two five-membered rings bonded to the central copper atom with the cis configuration (Tomita and Nitta 1961). Its stability constants are log K_1 = 8.3, log K_2 = 6.9 (Chukwumerije and Nash 1987). This complex was chosen to model the function of a neutral species (eliminating the charge effect) involving a nontoxic ligand, for which - in contrast to the hydrated Cu^{2+} species - direct permeation through the cell wall is conceivable.

MATERIALS AND METHODS

For two series of experiments, duckweed was sampled in early June and in early September on an one pond which was out of direct reach of agricultural activity and whose water was not excessively burdened by inputs of transition or heavy metals (Na = 5.3, K = 1.8, Mg = 3.4, Ca =12.5, Fe= 0.17, Al = 0.006, Cu = 0.001, Mn = 0.034, Zn = 0.009, Pb < 0.001, Cd < 0.001 $mg.l^{-1}$). Immediately after transportation, the samples were

multiply washed with cultivation medium, whose composition was as follows (mg.l^{-1}) : NH_4NO_3 200, KH_2PO_4 100, $MgSO_4.7H_2O$ 101, $CaCl_2$ 111, $FeCl_3$ 2.6. The plants were then formed into a layer on the medium level (0.5 m^2 area) in a 10 l vessel, and placed in a thermostatically controlled (18°C) box fitted with artificial light (Philips TL 40 W, 6 400 lux) with 12 h - 12 h light - dark cycles. The medium was circulated and spent medium was replaced with fresh one so that the entire volume of 10 l had been completely replaced before adding copper. After nine days, 1 M solution of copper(II) was added (3 tanks of $CuSO_4$ and 3 tanks of $[Cu(Gly)_2]$); the Cu(II) solution was pumped gradually so that it was homogenized in the medium as rapidly as possible while preventing a direct contact of the concentrated copper(II) solution with the plants. The experiments with the different copper concentrations (10-100 mg.l^{-1}) were invariably conducted for a standard period of 3 days.

The total content of copper added, $CuSO_4$ or $[Cu(Gly)_2]$, was determined by flame atomic absorption spectrometry, and the concentration of the so-called ionic form determined by means of an ion selective electrode (Cu-ISE; Crytur, Czechoslovakia) in 0.1 M $NaClO_4$, if this concentration was above the detection limit of this technique.

For the determination of copper in duckweed after the experiment, the plants were rinsed with water, dilute HNO_3 (pH 4.2) and water again.

Lyophilized samples of the plant (0.5 g) were mineralized with a mixture of concentrated H_2SO_4 and $HClO_4$ (4 : 1) in 15 ml pressure vessels at 120°C for 1 h and, subsequently, at 150°C for 4 h. Copper was determined on an IL 457 flame atomic absorption spectrometer.

Cultivation medium was prepared from chemicals of reagent grade purity and deionized water.

$[Cu(Gly)_2].H_2O$ was synthesized by reacting $Cu(OH)_2$, prepared in situ, with glycine (Tomita and Nitta 1961) and characterized by elemental analysis.

RESULTS AND DISCUSSION

The results given in Table 1 indicate that over the region of copper concentrations in the medium of 10 - 100 $mg.l^{-1}$, the copper content of the duckweed biomass is a roughly linear function of this concentration (in otherwise comparable conditions). In the design of the experiment, stress was paid particularly to standardization to enable the effects of the "ionic"and complex species to be compared; therefore, neither the effect of the physico-chemical and physiological parameters nor the process dynamics or kinetics were investigated.

The effect of $[Cu(Gly)_2]$ is substantially different from that of "Cu^{2+}" ($CuSO_4$). The established dependence of accumulation on the "external" concentration of copper(II), determined from triple repetitions always of three parallel experiments and with samples taken in two seasons of the year, demonstrates that for $[Cu(Gly)_2]$ the copper content of the plants is no unique function of the copper(II) concentration in the medium. The fact that increased concentration of $[Cu(Gly)_2]$ is not associated with increased accumulation indicates that in this case the copper intake is not related to the "Cu^{2+}" released by dissociation of the complex species. Very likely, $[Cu(Gly)_2]$ is taken in directly, and its toxicity only manifests itself after its degradation in the cell. The dependence of accumulation on the concentration of Cu(II) in the medium in the"Cu^{2+}"($CuSO_4$) form (Table 1, Fig. 1), warrants the concept of the "nonmetabolic" diffusion nature of the accumulation of the "ionic" form, which is controlled by the medium-cell concentration gradient. On the other hand, the appreciably high accumulation of copper in the form of $[Cu(Gly)_2]$ at a copper concentration of 10 $mg.l^{-1}$ in the medium (Table 1) is indicative of a different

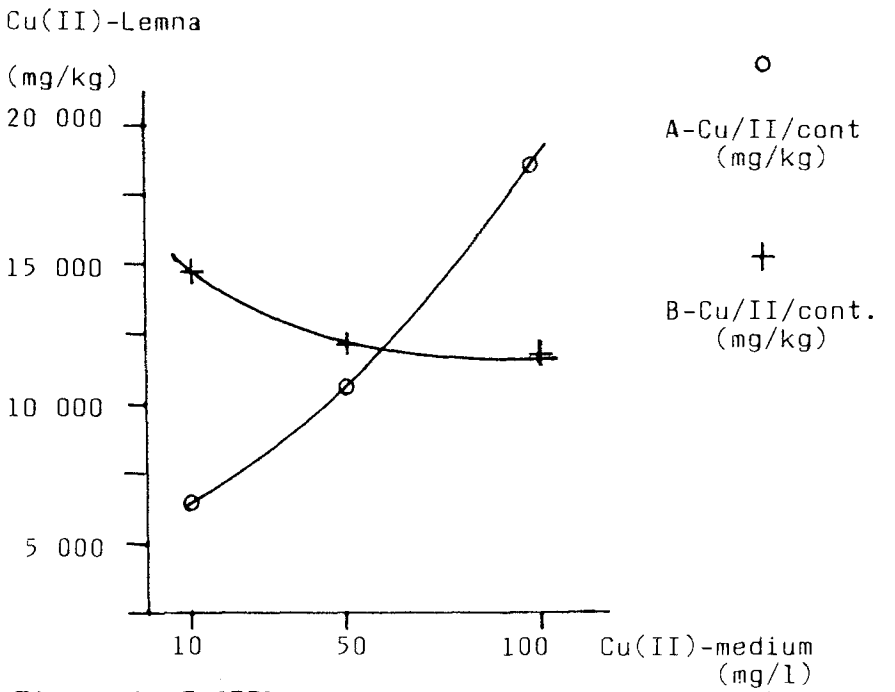

Figure 1. Cu(II) - accumulation in Lemna species.
A - $CuSO_4$ B - $[Cu(Gly)_2]$

Table 1. Cu(II) accumulation by duckweed (Lemna species) as a function of the chemical form of Cu(II) in the cultivation medium.

Total conc. of Cu(II) in the medium. ($mg.l^{-1}$)	$CuSO_4$ Average cont. of Cu(II) in the lyophil. sample. ($mg.kg^{-1}$)	$[Cu(Gly)_2]$ Average cont. of Cu(II) in the lyophil. sample. ($mg.kg^{-1}$)
10	6600 + 520	14900 + 890
50	10600 + 640	12200 + 860
100	18500 + 1110	11800 + 820

intake mechanism, whose transport section probably includes direct diffusion of $[Cu(Gly)_2]$. Owing to its zero charge and high thermodynamic stability, it does not enter into substantial interactions with the cell wall, in contrast to "Cu^{2+}". The dependence of the accumulation on the concentration of $[Cu(Gly)_2]$ in the medium suggests that rather than diffusion, the degradation process inside the cell is the controlling phenomenon. This, on the one hand,renders the resulting effect (accumulation) more intense at lower copper concentrations (10 $mg.l^{-1}$); on the other hand, the toxic effect is also enhanced, and this acts in the opposite way. Namely, it prevents the resulting accumulation from increasing with increasing concentration of $[Cu(Gly)_2]$ in the medium. This concept is borne out by the fact that the copper content of the biomass of dead as well as living plants was lower for $[Cu(Gly)_2]$ than for "Cu^{2+}"($CuSO_4$) (Benda et al.).

Acknowledgments. The authors wish to thank Dr.J.Květ, Botanical Institute in Třeboň, and Dr.V.Říha, Institute of Landscape Ecology in České Budějovice, for stimulating comments on the experimental part of this work, and Mrs J.Marková and Mr P.Láznička, Institute of Landscape Ecology, for technical assistence.

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Received June 26, 1990; accepted September 13, 1990.